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(54) Title: COMPOSITIONS AND METHODS USEFUL FOR REDUCING THE VISCOSITY OF PROTEIN-CONTAINING FORMULATIONS

(57) Abstract: The invention relates to use of certain compounds including, for example, certain charged amino acids and structural analogs thereof, for reducing the viscosity of aqueous protein-containing formulations. Associated compositions of matter and methods of use are also contemplated within the present invention.

COMPOSITIONS AND METHODS USEFUL FOR REDUCING THE VISCOSITY OF PROTEIN-CONTAINING FORMULATIONS

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application Serial No. 61/330,689, filed on May 3, 2010, which application is fully incorporated herein by reference.

FIELD OF THE INVENTION

The invention relates to use of certain compounds including, for example, certain charged amino acids and structural analogs thereof, for reducing the viscosity of aqueous protein-containing formulations. Associated compositions of matter and methods of use are also contemplated within the present invention.

BACKGROUND OF THE INVENTION

Protein-based therapy (including antibody-based therapy) is usually administered on a regular basis and requires several mg/kg dosing by injection. Subcutaneous injection is a typical route of administration of these therapies. Because of the small volumes used for subcutaneous injection (usually 1.0 ml- 1.2 ml), for high dose antibody therapies, this route of administration requires the creation of high concentration protein formulations (e.g., 50 mg/ml - 300 mg/ml).

The creation of highly concentrated protein formulations, however, pose challenges relating to the physical and chemical stability of the protein, and difficulty with manufacture, storage, and delivery of the protein formulation. One problem is the tendency of proteins to form particulates during processing and/or storage, which make manipulation during further processing difficult. To attempt to obviate this problem, surfactants and/or sugars have been added to protein formulations. Although surfactants and sugars may reduce the degree of particulate formation of proteins, they do not address another problem associated with manipulating and administering concentrated protein formulations, i.e., increased viscosity. In fact, sugars may enhance the intermolecular interactions within a protein or between proteins, or may create interactions between sugar molecules, and increase the viscosity of the protein formulation.

Increased viscosity of protein formulations has negative ramifications from processing through drug delivery to the patient. Various attempts have been made to study the effect of viscosity-reducing agents on highly concentrated aqueous protein-containing formulations (e.g., see US Patent No. 6,875,432). Notwithstanding these attempts, there is a continued need in the art to identify novel protein viscosity reducing agents and to employ those agents for the generation of relatively high concentration protein formulations with suitably low viscosities that are suitable for manufacture, storage, and therapeutic, particularly subcutaneous, administration.

SUMMARY OF THE INVENTION

The present invention is based upon the novel finding that certain molecules, including certain charged amino acids and derivitives, precursors or structural analogs thereof, are useful as additives to protein-containing formulations for the purpose of reducing the viscosity of those formulations in aqueous form.

Accordingly, in one aspect, the invention relates to a composition of matter comprising a protein and a compound that is capable of reducing the viscosity of an aqueous formulation comprising said protein. In one embodiment, the protein is an antibody. In another embodiment, the compound that is capable of reducing the viscosity of an aqueous formulation comprising said protein is selected from the group consisting of arginine (either arginine-HCl or arginine in the presence of a succinate counterion, e.g., arginine succinate), arginine dipeptide, arginine tripeptide, polyarginine, homoarginine, 2-amino-3-guanidino-propionic acid, guanidine, ornithine, agmatine, guanidobutyric acid, urea, citrulline, N-hydroxy-L-nor-arginine, nitroarginine methyl ester, argininamide, arginine methyl ester, arginine ethyl ester, lysine, lysinamide, lysine methyl ester, histidine, histidine methyl ester, histamine, alanine, alaninamide, alanine methyl ester, putrescine, cadaverine, spermidine, spermine, and methionine. Such compounds may be present in the formulation at a concentration which is at least 10 mM, preferably at least 20 mM, more preferably at least 50 mM, yet more preferably at least 100 mM, yet more preferably at a concentration between about 10 mM and 1 M. The composition may be in either aqueous or lyophilized form. In aqueous form, the composition of matter may have a viscosity of no greater than about 150 cP, preferably no greater than about 120 cP, preferably no greater than about 100 cP, preferably no greater than about 90 cP, preferably no greater than about 80 cP, preferably no greater than about 70 cP, preferably

no greater than about 60 cP, preferably no greater than about 50 cP, preferably no greater than about 40 cP. Total protein concentration present in the composition of matter is at least 50 mg/ml, preferably at least 75 mg/ml, more preferably at least 100 mg/ml, more preferably at least 150 mg/ml, more preferably at least 200 mg/ml, more preferably at least 250 mg/ml, more preferably at least 300 mg/ml.

Another aspect of the present invention is directed to an article of manufacture comprising a container holding any of the herein described compositions of matter.

In another aspect, a method is provided for reducing the viscosity of a protein-containing formulation, wherein the method comprises the step of adding to the formulation a viscosity reducing amount of a compound that is capable of reducing the viscosity of an aqueous formulation comprising said protein. In one embodiment, the protein is an antibody. In another embodiment, the compound that is capable of reducing the viscosity of an aqueous formulation comprising said protein is selected from the group consisting of arginine (either arginine-HCl or arginine in the presence of a succinate counterion, e.g., arginine succinate), arginine dipeptide, arginine tripeptide, polyarginine, homoarginine, 2-amino-3-guanidino-propionic acid, guanidine, ornithine, agmatine, guanidobutyric acid, urea, citrulline, N-hydroxy-L-nor-arginine, nitroarginine methyl ester, argininamide, arginine methyl ester, arginine ethyl ester, lysine, lysinamide, lysine methyl ester, histidine, histidine methyl ester, histamine, alanine, alaninamide, alanine methyl ester, putrescine, cadaverine, spermidine, spermine, and methionine. Such compounds may be added to the formulation to reach a final concentration which is at least 10 mM, preferably at least 20 mM, more preferably at least 50 mM, yet more preferably at least 100 mM, yet more preferably at a concentration between about 10 mM and 1 M. In one embodiment, the method further comprises the step of lyophilizing the formulation after the compound that is capable of reducing the viscosity of an aqueous formulation comprising said protein is added. In aqueous form, the formulation may have a viscosity of no greater than about 150 cP, preferably no greater than about 120 cP, preferably no greater than about 100 cP, preferably no greater than about 90 cP, preferably no greater than about 80 cP, preferably no greater than about 70 cP, preferably no greater than about 60 cP, preferably no greater than about 50 cP, preferably no greater than about 40 cP. Total protein concentration present in the formulation is at least 50 mg/ml, preferably at least 75 mg/ml, more preferably at least 100 mg/ml, more preferably

at least 150 mg/ml, more preferably at least 200 mg/ml, more preferably at least 250 mg/ml, more preferably at least 300 mg/ml.

In yet another aspect, a method is provided for preparing an aqueous protein-containing formulation, wherein the method comprises the step of adding to the formulation a viscosity reducing amount of a compound that is capable of reducing the viscosity of an aqueous formulation comprising said protein. In one embodiment, the protein is an antibody. In another embodiment, the compound that is capable of reducing the viscosity of an aqueous formulation comprising said protein is selected from the group consisting of arginine (either arginine-HCl or arginine in the presence of a succinate counterion, e.g., arginine succinate), arginine dipeptide, arginine tripeptide, polyarginine, homoarginine, 2-amino-3-guanidino-propionic acid, guanidine, ornithine, agmatine, guanidobutyric acid, urea, citrulline, N-hydroxy-L-nor-arginine, nitroarginine methyl ester, argininamide, arginine methyl ester, arginine ethyl ester, lysine, lysinamide, lysine methyl ester, histidine, histidine methyl ester, histamine, alanine, alaninamide, alanine methyl ester, putrescine, cadaverine, spermidine, spermine, and methionine. Such compounds may be added to the formulation to reach a final concentration which is at least 10 mM, preferably at least 20 mM, more preferably at least 50 mM, yet more preferably at least 100 mM, yet more preferably at a concentration between about 10 mM and 1 M. In aqueous form, the formulation may have a viscosity of no greater than about 150 cP, preferably no greater than about 120 cP, preferably no greater than about 100 cP, preferably no greater than about 90 cP, preferably no greater than about 80 cP, preferably no greater than about 70 cP, preferably no greater than about 60 cP, preferably no greater than about 50 cP, preferably no greater than about 40 cP. Total protein concentration present in the formulation is at least 50 mg/ml, preferably at least 75 mg/ml, more preferably at least 100 mg/ml, more preferably at least 150 mg/ml, more preferably at least 200 mg/ml, more preferably at least 250 mg/ml, more preferably at least 300 mg/ml.

Other embodiments will become apparent upon reading this patent specification.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

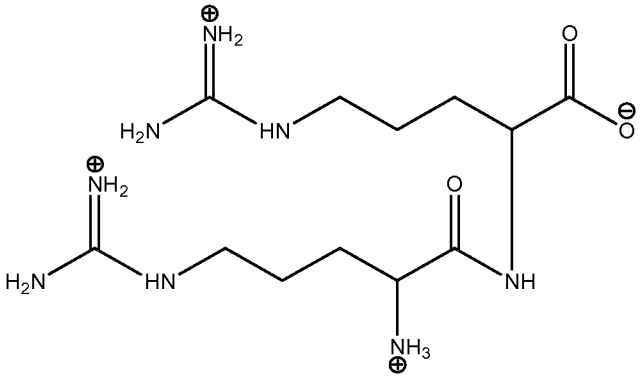
The present invention may be understood more readily by reference to the following detailed description of specific embodiments and the Examples included therein.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this

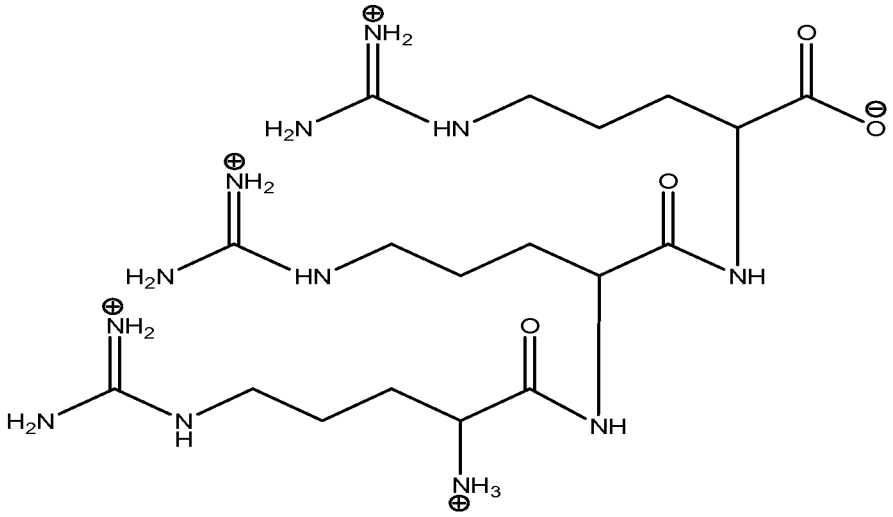
invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference in their entirety.

The present invention is based upon the novel finding that certain compounds including, for example, certain charged amino acids and structural analogs thereof, for reducing the viscosity of aqueous protein-containing formulations. Accordingly, in one aspect, the present invention describes compositions of matter comprising a protein and a compound capable of reducing the viscosity of an aqueous formulation comprising the protein. In certain embodiments, compounds identified herein as being capable of reducing the viscosity of an aqueous formulation comprising a protein include, for example:

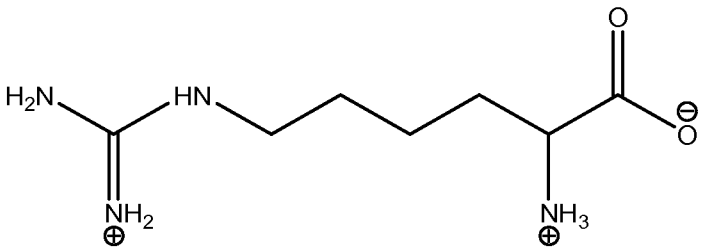
arginine dipeptide



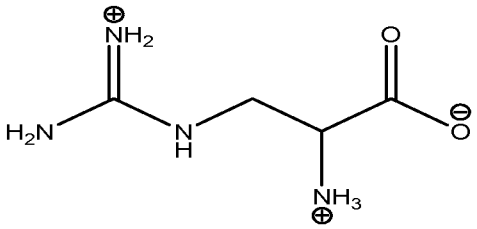
arginine tripeptide



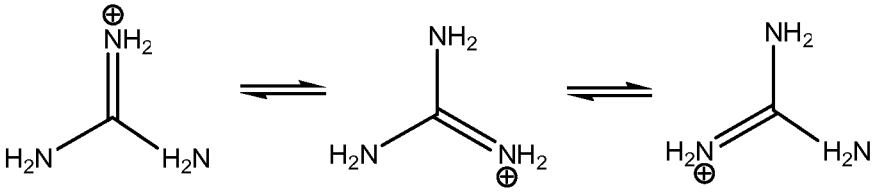
homoarginine



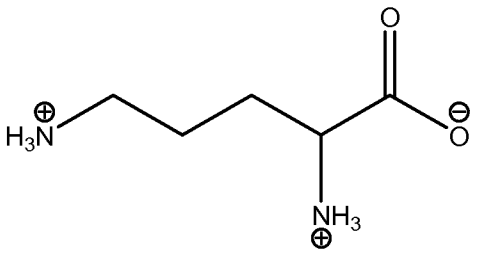
2-amino-3-guanidino-propionic acid



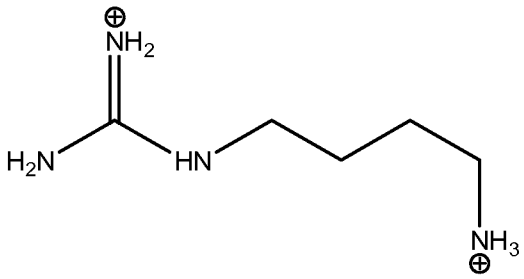
guanidine



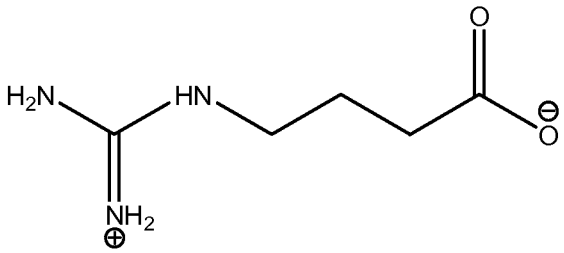
ornithine



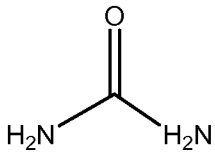
agmatine



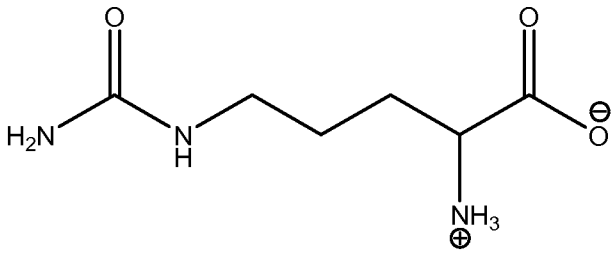
guanidobutyric acid



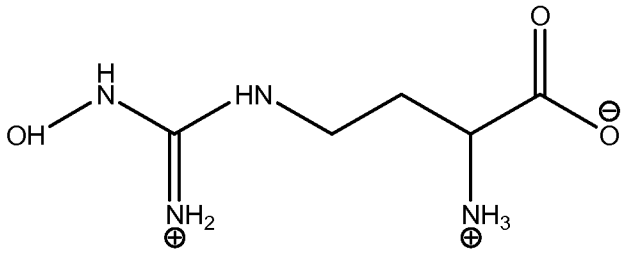
urea



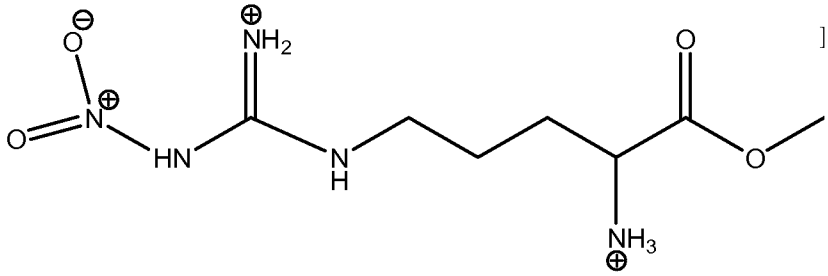
citrulline



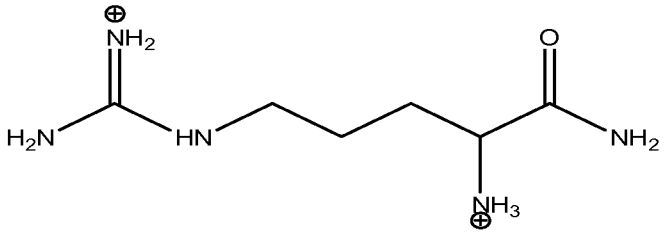
N-hydroxy-L-nor-arginine



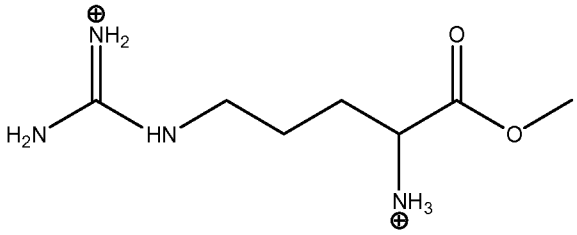
nitroarginine methyl ester



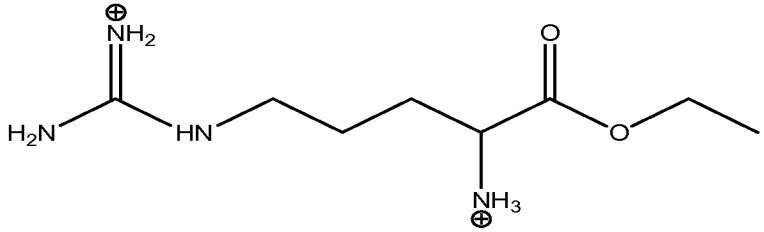
argininamide



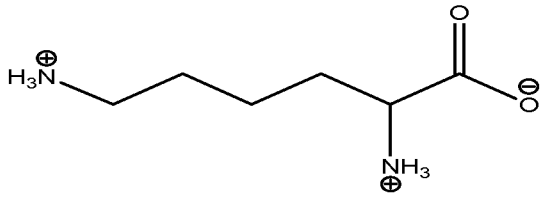
arginine methyl ester



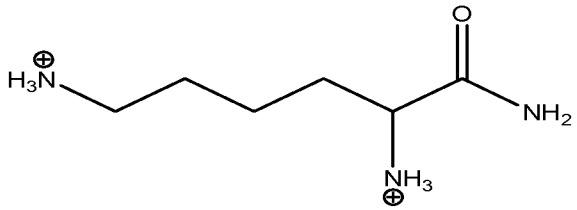
arginine ethyl ester



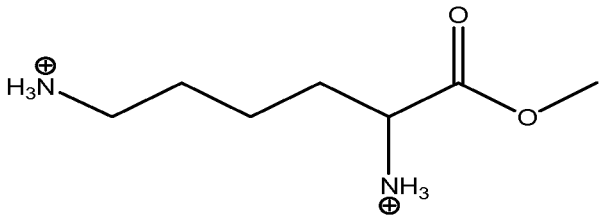
lysine



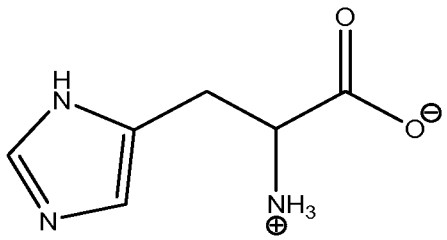
lysinamide



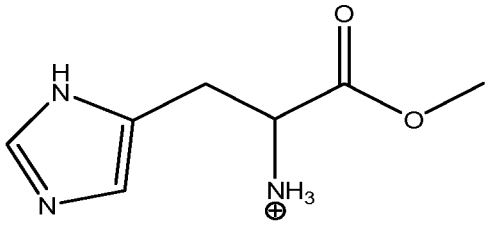
lysine methyl ester



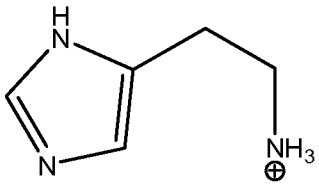
histidine



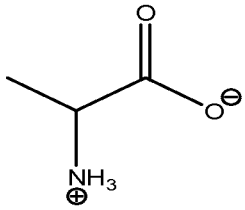
histidine methyl ester



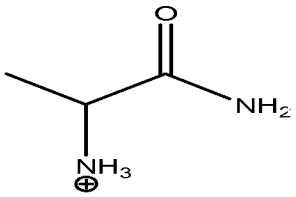
histamine



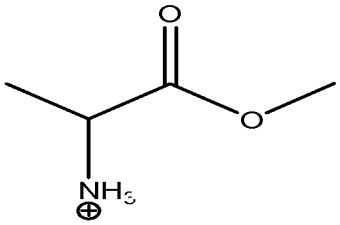
alanine



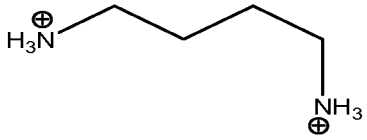
alaninamide



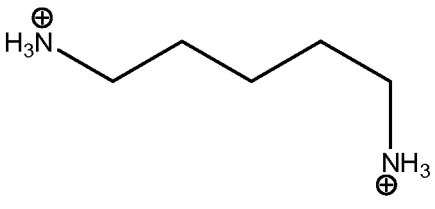
alanine methyl ester



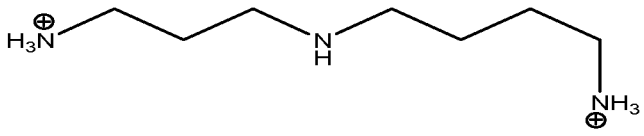
putrescine



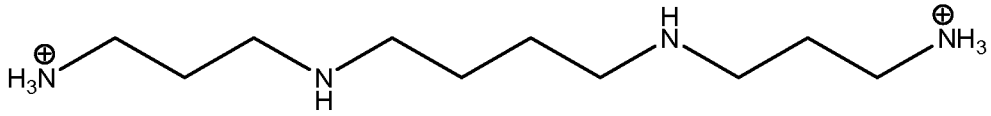
cadaverine



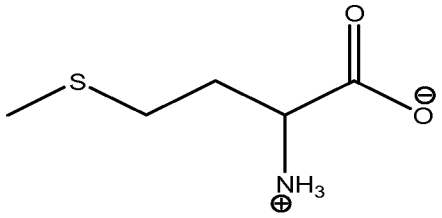
spermidine



spermine



methionine



The above described compounds may be employed singly as a viscosity reducing agent, or may be employed in combination with other viscosity reducing agents. Such compounds may be added to the protein-containing formulation to reach a final concentration (either singly or in combination) which is at least 10 mM, preferably at least 20 mM, more preferably at least 50 mM, yet more preferably at least 100 mM, yet more preferably at a concentration between about 10 mM and 1 M.

Generally, the viscosity reducing agents of the present invention find use in reducing the viscosity of protein-containing formulations, wherein the protein concentration in the formulation is at least about 50 mg/ml, preferably at least 75 mg/ml, more preferably at least 100 mg/ml, more preferably at least 150 mg/ml, more preferably at least 200 mg/ml, more preferably at least 250 mg/ml, more preferably at least 300 mg/ml.

In aqueous form, the protein-containing formulation (after addition of the compound capable of reducing the viscosity of an aqueous protein-containing

formulation) may have a viscosity of no greater than about 150 cP, preferably no greater than about 120 cP, preferably no greater than about 100 cP, preferably no greater than about 90 cP, preferably no greater than about 80 cP, preferably no greater than about 70 cP, preferably no greater than about 60 cP, preferably no greater than about 50 cP, preferably no greater than about 40 cP.

By "polypeptide" or "protein" is meant a sequence of amino acids for which the chain length is sufficient to produce the higher levels of tertiary and/or quaternary structure. Thus, proteins are distinguished from "peptides" which are also amino acid-based molecules that do not have such structure. Typically, a protein for use herein will have a molecular weight of at least about 5-20 kD, alternatively at least about 15-20 kD, preferably at least about 20 kD. "Peptide" is meant a sequence of amino acids that generally does not exhibit a higher level of tertiary and/or quaternary structure. Peptides generally have a molecular weight of less than about 5 kD.

Examples of polypeptides encompassed within the definition herein include mammalian proteins, such as, *e.g.*, renin; a growth hormone, including human growth hormone and bovine growth hormone; growth hormone releasing factor; parathyroid hormone; thyroid stimulating hormone; lipoproteins; alpha-1-antitrypsin; insulin A-chain; insulin B-chain; proinsulin; follicle stimulating hormone; calcitonin; luteinizing hormone; glucagon; clotting factors such as factor VIIIC, factor IX, tissue factor, and von Willebrands factor; anti-clotting factors such as Protein C; atrial natriuretic factor; lung surfactant; a plasminogen activator, such as urokinase or human urine or tissue-type plasminogen activator (t-PA); bombesin; thrombin; hemopoietic growth factor; tumor necrosis factor-alpha and -beta; enkephalinase; RANTES (regulated on activation normally T-cell expressed and secreted); human macrophage inflammatory protein (MIP-1-alpha); a serum albumin such as human serum albumin; Muellerian-inhibiting substance; relaxin A-chain; relaxin B-chain; prorelaxin; mouse gonadotropin-associated peptide; a microbial protein, such as beta-lactamase; DNase; IgE; a cytotoxic T-lymphocyte associated antigen (CTLA), such as CTLA-4; inhibin; activin; vascular endothelial growth factor (VEGF); receptors for hormones or growth factors; protein A or D; rheumatoid factors; a neurotrophic factor such as bone-derived neurotrophic factor (BDNF), neurotrophin-3, -4, -5, or -6 (NT-3, NT-4, NT-5, or NT-6), or a nerve growth factor such as NGF-β; platelet-derived growth factor (PDGF); fibroblast growth factor such as aFGF and bFGF; epidermal growth factor (EGF); transforming growth factor

(TGF) such as TGF-alpha and TGF-beta, including TGF-βI, TGF-p2, TGF-p3, TGF-p4, or TGF-β5; insulin-like growth factor-I and -II (IGF-I and IGF-II); des(l-3)-IGF-I (brain IGF-I), insulin-like growth factor binding proteins (IGFBPs); CD proteins such as CD3, CD4, CD8, CD19 and CD20; erythropoietin; osteoinductive factors; immunotoxins; a bone morphogenetic protein (BMP); an interferon such as interferon-alpha, -beta, and -gamma; colony stimulating factors (CSFs), *e.g.,* M-CSF, GM-CSF, and G-CSF; interleukins (ILs), *e.g.,* IL-1 to IL-10; superoxide dismutase; T-cell receptors; surface membrane proteins; decay accelerating factor; viral antigen such as, for example, a portion of the AIDS envelope; transport proteins; homing receptors; addressins; regulatory proteins; integrins such as CD11a, CDlib, CD11c, CD18, an ICAM, VLA-4 and VCAM; a tumor associated antigen such as CA125 (ovarian cancer antigen) or HER2, HER3 or HER4 receptor; immunoadhesins; and fragments and/or variants of any of the above-listed proteins as well as antibodies, including antibody fragments, binding to any of the above-listed proteins.

The protein which is formulated is preferably essentially pure and desirably essentially homogeneous *(i.e.,* free from contaminating proteins). "Essentially pure" protein means a composition comprising at least about 90% by weight of the protein, based on total weight of the composition, preferably at least about 95% by weight. "Essentially homogeneous" protein means a composition comprising at least about 99% by weight of protein, based on total weight of the composition.

In certain embodiments, the protein is an antibody. The antibody herein is directed against an "antigen" of interest. Preferably, the antigen is a biologically important protein and administration of the antibody to a mammal suffering from a disease or disorder can result in a therapeutic benefit in that mammal. However, antibodies directed against non-protein antigens (such as tumor-associated glycolipid antigens; see US Patent 5,091,178) are also contemplated. Where the antigen is a protein, it may be a transmembrane molecule *(e.g.,* receptor) or ligand such as a growth factor. Exemplary antigens include those proteins discussed above. Preferred molecular targets for antibodies encompassed by the present invention include CD polypeptides such as CD3, CD4, CD8, CD19, CD20 and CD34; members of the HER receptor family such as the EGF receptor (HER1), HER2, HER3 or HER4 receptor; cell adhesion molecules such as LFA-1, Macl, pl50,95, VLA-4, ICAM-1, VCAM and av/b3 integrin including either a or b subunits thereof *(e.g.,* anti-CD11a, anti-CD18 or anti-CDlib antibodies); growth factors such as VEGF; IgE;

blood group antigens; flk2/flt3 receptor; obesity (OB) receptor; *mpl* receptor; CTLA-4; polypeptide C etc. Soluble antigens or fragments thereof, optionally conjugated to other molecules, can be used as immunogens for generating antibodies. For transmembrane molecules, such as receptors, fragments of these *{e.g.,* the extracellular domain of a receptor) can be used as the immunogen. Alternatively, cells expressing the transmembrane molecule can be used as the immunogen. Such cells can be derived from a natural source *{e.g.,* cancer cell lines) or may be cells which have been transformed by recombinant techniques to express the transmembrane molecule.

Examples of antibodies to be purified herein include, but are not limited to: HER2 antibodies including trastuzumab (HERCEPTIN®) (Carter *et al, Proc. Natl. Acad. Sci. USA,* 89:4285-4289 (1992), U.S. Patent No. 5,725,856) and pertuzumab **(OMNITAPvG™)** (WOO 1/00245); CD20 antibodies (see below); IL-8 antibodies (St John *et al, Chest,* 103:932 (1993), and International Publication No. WO 95/23865); VEGF or VEGF receptor antibodies including humanized and/or affinity matured VEGF antibodies such as the humanized VEGF antibody huA4.6.1 bevacizumab (AVASTIN®) and ranibizumab (LUCENTIS®) (Kim *et al, Growth Factors,* 7:53-64 (1992), International Publication No. WO 96/30046, and WO 98/45331, published October 15, 1998); PSCA antibodies (WOO 1/40309); CDl la antibodies including efalizumab (RAPTIVA®) (US Patent No. 6,037,454, US Patent No. 5,622,700, WO 98/23761, Stoppa *et al, Transplant Intl.* 4:3-7 (1991), and Hourmant *et al, Transplantation* 58:377-380 (1994)); antibodies that bind IgE including omalizumab (XOLAIR®) (Presta *et al, J. Immunol.* 151:2623-2632 (1993), and International Publication No. WO 95/19181;US Patent No. 5,714,338, issued February 3, 1998 or US Patent No. 5,091,313, issued February 25, 1992, WO 93/04173 published March 4, 1993, or International Application No. PCT/US98/13410 filed June 30, 1998, US Patent No. 5,714,338); CD18 antibodies (US Patent No. 5,622,700, issued April 22, 1997, or as in WO 97/26912, published July 31, 1997); Apo-2 receptor antibody antibodies (WO 98/51793 published November 19, 1998); Tissue Factor (TF) antibodies (European Patent No. 0 420 937 Bl granted November 9, 1994); α_4-α_7 integrin antibodies (WO 98/06248 published February 19, 1998); EGFR antibodies *{e-g-* > chimerized or humanized 225 antibody, cetuximab, ERBUTIX ®as in WO 96/40210 published December 19, 1996); CD3 antibodies such as OKT3 (US Patent No. 4,515,893 issued May 7, 1985); CD25 or Tac antibodies such as CHI-621 (SIMULECT®) and ZENAPAX® (See US Patent No. 5,693,762 issued December 2, 1997); CD4 antibodies

such as the cM-7412 antibody (Choy *et al, Arthritis Rheum* 39(l):52-56 (1996)); CD52 antibodies such as CAMPATH-1H (ILEX/Berlex) (Riechmann *et al, Nature* 332:323-337 (1988)); Fc receptor antibodies such as the M22 antibody directed against FcyRI as in Graziano *et al, J. Immunol.* 155(10):4996-5002 (1995)); carcinoembryonic antigen (CEA) antibodies such as hMN-14 (Sharkey *et al, Cancer Res.* 55(23Suppl): 5935s-5945s (1995)); antibodies directed against breast epithelial cells including huBrE-3, hu-Mc 3 and CHL6 (Ceriani *et al, Cancer Res.* 55(23): 5852s-5856s (1995); and Richman *et al, Cancer Res.* 55(23 Supp): 5916s-5920s (1995)); antibodies that bind to colon carcinoma cells such as C242 (Litton *et al, Eur J. Immunol.* 26(1): 1-9 (1996)); CD38 antibodies, *e.g.,* AT 13/5 (Ellis *et al, J. Immunol.* 155(2):925-937 (1995)); CD33 antibodies such as Hu M195 (Jurcic *et al, Cancer Res* 55(23 Suppl):5908s-5910s (1995)) and CMA-676 or CDP771; EpCAM antibodies such as 17-1A (PANOREX®); GpIIb/IIIa antibodies such as abciximab or c7E3 Fab (REOPRO®); RSV antibodies such as MEDI-493 (SYNAGIS®); CMV antibodies such as PROTOVIR®; HIV antibodies such as PR0542; hepatitis antibodies such as the Hep B antibody OSTAVIR®; CA125 antibody including anti-MUC16 (WO2007/001851; Yin, BWT and Lloyd, KO, *J. Biol. Chem.* 276:27371-27375 (2001)) and OvaRex; idiotypic GD3 epitope antibody BEC2; αvβ3 antibody *(e.g.,* VITAXIN®; Medimmune); human renal cell carcinoma antibody such as ch-G250; ING-1; anti-human 17-lAn antibody (3622W94); anti-human colorectal tumor antibody (A33); anti-human melanoma antibody R24 directed against GD3 ganglioside; anti-human squamous-cell carcinoma (SF-25); human leukocyte antigen (HLA) antibody such as Smart ID 10 and the anti-HLA DR antibody Oncolym (Lym-1); CD37 antibody such as TRU 016 (Trubion); IL-21 antibody (Zymogenetics/Novo Nordisk); anti-B cell antibody (Impheron); B cell targeting MAb (Immunogen/Aventis); 1D09C3 (Morphosys/GPC); LymphoRad 131 (HGS); Lym-1 antibody, such as Lym -1Y-90 (USC) or anti-Lym-1 Oncolym (USC/Peregrine); LIF 226 (Enhanced Lifesci.); BAFF antibody *(e.g.,* WO 03/33658); BAFF receptor antibody (see *e.g.,* WO 02/24909); BR3 antibody; Blys antibody such as belimumab; LYMPHOSTAT -B™; ISF 154 (UCSD/Roche/Tragen); gomiliximа (Idee 152; Biogen Idee); IL-6 receptor antibody such as atlizumab (ACTEMRA™; Chugai/Roche); IL-15 antibody such as HuMax-Il-15 (Genmab/Amgen); chemokine receptor antibody, such as a CCR2 antibody *(e.g.,* MLN1202; Millieneum); anti-complement antibody, such as C5 antibody *(e.g.,* eculizumab, 5G1.1; Alexion); oral formulation of human immunoglobulin *(e.g.,* IgPO;

Protein Therapeutics); IL-12 antibody such as ABT-874 (CAT/Abbott); Teneliximab (BMS-224818; BMS); CD40 antibodies, including S2C6 and humanized variants thereof (WO00/75348) and TNX 100 (Chiron/Tanox); TNF-a antibodies including cA2 or infliximab (REMICADE®), CDP571, MAK-195, adalimumab (HUMIRA™), pegylated TNF-a antibody fragment such as CDP-870 (Celltech), D2E7 (Knoll), anti-TNF-a polyclonal antibody *(e.g.,* PassTNF; Verigen); CD22 antibodies such as LL2 or epratuzumab (LYMPHOCIDE®; Immunomedics), including epratuzumab Y-90 and epratzumab 1-131, Abiogen's CD22 antibody (Abiogen, Italy), CMC 544 (Wyeth/Celltech), combotox (UT Soutwestern), BL22 (NIH), and LympoScan Tc99 (Immunomedics) .

Examples of CD20 antibodies include: "C2B8," which is now called "rituximab" ("RITUXAN®") (US Patent No. 5,736,137); the yttrium-[90]-labelled 2B8 murine antibody designated "Y2B8" or "Ibritumomab Tiuxetan" (ZEVALIN®) commercially available from IDEC Pharmaceuticals, Inc. (US Patent No. 5,736,137; 2B8 deposited with ATCC under accession no. HB1 1388 on June 22, 1993); murine IgG2a "Bl," also called "Tositumomab," optionally labelled with ^{131}I to generate the "131I-B1" or "iodine 1131 tositumomab" antibody (BEXXAR™) commercially available from Corixa (see, also, US Patent No. 5,595,721); murine monoclonal antibody "1F5" (Press *et al., Blood* 69(2):584-591 (1987)) and variants thereof including "framework patched" or humanized 1F5 (WO 2003/002607, Leung, S.; ATCC deposit HB-96450); murine 2H7 and chimeric 2H7 antibody (US Patent No. 5,677,180); humanized 2H7 (WO 2004/056312, Lowman *et al.,);* 2F2 (HuMax-CD20), a fully human, high-affinity antibody targeted at the CD20 molecule in the cell membrane of B-cells (Genmab, Denmark; see, for example, Glennie and van de Winkel, *Drug Discovery Today* 8: 503-510 (2003) and Cragg *et al., Blood* 101: 1045-1052 (2003); WO 2004/035607; US2004/0167319); the human monoclonal antibodies set forth in WO 2004/035607 and US2004/0167319 (Teeling *et al.,);* the antibodies having complex N-glycoside-linked sugar chains bound to the Fc region described in US 2004/0093621 (Shitara *et al.,);* monoclonal antibodies and antigen-binding fragments binding to CD20 (WO 2005/000901, Tedder *et al.,)* such as HB20-3, HB20-4, HB20-25, and MB20-1 1; CD20 binding molecules such as the AME series of antibodies, *e.g.,* AME 33 antibodies as set forth in WO 2004/103404 and US2005/0025764 (Watkins *et al.,* Eli Lilly/Applied Molecular Evolution, AME); CD20 binding molecules such as those described in US 2005/0025764 (Watkins *et al.,);* A20

antibody or variants thereof such as chimeric or humanized A20 antibody (cA20, bA20, respectively) or IMMU-106 (US 2003/0219433, Immunomedics); CD20-binding antibodies, including epitope-depleted Leu-16, 1H4, or 2B8, optionally conjugated with IL-2, as in US 2005/0069545A1 and WO 2005/16969 (Carr *et al.,);* bispecific antibody that binds CD22 and CD20, for example, hLL2xhA20 (WO2005/14618, Chang *et al.,);* monoclonal antibodies L27, G28-2, 93-1B3, B-Cl or NU-B2 available from the International Leukocyte Typing Workshop (Valentine *et al.,* In: *Leukocyte Typing* III (McMichael, Ed., p. 440, Oxford University Press (1987)); 1H4 (Haisma *et al, Blood* 92:184 (1998)); anti-CD20 auristatin E conjugate (Seattle Genetics); anti-CD20-IL2 (EMD/Biovation/City of Hope); anti-CD20 MAb therapy (EpiCyte); anti-CD20 antibody TRU 015 (Trubion).

The term "antibody" as used herein includes monoclonal antibodies (including full length antibodies which have an immunoglobulin Fc region), antibody compositions with polyepitopic specificity, multispecific antibodies *{e.g.,* bispecific antibodies), diabodies, peptibodies, and single-chain molecules, as well as antibody fragments *{e.g.,* Fab, $F(ab')_2$, and Fv), any of which may optionally be conjugated to another component, e.g., a toxin. The term "immunoglobulin" (Ig) is used interchangeably with "antibody" herein.

The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. An IgM antibody consists of 5 of the basic heterotetramer unit along with an additional polypeptide called a J chain, and contains 10 antigen binding sites, while IgA antibodies comprise from 2-5 of the basic 4-chain units which can polymerize to form polyvalent assemblages in combination with the J chain. In the case of IgGs, the 4-chain unit is generally about 150,000 daltons. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain (V_H) followed by three constant domains (C_H) for each of the a and γ chains and four C_H domains for μ and ε isotypes. Each L chain has at the N-terminus, a variable domain (V_L) followed by a constant domain at its other end. The V_L is aligned with the V_H and the C_L is aligned with the first constant domain of the heavy chain (C_HI). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a V_H and V_L together forms a single antigen-binding site. For the structure

and properties of the different classes of antibodies, see *e.g., Basic and Clinical Immunology*, 8th Edition, Daniel P. Sties, Abba I. Terr and Tristram G. Parsolw (eds), Appleton & Lange, Norwalk, Conn., 1994, page 71 and Chapter 6.

The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (CH), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, having heavy chains designated α, δ, ε, γ. and μ, respectively. The γ and a classes are further divided into subclasses on the basis of relatively minor differences in the CH sequence and function, *e.g.*, humans express the following subclasses: IgGl, IgG2, IgG3, IgG4, IgAl and IgA2.

The term "variable" refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies. The V domain mediates antigen binding and defines the specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the entire span of the variable domains. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of about 15-30 amino acid residues separated by shorter regions of extreme variability called "hypervariable regions" or sometimes "complementarity determining regions" (CDRs) that are each approximately 9-12 amino acid residues in length. The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen binding site of antibodies (see Kabat *et al., Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody dependent cellular cytotoxicity (ADCC).

The term "hypervariable region" (also known as "complementarity determining regions" or CDRs) when used herein refers to the amino acid residues of an antibody which are (usually three or four short regions of extreme sequence variability) within the

V-region domain of an immunoglobulin which form the antigen-binding site and are the main determinants of antigen specificity. There are at least two methods for identifying the CDR residues: (1) An approach based on cross-species sequence variability *(i.e.,* Kabat *et al., Sequences of Proteins of Immunological Interest* (National Institute of Health, Bethesda, M S 1991); and (2) An approach based on crystallographic studies of antigen-antibody complexes (Chothia, C. *et al., J. Mol. Biol.* 196: 901-917 (1987)). However, to the extent that two residue identification techniques define regions of overlapping, but not identical regions, they can be combined to define a hybrid CDR.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.,* the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translation modifications *(e.g.,* isomerizations, amidations) that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al., Nature,* 256: 495 (1975), or may be made by recombinant DNA methods (see, *e.g.,* U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al., Nature,* 352:624-628 (1991) and Marks *et al., J. Mol. Biol.,* 222:581-597 (1991), for example.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is (are) identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of

such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; Morrison *et al, Proc. Natl. Acad. Sci. USA,* 81:6851-6855 (1984)). Chimeric antibodies of interest herein include "primitized" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate *{e.g.,* Old World Monkey, Ape etc.) and human content region sequences.

An "intact" antibody is one which comprises an antigen-binding site as well as a CL and at least the heavy chain domains, C_H1, C_H2 and C_H3. The constant domains may be native sequence constant domains *{e.g.,* human native sequence constant domains) or amino acid sequence variants thereof. Preferably, the intact antibody has one or more effector functions.

An "antibody fragment" comprises a portion of an intact antibody, preferably the antigen binding and/or the variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$ and Fv fragments; diabodies; linear antibodies (see U.S. Pat. No. 5,641,870, Example 2; Zapata *et al, Protein Eng.* 8(10): 1057-1062 [1995]); single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

Papain digestion of antibodies produced two identical antigen-binding fragments, called "Fab" fragments, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (V_H), and the first constant domain of one heavy chain (C_H1). Each Fab fragment is monovalent with respect to antigen binding, *i.e.,* it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large $F(ab')_2$ fragment which roughly corresponds to two disulfide linked Fab fragments having different antigen-binding activity and is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by having a few additional residues at the carboxy terminus of the C_H1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The Fc fragment comprises the carboxy-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences

in the Fc region, the region which is also recognized by Fc receptors (FcR) found on certain types of cells.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervarible loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

"Single-chain Fv" also abbreviated as "sFv" or "scFv" are antibody fragments that comprise the VH and VL antibody domains connected into a single polypeptide chain. Preferably, the sFv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of the sFv, see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments prepared by constructing sFv fragments (see preceding paragraph) with short linkers (about 5-10) residues) between the V_H and V_L domains such that inter-chain but not intra-chain pairing of the V domains is achieved, thereby resulting in a bivalent fragment, *i.e.*, a fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two "crossover" sFv fragments in which the V_H and V_L domains of the two antibodies are present on different polypeptide chains. Diabodies are described in greater detail in, for example, EP 404,097; WO 93/1 1161; Hollinger *et al, Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993).

The antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')2 or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of

the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol, 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al, Nature, 321:522-525 (1986); Riechmann et al, Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity and HAMA response (human anti-mouse antibody) when the antibody is intended for human therapeutic use. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable domain sequences. The human V domain sequence which is closest to that of the rodent is identified and the human framework region (FR) within it accepted for the humanized antibody (Sims et al, J. Immunol. 151:2296 (1993); Chothia et al, J. Mol. Biol, 196:901 (1987)). Another method uses a particular framework region derived from the consensus

sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter et al., Proc. Natl. Acad. Sci. USA, 89:4285 (1992); Presta et al, J. Immunol. 151:2623 (1993)).

It is further important that antibodies be humanized with retention of high binding affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

Various forms of a humanized antibody are contemplated. For example, the humanized antibody may be an antibody fragment, such as a Fab, which is optionally conjugated with one or more cytotoxic agent(s) in order to generate an immunoconjugate. Alternatively, the humanized antibody may be an intact antibody, such as an intact IgGl antibody.

As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (JH) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array into such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al, Proc. Natl. Acad. Sci. USA, 90:2551 (1993); Jakobovits et al, Nature, 362:255-258 (1993); Bruggemann et al, Year in Immuno. 7:33

(1993); U.S. Patent Nos. 5,545,806, 5,569,825, 5,591,669 (all of GenPharm); 5,545,807; and WO 97/17852.

Alternatively, phage display technology (McCafferty et al, Nature 348:552-553 [1990]) can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats, reviewed in, e.g., Johnson, Kevin S. and Chiswell, David J., Current Opinion in Structural Biology 3:564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson et al., Nature, 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks et al, J. Mol. Biol. 222:581-597 (1991), or Griffith et al, EMBO J. 12:725-734 (1993). See, also, U.S. Patent Nos. 5,565,332 and 5,573,905.

Human antibodies may also be generated by in vitro activated B cells (see U.S. Patents 5,567,610 and 5,229,275).

Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies may bind to two different epitopes of a protein as described herein. Other such antibodies may combine a protein binding site with a binding site for another protein. Alternatively, an anti-protein arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (e.g. CD3) (see, e.g., Baeuerle, et al, Curr. Opin. Mol. Ther. 11(1):22-30 (2009)), or Fc receptors for IgG (FcyR), such as FcyRI (CD64), FcyRII (CD32) and FcyRIII (CD16), so as to focus and localize cellular defense mechanisms to the TAT-expressing cell. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express a target protein. These antibodies possess a protein-binding

arm and an arm which binds the cytotoxic agent (e.g., saporin, anti-interferon-a, vinca alkaloid, ricin A chain, methotrexate or radioactive isotope hapten). Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g., F(ab')2 bispecific antibodies).

WO 96/16673 describes a bispecific anti-ErbB2/anti-FcYRIII antibody and U.S. Patent No. 5,837,234 discloses a bispecific anti-ErbB2/anti-FcyRI antibody. A bispecific anti-ErbB2/Fca antibody is shown in WO98/02463. U.S. Patent Nos. 5,821,337 and 6,407,213 teach bispecific anti-ErbB2/anti-CD3 antibodies. Additional bispecific antibodies that bind an epitope on the CD3 antigen and a second epitope have been described. See, for example, U.S. Patent Nos. 5,078,998 (anti-CD3/tumor cell antigen); 5,601,819 (anti-CD3/IL-2R; anti-CD3/CD28; anti-CD3/CD45); 6,129,914 (anti-CD3/malignant B cell antigen); 7,1 12,324 (anti-CD3/CD19); 6,723,538 (anti-CD3/CCR5); 7,235,641 (anti-CD3/EpCAM); 7,262,276 (anti-CD3/ovarian tumor antigen); and 5,731,168 (anti-CD3/CD4IgG).

Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein et al, Nature 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and in Traunecker et al, EMBO J. 10:3655-3659 (1991).

According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. Preferably, the fusion is with an Ig heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CHI) containing the site necessary for light chain bonding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host cell. This provides for greater flexibility in adjusting the mutual proportions of the three

polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yield of the desired bispecific antibody. It is, however, possible to insert the coding sequences for two or all three polypeptide chains into a single expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios have no significant affect on the yield of the desired chain combination.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies see, for example, Suresh et al, Methods in Enzymology 121 :210 (1986).

According to another approach described in U.S. Patent No. 5,731,168, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g., tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g., alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Patent No. 4,676,980, along with a number of cross-linking techniques.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al, Science 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')2 fragments. These fragments are reduced in the presence of the dithiol complexing agent, sodium arsenite, to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Recent progress has facilitated the direct recovery of Fab'-SH fragments from E. coli, which can be chemically coupled to form bispecific antibodies. Shalaby et al., J. Exp. Med. 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')2 molecule. Each Fab' fragment was separately secreted from E. coli and subjected to directed chemical coupling in vitro to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets. Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., J. Immunol. 148(5): 1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al, Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a VH connected to a VL by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one fragment are forced to pair with the complementary VL and VH domains of another fragment, thereby forming two antigen-binding sites. Another strategy for

making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber et al, J. Immunol, 152:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt et al., J. Immunol. 147:60 (1991).

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells [U.S. Patent No. 4,676,980], and for treatment of HIV infection [WO 91/00360; WO 92/200373; EP 03089]. It is contemplated that the antibodies may be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

A multivalent antibody may be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind. The antibodies of the present invention can be multivalent antibodies (which are other than of the IgM class) with three or more antigen binding sites (e.g. tetravalent antibodies), which can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody. The multivalent antibody can comprise a dimerization domain and three or more antigen binding sites. The preferred dimerization domain comprises (or consists of) an Fc region or a hinge region. In this scenario, the antibody will comprise an Fc region and three or more antigen binding sites amino-terminal to the Fc region. The preferred multivalent antibody herein comprises (or consists of) three to about eight, but preferably four, antigen binding sites. The multivalent antibody comprises at least one polypeptide chain (and preferably two polypeptide chains), wherein the polypeptide chain(s) comprise two or more variable domains. For instance, the polypeptide chain(s) may comprise VDl-(Xl)n-VD2-(X2)n-Fc, wherein VD1 is a first variable domain, VD2 is a second variable domain, Fc is one polypeptide chain of an Fc region, XI and X2 represent an amino acid or polypeptide, and n is 0 or 1. For instance, the polypeptide chain(s) may comprise: VH-CH1-flexible linker-VH-CHl-Fc region chain; or VH-CHl-VH-CHl-Fc region chain. The multivalent antibody herein preferably further comprises at least two (and preferably four) light chain

variable domain polypeptides. The multivalent antibody herein may, for instance, comprise from about two to about eight light chain variable domain polypeptides. The light chain variable domain polypeptides contemplated here comprise a light chain variable domain and, optionally, further comprise a CL domain.

An antibody that "specifically binds to" or is "specific for" a particular polypeptide or an epitope on a particular polypeptide is one that binds to that particular polypeptide or epitope on a particular polypeptide without substantially binding to any other polypeptide or polypeptide epitope.

The term "solid phase" describes a non-aqueous matrix to which the antibody of the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass *(e.g.,* controlled pore glass), polysaccharides *(e.g.,* agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column *(e.g.,* an affinity chromotography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Pat. No. 4,275,149.

A "species-dependent antibody", *e.g.,* a mammalian anti-human IgE antibody, is an antibody which has a stronger binding affinity for an antigen from a first mammalian species than it has for a homologue of that antigen from a second mammalian species. Normally, the species-dependent antibody "bind specifically" to a human antigen *(i.e.,* has a binding affinity (Kd) value of no more than about lxl 0^{-7} M, alternatively no more than about $1x10^{-8}$ M, alternatively no more than about $1x10^{-9}$ M) but has a binding affinity for a homologue of the antigen from a second non-human mammalian species which is at least about 50 fold, at least about 500 fold, or at least about 1000 fold, weaker than its binding affinity for the non-human antigen. The species-dependent antibody can be of any of the various types of antibodies as defined above, but preferably is a humanized or human antibody.

Antibody "effector functions" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: Clq binding and complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors *(e.g.,* B cell receptors); and B cell activation.

"Antibody-dependent cell-mediated cytotoxicity" or ADCC refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells *(e.g.*, natural killer (NK) cells, neutrophils and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies "arm" the cytotoxic cells and are required for killing of the target cell by this mechanism. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. Fc expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9: 457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in U.S. Pat. No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and natural killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo, e.g.*, in an animal model such as that disclosed in Clynes *et al., PNAS USA* 95:652-656 (1998).

"Fc receptor" or "FcR" describes a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors, FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (see M. Daeron, *Annu. Rev. Immunol.* 15:203-234 (1997). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol.* 9: 457-92 (1991); Capel *et al.*, Immunomethods 4: 25-34 (1994); and de Haas *et al., J. Lab. Clin. Med.* 126: 330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus. Guyer *et al., J. Immunol.* 117: 587 (1976) and Kim *et al., J. Immunol.* 24: 249 (1994).

"Human effector cells" are leukocytes which express one or more FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and perform

ADCC effector function. Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils, with PBMCs and MNK cells being preferred. The effector cells may be isolated from a native source, *e.g.*, blood.

"Complement dependent cytotoxicity" of "CDC" refers to the lysis of a target cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (Clq) to antibodies (of the appropriate subclass) which are bound to their cognate antigen. To assess complement activation, a CDC assay, *e.g.,* as described in Gazzano-Santoro *et al.*, *J. Immunol. Methods* 202: 163 (1996), may be performed.

"Isolated" when used to describe the various polypeptides and antibodies disclosed herein, means a polypeptide or antibody that has been identified, separated and/or recovered from a component of its production environment. Preferably, the isolated polypeptide is free of association with all other components from its production environment. Contaminant components of its production environment, such as that resulting from recombinant transfected cells, are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Ordinarily, however, an isolated polypeptide or antibody will be prepared by at least one purification step.

An "isolated" nucleic acid molecule encoding the polypeptides and antibodies herein is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the environment in which it was produced. Preferably, the isolated nucleic acid is free of association with all components associated with the production environment. The isolated nucleic acid molecules encoding the polypeptides and antibodies herein is in a form other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from nucleic acid encoding the polypeptides and antibodies herein existing naturally in cells.

The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising a polypeptide or antibody described herein fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with activity of the polypeptide to which it is fused. The tag polypeptide preferably also is fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody *(i.e.,* is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any

immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM. The Ig fusions preferably include the substitution of a domain of a polypeptide or antibody described herein in the place of at least one variable region within an Ig molecule. In a particularly preferred embodiment, the immunoglobulin fusion includes the hinge, CH2 and CH3, or the hinge, CHI, CH2 and CH3 regions of an IgGl molecule. For the production of immunoglobulin fusions see also U.S. Pat. No. 5,428,130 issued Jun. 27, 1995.

The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

An antibody possesses "biological activity" in a pharmaceutical formulation, if the biological activity of the antibody at a given time is within about 10% (within the errors of the assay) of the biological activity exhibited at the time the pharmaceutical formulation was prepared, as determined by the ability of the antibody *in vitro* or *in vivo* to bind to antigen and result in a measurable biological response.

A "stable" or "stabilized" formulation is one in which the protein therein essentially retains its physical and/or chemical stability upon storage. Stability can be measured at a selected temperature for a selected time period. Preferably, the formulation is stable at room temperature (~30°C) or at 40°C for at least 1 month and/or stable at about 2-8°C for at least 1 year and preferably for at least 2 years. For example, the extent of aggregation during storage can be used as an indicator of protein stability. Thus, a "stable" formulation may be one wherein less than about 10% and preferably less than about 5% of the protein is present as an aggregate in the formulation. Various analytical techniques for measuring protein stability are available in the art and are reviewed, for example, in *Peptide and Protein Drug Delivery*, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones, A. *Adv. Drug Delivery Rev.* 10: 29-90 (1993).

The term "aqueous solution" refers to a solution in which water is the dissolving medium or solvent. When a substance dissolves in a liquid, the mixture is termed a solution. The dissolved substance is the solute, and the liquid that does the dissolving (in this case water) is the solvent.

The term, "stabilizing agent" or "stabilizer" as used herein is a chemical or compound that is added to a solution or mixture or suspension or composition or therapeutic composition to maintain it in a stable or unchanging state; or is one which is used because it produces a reaction involving changes in atoms or molecules leading to a more stable or unchanging state.

A "viscosity reducing amount" of a compound that is capable of reducing viscosity of an aqueous protein-containing formulation is the amount that measurably reduces the viscosity of the formulation after addition thereto.

An "isotonic" formulation is one which has essentially the same osmotic pressure as human blood. Isotonic formulations will generally have an osmotic pressure from about 250 to 350 mOsm. The term "hypotonic" describes a formulation with an osmotic pressure below that of human blood. Correspondingly, the term "hypertonic" is used to describe a formulation with an osmotic pressure above that of human blood. Isotonicity can be measured using a vapor pressure or ice-freezing type osmometer, for example.

A "reconstituted" formulation is one which has been prepared by dissolving a lyophilized protein or antibody formulation in a diluent such that the protein is dispersed in the reconstituted formulation. The reconstituted formulation is suitable for administration *(e.g.,* parenteral administration) to a patient to be treated with the protein of interest and, in certain embodiments of the invention, may be one which is suitable for subcutaneous administration.

"Surfactants" are surface active agents that can exert their effect at surfaces of solid-solid, solid-liquid, liquid-liquid, and liquid-air because of their chemical composition, containing both hydrophilic and hydrophobic groups. These materials reduce the concentration of proteins in dilute solutions at the air-water and/or water-solid interfaces where proteins can be adsorbed and potentially aggregated. Surfactants can bind to hydrophobic interfaces in protein formulations. Proteins on the surface of water will aggregate, particularly when agitated, because of unfolding and subsequent aggregation of the protein monolayer.

"Surfactants" can denature proteins, but can also stabilize them against surface denaturation. Generally, ionic surfactants can denature proteins. However, nonionic surfactants usually do not denature proteins even at relatively high concentrations (1% w/v). Most parentally acceptable nonionic surfactants come from either the polysorbate or polyether groups. Polysorbate 20 and 80 are contemporary surfactant stabilizers in

marketed protein formulations. However, other surfactants used in protein formulations include Pluronic F-68 and members of the "Brij" class. Non-ionic surfactants can be sugar based. Sugar based surfactants can be alkyl glycosides. The general structure of the alkyl glycoside is Ri-0-(CH_2)x-R , where R is independently CH_3 or cyclohexyl (C_6H_{11}) and Ri is independently glucose or maltose. Exemplary alkyl glycosides include those in which Ri is glucose, R is CH_3, and x is 5 (n-hexyl -P-D-glucopyranoside), x is 6 (n-heptyl-β-D-glucopyranoside), x is 7 (n-octyl -P-D-glucopyranoside), x is 8 (n-nonyl- β-D-glucopyranoside), x is 9 (n-decyl -P-D-glucopyranoside), and x is 11 (n-dodecyl- β-D-glucopyranoside). Sometimes glucopyranosides are called glucosides. Exemplary alkyl glycosides additionally include those in which Ri is maltose, R is CH_3, and x is 5 (n-hexyl -P-D-maltopyranoside), x is 7 (n-octyl -P-D-maltopyranoside), x is 8 (n-nonyl- β-D-maltopyranoside), x is 9 (n-decyl -P-D-maltopyranoside), x is 10 (n-undecyl- β-D-maltopyranoside), x is 11 (n-dodecyl -P-D-maltopyranoside), x is 12 (n-tridecyl -P-D-maltopyranoside), x is 13 (n-tetradecyl -P-D-maltopyranoside), and x is 15 (n-hexadecyl-β-D-maltopyranoside). Sometimes maltopyranosides are called maltosides. Exemplary alkyl glycosides further include those in which Ri is glucose, x is 3, and R is cyclohexyl (3-cyclohexyl- 1-propyl- β-D-glucoside); and in which Ri is maltose, x is 4, and R is cyclohexyl (4-cyclohexyl-l -butyl- β-D-maltoside).

A "pharmaceutically acceptable acid" includes inorganic and organic acids which are non toxic at the concentration and manner in which they are formulated. For example, suitable inorganic acids include hydrochloric, perchloric, hydrobromic, hydroiodic, nitric, sulfuric, sulfonic, sulfinic, sulfanilic, phosphoric, carbonic, etc. Suitable organic acids include straight and branched-chain alkyl, aromatic, cyclic, cyloaliphatic, arylaliphatic, heterocyclic, saturated, unsaturated, mono, di- and tri-carboxylic, including for example, formic, acetic, 2-hydroxyacetic, trifluoroacetic, phenylacetic, trimethylacetic, t-butyl acetic, anthranilic, propanoic, 2-hydroxypropanoic, 2-oxopropanoic, propandioic, cyclopentanepropionic, cyclopentane propionic, 3-phenylpropionic, butanoic, butandioic, benzoic, 3-(4-hydroxybenzoyl)benzoic, 2-acetoxy-benzoic, ascorbic, cinnamic, lauryl sulfuric, stearic, muconic, mandelic, succinic, embonic, fumaric, malic, maleic, hydroxymaleic, malonic, lactic, citric, tartaric, glycolic, glyconic, gluconic, pyruvic, glyoxalic, oxalic, mesylic, succinic, salicylic, phthalic, palmoic, palmeic, thiocyanic, methanesulphonic, ethanesulphonic, 1,2-ethanedisulfonic, 2-hydroxyethanesulfonic, benzenesulphonic, 4-chorobenzenesulfonic, napthalene-2-sulphonic, p-toluenesulphonic,

camphorsulphonic, 4-methylbicyclo[2.2.2]-oct-2-ene-l-carboxylic, glucoheptonic, 4,4'-methylenebis-3-(hydroxy-2-ene-l-carboxylic acid), hydroxynapthoic.

"Pharmaceutically-acceptable bases" include inorganic and organic bases which are non-toxic at the concentration and manner in which they are formulated. For example, suitable bases include those formed from inorganic base forming metals such as lithium, sodium, potassium, magnesium, calcium, ammonium, iron, zinc, copper, manganese, aluminum, N-methylglucamine, morpholine, piperidine and organic nontoxic bases including, primary, secondary and tertiary amine, substituted amines, cyclic amines and basic ion exchange resins, *[e.g.,* $N(R')_4+$ (where R' is independently H or Ci_{-4} alkyl, *e.g.,* ammonium, Tris)], for example, isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-diethylaminoethanol, trimethamine, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, N-ethylpiperidine, polyamine resins and the like. Particularly preferred organic non-toxic bases are isopropylamine, diethylamine, ethanolamine, trimethamine, dicyclohexylamine, choline, and caffeine.

Additional pharmaceutically acceptable acids and bases useable with the present invention include those which are derived from the amino acids, for example, histidine, glycine, phenylalanine, aspartic acid, glutamic acid, lysine and asparagine.

"Pharmaceutically acceptable" buffers and salts include those derived from both acid and base addition salts of the above indicated acids and bases. Specific buffers and/or salts include histidine, succinate and acetate.

A "lyoprotectant" is a molecule which, when combined with a protein of interest, significantly prevents or reduces physicochemical instability of the protein upon lyophilization and subsequent storage. Exemplary lyoprotectants include sugars and their corresponding sugar alcohols; an amino acid such as monosodium glutamate or histidine; a methylamine such as betaine; a lyotropic salt such as magnesium sulfate; a polyol such as trihydric or higher molecular weight sugar alcohols, *e.g.* , glycerin, dextran, erythritol, glycerol, arabitol, xylitol, sorbitol, and mannitol; propylene glycol; polyethylene glycol; Pluronics®; and combinations thereof. Additional exemplary lyoprotectants include glycerin and gelatin, and the sugars mellibiose, melezitose, raffinose, mannotriose and stachyose. Examples of reducing sugars include glucose, maltose, lactose, maltulose, iso-maltulose and lactulose. Examples of non-reducing sugars include non-reducing

glycosides of polyhydroxy compounds selected from sugar alcohols and other straight chain polyalcohols. Preferred sugar alcohols are monoglycosides, especially those compounds obtained by reduction of disaccharides such as lactose, maltose, lactulose and maltulose. The glycosidic side group can be either glucosidic or galactosidic. Additional examples of sugar alcohols are glucitol, maltitol, lactitol and iso-maltulose. The preferred lyoprotectant are the non-reducing sugars trehalose or sucrose.

The lyoprotectant is added to the pre-lyophilized formulation in a "lyoprotecting amount" which means that, following lyophilization of the protein in the presence of the lyoprotecting amount of the lyoprotectant, the protein essentially retains its physicochemical stability upon lyophilization and storage.

A "pharmaceutically acceptable sugar" is a molecule which, when combined with a protein of interest, significantly prevents or reduces physicochemical instability of the protein upon storage. When the formulation is intended to be lyophilized and then reconstituted, "pharmaceutically acceptable sugars" may also be known as a "lyoprotectant". Exemplary sugars and their corresponding sugar alcohols includes: an amino acid such as monosodium glutamate or histidine; a methylamine such as betaine; a lyotropic salt such as magnesium sulfate; a polyol such as trihydric or higher molecular weight sugar alcohols, *e.g.,* glycerin, dextran, erythritol, glycerol, arabitol, xylitol, sorbitol, and mannitol; propylene glycol; polyethylene glycol; Pluronics®; and combinations thereof. Additional exemplary lyoprotectants include glycerin and gelatin, and the sugars mellibiose, melezitose, raffinose, mannotriose and stachyose. Examples of reducing sugars include glucose, maltose, lactose, maltulose, iso-maltulose and lactulose. Examples of non-reducing sugars include non-reducing glycosides of polyhydroxy compounds selected from sugar alcohols and other straight chain polyalcohols. Preferred sugar alcohols are monoglycosides, especially those compounds obtained by reduction of disaccharides such as lactose, maltose, lactulose and maltulose. The glycosidic side group can be either glucosidic or galactosidic. Additional examples of sugar alcohols are glucitol, maltitol, lactitol and iso-maltulose. The preferred pharmaceutically-acceptable sugars are the non-reducing sugars trehalose or sucrose.

Pharmaceutically acceptable sugars are added to the formulation in a "protecting amount" *{e.g.,* pre-lyophilization) which means that the protein essentially retains its physicochemical stability during storage *{e.g.,* after reconstitution and storage).

The "diluent" of interest herein is one which is pharmaceutically acceptable (safe and non-toxic for administration to a human) and is useful for the preparation of a liquid formulation, such as a formulation reconstituted after lyophilization. Exemplary diluents include sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution (*e.g.*, phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution. In an alternative embodiment, diluents can include aqueous solutions of salts and/or buffers.

A "preservative" is a compound which can be added to the formulations herein to reduce bacterial activity. The addition of a preservative may, for example, facilitate the production of a multi-use (multiple-dose) formulation. Examples of potential preservatives include octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride (a mixture of alkylbenzyldimethylammonium chlorides in which the alkyl groups are long-chain compounds), and benzethonium chloride. Other types of preservatives include aromatic alcohols such as phenol, butyl and benzyl alcohol, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol. The most preferred preservative herein is benzyl alcohol.

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, rabbits, cattle, pigs, hamsters, gerbils, mice, ferrets, rats, cats, etc. Preferably, the mammal is human.

A "disorder" is any condition that would benefit from treatment with the protein. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include carcinomas and inflammations.

A "therapeutically effective amount" is at least the minimum concentration required to effect a measurable improvement or prevention of a particular disorder. Therapeutically effective amounts of known proteins are well known in the art, while the effective amounts of proteins hereinafter discovered may be determined by standard techniques which are well within the skill of a skilled artisan, such as an ordinary physician.

"Viscosity," as used herein, may be "absolute viscosity" or "kinematic viscosity." "Absolute viscosity," sometimes called dynamic or simple viscosity, is a quantity that describes a fluid's resistance to flow. "Kinematic viscosity" is the quotient of absolute viscosity and fluid density. Kinematic viscosity is frequently reported when characterizing the resistive flow of a fluid using a capillary viscometer. When two fluids of equal volume are placed in identical capillary viscometers and allowed to flow by gravity, a viscous fluid takes longer than a less viscous fluid to flow through the capillary. If one fluid takes 200 seconds to complete its flow and another fluid takes 400 seconds, the second fluid is twice as viscous as the first on a kinematic viscosity scale. If both fluids have equal density, the second fluid is twice as viscous as the first on an absolute viscosity scale. The dimensions of kinematic viscosity are L^2/T where L represents length and T represents time. The SI units of kinematic viscosity are m^2/s. Commonly, kinematic viscosity is expressed in centistokes, cSt, which is equivalent to mm^2/s. The dimensions of absolute viscosity are M/L/T, where M represents mass and L and T represent length and time, respectively. The SI units of absolute viscosity are Pa•s, which is equivalent to kg/m/s. The absolute viscosity is commonly expressed in units of centiPoise, cP, which is equivalent to milliPascal-second, mPa•s.

Methods for the preparation of antibodies (including antibodies that are conjugated to a toxin) and other proteins which may be formulated as described herein are well known in the art and are described in detail in, for example, WO2007/00 1851.

Antibodies and other proteins may be formulated in accordance with the present invention in either aqueous or lyophilized form, the latter being capable if being reconstituted into an aqueous form.

The formulations described herein may be prepared as reconstituted lyophilized formulations. The proteins or antibodies described herein are lyophilized and then reconstituted to produce the liquid formulations of the invention. In this particular embodiment, after preparation of the protein of interest as described above, a "pre-lyophilized formulation" is produced. The amount of protein present in the pre-lyophilized formulation is determined taking into account the desired dose volumes, mode(s) of administration etc. For example, the starting concentration of an intact antibody can be from about 2 mg/ml to about 50 mg/ml, preferably from about 5 mg/ml to about 40 mg/ml and most preferably from about 20-30 mg/ml.

The protein to be formulated is generally present in solution. For example, in the liquid formulations of the invention, the protein may be present in a pH-buffered solution at a pH from about 4-8, and preferably from about 5-7. The buffer concentration can be from about 1 mM to about 200 mM, alternatively from about 1 mM to about 100 mM, alternatively from about 1 mM to about 50 mM, alternatively from about 3 mM to about 15 mM, depending, for example, on the buffer and the desired tonicity of the formulation *(e.g.,* of the reconstituted formulation). Exemplary buffers and/or salts are those which are pharmaceutically acceptable and may be created from suitable acids, bases and salts thereof, such as those which are defined under "pharmaceutically acceptable" acids, bases or buffers.

In one embodiment, a lyoprotectant is added to the pre-lyophilized formulation. The amount of lyoprotectant in the pre-lyophilized formulation is generally such that, upon reconstitution, the resulting formulation will be isotonic. However, hypertonic reconstituted formulations may also be suitable. In addition, the amount of lyoprotectant must not be too low such that an unacceptable amount of degradation/aggregation of the protein occurs upon lyophilization. However, exemplary lyoprotectant concentrations in the pre-lyophilized formulation are from about 10 mM to about 400 mM, alternatively from about 30 mM to about 300 mM, alternatively from about 50 mM to about 100 mM. Exemplary lyoprotectants include sugars and sugar alcohols such as sucrose, mannose, trehalose, glucose, sorbitol, mannitol. However, under particular circumstances, certain lyoprotectants may also contribute to an increase in viscosity of the formulation. As such, care should be taken so as to select particular lyoprotectants which minimize or neutralize this effect. Additional lyoprotectants are described above under the definition of "lyoprotectants", also referred herein as "pharmaceutically-acceptable sugars".

The ratio of protein to lyoprotectant can vary for each particular protein or antibody and lyoprotectant combination. In the case of an antibody as the protein of choice and a sugar *(e.g.,* sucrose or trehalose) as the lyoprotectant for generating an isotonic reconstituted formulation with a high protein concentration, the molar ratio of lyoprotectant to antibody may be from about 100 to about 1500 moles lyoprotectant to 1 mole antibody, and preferably from about 200 to about 1000 moles of lyoprotectant to 1 mole antibody, for example from about 200 to about 600 moles of lyoprotectant to 1 mole antibody.

A mixture of the lyoprotectant (such as sucrose or trehalose) and a bulking agent *(e.g.,* mannitol or glycine) may be used in the preparation of the pre-lyophilization formulation. The bulking agent may allow for the production of a uniform lyophilized cake without excessive pockets therein etc. Other pharmaceutically acceptable carriers, excipients or stabilizers such as those described in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980) may be included in the pre-lyophilized formulation (and/or the lyophilized formulation and/or the reconstituted formulation) provided that they do not adversely affect the desired characteristics of the formulation. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed and include; additional buffering agents; preservatives; co-solvents; antioxidants including ascorbic acid and methionine; chelating agents such as EDTA; metal complexes *{e.g.,* Zn-protein complexes); biodegradable polymers such as polyesters; and/or salt-forming counterions such as sodium.

The formulation herein may also contain more than one protein as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect the other protein. For example, it may be desirable to provide two or more antibodies which bind to the desired target *{e.g.,* receptor or antigen) in a single formulation. Such proteins are suitably present in combination in amounts that are effective for the purpose intended.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to, or following, lyophilization and reconstitution. Alternatively, sterility of the entire mixture may be accomplished by autoclaving the ingredients, except for protein, at about 120°C for about 30 minutes, for example.

After the protein, optional lyoprotectant and other optional components are mixed together, the formulation is lyophilized. Many different freeze-dryers are available for this purpose such as Hull50™ (Hull, USA) or GT20 ™ (Leybold-Heraeus, Germany) freeze-dryers. Freeze-drying is accomplished by freezing the formulation and subsequently subliming ice from the frozen content at a temperature suitable for primary drying. Under this condition, the product temperature is below the eutectic point or the collapse temperature of the formulation. Typically, the shelf temperature for the primary drying will range from about -30 to 25°C (provided the product remains frozen during primary drying) at a suitable pressure, ranging typically from about 50 to 250 mTorr. The

formulation, size and type of the container holding the sample *(e.g.,* glass vial) and the volume of liquid will mainly dictate the time required for drying, which can range from a few hours to several days *(e.g.,* 40-60 hrs). Optionally, a secondary drying stage may also be performed depending upon the desired residual moisture level in the product. The temperature at which the secondary drying is carried out ranges from about 0-40°C, depending primarily on the type and size of container and the type of protein employed. For example, the shelf temperature throughout the entire water removal phase of lyophilization may be from about 15-30°C *(e.g.,* about 20°C). The time and pressure required for secondary drying will be that which produces a suitable lyophilized cake, dependent, *e.g.,* on the temperature and other parameters. The secondary drying time is dictated by the desired residual moisture level in the product and typically takes at least about 5 hours *(e.g.,* 10-15 hours). The pressure may be the same as that employed during the primary drying step. Freeze-drying conditions can be varied depending on the formulation and vial size.

Prior to administration to the patient, the lyophilized formulation is reconstituted with a pharmaceutically acceptable diluent such that the protein concentration in the reconstituted formulation is at least about 50 mg/ml, for example from about 50 mg/ml to about 400 mg/ml, alternatively from about 80 mg/ml to about 300 mg/ml, alternatively from about 90 mg/ml to about 150 mg/ml. Such high protein concentrations in the reconstituted formulation are considered to be particularly useful where subcutaneous delivery of the reconstituted formulation is intended. However, for other routes of administration, such as intravenous administration, lower concentrations of the protein in the reconstituted formulation may be desired (for example from about 5-50 mg/ml, or from about 10-40 mg/ml protein in the reconstituted formulation). In certain embodiments, the protein concentration in the reconstituted formulation is significantly higher than that in the pre-lyophilized formulation. For example, the protein concentration in the reconstituted formulation may be about 2-40 times, alternatively 3-10 times, alternatively 3-6 times *(e.g.,* at least three fold or at least four fold) that of the pre-lyophilized formulation.

Reconstitution generally takes place at a temperature of about 25°C to ensure complete hydration, although other temperatures may be employed as desired. The time required for reconstitution will depend, *e.g.,* on the type of diluent, amount of excipient(s) and protein. Exemplary diluents include sterile water, bacteriostatic water for injection

(BWF), a pH buffered solution *(e.g.,* phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution. The diluent optionally contains a preservative. Exemplary preservatives have been described above, with aromatic alcohols such as benzyl or phenol alcohol being the preferred preservatives. The amount of preservative employed is determined by assessing different preservative concentrations for compatibility with the protein and preservative efficacy testing. For example, if the preservative is an aromatic alcohol (such as benzyl alcohol), it can be present in an amount from about 0.1-2.0% and preferably from about 0.5-1.5%, but most preferably about 1.0-1.2%.

Preferably, the reconstituted formulation has less than 6000 particles per vial which are ≥10 μm in size.

Therapeutic formulations are prepared for storage by mixing the active ingredient having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers *(Remington's Pharmaceutical Sciences* 18th edition, Mack Publishing Co., Easton, Pa. 18042 [1990]). Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers, antioxidants including ascorbic acid, methionine, Vitamin E, sodium metabisulfite, preservatives, isotonicifiers, stabilizers, metal complexes *(e.g.,* Zn-protein complexes), and/or chelating agents such as EDTA.

When the therapeutic agent is an antibody fragment, the smallest fragment which specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable region sequences of an antibody, antibody fragments or even peptide molecules can be designed which retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology (see, *e.g.,* Marasco *et al, Proc. Natl. Acad. Sci. USA* 90: 7889-7893 [1993]).

Buffers are used to control the pH in a range which optimizes the therapeutic effectiveness, especially if stability is pH dependent. Buffers are preferably present at concentrations ranging from about 1 mM to about 200 mM, alternatively from about 1 mM to about 100 mM, alternatively from about 1 mM to about 50 mM, alternatively from about 3 mM to about 15 mM. Suitable buffering agents for use with the present invention include both organic and inorganic acids and salts thereof. For example, citrate,

phosphate, succinate, tartrate, fumarate, gluconate, oxalate, lactate, acetate. Additionally, buffers may be comprised of histidine and trimethylamine salts such as Tris.

Preservatives are added to retard microbial growth, and are typically present in a range from 0.2%- 1.0% (w/v). Suitable preservatives for use with the present invention include octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium halides *(e.g.,* chloride, bromide, iodide), benzethonium chloride; thimerosal, phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol, 3-pentanol, and m-cresol.

Tonicity agents, sometimes known as "stabilizers" are present to adjust or maintain the tonicity of a liquid composition. When used with large, charged biomolecules such as proteins and antibodies, they are often termed "stabilizers" because they can interact with the charged groups of the amino acid side chains, thereby lessening the potential for inter and intra-molecular interactions. Tonicity agents can be present in any amount between 0.1% to 25% by weight, preferably 1 to 5%, taking into account the relative amounts of the other ingredients. Preferred tonicity agents include polyhydric sugar alcohols, preferably trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol.

Additional excipients include agents which can serve as one or more of the following: (1) bulking agents, (2) solubility enhancers, (3) stabilizers and (4) and agents preventing denaturation or adherence to the container wall. Such excipients include: polyhydric sugar alcohols (enumerated above); amino acids such as alanine, glycine, glutamine, asparagine, histidine, arginine, lysine, ornithine, leucine, 2-phenylalanine, glutamic acid, threonine, etc.; organic sugars or sugar alcohols such as sucrose, lactose, lactitol, trehalose, stachyose, mannose, sorbose, xylose, ribose, ribitol, myoinisitose, myoinisitol, galactose, galactitol, glycerol, cyclitols *(e.g.,* inositol), polyethylene glycol; sulfur containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, a-monothioglycerol and sodium thio sulfate; low molecular weight proteins such as human serum albumin, bovine serum albumin, gelatin or other immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides *(e-g-*, xylose, mannose, fructose, glucose; disaccharides *(e.g.,* lactose, maltose, sucrose); trisaccharides such as raffinose; and polysaccharides such as dextrin or dextran.

In order for the formulations to be used for *in vivo* administration, they must be sterile. The formulation may be rendered sterile by filtration through sterile filtration

membranes. The therapeutic compositions herein generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The route of administration is in accordance with known and accepted methods, such as by single or multiple bolus or infusion over a long period of time in a suitable manner, *e.g.*, injection or infusion by subcutaneous, intravenous, intraperitoneal, intramuscular, intraarterial, intralesional or intraarticular routes, topical administration, inhalation or by sustained release or extended-release means.

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise a cytotoxic agent, cytokine or growth inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 18th edition, *supra.*

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ-ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. Microencapsulation of recombinant proteins for sustained release has been successfully performed with human growth hormone (rhGH), interferon- (rhIFN-), interleukin-2, and MN rpg 120. Johnson *et al*, *Nat. Med.* 2: 795-799 (1996); Yasuda *et al*, *Biomed. Ther.*

27: 1221-1223 (1993); Hora *et al, Bio/Technology* 8: 755-758 (1990); Cleland, "Design and Production of Single Immunization Vaccines Using Polylactide Polyglycolide Microsphere Systems," in *Vaccine Design: The Subunit and Adjuvant Approach*, Powell and Newman, eds., (Plenum Press: New York, 1995), pp. 439-462; WO 97/03692; WO 96/40072; WO 96/07399; and U.S. Pat. No. 5,654,010.

The sustained-release formulations of these proteins may be developed using poly lactic-coglycolic acid (PLGA) polymer due to its biocompatibility and wide range of biodegradable properties. The degradation products of PLGA, lactic and glycolic acids, can be cleared quickly within the human body. Moreover, the degradability of this polymer can be adjusted from months to years depending on its molecular weight and composition. Lewis, "Controlled release of bioactive agents from lactide/glycolide polymer", in *Biodegradable Polymers as Drug Delivery Systems* (Marcel Dekker; New York, 1990), M. Chasin and R. Langer (Eds.) pp. 1-41.

While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S--S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

Liposomal or proteinoid compositions may also be used to formulate the proteins or antibodies disclosed herein. See U.S. Pat. Nos. 4,925,673 and 5,013,556.

Stability of the proteins and antibodies described herein may be enhanced through the use of non-toxic "water-soluble polyvalent metal salts". Examples include Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}, Fe^{3+}, Cu^{2+}, Sn^{2+}, Sn^{3+}, Al^{2+} and Al^{3+}. Example anions that can form water soluble salts with the above polyvalent metal cations include those formed from inorganic acids and/or organic acids. Such water-soluble salts have a solubility in water (at 20°C) of at least about 20 mg/ml, alternatively at least about 100 mg/ml, alternative at least about 200 mg/ml.

Suitable inorganic acids that can be used to form the "water soluble polyvalent metal salts" include hydrochloric, sulfuric, nitric, thiocyanic and phosphoric acid. Suitable organic acids that can be used include aliphatic carboxylic acid and aromatic acids. Aliphatic acids within this definition may be defined as saturated or unsaturated C2-9 carboxylic acids *(e.g.,* aliphatic mono-, di- and tri-carboxylic acids). For example, exemplary monocarboxylic acids within this definition include the saturated C2-9 monocarboxylic acids acetic, proprionic, butyric, valeric, caproic, enanthic, caprylic pelargonic and capryonic, and the unsaturated C2-9 monocarboxylic acids acrylic, propriolic methacrylic, crotonic and isocrotonic acids. Exemplary dicarboxylic acids include the saturated C2-9 dicarboxylic acids malonic, succinic, glutaric, adipic and pimelic, while unsaturated C2-9 dicarboxylic acids include maleic, fumaric, citraconic and mesaconic acids. Exemplary tricarboxylic acids include the saturated C2-9 tricarboxylic acids tricarballylic and 1,2,3-butanetricarboxylic acid. Additionally, the carboxylic acids of this definition may also contain one or two hydroxyl groups to form hydroxy carboxylic acids. Exemplary hydroxy carboxylic acids include glycolic, lactic, glyceric, tartronic, malic, tartaric and citric acid. Aromatic acids within this definition include benzoic and salicylic acid.

Commonly employed water soluble polyvalent metal salts which may be used to help stabilize the encapsulated polypeptides of this invention include, for example: (1) the inorganic acid metal salts of halides *(e.g.,* zinc chloride, calcium chloride), sulfates, nitrates, phosphates and thiocyanates; (2) the aliphatic carboxylic acid metal salts *(e.g.,* calcium acetate, zinc acetate, calcium proprionate, zinc glycolate, calcium lactate, zinc lactate and zinc tartrate); and (3) the aromatic carboxylic acid metal salts of benzoates *(e.g.,* zinc benzoate) and salicylates.

For the prevention or treatment of disease, the appropriate dosage of an active agent will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the agent, and the discretion of the attending physician. The agent is suitably administered to the patient at one time or over a series of treatments.

The method of the invention can be combined with known methods of treatment for a disorder, either as combined or additional treatments steps or as additional components of a therapeutic formulation.

Dosages and desired drug concentration of pharmaceutical compositions of the present invention may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary artisan. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles laid down by Mordenti, J. and Chappell, W. "The Use of Interspecies Scaling in Toxicokinetics," In *Toxicokinetics and New Drug Development*, Yacobi *et al.*, Eds, Pergamon Press, New York 1989, pp. 42-46.

When *in vivo* administration of the polypeptides or antibodies described herein are used, normal dosage amounts may vary from about 10 ng/kg up to about 100 mg/kg of mammal body weight or more per day, preferably about 1 mg/kg/day to 10 mg/kg/day, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature; see, for example, U.S. Pat. No. 4,657,760; 5,206,344; or 5,225,212. It is within the scope of the invention that different formulations will be effective for different treatments and different disorders, and that administration intended to treat a specific organ or tissue may necessitate delivery in a manner different from that to another organ or tissue. Moreover, dosages may be administered by one or more separate administrations, or by continuous infusion. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

The formulations of the present invention, including but not limited to reconstituted formulations, are administered to a mammal in need of treatment with the protein, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes.

In preferred embodiments, the formulations are administered to the mammal by subcutaneous *{i.e.*, beneath the skin) administration. For such purposes, the formulation may be injected using a syringe. However, other devices for administration of the formulation are available such as injection devices *{e.g.*, the Inject-ease™ and Genject™

devices); injector pens (such as the GenPen™); auto-injector devices, needleless devices *(e.g.,* MediJector™ and BioJector™); and subcutaneous patch delivery systems.

In a specific embodiment, the present invention is directed to kits for a single dose-administration unit. Such kits comprise a container of an aqueous formulation of therapeutic protein or antibody, including both single or multi-chambered pre-filled syringes. Exemplary pre-filled syringes are available from Vetter GmbH, Ravensburg, Germany.

The appropriate dosage ("therapeutically effective amount") of the protein will depend, for example, on the condition to be treated, the severity and course of the condition, whether the protein is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the protein, the type of protein used, and the discretion of the attending physician. The protein is suitably administered to the patient at one time or over a series of treatments and may be administered to the patient at any time from diagnosis onwards. The protein may be administered as the sole treatment or in conjunction with other drugs or therapies useful in treating the condition in question.

Where the protein of choice is an antibody, from about 0.1-20 mg/kg is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques.

In another embodiment of the invention, an article of manufacture is provided which contains the formulation and preferably provides instructions for its use. The article of manufacture comprises a container. Suitable containers include, for example, bottles, vials *(e.g.,* dual chamber vials), syringes (such as single or dual chamber syringes) and test tubes. The container may be formed from a variety of materials such as glass or plastic. The label, which is on, or associated with, the container holding the formulation may indicate directions for reconstitution and/or use. The label may further indicate that the formulation is useful or intended for subcutaneous administration. The container holding the formulation may be a multi-use vial, which allows for repeat administrations *(e-g-*, from 2-6 administrations) of the reconstituted formulation. The article of manufacture may further comprise a second container comprising a suitable diluent *(e.g.,* BWFI). Upon mixing of the diluent and the lyophilized formulation, the final protein concentration in the reconstituted formulation will generally be at least 50 mg/ml. The

article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. All citations throughout the disclosure are hereby expressly incorporated by reference.

EXAMPLE 1 - Investigation of Protein Viscosity in Solution

This example illustrates measurements of viscosity of various antibody-containing formulations.

The viscosity of various aqueous formulations of an anti-CD4 monoclonal antibody in solution was evaluated. Specifically, in this study, buffered solutions containing various concentrations of anti-CD4 monoclonal antibody (20 mM Histidine-succinate, pH 6.3) were prepared and the viscosity of the resulting solution was determined. In this regard, viscosity was measured using a standard cone-and-plate rheometer (TA Instruments AR-G2 stress rheometer using a 20 mm diameter, 1 degree cone, and water solvent trap) at a temperature of 25°C and a shear rate of 1000 1/s. Upon loading, each sample was allowed to equilibrate for 2 minutes at 25°C prior to the start of data collection. Data was collected for a minimum of 2 minutes to ensure steady state was reached. Solutions were prepared by dialysis and/or addition of the dry excipient into a concentrated protein solution to achieve the desired final excipient concentration. Samples were stored at 2-8°C until being brought to room temperature prior to sample loading. Protein concentration measurements of each sample were made using UV absorbance spectroscopy by gravimetric dilution. Samples were measured within 2 weeks of preparation (usually within 2-3 days). The results of these initial analyses are shown in Table I below.

Table I

Antibody Concentration (mg/ml)	Excipient	Absolute Viscosity (cP)
195.4 mg/ml anti-CD4 antibody	none	75.3 cP
219.2 mg/ml anti-CD4 antibody	none	145.2 cP
228.8 mg/ml anti-CD4 antibody	none	193.7 cP
245.8 mg/ml anti-CD4 antibody	none	328.6 cP

EXAMPLE 2 - Investigation of the Effect of Arginine on the Viscositv of an Aqueous Antibody-Containing Formulation

This example illustrates how arginine-HCl and arginine succinate (arginine-S) effect the viscosity of an aqueous monoclonal antibody-containing formulation.

The viscosity-reducing effect of arginine-HCl and arginine succinate in an aqueous formulation of an anti-CD4 monoclonal antibody in solution was evaluated. Specifically, in this study, buffered solutions containing various concentrations of anti-CD4 monoclonal antibody (20 mM Histidine-succinate, pH 6.3) were prepared in combination with various concentrations of free arginine and the viscosity of the resulting solution was determined as described above. The results of these analyses are shown in Table II below.

Table II

Antibody Concentration (mg/ml)	Excipient	Absolute Viscositv (cP)
243.3 mg/ml anti-CD4 antibody	30 mM arginine-HCl	128.8 cP
228.0 mg/ml anti-CD4 antibody	200 mM arginine-S	34.4 cP
228.0 mg/ml anti-CD4 antibody	410 mM arginine-S	34.8 cP
235.5 mg/ml anti-CD4 antibody	1000 mM arginine-S	49.9 cP

The data shown in Table II demonstrate that the buffered anti-CD4 antibody-containing aqueous formulation is highly viscous and that addition of 30 mM arginine-HCl functions to significantly reduce the viscosity of the resulting solution. Also, addition of increasing amounts of arginine succinate has a viscosity-reducing effect. Hence, these data demonstrate that arginine-HCl and arginine with a succinate counterion, e.g., arginine succinate, serve as effective excipients/additives for use in reducing the viscosity of high concentration protein-containing formulations, thereby making those formulations more amenable to administration via the subcutaneous route.

EXAMPLE 3 - Investigation of the Effect of Various Arginine Derivatives, Precursors, and Structural Analogs on the Viscositv of an Aqueous Antibody-Containing Formulation

This example illustrates how various arginine derivatives, precursors and structural analogs effect the viscosity of an aqueous monoclonal antibody-containing formulation.

Given that the data in Example 2 demonstrated that arginine-HCl and arginine succinate have a beneficial effect on reducing the viscosity of high concentration antibody-containing formulations, we next sought to determine the effect that various different arginine derivatives, precursors and structural analogs would have on such protein-containing formulations. Specifically, in the following studies, buffered solutions containing various concentrations of anti-CD4 monoclonal antibody (20 mM Histidine-succinate, pH 6.3) were prepared in combination with various concentrations of different derivatives, precursors or analogs of arginine and the viscosity of the resulting solution was determined using a standard cone and plate rheometer as described above. More specifically, viscosity was measured using a standard cone-and-plate rheometer (TA Instruments AR-G2 stress rheometer using a 20 mm diameter, 1 degree cone, and water solvent trap) at a temperature of 25°C and a shear rate of 1000 1/s. Upon loading, each sample was allowed to equilibrate for 2 minutes at 25°C prior to the start of data collection. Data was collected for a minimum of 2 minutes to ensure steady state was reached. Solutions were prepared by dialysis and/or addition of the dry excipient into a concentrated protein solution to achieve the desired final excipient concentration. Samples were stored at 2-8°C until being brought to room temperature prior to sample loading. Protein concentration measurements of each sample were made using UV absorbance spectroscopy by gravimetric dilution.

A. Arginine Oligopeptides

The effect of adding arginine dipeptide, arginine tripeptide or polyarginine to aqueous anti-CD4 monoclonal antibody formulations was determined as described above. The results of these analyses are shown in Table III below.

Table III

Antibody Concentration (mg/ml)	Excipient	Absolute Viscosity (cP)
243.9 mg/ml anti-CD4 antibody	30 mM arginine dipeptide	85.3 cP
243.9 mg/ml anti-CD4 antibody	30 mM arginine tripeptide	67.3 cP
221.6 mg/ml anti-CD4 antibody	150 mM arginine dipeptide	40.8 cP
227.5 mg/ml anti-CD4 antibody	150 mM arginine tripeptide	34.7 cP
206.8 mg/ml anti-CD4 antibody	0.1 mg/ml polyarginine (MW = 5,000 - 15,000)	89.6 cP

B. Varying Arginine Side Chain Length

The effect of altering side chain length of the arginine-based excipient on aqueous anti-CD4 monoclonal antibody formulations was determined as described above. The results of these analyses are shown in Table IV below.

Table IV

Antibody Concentration (mg/ml)	Excipient	Absolute Viscosity (cP)
226.4 mg/ml anti-CD4 antibody	200 mM homoarginine	32.9 cP
230.0 mg/ml anti-CD4 antibody	200 mM 2-amino-3-guanidinopropionic acid	33.5 cP

C. Removing Arginine Functional Groups

The effect of removing various functional groups from the arginine-based excipient on aqueous anti-CD4 monoclonal antibody formulations was determined as described above. The results of these analyses are shown in Table V below.

Table V

Antibody Concentration (mg/ml)	Excipient	Absolute Viscosity (cP)
239.4 mg/ml anti-CD4 antibody	200 mM guanidine	74.4 cP
243.4 mg/ml anti-CD4 antibody	200 mM ornithine	67.3 cP
220.4 mg/ml anti-CD4 antibody	200 mM agmatine	27.4 cP
231.5 mg/ml anti-CD4 antibody	200 mM guanidobutyric acid	82.3 cP

D. Other Related Compounds

The effect of other arginine-related compounds on formulation viscosity was also analyzed and the results shown in Table VI below.

Table VI

Antibody Concentration (mg/ml)	Excipient	Absolute Viscosity (cP)
233.8 mg/ml anti-CD4 antibody	200 mM urea	66.4 cP
235.5 mg/ml anti-CD4 antibody	200 mM citrulline	131.3 cP
218.8 mg/ml anti-CD4 antibody	200 mM canavanine	842.6 cP
230.2 mg/ml anti-CD4 antibody	200 mM N-hydroxy-nor-arginine	44.1 cP
225.0 mg/ml anti-CD4 antibody	200 mM nitroarginine methyl ester	28.2 cP
227.4 mg/ml anti-CD4 antibody	200 mM NG-NG-dimethyl-arginine dihydrochloride	419.9 cP
236.2 mg/ml anti-CD4 antibody	200 mM argininamide	34.6 cP
224.2 mg/ml anti-CD4 antibody	200 mM arginine methyl ester	25.1 cP
239.3 mg/ml anti-CD4 antibody	200 mM arginine ethyl ester	35.9 cP
236.5 mg/ml anti-CD4 antibody	200 mM lysine methyl ester	39.0 cP
245.7 mg/ml anti-CD4 antibody	200 mM lysine	78.7 cP
243.5 mg/ml anti-CD4 antibody	200 mM lysinamide	55.1 cP
245.1 mg/ml anti-CD4 antibody	200 mM histidine	63.6 cP
246.5 mg/ml anti-CD4 antibody	200 mM histidine methyl ester	109.0 cP
245.9 mg/ml anti-CD4 antibody	200 mM histamine	46.3 cP
249.2 mg/ml anti-CD4 antibody	200 mM alanine	35.3 cP
247.1 mg/ml anti-CD4 antibody	200 mM alaninamide	88.0 cP
247.9 mg/ml anti-CD4 antibody	200 mM alanine methyl ester	84.6 cP
248.1 mg/ml anti-CD4 antibody	200 mM glutamic acid amide	206.3 cP
248.4 mg/ml anti-CD4 antibody	200 mM gamma-amino butyric acid	197.6 cP
240.7 mg/ml anti-CD4 antibody	200 mM glutamine methyl ester	1396.0 cP
227.4 mg/ml anti-CD4 antibody	200 mM putrescine	31.5 cP
239.8 mg/ml anti-CD4 antibody	200 mM cadaverine	39.5 cP

Table VI (conf)

Antibody Concentration (mg/ml)	Excipient	Absolute Viscosity (cP)
232.7 mg/ml anti-CD4 antibody	200 mM spermidine	36.8 cP
238.6 mg/ml anti-CD4 antibody	200 mM spermine	35.0 cP
230.1 mg/ml anti-CD4 antibody	200 mM methionine	110.8 cP
250.2 mg/ml anti-CD4 antibody	200 mM guanidine, 200 mM ammonium HC1	67.0 cP
251.2 mg/ml anti-CD4 antibody	100 mM guanidine, 100 mM ammonium HC1	105.7 cP

E. Summary

The data presented in Table I above demonstrates that arginine (either arginine-HC1 or arginine succinate) is an excipient that effectively reduces the viscosity of high concentration protein-containing solutions. Based upon this data, additional experiments were conducted to test the effect of various other "arginine-related" excipients on the viscosity of aqueous high concentration protein-containing solutions. As shown in Tables II-VI, many of the additional excipients tested demonstrated a viscosity-lowering effect. Interestingly, other structurally-related excipients (e.g., canavanine and NG-NG-dimethyl-arginine dihydrochloride) actually functioned to increase the viscosity of the high concentration protein-containing solution, demonstrating that structural homology to arginine is not predictive of the effect that the compound may have on a protein-containing solution.

EXAMPLE 4 - Investigation of the Dependence of Viscosity on Excipient Concentration

This example illustrates the effect of varying excipient concentration on the viscosity of an aqueous monoclonal antibody-containing formulation.

The viscosity-reducing effect of various different concentrations of two excipients shown in Example 3 above as being capable of reducing the viscosity of high concentration protein-containing solutions was evaluated. Specifically, in this study, buffered solutions containing various concentrations of anti-CD4 monoclonal antibody (20 mM Histidine-succinate, pH 6.3) were prepared in combination with various different concentrations of either agmatine or homoarginine and the viscosity of the resulting

solution was determined as described above. The results of these analyses are shown in Table VII, where viscosity measurements presented represent the average of that obtained from two independent analyses of the same aqueous formulation.

Table VII

Antibody Concentration (mg/ml)	Excipient	Viscosity (cP)
234.4 mg/ml anti-CD4 antibody	11 mM arginine	149.1 cP
232.0 mg/ml anti-CD4 antibody	52 mM arginine	70.5 cP
234.0 mg/ml anti-CD4 antibody	11 mM agmatine	122.2 cP
232.7 mg/ml anti-CD4 antibody	55 mM agmatine	59.7 cP
231.7 mg/ml anti-CD4 antibody	107 mM agmatine	46.4 cP
230.8 mg/ml anti-CD4 antibody	204 mM agmatine	36.1 cP
224.5 mg/ml anti-CD4 antibody	469 mM agmatine	28.8 cP
215.3 mg/ml anti-CD4 antibody	895 mM agmatine	27.0 cP
234.2 mg/ml anti-CD4 antibody	10 mM homoarginine	153.9 cP
232.0 mg/ml anti-CD4 antibody	50 mM homoarginine	71.7 cP
229.5 mg/ml anti-CD4 antibody	101 mM homoarginine	44.5 cP
224.3 mg/ml anti-CD4 antibody	196 mM homoarginine	29.6 cP
216.5 mg/ml anti-CD4 antibody	449 mM homoarginine	21.8 cP
200.9 mg/ml anti-CD4 antibody	819 mM homoarginine	21.1 cP

The data presented in Table VII above demonstrates that the viscosity-lowering effect of excipients shown in Example 3 above as having a viscosity lowering effect occurs over a broad range of concentrations. More specifically, it is apparent from the data presented in Table VII that viscosity lowering effects generally become apparent at around a concentration of about 10 mM and are enhanced and maintained through concentrations approaching 900 mM to 1 M. Given these data, one would expect that excipients demonstrated herein as having a viscosity lowering effect wold exhibit that effect over a broad range of concentrations between and including from about 10 mM to about 1 M.

WHAT IS CLAIMED IS:

1. A composition of matter comprising a protein and a compound capable of reducing the viscosity of an aqueous formulation comprising said protein.

2. The composition of matter of Claim 1, wherein the protein is an antibody.

3. The composition of matter of Claim 1, wherein said compound capable of reducing the viscosity of an aqueous formulation comprising said protein is selected from the group consisting of arginine-HCl, arginine succinate, arginine dipeptide, arginine tripeptide, polyarginine, homoarginine, 2-amino-3-guanidino-propionic acid, guanidine, ornithine, agmatine, guanidobutyric acid, urea, citrulline, N-hydroxy-L-nor-arginine, nitroarginine methyl ester, argininamide, arginine methyl ester, arginine ethyl ester, lysine, lysinamide, lysine methyl ester, histidine, histidine methyl ester, histamine, alanine, alaninamide, alanine methyl ester, putrescine, cadaverine, spermidine, spermine, and methionine.

4. The composition of matter of Claim 3, wherein said compound capable of reducing the viscosity of said aqueous formulation is present at a concentration of at least 10 mM.

5. The composition of matter of Claim 3, wherein said compound capable of reducing the viscosity of said aqueous formulation is present at a concentration of at least 20 mM.

6. The composition of matter of Claim 3, wherein said compound capable of reducing the viscosity of said aqueous formulation is present at a concentration of at least 50 mM.

7. The composition of matter of Claim 3, wherein said compound capable of reducing the viscosity of said aqueous formulation is present at a concentration of at least 100 mM.

8. The composition of matter of Claim 3, wherein said compound capable of reducing the viscosity of said aqueous formulation is present at a concentration of from about 10 mM to about 1 M.

9. The composition of matter of Claim 1 which is in aqueous form.

10. The composition of matter of Claim 1 which is in lyophilized form.

11. The composition of matter of Claim 1, wherein the protein concentration is at least 100 mg/ml.

12. The composition of matter of Claim 1, wherein the viscosity is no greater than 150 cP.

13. An article of manufacture comprising a container holding the composition of matter of Claim 1.

14. A method of reducing the viscosity of a protein-containing formulation, said method comprising the step of adding to said formulation a viscosity reducing amount of a compound capable of reducing the viscosity of an aqueous formulation comprising said protein.

15. The method of Claim 14, wherein said compound capable of reducing the viscosity of an aqueous formulation comprising said protein is selected from the group consisting of arginine-HCl, arginine succinate, arginine dipeptide, arginine tripeptide, polyarginine, homoarginine, 2-amino-3-guanidino-propionic acid, guanidine, ornithine, agmatine, guanidobutyric acid, urea, citrulline, N-hydroxy-L-nor-arginine, nitroarginine methyl ester, argininamide, arginine methyl ester, arginine ethyl ester, lysine, lysinamide, lysine methyl ester, histidine, histidine methyl ester, histamine, alanine, alaninamide, alanine methyl ester, putrescine, cadaverine, spermidine, spermine, and methionine.

16. The method of Claim 14, wherein said compound is added to a final concentration of at least 10 mM.

17. The method of Claim 14, wherein said compound is added to a final concentration of at least 20 mM.

18. The method of Claim 14, wherein said compound is added to a final concentration of at least 50 mM.

19. The method of Claim 14, wherein said compound is added to a final concentration of at least 100 mM.

20. The method of Claim 14, wherein said compound is added to a final concentration of between about 10 mM and about 1 M.

21. The method of Claim 14, wherein said protein is an antibody.

22. The method of Claim 14 further compising the step of lyophilizing said formulation.

23. The method of Claim 14, wherein the protein concentration present in said formulation is at least 100 mg/ml.

24. The method of Claim 14, wherein the viscosity of said formulation is no greater than 150 cP.

25. A method of preparing an aqueous protein-containing formulation, said method comprising the step of adding to a protein-containing solution a viscosity reducing amount of a compound capable of reducing the viscosity of an aqueous formulation comprising said protein.

26. The method of Claim 25, wherein said compound capable of reducing the viscosity of an aqueous formulation comprising said protein is selected from the group consisting of arginine-HCl, arginine succinate, arginine dipeptide, arginine tripeptide, polyarginine, homoarginine, 2-amino-3-guanidino-propionic acid, guanidine, ornithine, agmatine, guanidobutyric acid, urea, citrulline, N-hydroxy-L-nor-arginine, nitroarginine methyl ester, argininamide, arginine methyl ester, arginine ethyl ester, lysine, lysinamide, lysine methyl ester, histidine, histidine methyl ester, histamine, alanine, alaninamide, alanine methyl ester, putrescine, cadaverine, spermidine, spermine, and methionine.

27. The method of Claim 25, wherein said compound is added to a final concentration of at least 10 mM.

28. The method of Claim 25, wherein said compound is added to a final concentration of at least 20 mM.

29. The method of Claim 25, wherein said compound is added to a final concentration of at least 50 mM.

30. The method of Claim 25, wherein said compound is added to a final concentration of at least 100 mM.

31. The method of Claim 25, wherein said compound is added to a final concentration of between about 10 mM and about 1 M.

32. The method of Claim 25, wherein said protein is an antibody.

33. The method of Claim 25, wherein the protein concentration present in said formulation is at least 100 mg/ml.

34. The method of Claim 25, wherein the viscosity of said formulation is no greater than 150 cP.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/034001

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 C07K16/28 A61K47/18
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal , BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/065746 A2 (GENENTECH INC [US] ; NOVARTIS AG [CH] ; LIU JUN [US] ; SHIRE STEVEN J [US) 22 June 2006 (2006-06-22) e.g. claim 1; the whole document	1-34
X	WO 2004/091658 Al (GENENTECH INC [US] ; LIU JUN [US] ; SHIRE STEVEN [US]) 28 October 2004 (2004-10-28) e.g. claim 1; the whole document	1-34
X	EP 1 688 432 Al (CHUGAI PHARMACEUTICAL CO LTD [JP]) 9 August 2006 (2006-08-09) e.g. paragraph 15 , 50; the whole document	1-34

-/- .

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" documentwhich may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such docu¬ments, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 July 2011	22/09/2011

Name and mailing address of the ISA/	Authorized officer
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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/034001

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 1 977 763 Al (CHUGAI PHARMACEUTICAL CO LTD [JP]) 8 October 2008 (2008-10-08) e.g. paragraph 55; claim 1; the whole document -----	1-34
A	WO 2007/076062 A2 (WYETH CORP [US]; WARNE NICHOLAS W [US]; NICHOLS PILARIN E [US]; L0UREI) 5 July 2007 (2007-07-05) e.g. claim 1, 13-21; the whole document -----	1-34

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2011/034001

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-34(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: l-34(partially)

composition according to claim 1, article of manufacture according to claim 13, method according to claim 14,25, all of the aforementioned comprising as compound capable of reducing the viscosity of an aqueous formulation comprising a protein is arginine-HCl

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2-32. claims: l-34(partially)

composition according to claim 1, article of manufacture according to claim 13, method according to claim 14,25, all of the aforementioned comprising as compound capable of reducing the viscosity of an aqueous formulation comprising a protein is arginine succinate, arginine dipeptide, arginine tripeptide, polyarginine, homoarginine, 2-amino-3-guanidino-propionic acid, guanidine, ornithine, agmatine, guanidobutyric acid, urea, citrulline, N-hydroxy-L-nor-arginine, nitroarginine methyl ester, argininamide, arginine methyl ester, arginine ethyl ester, lysine, lysinamide, lysine methyl ester, histidine, histidine methyl ester, histamine, alanine, alaninamide, alanine methyl ester, putrescine, cadaverine, spermidine, spermine, or methionine; i.e. each compound being one invention, e.g. invention 2: arginine succinate; invention 3: arginine dipeptide; ...; invention 32: methionine

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2011/034001

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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