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(71) Applicants: PROTEIN SPECIALTIES, LTD. [CA/CA]; 33 Harbour Square #2018, Toronto, Ontario M5J 2G2 (CA). THE HOSPITAL FOR SICK CHILDREN [CA/CA]; 555 University Avenue, Toronto, Ontario M5G 1X8 (CA).

(72) Inventors: ROTHSTEIN, Aser; Apartment 2018, 33 Harbour Square, Toronto, Ontario M5J 2G2 (CA). KEELEY, Fred, W.; 17 Minton Place, Toronto, Ontario M4K 3X7 (CA). ROTHSTEIN, Steven, J.; 15 Fieldstone Road, Guelph, Ontario N1L 1A5 (CA).

(74) Agents: COTE, France et al.; Swabey Ogilvy Renault, Suite 1600, 1981 McGill College Avenue, Montréal, Québec H3A 2Y3 (CA).

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(54) Title: SELF-ALIGNING PEPTIDES DERIVED FROM ELASTIN AND OTHER FIBROUS PROTEINS

(57) Abstract

A polypeptide is provided that has a secondary structure characterized by at least three beta-sheet/beta-turn structures, and that is not a naturally occurring fibrous protein. Such polypeptides, illustrated by one modeled on elastin, are useful in prosthesis.

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SELF-ALIGNING PEPTIDES DERIVED FROM ELASTIN AND OTHER FIBROUS PROTEINS

BACKGROUND OF THE INVENTION

The present invention relates to self-aligning peptides modeled on human elastin and other fibrous proteins. The peptides are useful, for example, as biocompatible material for implantation into humans, or for elastic materials.

Currently available synthetic implant materials for soft tissue prosthesis fall short of optimal biocompatibility. The ideal material would provide appropriate structural support, would be biocompatible, in the sense of causing no immunogenic or thrombogenic response, would mimic the physical properties of the tissue replaced, and would provide a friendly environment for normal cell infiltration and growth.

While tissue can sometimes be borrowed from another part of the patient's body, such as by skin grafting or blood vessel replacement, this approach has several limitations, including the limited availability of appropriate donor tissue. Synthetic materials such as dacron, teflon (Gortex) and polyurethane, as well as metals (such as stainless steel and titanium), often are used for prostheses of soft tissues. While these materials can meet the requirements of strength, durability, and flexibility, as foreign materials they are not maximally biocompatible for long term use.

One approach to dealing with this problem has been to coat non-biological materials with proteins or other natural substances. Another approach has been to use biological materials from animal tissue preparations. For example, animal skin preparations have been used to cover burns, and processed animal blood vessels have been

used to provide potential blood vessel replacements for humans.

Elastin, a natural structural protein, has received considerable attention for potential use in prostheses, both in soluble forms for coating non-biological prostheses, and in solid forms to produce biologically-derived prostheses. Elastin has structural properties which make it suitable for use in prosthesis and it provides a biocompatible, non-thrombogenic surface for cell infiltration. It is a durable, extremely stable, and highly insoluble extracellular matrix protein which imparts the properties of extensibility and elastic recoil to tissues in which it is found, including large blood vessels, elastic ligaments, lung parenchyma, and skin.

Large arteries are a good source of elastin. Because human arteries are not available in quantity, however, animal arteries have been the primary source of elastin. Because arterial elastin is a highly insoluble matrix, soluble elastin-derived material is generated by treating the insoluble protein with acid or alkali, producing hydrolyzates such as alpha- and kappa-elastin. These are relatively undefined mixtures of peptides of mixed sizes.

In attempts to develop biocompatible materials, soluble animal elastin materials have been used to coat non-biological prosthetic materials, usually with fixation by chemical cross-linking agents. For example, U.S. Patent No. 4,960,423 (Smith) is directed to a synthetic vascular prosthesis coated with a water-soluble peptide derived from animal elastin.

U.S. Patent No. 5,416,074 (Rabaud) is directed to a composition comprising elastin or a solubilized elastin peptide and another connective tissue protein, such as fibrin. The solubilized elastin peptide has a molecular weight of greater than 10,000.

U.S. Patent No. 4,474,851 (Urry) is directed to an elastomeric composite material comprising an artificial core fiber, such as Dacron, and a polypeptide comprising

repeating tetrapeptide or pentapeptide units. The units are derived from units observed to be repeated in the tropoelastin molecule, Val-Pro-Gly-Val-Gly (VPGVG) and Val-Pro-Gly-Gly (VPGG). The polypeptide comprises a series of beta-turns and is proposed to have a beta-coil structure. The polypeptide provides elastomeric properties to the composite material, but has little structural strength or integrity. The artificial core fiber provides these latter properties to the composite material.

U.S. Patent No. 4,979,959 (Guire) is directed to a method of improving the biocompatibility of solid biomaterials by coating them with biocompatible agents and chemically linking the biocompatible agents to the surface via a photochemical reaction.

Elastin-based materials also have been used to produce solid materials from which prostheses can be manufactured. These include soluble animal elastin co-aggregated with other proteins such as collagen, fibrin, fibronectin and laminin, to produce gel-like materials, and polymerized materials derived from short hydrophobic sequences of human elastin (such as PGVGVA). In some cases, these synthetic peptides also include short alanine-rich sequences containing lysine residues, allowing cross-linking between the elastin-like peptides or to other proteins such as collagen. Both elastin and collagen contain crosslinks derived from lysine. For example, U.S. Patent No. 5,223,420 (Rabaud) is directed to an elastin-based product comprising an adduct containing elastin and at least one other protein, such as fibrin.

U.S. Patent No. 4,589,882 (Urry) is directed to an artificial elastomeric copolymer comprising an elastomeric component of repeating units of tetrapeptides and pentapeptides and a crosslinking component which may comprise amino acid residues. The repeating units are derived from elastin. U.S. Patent No. 4,132,746 (Urry) is directed to a synthetic, insoluble, crosslinked

polypentapeptide. The pentapeptide is the VPGVG peptide present in tropoelastin. See also U.S. Patent No. 4,500,700, U.S. Patent No. 4,870,055, and U.S. Patent No. 5,250,516 (all to Urry) for other materials derived from this peptide. The polypeptides described in these patents comprise a series of beta-turns and are proposed to have a beta-coil structure.

Animal arteries also have been stripped of extraneous material, leaving largely a matrix of elastin and collagen in tubular form that can be used for blood vessel replacement. For example, U.S. Patent No. 4,776,853 (Klement) is directed towards a process for preparing an implantable biological material from suitable donor tissue.

The respective contents of the above-described patents and publications are incorporated by reference herein in their entirety.

The materials discussed above were developed to satisfy the need for prostheses suitable for implantation into humans. These materials are not completely satisfactory, however, and there remains a need for prosthesis which have appropriate mechanical properties and which can be used in contact with blood, tissue fluids and cells without adverse effects.

SUMMARY OF THE INVENTION

It is an object of the present invention, therefore, to provide a material that can be used in prostheses that are implanted into humans. It is another object of the invention to provide prostheses suitable for implantation into humans.

In accordance with these and other objects, the invention provides a polypeptide that comprises at least three beta-sheet/beta-turn structures and that is not a naturally occurring fibrous protein. In accordance with one embodiment, the polypeptide consists essentially of a portion of the amino acid sequence set forth in Figure

1B. In accordance with another embodiment, the polypeptide consists essentially of a portion of the amino acid sequence of an animal elastin. In accordance with yet another embodiment, the polypeptide consists essentially of a portion of the amino acid sequence of lamprin. In accordance with another embodiment, the polypeptide consists essentially of a portion of the amino acid sequence of a spider silk protein.

The invention also provides a material suitable for implantation into humans, wherein the material consists essentially of a polypeptide consisting essentially of a portion of the amino acid sequence set forth in Figure 1B. In accordance with another embodiment, the invention provides a prosthesis comprising an animal material, wherein a surface of the animal material is coated with a polypeptide consisting essentially of a portion of the amino acid sequence set forth in Figure 1B. In accordance with another embodiment, the invention provides a prosthesis comprising a synthetic material, wherein a surface of the synthetic material is coated with a polypeptide consisting essentially of a portion of the amino acid sequence set forth in Figure 1B. In accordance with yet another embodiment, the invention provides a prosthesis comprising a metal, wherein a surface of the metal is coated with a polypeptide consisting essentially of a portion of the amino acid sequence set forth in Figure 1B.

The invention also provides a cosmetic material comprising a polypeptide consisting essentially of a portion of the amino acid sequence set forth in Figure 1B.

The invention also provides elastic material and high tensile-strength material comprising a polypeptide that comprises at least three beta-sheet/beta-turn structures and that is not a naturally occurring fibrous protein.

The invention also provides a material comprising two or more polypeptides selected from the group consisting of (A) a polypeptide consisting essentially of a portion

of the amino acid sequence set forth in Figure 1B comprising at least three beta-sheet/beta-turn structures; (B) a polypeptide consisting essentially of a portion of the amino acid sequence of an animal elastin comprising at least three beta-sheet/beta-turn structures; (C) a polypeptide consisting essentially of a portion of the amino acid sequence of lamprin comprising at least three beta-sheet/beta-turn structures; and (D) a polypeptide consisting essentially of a portion of the amino acid sequence of a spider silk protein comprising at least three beta-sheet/beta-turn structures, wherein the two or more polypeptides may be the same or different.

The invention also provides a polypeptide having the primary structure of a portion of a naturally occurring fibrous protein and a secondary structure comprising at least three beta-sheet/beta-turn structures, wherein (A) each of the beta-sheet/beta-turn structures comprises from 3 to about 7 amino acid residues and (B) the polypeptide is not a naturally occurring fibrous protein.

Additional objects and advantages of the invention are set forth in part in the description that follows, and in part will be obvious from the description, or may be learned by practice of the invention. The objects and advantages may be realized and obtained by means of the invention recited in the appended claims.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A shows the domain structure of human elastin. The location of the domains used in the expressed construct described in Example 2 is indicated by the bracketed region.

Figure 1B shows the amino acid sequence of human elastin, without the signal peptide. The underlined amino acid residues comprise the polypeptide of the present invention named MFU-1.

Figure 1C shows the GST fusion construct used to express MFU-1.

Figure 1D is a cartoon representation of the hydrophobic and crosslinking domains corresponding to the expressed exons described in Example 1.

Figure 1E is a schematic diagram of a peptide with beta-sheet/beta-turn structures.

Figure 2 depicts the chromatography on BioGel P-30 in 0.05M acetic acid of cleavage products after cyanogen bromide treatment to release MFU-1 from the GST fusion protein, as described in Example 2. The MFU-1 is contained in Fraction 1.

Figure 3 illustrates the coacervation (self-aggregation) of MFU-1.

Figure 4A shows the GST fusion construct used to express the polypeptide of the present invention named MFU-2.

Figure 4B is a cartoon representation of the hydrophobic and crosslinking domains of MFU-2.

Figure 4C shows the amino acid sequence of MFU-2.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention is directed to unique polypeptides modeled on human elastin and other naturally occurring fibrous proteins. While the discussion below often refers to human elastin as the exemplary parent protein, polypeptides modeled on other naturally occurring fibrous proteins are contemplated by the present invention, and can be made and used in manners analogous to those described for polypeptides modeled on human elastin.

The phrase "parent protein" here denotes the protein on which a polypeptide of the invention is modeled. For example, a polypeptide modeled on human elastin comprises a portion of the human tropoelastin amino acid sequence. A "naturally occurring fibrous protein" is any fibrous protein found in nature, where the phrase "fibrous

protein" has the conventional meaning in the art. Thus, a fibrous protein is a protein that consists of polypeptide chains arranged in a matrix so as to form long fibers or sheets. See Lehninger, BIOCHEMISTRY 60 (1975). Examples of fibrous proteins include, but are not limited to, elastin, lamprin and spider silk protein. Robson *et al.*, *J. Biol. Chem.* 268: 1440-47 (1993), incorporated by reference herein in its entirety, discloses additional proteins on which polypeptides of the present invention may be modeled.

Amino acid sequence information is available for elastin and other fibrous extracellular matrix proteins, such as spider silks and lamprin. Together with analyses of secondary and tertiary structures, this information has led to general theories concerning their mechanical properties and, in particular, mechanisms for their assembly into insoluble fibers.

Elastin is synthesized *in vivo* as a monomer called tropoelastin which, upon secretion from the cell, assembles into a branched polymeric network through the formation of covalent crosslinks called desmosines. Mecham *et al.*, *in* CELL BIOLOGY OF EXTRACELLULAR MATRIX, 2D ED. (New York, 1991). Desmosine crosslinks are generated enzymically through the action of lysyl oxidase. Each desmosine incorporates the side chains of four lysine residues, two from each of the polypeptide chains involved. Although the principles underlying the elastomeric properties of elastin remain a matter of debate, there is agreement that this unusual property is dependent on the strongly hydrophobic nature of the protein.

Tropoelastin consists predominantly of alternating hydrophobic and crosslinking domains. Indik *et al.*, *Proc. Nat'l Acad. Sci. USA* 84: 5680-84 (1986). Crosslinking domains are rich in alanine (A), with the lysines (K) destined for involvement in crosslink formation present in KAAK and KAAAK spacings. The domains separating these crosslinking regions are

strongly hydrophobic in character, and contain many tandemly repeated penta- and hexa-peptide sequences. In human elastin the most striking of these is the sequence PGVGVA, repeated 7 times in exon 24. Indik *et al.*, *supra*.

Structural studies on repeat hydrophobic sequences indicate an exclusively beta-sheet/beta turn structure. That is, they comprise beta-sheets with intervening beta-turns. Analogous beta-sheet/beta-turn structures also contribute to the structures of other self-aggregating, polymeric matrix proteins, including spider silks, lamprin, and silk moth chorion, all of which form stable fibers or matrices with high tensile strength. These structures have been proposed to be crucial for the ability of these proteins to self-assemble. Robson *et al.*, *supra*.

There is evidence that the periodically spaced hydrophobic domains direct the assembly of tropoelastin into higher order structures. Tropoelastin, as well as solubilized fragments of elastin (*i.e.*, kappa-elastin and alpha-elastin), and synthetic peptides corresponding to the hydrophobic repeat sequences can all undergo coacervation, a process in which hydrophobic interactions between polypeptide chains result in the formation of oligomeric, fibrillar structures. This self-aggregation is not random: the hydrophobic domains facilitate the alignment of tropoelastin monomers for crosslinking into the fibrillar elastic matrix. Robson *et al.*, *supra*; Bressan *et al.*, *J. Ultrastr. & Mol. Struct. Res.* 94: 209-16 (1986).

As shown in Figure 1A, human elastin consists for most of its length of alternating crosslinking domains and hydrophobic domains. The crosslinking domains consist mainly of lysine (K) and alanine (A) residues in KAAK and KAAAK sequences, wherein the lysine residues are in a suitable conformation for oxidative deamination by lysyl oxidase and subsequent formation of the covalent desmosine crosslinks. Indik *et al.*, *supra*. The

hydrophobic domains are rich in hydrophobic pentapeptide and hexapeptide sequences believed to be in beta-sheet/beta-turn structures. Tamburro *et al.*, ADVANCES IN LIFE SCIENCES 115-27 (1990). These hydrophobic regions are believed to be important to elastin's physical properties of extensibility and elastic recoil, and to the ability of tropoelastin (the monomeric precursor of elastin) to self-aggregate into fibrillar structures. Robson *et al.*, *supra*; Tamburro *et al.*, *supra*. Other proteins capable of self-aggregation and self-alignment into stable fibrillar matrices, including eggshell chorion proteins of insects, spider dragline silk, and lamprin from lamprey cartilage, all possess similar regions of hydrophobic repeat peptides with beta-sheet/beta-turn structures. Hamodrakas *et al.*, *Int. J. Biol. Macromol.* 11: 307-13 (1989); Simmons *et al.*, *Science* 271: 84-87 (1996); Robson *et al.*, *supra*.

The polypeptides of the present invention are modeled on elastin and other fibrous proteins, such as spider silk and lamprin, and comprise the number and kinds of amino acid residues necessary for self-alignment, which is a first step in fiber formation. For convenience, each polypeptide of the present invention is referred to as a minimal functional unit, or MFU. The secondary structure of an MFU according to the present invention comprises at least three beta-sheet/beta-turn structures.

As discussed above, beta-sheet and beta-turn structures are well known in the art. Beta-sheet structures in accordance with the present invention are typically comprised of several amino acid residues, for example, from 3 to about 7 amino acid residues, acceptably from about 5 to about 7 amino acid residues, and, in particular, from 5 to 7 amino acid residues. The amino acid residues of the beta-sheet structures may have hydrophobic side chains. Beta-turn structures in accordance with the present invention are typically initiated by two amino acid residues, often GG or PG, and may comprise additional amino acid residues. For

example, a beta-turn structure in accordance with the present invention may comprise from about 2 to about 4 amino acid residues, acceptably from 2 to 4 amino acid residues, and, in particular, four amino acid residues.

Figure 1E is a schematic diagram of a peptide with beta-sheet/beta-turn structures. The shaded ribbon represents a peptide. The six straight portions of the ribbon represent the beta-sheet structures and the five curved portions of the ribbon represent the beta-turn structures. The empty circles represent hydrophobic side chains which are directed below the beta-sheets, and the shaded circles represent hydrophobic side chains which are directed above the beta-sheets. These hydrophobic side chains are on amino acid residues such as alanine, valine, isoleucine, leucine, tyrosine and phenylalanine. The rectangles indicate hydrogen bonds which stabilize the beta-turn structures. See also Robson *et al.*, *supra*; Lehninger, *supra*, at pages 133-35.

The MFUs of the present invention are soluble, and exhibit the property of coacervation, aligning themselves in the same manner as the parent protein. For example, the hydrophobic sequences of the MFUs align in the same manner as the hydrophobic sequences of the parent proteins. When considering the secondary structure of the MFUs, this means that the beta-sheets of the MFUs are aligned with each other. This alignment occurs in the same manner as in the parent proteins, with the beta-sheets being stacked in a "lego"-type motif. See Robson, *et al.*, *supra*. In elastin-derived MFUs, the alignment also results in the lysine residues aligning in a manner that permits crosslinking between the MFUs.

One embodiment of the present invention provides a polypeptide having the primary structure (that is, the amino acid sequence) of a portion of a naturally occurring fibrous protein and a secondary structure comprising at least three beta-sheet/beta-turn structures, wherein the polypeptide is not a naturally occurring fibrous protein. Preferably, each of the

beta-sheet/beta-turn structures comprises from 3 to about 7 amino acid residues. The polypeptide is long enough to identify the parent protein to which it corresponds. It is believed that a length of at least about 10 amino acid residues is sufficient in this regard. The polypeptide may be longer and, for example, can be up to the length of the entire parent protein.

Also contemplated as part of the present invention is a polypeptide comprising the primary structure of a portion of a naturally occurring fibrous protein wherein the primary structure is modified by the addition, substitution and/or deletion of one or more amino acid residues. The polypeptide has a secondary structure comprising at least three beta-sheet/beta-turn structures and exhibits the properties of self-alignment described herein. While there is no set limit on the number of modifications that could be made, it is believed that modifications involving the addition, substitution and/or deletion of from 1 to about 20, particularly from 1 to about 10, specifically from 1 to about 5, amino acid residues can be effected while maintaining the above-described properties of the polypeptide.

Preferably, only conservative amino acid alterations are undertaken. Illustrative amino acid substitutions include the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; glycine to proline; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine, glutamine, or glutamate; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; valine to isoleucine or leucine.

For example, modifications in the hydrophobic regions of the polypeptide may comprise substituting one or more of the amino acids residues at the beta-turns with other

amino acids that initiate beta-turns. For example, one or more of the P or G residues may be replaced with a G or P residue, respectively, or may be replaced with a serine residue. Additionally or alternatively, modifications may be made to the amino acid residues in the beta-sheet structure, such as the addition, deletion or substitution of one or more amino acid residues. For example, an amino acid residue having a hydrophobic side chain can be replaced by a different amino acid residue having a hydrophobic side chain, or having a side chain with similar properties. Exemplary substitutions include intersubstitutions of alanine, valine, isoleucine, leucine, tyrosine and phenylalanine.

For polypeptides comprising a crosslinking domain, any number of additions, substitutions and deletions can be made that do not interfere with the alpha-helical structure of the crosslinking domain, such as additions, deletions, and conservative amino acid substitutions, as discussed above. Also, lysine residues can be replaced with any other amino acid residue that participates in crosslinking, such as acidic or basic residues, including arginine, aspartic acid and glutamic acid.

In accordance with one embodiment of the invention, a polypeptide is provided whose amino acid sequence is a variant of a portion of the amino acid sequence set forth in Figure 1B. The amino acid sequence of such a polypeptide corresponds to a portion of the amino acid sequence set forth in Figure 1B, wherein the amino acid sequence set forth in the Figure is modified by the addition, deletion, or substitution of from 1 to about 10 amino acid residues. Such a polypeptide has a secondary structure comprising at least three beta-sheet/beta-turn structures and exhibits the properties of self-alignment described herein. In accordance with another embodiment of the invention, a polypeptide is provided whose amino acid sequence is a variant of the amino acid sequence set forth in Figure 4C. The amino acid sequence of such a polypeptide corresponds to a portion of the amino acid

sequence set forth in Figure 4C, wherein the amino acid sequence set forth in the Figure is modified by the addition, deletion, or substitution of from 1 to about 10 amino acid residues. Such a polypeptide has a secondary structure comprising at least three beta-sheet/beta-turn structures and exhibits the properties of self-alignment described herein.

While the description below uses MFUs modeled on elastin as exemplary MFUs, peptides derived from other proteins are encompassed by the present invention. For example, peptides derived from any other fiber-forming proteins, including spider silk and lamprin, are contemplated as part of the present invention. These MFUs can be obtained as described herein for MFUs modeled on elastin. Moreover, mixtures of MFUs from different parent proteins (*e.g.*, MFUs modeled on lamprin and elastin) can be used together to produce a variety of materials.

The domain structure of human elastin is illustrated in Figure 1A. As shown in this Figure, there are a number of alternating crosslinking and hydrophobic domains. The hydrophobic domains each are believed to comprise a number of beta-sheet/beta-turn-forming sequences. These domains represent probable MFUs of elastin. One of these, used in further experimentation, is designated by the bracket and is named MFU-1 (see Example 1 below). Figure 1B sets forth the amino acid sequence of human elastin. The underlined amino acid residues, residues 374-499, comprise MFU-1. Other MFUs modeled on human elastin include polypeptides comprising amino acid residues 19-160, 188-367 and 607-717, respectively.

MFUs modeled on human elastin comprise a portion of the amino acid sequence of the tropoelastin molecule (Figure 1B) and have at least three beta-sheet/beta-turn structures in their secondary structure. They also may comprise amino acids residues which are capable of participating in crosslinking, such as lysine residues.

In one embodiment of the invention, the MFU comprises two amino acid residues capable of participating in crosslinking in such a manner as to form a desmosine-type linkage. For example, the MFU may comprise a KAAK or KAAAK amino acid sequence.

In a preferred embodiment, a polypeptide modeled on human elastin consists essentially of a portion of the amino acid sequence set forth in Figure 1B. The phrase "A consists essentially of B" herein denotes that A comprises B and possibly other components that do not materially affect the characteristics of the A-B material. For example, a polypeptide consisting essentially of a portion of the amino acid sequence set forth in Figure 1B denotes a polypeptide which comprises a portion of the amino acid sequence set forth in Figure 1B and which also may comprise other amino acid residues that do not materially alter the characteristics of the polypeptide. That is, the polypeptide maintains the characteristics of having at least three beta-sheet/beta-turn structures, and self-aligning in the same manner as tropoelastin peptides.

As described above, the secondary (beta-sheet/beta-turn) structure of the MFUs is believed to guide the self-aggregation and self-alignment of the MFUs such that the MFUs align themselves in a manner that mimics the structure of aggregates of the parent protein. For example, the beta-sheets of the MFUs are aligned, and the lysine residues of elastin-modeled MFUs are aligned for enzymic or chemical crosslinking into stable polymeric structures, mimicking the way tropoelastin monomers form the elastin protein.

An MFU can be obtained by any method, including direct synthesis or recombinant production of the peptide. For example, the DNA for an MFU modeled on human elastin can be obtained directly from DNA coding for human elastin either by cleavage of the DNA and selection of the appropriate segment, or by synthesis of the DNA via a variety of well-known methods.

By means of available technology, DNA sequences coding for tandem repeats of any human elastin MFU, or for MFUs containing larger domains of human elastin, up to and including the entire tropoelastin molecule, can be constructed. These larger elastin sequences may offer advantages in terms of their kinetics of assembly or their mechanical properties. For example, MFU-2, which consists of exons 20, 21, 23, 24, 21, 23, and 24 of human elastin, has been expressed and purified. The amino acid sequence of this peptide is set forth in Figure 4C. MFU-2 demonstrates an increased tendency towards spontaneous self-aggregation than MFU-1, as evidenced by a lower coacervation temperature. See Example 6 below.

While the MFUs of the present invention are normally soluble in solution, simple manipulations of pH, salt content and temperature of these solutions initiate coacervation and self-alignment of the polypeptides, resulting in aggregates of elastin-like fibers. The exact conditions that will bring about coacervation and self-alignment of the MFUs varies depending on the MFU polypeptide and the MFU solution to be manipulated. Conditions that bring about coacervation are well-known to those skilled in the art, and those skilled in the art can induce coacervation and self-alignment of MFUs by following routine laboratory procedures.

Figure 3 illustrates the ability of the MFUs of the present invention to coacervate. In particular, Figure 3 illustrates the coacervation (self-aggregation) of MFU-1 of human elastin. The peptide was dissolved at a concentration of 0.25 mg/ml in phosphate-buffered saline, pH 7.4, containing 1.5M NaCl and 0.3 mM CaCl2, and the temperature of the solution was raised at a uniform rate. The onset of coacervation occurred at 53 °C, and is indicated by an increase in turbidity of the solution. The data set forth in Example 4 below illustrate the ability of MFUs to assemble with non-human elastin.

A characteristic property of the MFUs of the present invention is their ability to self-assemble in an ordered

manner, in the same manner as the tropoelastin monomers of human elastin. For example, the MFUs align themselves in an order that aligns their beta-sheet structures and that permits crosslinking between the individual MFU peptides, when the polypeptide is modeled on elastin. This process of self-alignment and self-aggregation is considered to be the first step in fiber formation. After enzymic crosslinking, the fibers can be made into a material that has chemical and structural properties similar to those of natural elastin polymers. This MFU material can be used to construct human elastin-like prostheses such as tubes for blood vessel replacement and sheets for other uses such as wound or burn healing. Alternatively, the MFUs can be co-aggregated with other proteins, for example collagen, to provide prosthesis material that resembles the natural structural materials of the body.

MFU-based material is subject to infiltration of cells growing in the patient, including endothelial cells, and the prosthesis can become a permanent, living, tissue replacement. This human-like MFU material is more biocompatible than other elastin-containing materials which have heretofore been proposed for prostheses, including the polymers produced from chemically synthesized sequences of elastin described in the Urry patents and the material produced from hydrolyzed non-human elastin co-aggregated with other proteins described above.

An MFU modeled on human elastin in accordance with the present invention offers distinct advantages over other elastin preparations. For example, in contrast to the solubilized fragments of elastin used before, an MFU is a single peptide of defined composition. The MFU is considerably smaller than the parent protein and simpler in structure, and therefore is easier to produce or express in quantity, to handle in solution, and to manipulate for experimental and practical purposes. Like other elastin preparations, the MFU is non-thrombogenic

and provides a friendly environment for cell infiltration. In addition, being composed entirely of a human elastin sequence, an MFU is non-immunogenic, thus providing a truly biocompatible material.

MFUs modeled on human elastin according to the present invention also can be used in any way that human or animal elastin is used. For example, the soluble MFUs of human elastin of the present invention can be used to coat the surfaces of non-biological materials, such as prothesis, in the same manner that solubilized (*i.e.*, hydrolyzed) non-human elastin preparations, such as animal alpha- and kappa-elastins, have been used. MFUs can be used to coat any prosthesis, including a prosthesis comprising a synthetic material, an animal materials, and/or a metal. The prostheses can be coated with many layers of MFUs. For example, from 1 layer to 500 or more layers of MFU can be coated onto a prosthesis. The MFUs can be crosslinked after being coated onto the prosthesis to improve the permanence of the coating. As used herein, the term prosthesis is meant to encompass any material that is implanted into the body, including material for blood vessel replacement, for heart valve replacement, cloth-like material, stents, and materials for use as coverings for burns or wounds to promote healing.

Because the MFU's of the present invention are non-thrombogenic, and provide a surface on which endothelial and other cells can adhere and grow, prostheses coated with MFUs are more biocompatible than an uncoated prosthesis. Moreover, prostheses coated with MFUs have the advantage over prosthesis coated with animal-derived elastin of containing a human sequence and, hence, being non-immunogenic. Also, the MFUs comprise a defined, homogeneous peptide rather than an undefined mixture of peptides of various sizes, like the animal-derived products previously described.

The MFUs of human elastin of the present invention also can be used in cosmetics, for example, in the manner

that hydrolyzed animal elastin are used. See U.S. Patents No. 4,179,333 (Braeumer), No. 4,659,740 (Usher), No. 4,474,763 (Lubowe), No. 4,419,288 (Cioca), No. 4,327,078 (Charlet) and No. 4,963,656 (Mitani), the respective contents of which are incorporated herein by reference in their entirety.

The MFUs of the present invention can be used in conjunction with animal elastin and collagen frameworks, as a human blood vessel replacement. The animal elastin/collagen material is obtained by extracting all other proteins, cellular and soluble components from animal blood vessels, leaving a tube consisting essentially of animal elastin and collagen. See, for example, U.S. Patent No. 4,776,853 (Klement), discussed above. The MFUs spontaneously associate with the animal elastin matrix of animal vessel preparations because of their inherent property of self-assembly and self-alignment. The entire surface of animal elastin vessels can therefore be covered with multiple layers of human elastin MFUs, with permanent association achieved by enzymic or chemical crosslinking. Animal vessels with a human MFU surface will have substantially decreased immunogenicity and improved biocompatibility over non-coated animal elastin prostheses.

As discussed above, solubilized (hydrolyzed) animal elastin has been co-aggregated with other proteins such as fibrin, and short repeated hydrophobic elastin sequences have been polymerized into high molecular weight material. The MFUs of the present invention can be used in a similar manner to create fibers for use in making prosthesis consisting essentially of MFUs. For example, MFUs can be co-aggregated with fibrin and other short, hydrophobic elastin sequences and polymerized into higher molecular weight material.

The present invention also provides MFUs modeled on animal elastin. Such MFUs are useful, for example, in elastic materials. The amino acid sequences of several

animal elastins are known, including mouse, rat, chicken bovine and porcine.

The present invention also relates to MFUs modeled on other fibrous, self-assembling proteins, including but not limited to lamprin and spider silk proteins. The MFUs of these proteins contain sufficient information (*i.e.*, sufficient beta-sheet/beta-turn structures) to direct their alignment into fibrillar polymeric structures. For example, the amino acid sequence of lamprin is known, and the secondary structure of this protein is believed to comprise a number of beta-sheet/beta-turn structures. Robson *et al.*, *supra*. An MFU modeled on lamprin in accordance with the present invention comprises a portion of the amino acid sequence of lamprin that has at least three beta-sheet/beta-turn structures, and which is not the naturally occurring lamprin protein. In a preferred embodiment, an MFU modeled on lamprin consists essentially of a portion of the amino acid sequence of lamprin. Alternatively, an MFU modeled on lamprin comprises a portion of the amino acid sequence of lamprin, wherein the amino acid sequence is modified by one or more additions, substitutions and/or deletions, as described above.

MFUs modeled on lamprin and other fibrous proteins can be used to make a variety of materials. The materials have the special properties of high tensile strength, elasticity and plasticity of their parent proteins, and thus are suitable for a number of different applications, for example, in cords and ropes for use in parachutes, which require high tensile strength.

The present invention also encompasses materials that include two or more MFUs derived from a single parent protein, wherein the MFUs may be the same or different, and materials which include two or more MFUs derived from different parent proteins. Such combinations of MFUs from the same or different parent proteins can be chosen to form a product with desired physical properties. For example, a combination of an MFU derived from elastin and

an MFU derived from a spider silk protein will have the high extensilibity of elastin and the high tensile strength of the spider silk protein. Appropriate selection of the MFUs and their relative amounts permits the production of a material with specified properties.

The combination may be in any form, such as a mixture of MFUs, a fusion protein comprising two or more MFUs, or two or more MFUs chemically linked together. For example, one embodiment of the present invention provides a polypeptide comprising an MFU modeled on elastin, such as animal or human elastin, and an MFU modeled on another fibrous protein, such as lamprin or a spider silk protein. Such a polypeptide can be made by methods known to those skilled in the art, for example, by methods used to make fusion proteins. An MFU comprising exons 21 and 22 of human elastin flanked on both sides by tandem repeat sequences from lamprin has been expressed as a fusion protein. See Example 7 below. In an alternative embodiment, a material is provided which comprises an MFU modeled on animal or human elastin chemically-linked to an MFU modeled on lamprin or a spider silk protein. Such chemically-linked polypeptides can be made by methods known to those skilled in the art. Other combinations of MFUs modeled on the same or different parent proteins also are encompassed by the present invention.

The present invention is further illustrated below by reference to the following examples. The examples are illustrative only, and are not to be construed as limiting the scope of the invention.

Example 1. Selection of Minimal Functional Unit-1 (MFU-1) of Human Elastin

As discussed above, the beta-sheet/beta-turn structure of the hydrophobic domains of fibrous proteins such as elastin are believed to play an important role in the self-alignment and self-assembly of the proteins. These structures are focused on for the selection of MFUs.

A specific MFU of human elastin (designated MFU-1) was selected for expression. This MFU is encoded by four exon regions of the human elastin gene: exons 20 (35 amnio acids), 21 (14 amino acids), 23 (19 amino acids) and 24 (53 amino acids). Figure 1C. The amino acid residues of this peptide are underlined in Figure 1B. The MFU comprises two adjacent central cross linking domains flanked on each side by hydrophobic domains. Figure 1D is a cartoon representation of the hydrophobic and cross linking domains corresponding to MFU-1. The crosslinking domain containing lysine residues, believed to be in an alpha-helical conformation, is represented by the cylinder. The flanking hydrophobic domains are represented as square planes with protruding hydrophobic side chains. These hydrophobic domains each comprise several beta-sheet/beta-turn structural units. This MFU constitutes only approximately one-sixth of the total mass of elastin, and has a size of about 10,000 daltons.

This particular unit was chosen because the flanking hydrophobic exon, exon 24, contains a seven-fold repeat of a PGVGVA sequence which is likely to play a role in elastin alignment and assembly. The importance of this domain is supported by the fact that domains of similar tandem repeats at this site are found in elastins of several species, and by the evidence that synthetic peptides mimicking this hydrophobic repeat sequence self-aggregate to form fibrillar structures. Also, the PGVGVA sequence interacts specifically with an elastin-binding protein, one of the functions of which is to prevent premature intracellular self-aggregation of tropoelastin. Hinek *et al.*, *J. Cell Biol.* 126: 563-73 (1994). This tropoelastin-binding protein has also been shown to inhibit *in vitro* self-aggregation of solubilized elastin fragments (kappa-elastin). Hinek, *Cell Adhesion & Comm.* 2: 1-9 (1994).

Peptides comprising other hydrophobic domains of human elastin are expected to posses similar abilities to self-assemble and self-align, and are suitable MFUs in

accordance with the present invention. For example, peptides comprising amino acid residues 19-160, 188-367 and 607-717 of the human elastin amino acid sequence set forth in Figure 1B are suitable MFUs.

Example 2. Expression of MFU-1 of Human Elastin

By the use of a human fetal aortic elastin cDNA, the region encompassing exons 20, 21, 23, and 24 of human elastin was cloned by PCR. The 5' primer contained a BamHI site, followed by a methionine codon and 15 bases homologous to the 5' end of exon 20. The methionine codon was inserted for subsequent use as a cyanogen bromide cleavage site, since no other methionines occur in the elastin sequence. The 3' primer contained an EcoRI site, followed by a stop codon and 15 bases complementary to the 3' end of exon 24 (see Figure 1C). The PCR product was ligated into a BamHI-EcoRI digested pGEX-2t vector (Pharmacia) and the sequence confirmed. The ligation product was transfected into *E. coli*. The expressed fusion product was isolated by glutathione affinity chromatography and the elastin MFU cleaved from the GST protein by cyanogen bromide treatment, yielding a ~10 kDa cleavage product. This cleavage product was purified by BioGel P-30 chromatography in 0.05M acetic acid. (Figure 2). The elastin MFU-1 is contained in Fraction 1.

The identity of the released MFU was confirmed by Western blotting with antibodies to elastin and to the PGVGVA sequence contained in one of the hydrophobic domains, and by amino acid analysis. In particular, Fraction 1 obtained from the BioGel P-30 chromatography depicted in Figure 2 was characterized by Western Blot analysis. Western blots using a monoclonal antibody to PGVGVA were performed on affinity-purified products before CNBr cleavage and on Fraction 1. A Western blot using a polyclonal antibody to human elastin also was performed on Fraction 1. The yield of the elastin polypeptide is estimated at 1-3 mg/L.

The following table sets forth the amino acid composition of Fraction 1 from chromatography on BioGel P-30. The predicted (expected) and actual (found) compositions are shown.

	Expected	Found
ASP	0	0.7
GLX	4	4.2
HYP	0	0.0
SER	1	1.3
GLY	33	33.8
HIS	0	0.2
ARG	0	0.5
THR	1	1.2
ALA	27	26.9
PRO	15	12.9
TYR	1	1.3
VAL	25	22.3
MET	0	0.3
CYS	0	0.1
ILU	3	3.4
LEU	1	2.4
PHE	3	3.2
LYS	4	5.3

Radioactively labelled MFU-1 was generated by conventional means for use in experimentation by incubating the transfected *E. coli* in the presence of radioactively labeled valine and glycine.

Example 3. Self-Aggregation of MFU-1 of Human Elastin

While the MFU-1 obtained as described above is soluble at room temperature, the ability of the MFU to self-aggregate (the first step in fiber formation) was readily induced by increasing the salt content of the solution and elevating the temperature, with the onset of coacervation occurring at 53 °C. See Figure 3. This behavior of MFU-1 is similar to the temperature-dependent self-aggregation of the parent molecule, tropoelastin.

Example 4. Use of MFU-1 to Humanize Animal-Based Prosthesis

The ability of MFU-1 to coat animal-based prosthetic materials was evaluated as follows:

A matrix of insoluble elastin was prepared from chicken aortic tissue by a cyanogen bromide extraction method. This insoluble, non-human elastin matrix was incubated for 16 hours at 37° in phosphate-buffered saline, at pH 7.4, in the presence of radioactively labeled human MFU-1 prepared as described above. The tissue was then washed extensively with phosphate-buffered saline at pH 7.4.

The association of human elastin MFU-1 with chicken elastin was demonstrated by fluorescence microscopy. Samples of chicken elastin were incubated in the presence of phosphate-buffered saline (PBS) alone and in PBS in the presence of radioactively labeled human elastin MFU-1 (MFU). Increased autofluorescence of elastin after incubation with the human MFU-1 indicated the association of the human MFU-1 with the surface of the chicken elastin. The MFU-1-coated chicken elastin matrix displayed enhanced surface auto-fluorescence which was uniform over the entire surface, suggesting a complete and continuous coating of the matrix by MFU-1.

The following table shows the calculation of the surface coating of chicken elastin with the human elastin MFU-1:

	Bound MFU (cpm)	Bound MFU (nmoles)	Total Area of Chicken Elastin (mm^2)	Total MFU Area (mm^2)	MFU Coverage
PBS	0	0.00	13.0	0	0
PBS	0	0.00	16.1	0	0
MFU	254	0.22	13.0	3708	285
MFU	147	0.13	6.7	2151	321

Estimation of surface coverage assumed a cross-sectional diameter of the human MFU-1 of 6 nm. It was estimated that about 1-3 μg of human MFU-1 per mg chicken elastin matrix remained firmly associated with the insoluble matrix of chicken elastin. This amount of MFU-1 is sufficient to cover the estimated surface area

of the chicken elastin matrix up to two hundred-fold, and indicates that the MFU-1 formed a multi-layer coating on the chicken elastin matrix.

Example 5. Coating Non-Biological Prosthetic Materials With MFU-1 of Human Elastin

The suitability of MFU-1 as a coating material was evaluated for non-biological prosthetic materials, including dacron, polyurethane and teflon.

Via techniques similar to those described in Example 4 above, exposure of non-biological prosthetic materials, including dacron, polyurethane, expanded polytetrafluoroethylene (ePTFE) (Gortex) or a Teflon-coated metal to soluble MFU-1 was shown to result in the coating of these materials with a surface layer of the human elastin MFU. For the Dacron, polyurethane and ePTFE coatings, the amount of bound MFU was sufficient to cover the material at least twenty-fold, based on the surface area of the material and the estimated cross-sectional area of MFU-1. For the teflon-coated metal, the surface coverage was estimated to be approximately 500-fold:

	Bound MFU (cpm)	Bound MFU (nmoles)	Total Area of Substrate (mm^2)	Total MFU Area (mm^2)	MFU Coverage
PBS	0	0.00	13.0	0	0
Polyurethane	32	0.03	13.0	472	36
Dacron	68	0.06	13.0	989	76
Teflon	87	0.08	14.0	1276	91
Teflon-Coated Metal	102	0.04	1.19	706	572

The formation of multiple layers of MFUs on these materials indicates that, once an initial layer of MFU forms on the prosthetic material, additional layers are formed by self-aggregation of the MFUs.

MFUs bound in this manner are not readily removed by washing. The coating can be made permanent by treating the material to crosslink the MFUs covalently to the prosthetic material, via methods previously described (for example, in U.S. Patent No. 4,474,851 (Urry), *supra*), and by crosslinking the MFUs to each other via their amino groups. This provides a permanent elastin matrix on the surface of the prosthesis.

Because elastin is inherently non-thrombogenic, coating these synthetic prostheses with human elastin MFUs reduces the tendency of prostheses made from these materials to bind and activate platelets. For example, we have demonstrated that ePTFE materials coated with MFU-1 do not exhibit platelet adherence or activation.

We also have demonstrated that materials coated with human elastin MFUs provide a surface for cell attachment and growth. In particular, vascular smooth muscle cells and endothelial cells were found to adhere, spread, and proliferate on surfaces coated with MFU-1. Similar results are expected with materials formed from human elastin MFUs.

Example 6. Expression of MFU-2 of Human Elastin

Via techniques similar to those described in Example 2 above, a second polypeptide modeled on human elastin (MFU-2) has been expressed and partially characterized. This polypeptide comprises a tandem duplicate of MFU-1, consisting of exons 20, 21, 23, 24, 21, 23, and 24. Figures 4A, 4C. It contains three hydrophobic domains and two crosslinking domains. Figure 4B. MFU-2 undergoes coacervation at approximately 34 °C, indicating an increased tendency for self-aggregation compared to MFU-1. This increased tendency arises from the duplication of hydrophobic and crosslinking domains.

Example 7. Expression of MFUs Based on Lamprin and Elastin/Lamprin

Via techniques similar to those described in Example 2 above, constructs consisting of the entire polypeptide sequence of lamprin were expressed. A chimeric construct consisting of a crosslinking domain of human elastin (exons 21 and 23) flanked on both sides by tandem repeat sequences from lamprin, (GGLGY)6, also was expressed.

Example 8. Formation of Fibrillar Matrices From MFUs and Their Use as a Prosthetic Material

MFUs can be used to produce fibrillar matrices useful, for example, for making prosthetic materials. This can be effected either during the process of self-aggregation or after self-aggregation, by extrusion into an appropriate medium or by other known procedures for making fibers. Self-assembly of MFU-1 into fibrillar structures similar to those formed by human tropoelastin can be confirmed by transmission electron microscopy of the coacervates.

Polypeptides comprising multiple repeats of MFUs or comprising a region of human elastin containing two or more MFUs, up to and including the entire tropoelastin molecule, also can be used to make fibers in accordance with the present invention. The larger MFU-containing peptides may demonstrate improved self-assembly or fiber-forming characteristics, or may produce fibers with superior mechanical properties.

Once formed, the fibrillar matrices can be stabilized by crosslinking either enzymically (for example, with lysyl oxidase) or chemically (via bi-functional aldehydes or other crosslinking agents) to produce material similar to natural elastin. Coacervation-generated polymers of MFU-1 have been stabilized by chemical crosslinking of lysine residue side chains via a catechol/peroxidase method described in Stahmann *et al.*, *Biopolymers* **16**: 1307-18 (1977). The ability to stabilize the polymers by

this method confirms that the process of self-aggregation (coacervation) aligns the lysine residues appropriately for crosslinking.

The MFUs also can be co-aggregated and co-crosslinked with other human proteins, such as collagens, to more closely mimic natural structural materials.

Material made from MFU fibers can be formed or woven into sheets or tubes for various prosthetic uses. This human-like material has superior biocompatibility compared to other elastin-containing materials heretofore proposed for prostheses.

It will be apparent to those skilled in the art that various modifications and variations can be made to the processes and compositions of this invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.

What Is Claimed Is:

1. A polypeptide that comprises at least three beta-sheet/beta-turn structures and that is not a naturally occurring fibrous protein.

2. The polypeptide of claim 1, wherein each of the beta-sheet structures comprises from 3 to about 7 amino acid residues.

3. The polypeptide of claim 2, wherein each of the beta-sheet structures comprises from about 5 to about 7 amino acid residues.

4. The polypeptide of claim 1, further comprising at least one amino acid residue capable of participating in cross-linking.

5. The polypeptide of claim 1, wherein the polypeptide consists essentially of a portion of the amino acid sequence set forth in Figure 1B.

6. The polypeptide of claim 5, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of amino acid residues 374-499, 19-160, 188-367 and 607-717, respectively, of Figure 1B.

7. The polypeptide of claim 5, wherein the portion of the amino acid sequence set forth in Figure 1B is modified by the addition, deletion or substitution of from 1 to about 10 amino acid residues.

8. The polypeptide of claim 5, wherein the polypeptide comprises tandem repeats of a portion of the amino acid sequence set forth in Figure 1B.

9. The polypeptide of claim 8, wherein the polypeptide consists essentially of the amino acid sequence set forth in Figure 4C.

10. The polypeptide of claim 8, wherein the polypeptide consists essentially of the amino acid sequence set forth in Figure 4C, modified by the addition, deletion or substitution of from 1 to about 10 amino acid residues.

11. A prosthesis comprising an animal material, wherein a surface of the animal material is coated with a polypeptide according to claim 5.

12. A prosthesis comprising a synthetic material, wherein a surface of the synthetic material is coated with a polypeptide according to claim 5.

13. A prosthesis comprising a metal, wherein a surface of the metal is coated with a polypeptide according to claim 5.

14. Material suitable for implantation into humans, wherein the material consists essentially of a polypeptide according to claim 5.

15. The material of claim 14, wherein the material is selected from the groups consisting of materials for blood vessel replacement, materials for heart valve replacement, materials for covering burns, materials for covering wounds, and stents.

16. A cosmetic material comprising the polypeptide of claim 5.

17. An elastic material comprising the polypeptide of claim 1.

18. A high tensile-strength material comprising the polypeptide of claim 1.

19. The polypeptide of claim 1, wherein the polypeptide consists essentially of a portion of the amino acid sequence of an animal elastin.

20. The polypeptide of claim 1, wherein the polypeptide consists essentially of a portion of the amino acid sequence of lamprin.

21. The polypeptide of claim 1, wherein the polypeptide consists essentially of a portion of the amino acid sequence of a spider silk protein.

22. A material comprising two or more polypeptides selected from the group consisting of:

(A) a polypeptide consisting essentially of a portion of the amino acid sequence set forth in Figure 1B comprising at least three beta-sheet/beta-turn structures;

(B) a polypeptide consisting essentially of a portion of the amino acid sequence of an animal elastin comprising at least three beta-sheet/beta-turn structures;

(C) a polypeptide consisting essentially of a portion of the amino acid sequence of lamprin comprising at least three beta-sheet/beta-turn structures; and

(D) a polypeptide consisting essentially of a portion of the amino acid sequence of a spider silk protein comprising at least three beta-sheet/beta-turn structures,

wherein the two or more polypeptides may be the same or different.

23. The material of claim 22, wherein the material comprises a mixture of the two or more polypeptides.

24. The material of claim 22, wherein the material comprises a fusion protein comprising the two or more polypeptides.

25. The material of claim 22, wherein the two or more polypeptides are chemically linked together.

26. A polypeptide having the primary structure of a portion of a naturally occurring fibrous protein and a secondary structure comprising at least three beta-sheet/beta-turn structures, wherein

(A) each of the beta-sheet/beta-turn structures comprises from 3 to about 7 amino acid residues and (B) the polypeptide is not a naturally occurring fibrous protein.

FIG.1A

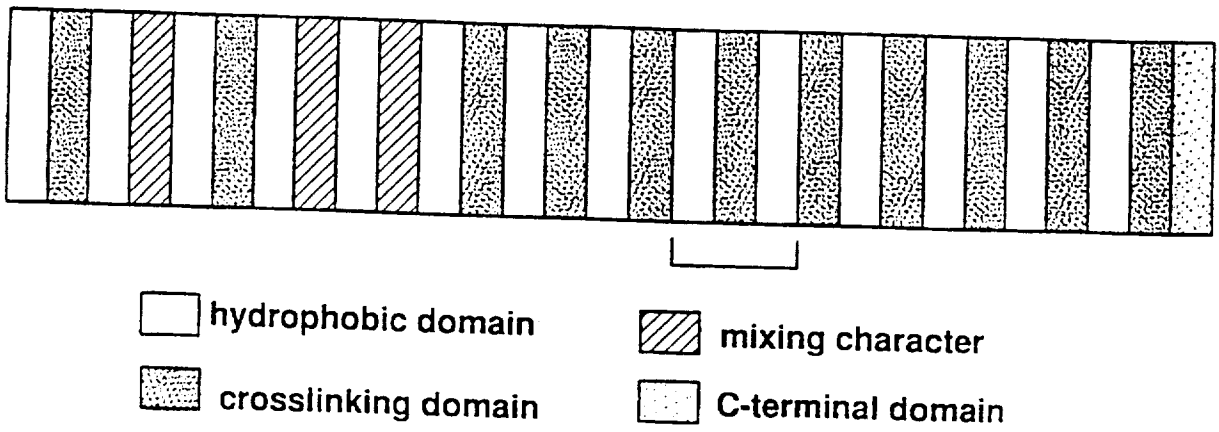

FIG.1C

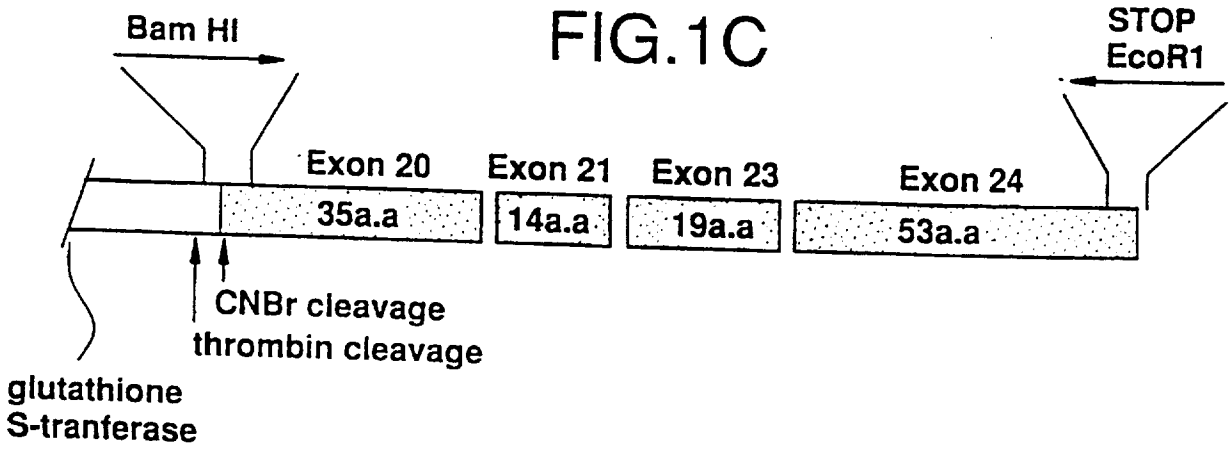

FIG.1D

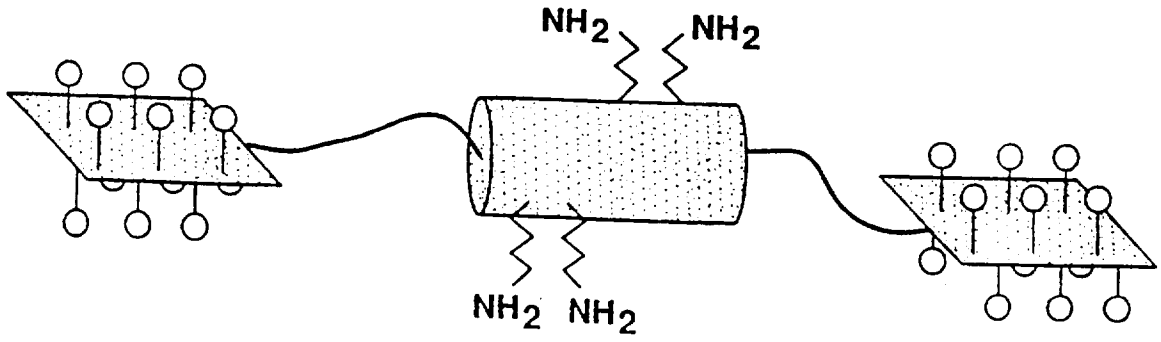

FIG.1B

1	11	21	31	41	51
GGVPGAIPGG	VPGGVFYPGA	GLGALGGGAL	GPGGKPLKPV	PGGLAGAGLG	AGLGAFPAVT
FPGALVPGGV	ADAAAAYKAA	KAGAGLGGVP	GVGGLGVSAG	AVVPQPGAGV	KPGKVPGVGL
PGVYPGGVLP	GARFPGVGVL	PGVPTGAGVK	PKAPGVGGAF	AGIPGVGPFG	GPQPGVPLGY
PIKAPKLPGG	YGLPYTTGKL	PYGYGPGGVA	GAAGKAGYPT	GTGVGPQAAA	AAAAKAAAKF
GAGAAGVLPG	VGGAGVPGVP	GAIPGIGGIA	GVGTPAAAAA	AAAAAKAAKY	GAAAGLVPGG
PGFGPGVVGV	PGAGVPGVGV	PGAGIPVVPG	AGIPGAAVPG	VVSPEAAAKA	AAKAAKYGAR
PGVGVGGIPT	YGVGAGGFPG	FGVGVGGIPG	VAGVPSVGGV	PGVGGVPGVG	ISPEAQAAAA
AKAAKYGVGT	PAAAAAKAAA	KAAQFGLVPG	VGVAPGVGVA	PGVGVAPGVG	LAPGVGVAPG
VGVAPGVGVA	PGIGPGGVAA	AAKSAAKVAA	KAQLRAAAGL	GAGIPGLGVG	VGVPGLGVGA
GVPGLGVGAG	VPGFGAGADE	GVRRSLSPEL	REGDPSSSQH	LPSTPSSPRV	PGALAAAKAA
KYGAAVPGVL	GGLGALGGVG	IPGGVVGAG	PAAAAAAAKAA	AKAAQFGLVG	AAGLGGLGVG
GLGVPGVGGL	GGIPPAAAAK	AAKYGAAGL	GGVLGGAQFP	LGGVAARPGF	GLSPIFPGGA
CLGKACGRKR	K				

FIG.1E

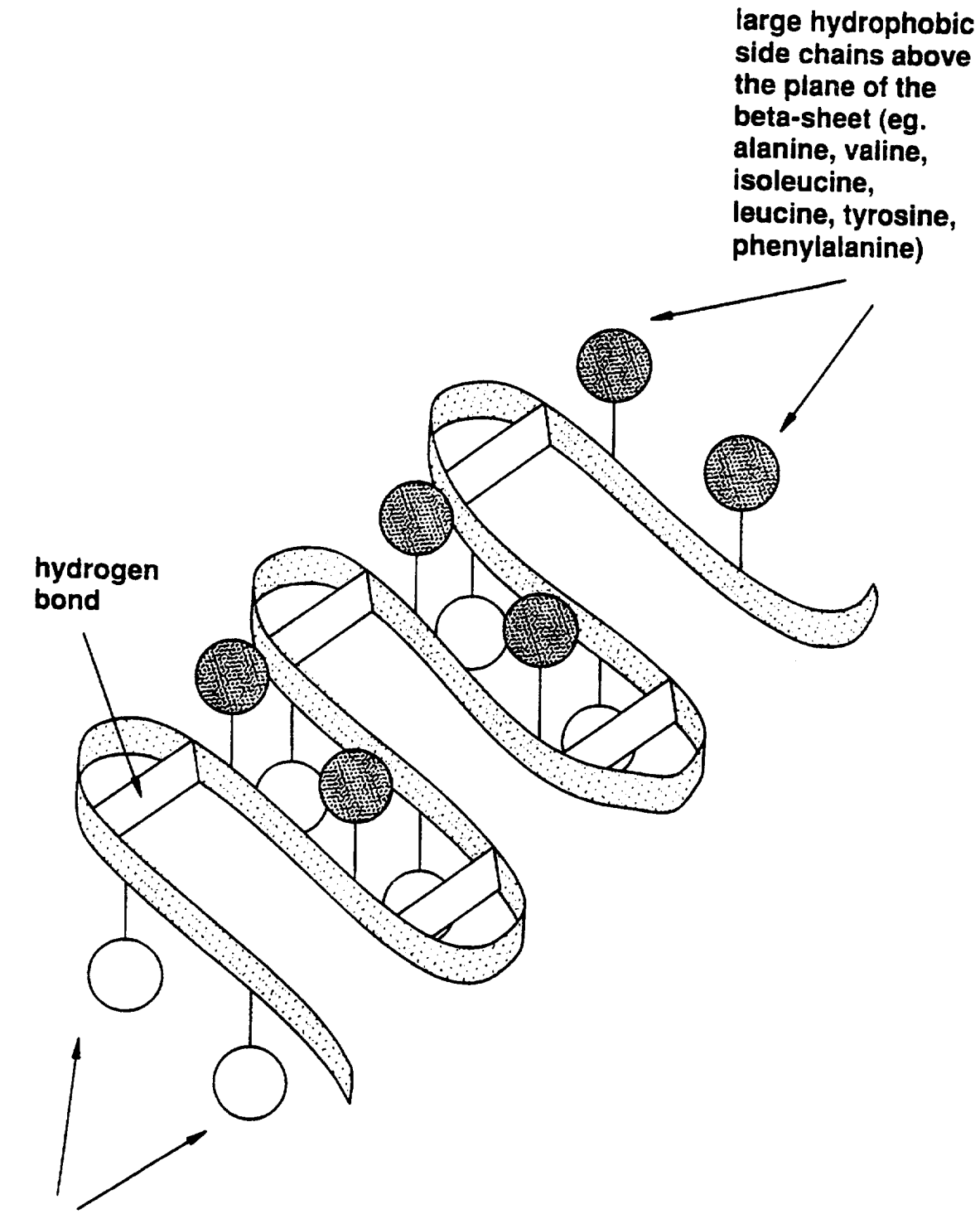
large hydrophobic side chains above the plane of the beta-sheet (eg. alanine, valine, isoleucine, leucine, tyrosine, phenylalanine)
hydrogen bond
large hydrophobic side chains below the plane of the beta-sheet (eg. alanine, valine, isoleucine, leucine, tyrosine, phenylalanine)

FIG.2

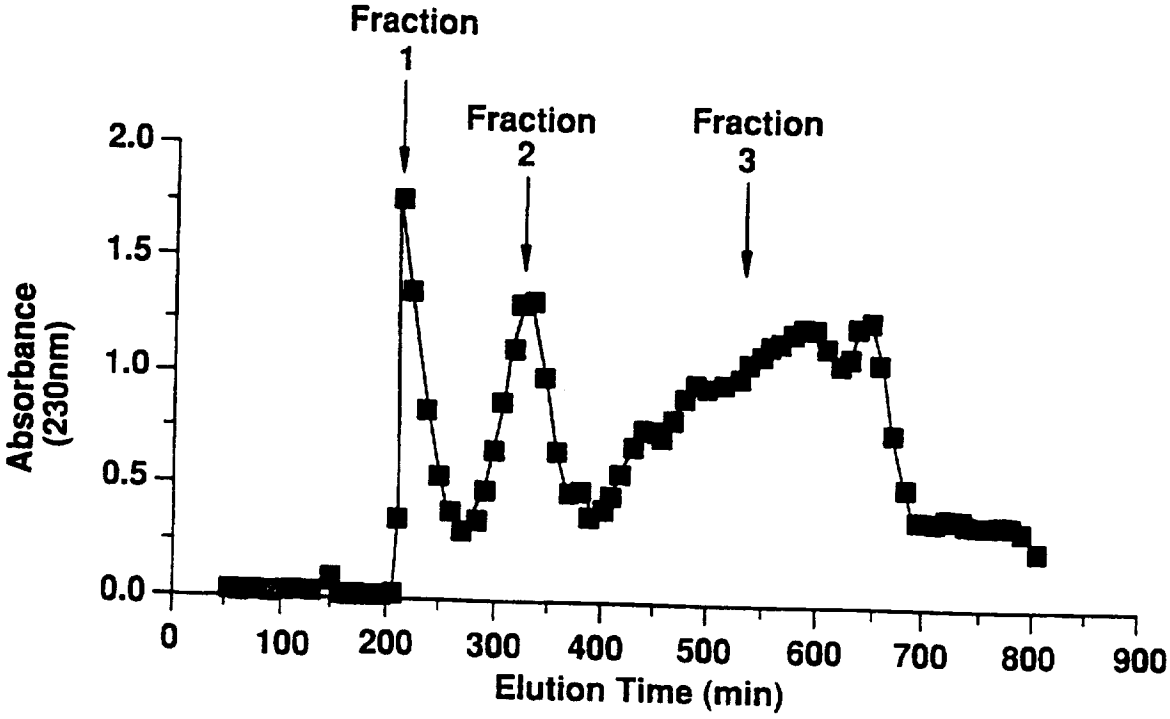
Fraction 1
Fraction 2
Fraction 3
Absorbance (230nm)
2.0
1.5
1.0
0.5
0.0
0
100
200
300
400
500
600
700
800
900
Elution Time (min)

FIG.3

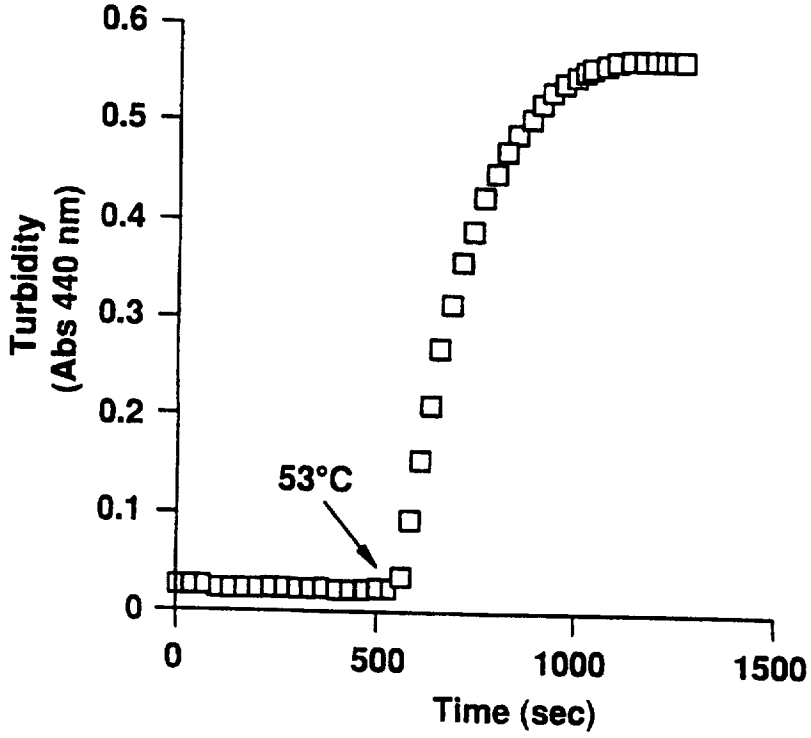
Turbidity (Abs 440 nm)
0.6
0.5
0.4
0.3
0.2
0.1
0
53°C
0
500
1000
1500
Time (sec)

FIG.4A

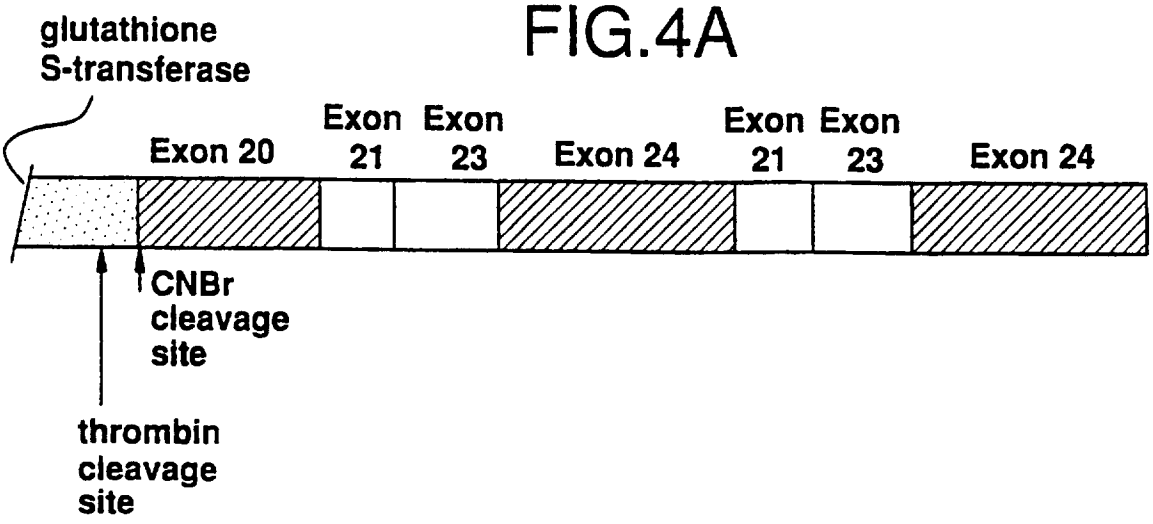

FIG.4B

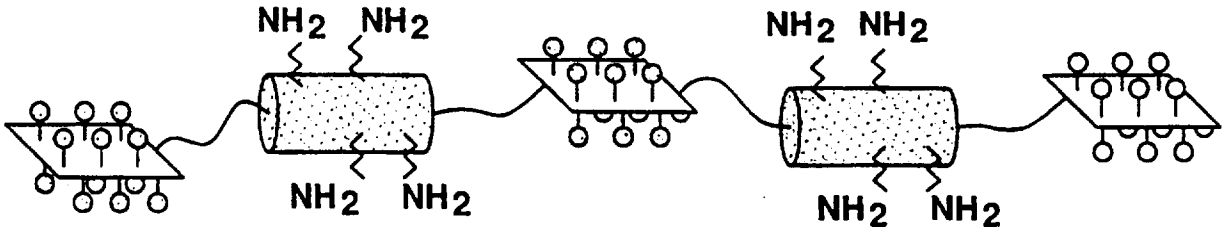

FIG.4C

FPGFGVGVGG IPGVAGVPGV GGVPGVGGVP GVGISPEAQA AAAAKAAKYG
VGTPAAAAAK AAAKAAQFGL VPGVGVAPGV GVAPGVGVAP GVGLAPGVGV
APGVGVAPGV GVAPAIGPPE AQAAAAAKAA KYGVGTPAAA AAKAAAKAAQ
FGLVPGVGVA PGVGVAPGVG VAPGVGLAPG VGVAPGVGVA PGVGVAPAIG P