Prevention and Reduction of Blood Loss

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Abstract
Methods are described for preventing or reducing ischemia and/or systemic inflammatory response in a patient such as perioperative blood loss and/or systemic inflammatory response in a patient subjected to cardiothoracic surgery, e.g. coronary artery bypass grafting and other surgical procedures, especially when such procedures involve extra-corporeal circulation, such as cardiopulmonary bypass.
**FIG. 2**
FIG. 3A
FIG. 3B
PREVENTION AND REDUCTION OF BLOOD LOSS

RELATED APPLICATION


[0002] The entire teachings of the above applications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0003] Proteases are involved in a broad range of biological pathways. In particular, serine proteases such as kalikrein, plasmin, elastase, urokinase plasminogen activator, thrombin, human lipoprotein-associated coagulation inhibitor, and coagulation factors such as factors VIIa, IXa, Xa, XIa, and XIIa have been implicated in pathways affecting blood flow, e.g. genetal and focal ischemia, tumor invasion, fibrinolysis, peroperative blood loss, and inflammation. Inhibitors of specific serine proteases, therefore, have received attention as potential drug targets for various ischemic maladies.

[0004] One such inhibitor, aprotinin (also called bovine pancreatic trypsin inhibitor or BPTI), obtained from bovine lung, has been approved in the United States for prophylactic use in reducing peroperative blood loss and the need for transfusion in patients undergoing cardiopulmonary bypass (CPB), e.g. in the course of a coronary artery bypass grafting procedure. Aprotinin is commercially available under the trade name TRASYLOL® RTM. (Bayer Corporation Pharmaceutical Division, West Haven, Conn.) and was previously approved for use to treat pancreatitis. The effectiveness of aprotinin is associated with its relatively non-specific abilities to inhibit a variety of serine proteases, including plasma kalikrein and plasmin. These proteases are important in a number of pathways of the contact activation system (CAS).

[0005] CAS is initially activated when whole blood contacts the surface of foreign substrates (e.g., kaolin, glass, dextran sulfate, or damaged bone surfaces). Kalikrein, a serine protease, is a plasma enzyme that initiates the CAS cascade leading to activation of neutrophils, plasmin, coagulation, and various kinins. Kalikrein is secreted as a zymogen (pre-kalikrein) that circulates as an inactive molecule until activated by a proteolytic event early in the contact activation cascade. Clearly, specific inhibition of kalikrein would be a very attractive approach to control blood loss associated with CPB and the onset of systemic inflammatory response (SIR) as would be encountered during, for example, various invasive surgical procedures.

[0006] Despite being the only licensed compound for preventing peroperative blood loss in CPB for coronary artery bypass grafting (CABG) procedures, aprotinin is not as widely used as would be expected. There are serious concerns regarding the use of this bovine polypeptide in patients who require CPB, and in particular the use of this compound in CABG procedures. Aprotinin is not specific for kalikrein, but interacts with additional enzymes (e.g., plasmin) in multiple pathways. Thus, the mechanism of action of aprotinin is largely speculative, and the lack of precise understanding of what is affected during aprotinin treatment produces the risk of complications during treatment. One frequently cited complication is uncontrolled thrombosis, due to aprotinin’s actions upon the fibrinolytic pathway. There is concern not only over such hyperacute events as major vessel thrombosis in the peroperative period, but also over graft patency after the CABG procedure. Furthermore, as a naturally occurring protein obtained from bovine lung, administration of aprotinin in humans can elicit severe hypersensitivity or anaphylactic or anaphylactoid reactions after the first and, more often, after repeat administration to patients. This is particularly of concern in the large number of patients who have repeat CABG procedures. In addition, there is an increasing public concern regarding use of material derived from bovine sources as a potential vector for the transmission of bovine spongiform encephalopathy to humans.

[0007] These concerns make clear that a need remains for more effective and more specific means and methods for preventing or reducing perioperative blood loss and the onset of SIR in a patient subjected to surgery resulting in activation of the CAS, such as CABG procedures in patients of CPB, or hip replacement.

SUMMARY OF THE INVENTION

[0008] This invention is based on the discovery of peptides that inhibit serine proteases. Serine proteases such as, for example, kalikrein, are involved in, for example, patients leading to excessive peroperative blood loss and the onset of systemic inflammatory response. Preferred kalikrein peptide inhibitors include those described in U.S. Pat. Nos. 6,333,402 and 6,057,287 to Markland et al., the contents of which are incorporated herein by reference in their entirety. The invention is directed in part to the use of the peptides in therapeutic methods and compositions suitable for use in eliminating or reducing various ischemias, including but not limited to peroperative blood loss, and the onset of systemic inflammatory response. Perioperative blood loss results from invasive surgical procedures that lead to contact activation of complement components and the coagulation/fibrinolysis systems. More specifically, the invention provides methods of using kalikrein inhibitors to reduce or prevent peroperative blood loss and a systemic inflammatory response in patients subjected to invasive surgical procedures, especially cardiothoracic surgery.

[0009] In one embodiment, the invention is directed to a method for preventing or reducing ischemia in a patient comprising administering to the patient a composition comprising a polypeptide comprising the amino acid sequence: Xaa1 Xaa2 Xaa3 Xaa4 Cys Xaa5 Xaa6 Xaa7 Xaa8 Xaa9 Xaa10 Xaa11 Gly Xaa13 Cys Xaa15 Xaa16 Xaa17 Xaa18 Xaa19 Xaa20 Xaa21 Xaa22 Xaa23 Xaa24 Xaa25 Xaa26 Xaa27 Xaa28 Xaa29 Cys Xaa31 Xaa32 Phe Xaa34 Xaa35 Gly Gly Cys Xaa39 Xaa40 Xaa41 Xaa42 Xaa43 Xaa44 Xaa45 Xaa46 Xaa47 Xaa48 Xaa49 Xaa50 Cys Xaa52 Xaa53 Xaa54 Cys Xaa56 Xaa57 Xaa58 (SEQ ID NO:1), wherein Xaa1, Xaa2, Xaa3, Xaa4, Xaa5, Xaa6, Xaa7 or Xaa8 are each individually an amino acid or absent; Xaa10 is an amino acid selected from the group consisting of: Asp and Gln; Xaa11 is an amino acid selected from the group consisting of: Asp, Gly, Ser, Val, Asn, Ile, Ala and Thr; Xaa13 is an amino acid selected from the group consisting of: Arg, His, Pro, Asn, Ser, Thr, Ala, Gly, Lys and Gln; Xaa15 is an amino acid selected from the group consisting of: Arg, Lys, Ala, Ser, Gly, Met, Asn and Gln; Xaa16 is an amino an acid selected from the group consisting of: Ala, Gly, Ser, Asp and Asn; Xaa17 is an amino acid selected from the group consisting of: Ala, Asn, Ser, Ile, Gly, Val, Gln and Thr; Xaa18 is an amino acid selected from the group consisting of: His, Leu, Gln and Ala; Xaa19 is an amino acid selected from the group consisting of: Tyr and Phe; Xaa20 is an amino acid selected from the group consisting of: Trp, Phe, Tyr, His and Ile; Xaa22 is an amino acid selected from the group consisting of: Tyr and Phe; Xaa23 is an amino acid selected from the group consisting of: Cys, Tyr and Phe;
acid selected from the group consisting of: Tyr and Phe; Xaa31 is an amino acid selected from the group consisting of: Glu, Asp, Gln, Asn, Ser, Ala, Val, Leu, Ile and Thr; Xaa32 is an amino acid selected from the group consisting of: Glu, Gln, Asp, Pro, Thr, Leu, Ser, Ala, Gly and Val; Xaa34 is an amino acid selected from the group consisting of: Thr, Ile, Ser, Val, Ala, Asn, Gly and Leu; Xaa35 is an amino acid selected from the group consisting of: Tyr, Phe and Phe; Xaa39 is an amino acid selected from the group consisting of: Glu, Gln, Ala, Ser and Asp; Xaa40 is an amino acid selected from the group consisting of: Gly and Ala; Xaa43 is an amino acid selected from the group consisting of: Asn and Gly; Xaa45 is an amino acid selected from the group consisting of: Phe and Tyr; and wherein the polypeptide inhibits kallikrein. In a particular embodiment, the surgical procedure can be a cardiothoracic surgery, such as, for example, cardiopulmonary bypass or coronary artery bypass grafting.

[0010] In a particular embodiment, the ischemia is perioperative blood loss due to a surgical procedure performed on the patient. The surgical procedure can be a cardiothoracic surgery, such as, for example, cardiopulmonary bypass or coronary artery bypass grafting.

[0011] In a particular embodiment, individual amino acid positions of SEQ ID NO:1 can be one or more of the following: Xaa10 is Asp, Xaa11 is Asp, Xaa13 is Pro, Xaa15 is Arg, Xaa16 is Ala, Xaa17 is Ala, Xaa18 is His, Xaa19 is Pro, Xaa21 is Trp, Xaa31 is Glu, Xaa32 is Glu, Xaa34 is Ile, Xaa35 is Tyr, Xaa39 is Glu.

[0012] In another embodiment, the invention is directed to a method for preventing or reducing the onset of systemic inflammatory response associated with a surgical procedure in a patient comprising administering to the patient a composition comprising a polypeptide consisting of the amino acid sequence: Glu Ala Met His Ser Phe Cys Ala Phe Lys Ala Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Glu Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Glu Asn Arg Phe Ghu Ser Leu Glu Glu Cys Lys Met Cys Thr Arg Asp (SEQ ID NO:2), wherein the polypeptide inhibits kallikrein. In one embodiment, the surgical procedure is a cardiothoracic surgery, such as, for example, cardiopulmonary bypass or coronary artery bypass grafting.

[0013] In yet another embodiment, the invention is directed to a method for preventing or reducing the onset of systemic inflammatory response associated with a surgical procedure in a patient comprising administering to the patient a composition comprising a polypeptide consisting of the amino acid sequence: Glu Ala Met His Ser Phe Cys Ala Phe Lys Ala Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Glu Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Glu Asn Arg Phe Ghu Ser Leu Glu Glu Cys Lys Met Cys Thr Arg Asp (SEQ ID NO:2), wherein the polypeptide inhibits kallikrein. In one embodiment, the surgical procedure is a cardiothoracic surgery, such as, for example, cardiopulmonary bypass or coronary artery bypass grafting.

[0014] In a particular embodiment, the invention is directed to a method for preventing or reducing ischemia in a patient comprising administering to the patient a composition comprising a polypeptide consisting of the amino acid sequence: Met His Ser Phe Cys Ala Phe Lys Ala Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Glu Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Glu Asn Arg Phe Ghu Ser Leu Glu Glu Cys Lys Met Cys Thr Arg Asp (amino acids 3-60 of SEQ ID NO:2), wherein the polypeptide inhibits kallikrein. In one embodiment, the surgical procedure is a cardiothoracic surgery, such as, for example, cardiopulmonary bypass or coronary artery bypass grafting.

[0015] In yet another embodiment, the invention is directed to a method for preventing or reducing the onset of systemic inflammatory response associated with a surgical procedure in a patient comprising administering to the patient a composition comprising a polypeptide consisting of the amino acid sequence: Met His Ser Phe Cys Ala Phe Lys Ala Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Glu Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Glu Asn Arg Phe Ghu Ser Leu Glu Glu Cys Lys Met Cys Thr Arg Asp (amino acids 3-60 of SEQ ID NO:2), wherein the polypeptide inhibits kallikrein. In one embodiment, the surgical procedure is a cardiothoracic surgery, such as, for example, cardiopulmonary bypass or coronary artery bypass grafting.

[0016] In another embodiment, the invention is directed to a method for preventing or reducing ischemia in a patient comprising administering to the patient a composition comprising a polypeptide consisting of the amino acid sequence: Met His Ser Phe Cys Ala Phe Lys Ala Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Glu Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Glu Asn Arg Phe Ghu Ser Leu Glu Glu Cys Lys Met Cys Thr Arg Asp (amino acids 3-60 of SEQ ID NO:2), wherein the polypeptide inhibits kallikrein. In one embodiment, the ischemia can be perioperative blood loss due to a surgical procedure performed on the patient. In one embodiment, the surgical procedure is a cardiothoracic surgery, such as, for example, cardiopulmonary bypass or coronary artery bypass grafting.

[0017] In another embodiment, the invention is directed to a method for preventing or reducing the onset of systemic inflammatory response associated with a surgical procedure in a patient comprising administering to the patient a composition comprising a polypeptide consisting of the amino acid sequence: Glu Ala Met His Ser Phe Cys Ala Phe Lys Ala Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Glu Cys Glu Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Glu Asn Arg Phe Ghu Ser Leu Glu Glu Cys Lys Met Cys Thr Arg Asp (SEQ ID NO:2), wherein the polypeptide inhibits kallikrein. In one embodiment, the surgical procedure is a cardiothoracic surgery, such as, for example, cardiopulmonary bypass or coronary artery bypass grafting.
procedure performed on the patient. In one embodiment, the surgical procedure is a cardiothoracic surgery, such as, for example, cardiopulmonary bypass or coronary artery bypass grafting.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[0017] FIG. 1 is a simplified diagram of major multiple pathways and related events involved in the contact activation system and systemic inflammatory response (SIR) that can arise in a patient subjected to soft and bone tissue trauma such as that associated with a coronary artery bypass grafting (CABG) procedure, especially when the CABG procedure involves extra-corpooreal blood circulation, such as cardiopulmonary bypass (Bypass Apparatus). Arrows indicate activation from one component or event to another component or event in the cascade. Arrows in both directions indicate activating effects of components or events in both directions. Broken arrows indicate likely participation of one component or event in the activation of another component or event. Abbreviations are as follows: "IPA"—tissue plasminogen activator; "Ca2+"—a protein component of the complement system; "Xa110"—activator protein of prekallikrein to form active kallikrein; "Extrinsic"—extrinsic coagulation system; "Intrinsic"—intrinsic coagulation system.

[0018] FIG. 2 shows a portion of a DNA and corresponding deduced amino acid for a KI polypeptide of the invention in plasmid pPIC-K503. The inserted DNA encodes the mat. alpha. propre signal peptide of *Saccharomyces cerevisiae* (underlined) fused in frame to the amino terminus of the PEP-1 KI polypeptide having the amino acid sequence enclosed by the boxed area. The amino acid sequence of the PEP-1 KI polypeptide shown in the boxed region is SEQ ID NO.2, and the corresponding nucleotide coding sequence of the KI polypeptide is SEQ ID NO.3. The dashed arrows indicate the location and direction of two PCR primer sequences in AOX regions that were used to produce sequencing templates. DNA sequence for the entire nucleotide sequence of the figure comprises the structural coding sequence for the fusion protein and is designated SEQ ID NO.27. The entire amino acid sequence is SEQ ID NO.28. The double underlined portion of the sequence indicates a diagnostic probe sequence. BstII and EcoRI indicate locations of their respective palindromic, hexameric, restriction endonuclease sites in the sequence. Asterisks denote translational stop codons.

[0019] FIGS. 3A and 3B show an alignment of amino acid sequences of the preferred embodiments of the invention, the native LACI sequence from which these variants were derived (SEQ ID NO:32), and other known Kunitz domains (SEQ ID NOs:29-31 and 33-53). Cysteine residues are highlighted.

**DETAILED DESCRIPTION OF THE INVENTION**

[0020] A description of preferred embodiments of the invention follows.

[0021] The invention is based on the discovery of a group of kallikrein inhibitor (KI) polypeptides that inhibit plasma kallikrein with a specificity that permits their use in improved methods of preventing or reducing ischemia such as, for example, perioperative blood loss and/or a systemic inflammatory response (SIR) induced by kallikrein, especially, for example, in patients undergoing surgical procedures and particularly surgical procedures involving cardiothoracic surgery, e.g., cardiopulmonary bypass (CPB), such as a coronary artery bypass graft (CABG) procedures. K’s can be used specifically for, e.g., pediatric cardiac surgery, lung transplantation, total hip replacement and orthotopic liver transplantation, and to reduce or prevent perioperative stroke during CABG, extracorporeal membrane oxygenation (ECMO) and cerebrovascular accidents (CVA) during these procedures.

[0022] Cardiothoracic surgery is surgery of the chest area, most commonly the heart and lungs. Typical diseases treated by cardiothoracic surgery include coronary artery disease, tumors and cancers of the lung, esophagus and chest wall, heart vessel and valve abnormalities, and birth defects involving the chest or heart. Where cardiothoracic surgery is utilized for treatment, the risk of blood loss (e.g., surgery-induced ischemia) and the onset of a systemic inflammatory response (SIR) is incurred. Surgery-induced SIR can result in severe organ dysfunction (systemic inflammatory response syndrome; SIRS).

Polypeptides Useful in the Invention

[0023] KI polypeptides useful in the invention comprise Kunitz domain polypeptides. In one embodiment these Kunitz domains are variant forms of the looped structure comprising Kunitz domain 1 of human lipoprotein-associated coagulation inhibitor (LACI) protein. LACI contains three internal, well-defined, peptide loop structures that are paradigm Kunitz domains (Girard, T. et al., 1989. Nature, 338: 518-520). The three Kunitz domains of LACI confer the ability to bind and inhibit kallikrein, although not with exceptional affinity. Variants of Kunitz domain 1 of LACI described herein have been screened, isolated and bind kallikrein with enhanced affinity and specificity (see, for example, U.S. Pat. Nos. 5,795,865 and 6,057,287, incorporated herein by reference). An example of a preferred polypeptide useful in the invention has the amino acid sequence defined by amino acids 3-60 of SEQ ID NO:2.

[0024] Every polypeptide useful in the invention binds kallikrein, and preferred polypeptides are also kallikrein inhibitors (KI) as determined using kallikrein binding and inhibition assays known in the art. The enhanced affinity and specificity for kallikrein of the variant Kunitz domain polypeptides described herein provides the basis for their use in cardiothoracic surgery, e.g., CPB and especially CABG surgical procedures, to prevent or reduce perioperative blood loss and/or the onset of SIR in patients undergoing such procedures. The KI polypeptides used in the invention have or comprise the amino acid sequence of a variant Kunitz domain polypeptide originally isolated by screening phage display libraries for the ability to bind kallikrein.

[0025] KI polypeptides useful in the methods and compositions of the invention comprise a Kunitz domain polypeptide comprising the amino acid sequence:

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Xaa1 Xaa2 Xaa3 Xaa4 Cys Xaa6 Xaa7 Xaa8 Xaa9 Xaa10
Xaa11 Gly Xaa13 Cys Xaa15 Xaa16 Xaa17 Xaa18 Xaa19
Xaa20 Xaa21 Xaa22 Xaa23 Xaa24 Xaa25 Xaa26 Xaa27
Xaa28 Xaa29 Cys Xaa31 Xaa32 Phe Xaa34 Xaa35 Gly
Gly Cys Xaa39 Xaa40 Xaa41 Xaa42 Xaa43 Xaa44 Xaa45
Xaa46 Xaa47 Xaa48 Xaa49 Xaa50 Cys Xaa52 Xaa53
Xaa54 Cys Xaa56 Xaa57 Xaa58
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“Xaa” refers to a position in a peptide chain that can be any of a number of different amino acids. For example, for the KI peptides described herein, Xaa10 can be Asp or Glu; Xaa11 can be Asp, Gly, Ser, Val, Asn, Ile, Ala or Thr; Xaa13 can be Pro, Arg, His, Asn, Ser, Thr, Ala, Gly, Lys or Glu; Xaa15 can be Arg, Lys, Ala, Ser, Gly, Met, Asn or Glu; Xaa16 can be Ala, Gly, Ser, Asp or Asn; Xaa17 can be Ala, Asn, Ser, Ile, Gly, Val, Gln or Thr; Xaa18 can be His, Leu, Gln or Ala; Xaa19 can be Pro, Gln, Leu, Asn or Ile; Xaa21 can be Trp, Phe, Tyr or Ile; Xaa31 can be Glu, Asp, Gln, Asn, Ser, Ala, Val, Leu, Ile or Thr; Xaa32 can be Glu, Gln, Asp, Asn, Pro, Thr, Leu, Ser, Ala, Gly or Val; Xaa34 can be Ile, Thr, Ser, Val, Ala, Asn, Gly or Leu; Xaa35 can be Tyr, Trp or Phe; Xaa39 can be Glu, Gly, Ala, Ser or Asp. Amino acids Xaa6, Xaa7, Xaa8, Xaa9, Xaa20, Xaa24, Xaa25, Xaa26, Xaa27, Xaa28, Xaa29, Xaa41, Xaa42, Xaa44, Xaa46, Xaa47, Xaa48, Xaa49, Xaa50, Xaa52, Xaa53 and Xaa54 can be any amino acid. Additionally, each of the first four and at last three amino acids of SEQ ID NO: 1 can optionally be present or absent and can be any amino acid, if present.

Peptides defined according to SEQ ID NO: 1 form a set of polypeptides that bind to kallikrein. For example, in a preferred embodiment of the invention, a KI polypeptide useful in the methods and compositions of the invention has the following variable positions: Xaa1 can be Asp, Gly, Ser or Val; Xaa3 can be Pro, Arg, His or Asn; Xaa5 can be Arg or Lys; Xaa6 can be Ala or Gly; Xaa7 can be Ala, Asn, Ser or Ile; Xaa8 can be His, Leu or Gln; Xaa9 can be Pro, Gln or Leu; Xaa18 can be Trp or Phe; Xaa31 can be Glu, Gln or Asn; Xaa34 can be Ile, Thr or Ser; Xaa35 is Tyr; and Xaa39 can be Glu, Gly or Ala.

A more specific embodiment of the claimed invention is defined by the following amino acids at variable positions: Xaa10 is Asp; Xaa11 is Asp; Xaa13 can be Pro or Arg; Xaa15 is Arg; Xaa16 can be Ala or Gly; Xaa17 is Ala; Xaa18 is His; Xaa19 is Pro; Xaa21 is Trp; Xaa31 is Glu; Xaa32 is Glu; Xaa34 can be Ile or Ser; Xaa35 is Tyr, and Xaa39 is Gly.

Also encompassed within the scope of the invention are peptides that comprise portions of the polypeptides described herein. For example, polypeptides could comprise binding domains for specific kallikrein epitopes. Such fragments of the polypeptides described herein would also be encompassed.

KI polypeptides useful in the methods and compositions described herein comprise a Kunitz domain. A subset of the sequences encompassed by SEQ ID NO: 1 are described by the following (where not indicated, “Xaa” refers to the same set of amino acids that are allowed for SEQ ID NO: 1):

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly
Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn
Ile Phe Thr Arg Glu Cys Glu Phe Ile Tyr Gly
Gly Cys Glu Gly Asn Asn Arg Phe Glu Ser Leu
Glut Cys Lys Lys Met Cys Thr Arg Asp
(amino acids 3-60 of SEQ ID NO:2).

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly
Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn
Ile Phe Thr Arg Glu Cys Glu Phe Ile Tyr Gly
Gly Cys Glu Gly Asn Asn Arg Phe Glu Ser Leu
Glut Cys Lys Lys Met Cys Thr Arg Asp
(SEQ ID NO: 4)

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly
Pro Cys Lys Ala Asn His Leu Arg Phe Phe Asn
Ile Phe Thr Arg Glu Cys Glu Phe Ser Tyr Gly
Gly Cys Glu Gly Asn Asn Arg Phe Glu Ser Leu
Glut Cys Lys Lys Met Cys Thr Arg Asp
(SEQ ID NO: 5)

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly
Pro Cys Lys Ala Asn His Leu Arg Phe Phe Asn
Ile Phe Thr Arg Glu Cys Glu Phe Ser Tyr Gly
Gly Cys Glu Gly Asn Asn Arg Phe Glu Ser Leu
Glut Cys Lys Lys Met Cys Thr Arg Asp
(SEQ ID NO: 6)

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly
Pro Cys Lys Ala Asn His Leu Arg Phe Phe Asn
Ile Phe Thr Arg Glu Cys Glu Phe Ser Tyr Gly
Gly Cys Glu Gly Asn Asn Arg Phe Glu Ser Leu
Glut Cys Lys Lys Met Cys Thr Arg Asp
(SEQ ID NO: 7)

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly
Pro Cys Lys Ala Asn His Leu Arg Phe Phe Asn
Ile Phe Thr Arg Glu Cys Glu Phe Ser Tyr Gly
Gly Cys Glu Gly Asn Asn Arg Phe Glu Ser Leu
Glut Cys Lys Lys Met Cys Thr Arg Asp
(SEQ ID NO: 8)
Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly
His Cys Lys Gly Ala His Leu Arg Phe Phe Asn
Ile Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly
Gly Cys Glu Gly Asn Gln Asn Arg Phe Glu Ser Leu
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp,

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly
Ser Cys Arg Ala His Leu Arg Thr Phe Phe Asn
Ile Phe Thr Arg Gln Cys Glu Glu Phe Ser Tyr Gly
Gly Cys Gly Asn Gln Asn Arg Phe Glu Ser Leu
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp,

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly
Arg Cys Lys Gly Ala His Leu Arg Phe Phe Asn
Ile Phe Thr Arg Gln Cys Glu Glu Phe Ser Tyr Gly
Gly Cys Gly Asn Gln Asn Arg Phe Glu Ser Leu
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp,

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly
Arg Cys Arg Gly Ala His Pro Arg Trp Phe Phe Asn
Ile Phe Thr Arg Gln Cys Glu Glu Phe Ser Tyr Gly
Gly Cys Gly Asn Gln Asn Arg Phe Glu Ser Leu
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp,

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly
Pro Cys Arg Ala His Leu Arg Phe Phe Asn
Ile Phe Thr Arg Gln Cys Glu Glu Phe Ser Tyr Gly
Gly Cys Gly Asn Gln Asn Arg Phe Glu Ser Leu
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp,

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly
Arg Cys Arg Gly Ala His Pro Arg Trp Phe Phe Asn
Ile Phe Thr Arg Gln Cys Glu Glu Phe Ser Tyr Gly
Gly Cys Gly Asn Gln Asn Arg Phe Glu Ser Leu
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp,

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly
Arg Cys Arg Gly Ala His Pro Arg Trp Phe Phe Asn
Ile Phe Thr Arg Gln Cys Glu Glu Phe Ser Tyr Gly
Gly Cys Gly Asn Gln Asn Arg Phe Glu Ser Leu
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp,
[SEQ ID NO: 21]
Met His Ser Phe Cys Ala Phe Lys Ala Asp Gly Gly
Arg Cys Arg Ala Ile Gln Pro Arg Trp Phe Phe Aam
Ile Phe Thr Arg Gln Cys Glu Glu Phe Ser Tyr Gly
Gly Cys Gly Arg Aam Gln Aam Arg Phe Glu Ser Len
Gln Glu Cys Lys Lys Met Cys Thr Arg Asp.

[SEQ ID NO: 22]
Met His Ser Phe Cys Ala Phe Lys Ala Asp Gly Gly
Arg Cys Arg Gly Ala His Pro Arg Trp Phe Phe Aam
Ile Phe Thr Arg Glu Cys Glu Glu Phe Ser Tyr Gly
Gly Cys Gly Aam Gln Aam Arg Phe Glu Ser Leu
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp.

[0031] FIGS. 3A and 3B provides a amino acid sequence alignment of these sequences, the native LACI sequence from which these variants were derived (SEQ ID NO:32), and other known Kunitz domains (SEQ ID NOS: 29-31 and 33-53).

[0032] The KI polypeptides useful in the methods and compositions described herein can be made synthetically using any standard polypeptide synthesis protocol and equipment. For example, the stepwise synthesis of a KI polypeptide described herein can be carried out by the removal of an amino (N) terminal-protecting group from an initial (i.e., carboxy-terminal) amino acid, and coupling thereto of the carboxyl end of the next amino acid in the sequence of the polypeptide. This amino acid is also suitably protected. The carboxyl group of the incoming amino acid can be activated to react with the N-terminus of the bound amino acid by formation into a reactive group such as formation into a carbodiimide, a succinic anhydride, or an "active ester" group such as hydroxybenzotriazole or pentafluorophenyl esters. Preferred solid-phase peptide synthesis methods include the BOC method, which utilizes tert-butyloxycarbonyl as the alpha-amino protecting group, and the FMOC method, which utilizes 9-fluorenylemethoxycarbonyl to protect the alpha-amino of the amino acid residues. Both methods are well known to those of skill in the art (Stewart, J. and Young, J., Solid-Phase Peptide Synthesis (W. H. Freeman Co., San Francisco 1989); Merrifield, J., 1963. Am. Chem. Soc., 85:2149-2154; Bodanszky, M. and Bodanszky, A., The Practice of Peptide Synthesis (Springer-Verlag, New York 1984); the entire teachings of these references is incorporated herein by reference). If desired, additional amino- and/or carboxy-terminal amino acids can be designed into the amino acid sequence and added during polypeptide synthesis.

[0033] Alternatively, Kunitz domain polypeptides and KI polypeptides useful in the compositions and methods of the invention can be produced by recombinant methods using any of a number of cells and corresponding expression vectors, including but not limited to bacterial expression vectors, yeast expression vectors, baculovirus expression vectors, mammalian viral expression vectors, and the like. Kunitz domain polypeptides and KI polypeptides useful in the compositions and methods of the invention can also be produced transgenically using nucleic acid molecules comprising a coding sequence for a Kunitz domain or KI polypeptide described herein, wherein the nucleic acid molecule can be integrated into and expressed from the genome of a host animal using transgenic methods available in the art. In some cases, it could be necessary or advantageous to fuse the coding sequence for a Kunitz domain polypeptide or a KI polypeptide comprising the Kunitz domain to another coding sequence in an expression vector to form a fusion polypeptide that is readily expressed in a host cell. Preferably, the host cell that expresses such a fusion polypeptide also processes the fusion polypeptide to yield a Kunitz domain or KI polypeptide useful in the invention that contains only the desired amino acid sequence. Obviously, if any other amino acid(s) remain attached to the expressed Kunitz domain or KI polypeptide, such additional amino acid(s) should not diminish the kallikrein binding and/or kallikrein inhibitory activity of the Kunitz domain or KI polypeptide so as to preclude use of the polypeptide in the methods or compositions of the invention.

[0034] A preferred recombinant expression system for producing KI polypeptides useful in the methods and compositions described herein is a yeast expression vector, which permits a nucleic acid sequence encoding the amino acid sequence for a KI polypeptide or Kunitz domain polypeptide to be linked in the same reading frame with a nucleotide sequence encoding the mat.alpha. prepro leader peptide sequence of Saccharomyces cerevisiae, which in turn is under the control of an operable yeast promoter. The resulting recombinant yeast expression plasmid can then be transformed by standard methods into the cells of an appropriate, compatible yeast host, which cells are able to express the recombinant protein from the recombinant yeast expression vector. Preferably, a host yeast cell transformed with such a recombinant expression vector is also able to process the fusion protein to provide an active KI polypeptide useful in the methods and compositions of the invention. A preferred yeast host for producing recombinant Kunitz domain polypeptides and KI polypeptides comprising such Kunitz domains is Pichia pastoris.

[0035] As noted above, KI polypeptides that are useful in the methods and compositions herein can comprise a Kunitz domain polypeptide described herein. Some KI polypeptides can comprise an additional flanking sequence, preferably of one to six amino acids in length, at the amino and/or carboxy-terminal end, provided such additional amino acids do not significantly diminish kallikrein binding affinity or kallikrein inhibition activity so as to preclude use in the methods and compositions described herein. Such additional amino acids can be deliberately added to express a KI polypeptide in a particular recombinant host cell or can be added to provide an additional function, e.g., to provide a peptide to link the KI polypeptide to another molecule or to provide an affinity moiety that facilitates purification of the polypeptide. Preferably, the additional amino acid(s) do not include cysteine, which could interfere with the disulfide bonds of the Kunitz domain.

[0036] An example of a preferred Kunitz domain polypeptide useful in the methods and compositions of the invention has the amino acid sequence of residues 3-60 of SEQ ID NO:2. When expressed and processed in a yeast fusion protein expression system (e.g., based on the integrating expression plasmid pHIL-D2), such a Kunitz domain polypeptide retains an additional amino terminal Glu-Ala dipeptide from the fusion with the mat.alpha. prepro leader peptide sequence of S. cerevisiae. When secreted from the yeast host cell, most
of the leader peptide is processed from the fusion protein to yield a functional KI polypeptide (referred to herein as "PEP-1") having the amino acid sequence of SEQ ID NO:2 (see boxed region in FIG. 2).

[0037] Particularly preferred KI polypeptides useful in the methods and compositions described herein have a binding affinity for kallikrein that is on the order of 1000 times higher than that of aprotinin, which is currently approved for use in CABG procedures to reduce blood loss. The surprisingly high binding affinities of such KI polypeptides described herein indicate that such KI polypeptides exhibit a high degree of specificity for kallikrein to the exclusion of other molecular targets (see Table 1, below). Thus, use of such polypeptides according to the invention reduces much of the speculation as to the possible therapeutic targets in a patient. The lower degree of specificity exhibited by, for example, aprotinin, leads to possible pleiotropic side effects and ambiguity as to its therapeutic mechanism.

[0038] The polypeptides defined by, for example, SEQ ID NO:1 contain invariant positions, e.g., positions 5, 14, 30, 51 and 55 can be Cys only. Other positions such as, for example, positions 6, 7, 8, 9, 20, 24, 25, 26, 27, 28, 29, 41, 42, 44, 46, 47, 48, 49, 50, 52, 53 and 54 can be any amino acid (including non-naturally occurring amino acids). In a particularly preferred embodiment, one or more amino acids correspond to that of a native sequence (e.g., SEQ ID NO:32, see FIG. 3). In a preferred embodiment, at least one variable position is different from that of the native sequence. In yet another preferred embodiment, the amino acids can each be individually or collectively substituted by a conservative or non-conservative amino acid substitution. Conservative amino acid substitutions replace an amino acid with another amino acid of similar chemical structure and may have no affect on protein function. Non-conservative amino acid substitutions replace an amino acid with another amino acid of dissimilar chemical structure. Examples of conserved amino acid substitutions include, for example, Asn->Asp, Arg->Lys and Ser->Thr. In a preferred embodiment, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 and/or 21 of these amino acids can be independently or collectively, in any combination, selected to correspond to the corresponding position of SEQ ID NO:2.

[0039] Other positions, for example, positions 10, 11, 13, 15, 16, 17, 18, 19, 21, 22, 23, 31, 32, 34, 35, 39, 40, 43 and 45, can be any of a selected set of amino acids. Thus SEQ ID NO:1 defines a set of possible sequences. Each member of this set contains, for example, a cysteine at positions 5, 14, 30, 51 and 55, and any one of a specific set of amino acids at positions 10, 11, 13, 15, 16, 17, 18, 19, 21, 22, 23, 31, 32, 34, 35, 39, 40, 43 and 45. In a preferred embodiment, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18 and/or 19 of these amino acids can be independently or collectively, in any combination, selected to correspond to the corresponding position of SEQ ID NO:2. The peptide preferably has at least 80%, at least 85%, at least 90% or at least 95% identity to SEQ ID NO:2.

Methods and Compositions

[0040] The present invention is also directed to methods for preventing or reducing ischemia. Preferred in the invention are methods for preventing or reducing perioperative blood loss and/or a systemic inflammatory response (SIR) in a patient, especially associated with cardiothoracic surgery. A method for treatment involves the administration of a KI polypeptide comprising a kunitz domain. One embodiment of the method involves using a peptide containing an amino acid sequence of SEQ ID NO:1 that has an affinity for kallikrein that is approximately 1000-fold or higher than that of a broad range serine protease, e.g., aprotinin, which is isolated from bovine lung and currently approved for use in CABG procedures (TRASYLOL.R.TM., Bayer Corporation Pharmaceutical Division, West Haven, Conn.).

[0041] Patients subjected to any of a number of surgical procedures, especially those involving extra-corporeal circulation, e.g., cardiothoracic surgery, such as, for example, CPB, and/or bone trauma, such as sternal split or hip replacement, are at risk for perioperative blood loss and inflammation. Contact of a patient's blood with the cut surfaces of bone or CPB equipment is sufficient to activate one or several undesirable cascade responses, including a contact activation system (CAS), which can lead to extensive perioperative blood loss requiring immediate blood transfusion, as well as a systemic inflammatory response (SIR), which, in turn, can result in permanent damage to tissues and organs. While not desiring to be limited to any particular mechanism or theory, it appears that the blood loss that occurs associated with cardiothoracic surgery, e.g., CPB, as in a CABG procedure, probably results from extensive capillary leakage, which can result in significant loss of blood that must be replaced by immediate blood transfusion.

[0042] The methods described herein are useful for preventing or reducing various ischemias including, for example, perioperative blood loss and SIR in a patient subjected to a surgical procedure, and especially wherein the surgical procedure requires extra-corporeal circulation, e.g., cardiothoracic surgery, such as, for example, CPB. The methods of the invention are particularly useful for preventing or reducing perioperative blood loss and/or SIR in a patient subjected to a CABG procedure requiring CPB or other cardiac surgery.

[0043] Preferred compositions for medical use comprise a KI polypeptide described herein. Such compositions useful can further comprise one or more pharmacologically acceptable buffers, carriers, and excipients, which can provide a desirable feature to the composition including, but not limited to, enhanced administration of the composition to a patient, enhanced circulating half-life of the KI polypeptide of the composition, enhanced compatibility of the composition with patient blood chemistry, enhanced storage of the composition, and/or enhanced efficacy of the composition upon administration to a patient. In addition to a KI polypeptide described herein, compositions can further comprise one or more other pharmaceutically active compounds that provide an additional prophylactic or therapeutic benefit to a patient of an invasive surgical procedure.

[0044] Compositions useful in the methods of the invention comprise any of the Kunitz domain polypeptides or KI polypeptides comprising such Kunitz domain polypeptides described herein. Particularly preferred are KI polypeptides comprising a Kunitz domain polypeptide having a 58-amino acid sequence of amino acids 3-60 of SEQ ID NO:2. An example of such a particularly preferred KI polypeptide useful in the methods and compositions of the invention is the PEP-1 KI polypeptide having the 60-amino acid sequence of SEQ ID NO:2. A nucleotide sequence encoding the amino acid sequence of SEQ ID NO:2 is provided in SEQ ID NO:3 (see, e.g., nucleotides 309-488 in FIG. 2). It is understood that based on the known genetic code, the invention also provides
degenerate forms of the nucleotide sequence of SEQ ID NO:3 by simply substituting one or more of the known degenerate codons for each amino acid encoded by the nucleotide sequence. Nucleotides 7-180 of SEQ ID NO:3, and degenerate forms thereof, encode the non-naturally occurring Kunitz domain polypeptide having the 58-amino acid sequence of amino acids 3-60 of SEQ ID NO:2.

Any of a variety of nucleic acid molecules can comprise the nucleotide sequence of nucleotides 7-180 of SEQ ID NO:3, degenerate forms, and portions thereof, including but not limited to, recombinant plasmid genomes, recombinant mammalian viral vectors, recombinant insect viral vectors, yeast mini chromosomes, and various plasmids. Such plasmids include those used to clone and/or express such nucleotide coding sequences. Expression vectors provide a promoter, which can be operably linked to a particular nucleotide sequence and an appropriate host cell, which is able to transcribe the particular nucleotide coding sequence into a functional messenger RNA (mRNA) and also translate the mRNA into the corresponding polypeptide. A polypeptide so produced can then be isolated from the host cell. Nucleic acid molecules comprising a nucleic acid sequence encoding a Kunitz domain or KI polypeptide described herein can be made by standard nucleic acid synthesis methods, recombinant DNA methodologies, polymerase chain reaction (PCR) methods, and any combination thereof.

Perioperative Blood Loss and Reduced Heart Bloodflow

Due to the many advances in medicine, a number of highly invasive surgical procedures are carried out each day that result in blood loss, or place patients at a high risk for blood loss. Such patients must be carefully monitored to restore and maintain normal blood supply and hemostasis, and they may need blood transfusions. Surgical procedures that involve blood loss include those involving extra-coronary circulation methods such as cardiothoracic surgery, e.g., CPB. In such methods, a patient’s heart is stopped and the circulation, oxygenation, and maintenance of blood volume are carried out artificially using an extra-coronary circuit and a synthetic membrane oxygenator. These techniques are commonly used during cardiac surgery. Additionally, it is apparent that surgery involving extensive trauma to bone, such as the sternal split necessary in CABG or hip replacement procedures, is also associated with activation of the CAS, which can result in a variety of disruptions in the blood and vasculature.

Atherosclerotic coronary artery disease (CAD) causes a narrowing of the lumen of one or several of the coronary arteries; this limits the flow of blood to the myocardium (i.e., the heart muscle) and can cause angina, heart failure, and myocardial infarcts. In the end stage of coronary artery atherosclerosis, the coronary circulation can be almost completely occluded, causing life threatening angina or heart failure, with a very high mortality. CABG procedures may be required to bridge the occluded blood vessel and restore blood to the heart; these are potentially life saving. CABG procedures are among the most invasive of surgeries in which one or more healthy veins or arteries are implanted to provide a “bypass” around the occluded area of the diseased vessel. CABG procedures carry with them a small but important perioperative risk, but they are very successful in providing patients with immediate relief from the mortality and morbidity of atherosclerotic cardiovascular disease. Despite these very encouraging results, repeat CABG procedures are frequently necessary, as indicated by a clear increase in the number of patients who eventually undergo second and even third procedures; the perioperative mortality and morbidity seen in primary CABG procedures is increased in these re-do procedures.

There have been improvements in minimally invasive surgical techniques for uncomplicated CAD. However, nearly all CABG procedures performed for valvular and/or congenital heart disease, heart transplantation, and major aortic procedures, are still carried out on patients supported by CPB. In CPB, large cannulae are inserted into the great vessels of a patient to permit mechanical pumping and oxygenation of the blood using a membrane oxygenator. The blood is returned to the patient without flowing through the lungs, which are hypoperfused during this procedure. The heart is stopped using a cardioplegic solution, the patient cooled to help prevent brain damage, and the peripheral circulating volume increased by an extracorporeal circuit, i.e., the CPB circuit, which requires “priming” with donor blood and saline mixtures are used to fill the extracorporeal circuit. CPB has been extensively used in a variety of procedures performed for nearly half a century with successful outcomes. The interaction between artificial surfaces, blood cells, blood proteins, damaged vascular endothelium, and extravascular tissues, such as bone, disturbs hemostasis and frequently activates the CAS, which, as noted above, can result in a variety of disruptions in the blood and vasculature. Such disruption leads to excess perioperative bleeding, which then requires immediate blood transfusion. A consequence of circulating whole blood through an extracorporeal circuit in CPB can also include the systemic inflammatory response (SIR), which is initiated by contact activation of the coagulation and complement systems. Indeed, much of the morbidity and mortality associated with seemingly mechanically successful CPB surgical procedures is the result of the effects of activating coagulation, fibrinolysis, or complement systems. Such activation can damage the pulmonary system, leading to adult respiratory distress syndrome (ARDS), impairment of kidney and splanchic circulation, and induction of a general coagulopathy leading to blood loss and the need for transfusions. In addition to the dangers of perioperative blood loss, additional pathologies associated with SIR include neurocognitive deficits, stroke, renal failure, acute myocardial infarct, and cardiac tissue damage.

Blood transfusions also present a significant risk of infection and elevate the cost of CABG or other similar procedures that require CPB. In the absence of any pharmacological intervention, three to seven units of blood must typically be expended on a patient, even with excellent surgical techniques. Accordingly, there is considerable incentive for the development of new and improved pharmacologically effective compounds to reduce or prevent perioperative bleeding and SIR in patients subjected to CPB and CABG procedures.

Administration and Dosing Considerations for KI Polypeptides

K1 polypeptides described herein can be administered to a patient before, during, and/or after a surgical procedure in a pharmaceutically acceptable composition. The term “pharmaceutically acceptable” composition refers to a non-toxic carrier or excipient that may be administered to a patient, together with a compound of this invention, and
wherein the carrier or excipient not destroy the biological or pharmacological activity of the composition. KI polypeptides described herein can be administered locally or systemically by any suitable means for delivery of a kallikrein inhibitory amount of the KI polypeptides to a patient including but not limited to systemic administrations such as, for example, intravenous and inhalation. Parenteral administration is particularly preferred.

For parenteral administration, the polypeptides can be injected intravenously, intramuscularly, intraperitoneally, or subcutaneously. Intravenous administration is preferred. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Other pharmaceutically acceptable carriers include, but are not limited to, sterile water, saline solution, and buffered saline (including buffers like phosphate or acetate), alcohol, vegetable oils, polyethylene glycols, gelatin, lactose, amyllose, magnesium stearate, tate, silicic acid, paraffin, etc. Where necessary, the composition can also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection, preservatives, stabilizers, wetting agents, emulsifiers, salts, lubricants, etc. as long as they do not react deleteriously with the active compounds. Similarly, the composition can comprise conventional excipients, e.g., pharmaceutically acceptable organic or inorganic carrier substances suitable for parenteral, enteral or intranasal application which do not deleteriously react with the active compounds. Generally, the ingredients will be supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent in activity units. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade “water for injection” or saline. Where the composition is to be administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients can be mixed prior to administration.

Preferably, the methods of the invention comprise administering a KI polypeptide to a patient as an intravenous infusion according to any approved procedure. Thus, a KI polypeptide described herein can be administered to a patient subjected to a CABG procedure at the times similar to those currently used in approved protocols for administering aprotinin and in an amount necessary to provide a patient with a required number or concentration of kallikrein inhibitory units (KIU). According to the invention, a KI polypeptide described herein can also be administered to a patient in the immediate postoperative period, when bleeding abnormalities can occur as a consequence of downstream effects of SIR. For example, in a procedure involving CPB, a KI polypeptide described herein can be administered to a patient as an initial loading dose, e.g., an effective amount over the course of a convenient time, such as 10 minutes, prior to induction of anesthesia. Then, at induction of anesthesia, a second dose of KI polypeptide can be injected into the CPB priming fluid (“pump prime volume”). The patient can then be placed on a continuous and controlled intravenous infusion dose for the duration of the surgical procedure, and after the procedure if indicated.

Currently there are two regimens approved in the United States for administering aprotinin to a patient undergoing a CABG procedure (see, product label and insert for TRASYLOL.RTM., Bayer Corporation Pharmaceutical Division, West Haven, Conn.). One such approved regimen uses a 2 million KIU intravenous loading dose, 2 million KIU into the pump prime volume, and 500,000 KIU per hour of surgery. Another approved regimen uses 1 million KIU intravenous loading dose, 1 million KIU into the pump prime volume, and 250,000 KIU per hour of surgery. As these regimens are based on KIU, the regimens are readily adapted to any KI polypeptide described herein once the specific activity and KIU of a particular KI polypeptide has been determined by standard assays. Owing to the enhanced binding affinity and inhibitory activity in representative KI polypeptides described herein relative to aprotinin, it is expected that such compositions and methods of the invention are likely to require fewer milligrams (mg) per patient to provide a patient with the required number or concentration of KIU.

Several considerations regarding dosing with a KI polypeptide in methods of the invention can be illustrated by way of example with the representative PEP-1 KI polypeptide of the invention having the amino sequence of SEQ ID NO:2 (molecular weight of 7,054 Daltons).

Table 1, below, provides a comparison of the affinity (K.sub,1 app) of the PEP-1 KI polypeptide for kallikrein and eleven other known plasma proteases. **TABLE 1** Aprotinin Protease Substrate PEP-1 K.sub,1 app (PM) K.sub,1 app (PM) human plasma kallikrein 44 3.0 .times. 10.sup,4 human urine kallikrein1 .times. 10.sup,8 4.0 .times. 10.sup,3 porcine pancreatic kallikrein 2.7 .times. 10.sup,7 human C1r, activated2.0 .times. 10.sup,9 1.0 .times. 10.sup,4 7 human plasmin1.0 .times. 10.sup,9 1.0 .times. 10.sup,4 human factor XIIa2.0 .times. 10.sup,7 1.0 .times. 10.sup,8 human plasmin1.4 .times. 10.sup,5 594 human prostate trypsin1.0 .times. 10.sup,7 1.0 .times. 10.sup,8 human pancreatic chymotrypsin1.0 .times. 10.sup,7 1.0 .times. 10.sup,8 human neutrophil elastase2.0 .times. 10.sup,7 1.0 .times. 10.sup,8 human neutrophil elastase2.0 .times. 10.sup,7 1.0 .times. 10.sup,8 human plasma thrombin2.0 .times. 10.sup,7 1.0 .times. 10.sup,8

Clearly, the PEP-1 KI polypeptide is highly specific for human plasma kallikrein. Furthermore, the affinity (K.sub,1 app) of PEP-1 for kallikrein is 1000 times higher than the affinity of aprotinin for kallikrein: the K.sub,1 app of PEP-1 for kallikrein is about 44 pM (Table 1), whereas the K.sub,1 app of aprotinin for kallikrein is 30,000 pM. Thus, a dose of PEP-1 could be approximately 1000 times lower than that used for aprotinin on a per mole basis. However, consideration of several other factors may provide a more accurate estimation of the dose of PEP-1 required in practice. Such factors include the amount of kallikrein activated during CPB in a particular patient, the concentration of kallikrein required to elicit an SIR, and the bioavailability and pharmacological distribution of PEP-1 in a patient. Nevertheless, use of a KI polypeptide in methods according to the invention and provided in doses currently approved for the use of aprotinin is still expected to provide significant improvements over the current use of the less specific, lower affinity, bovine aprotinin.

For example, the total amount of circulating prekallikrein in plasma is estimated at approximately 500 nM (Silverberg, M. et al., “The Contact System and Its Disorders,” in Blood: Principles and Practice of Hematology, Handin, R. et al., eds., JB Lippincott Co., Philadelphia, 1995). If all of the prekallikrein were activated, then at least 500 nM of PEP-1 would be required for a stoichiometric inhibition of kall-
likrein. An individual having 5 liters of plasma would therefore require about 18 mg of PEP-1 to achieve a plasma concentration of 500 nM.

Another factor to consider is the threshold concentration of kallikrein required to induce a SIR in a patient. If the concentration of active kallikrein must be maintained below, e.g., 1 nM, then owing to its high affinity for kallikrein, PEP-1 offers a significant advantage over aprotinin in the amount of protein that would be required to inhibit SIR. In particular, a concentration of PEP-1 of 1 nM would inhibit 99.6% of kallikrein present at 1 nM (i.e., only 0.4 pM free kallikrein remaining in the blood), whereas, an aprotinin concentration of 1 nM would only inhibit 24.5% of the kallikrein present at 1 nM. For aprotinin to inhibit 99% of the kallikrein at 1 nM, an aprotinin concentration in the plasma of at least 3 μM is required (i.e., 3000 times higher concentration than for PEP-1).

For a patient undergoing CPB, an initial clinical dose of PEP-1 can be estimated from a recommended dose regimen of aprotinin (1 times 10 sup.6 KIU) mentioned above. Aprotinin is reported in a package insert to have as specific inhibitory activity of 7143 KIU/mg determined using a dog blood pressure assay. Therefore, 1 times 10 sup.6 KIU of aprotinin is equivalent to 140 mg of aprotinin (i.e., 1 times 10 sup.6 KIU/7143 KIU/mg=140 mg of aprotinin). In a patient having a blood plasma volume of 5 liters, 140 mg corresponds to approximately 4.3 μM aprotinin (molecular weight of aprotinin is 6212 Daltons). The specific activity of aprotinin in the standard inhibitory assay used for PEP-1 is 0.4 KIU/mg of polypeptide. A dose of 140 mg would correspond to a loading dose for aprotinin of 56 KIU (140 mg times 0.4 KIU/mg=56 KIU). In contrast, since the specific activity of the PEP-1 KI polypeptide is 10 KIU/mg in the standard inhibition assay, a dose of only 5.6 mg of PEP-1 would be required to provide the number of KIUs equivalent to 140 mg of aprotinin. In a patient with a plasma volume of 5 liters, this corresponds to about 160 nM PEP-1 (molecular weight of PEP-1 is 7054 Daltons), although a higher dose of the PEP-1 KI polypeptide can be required if all of the plasma kallikrein (500 nM) is activated and/or if this KI polypeptide is poorly distributed in a patient.

Furthermore, the KI polypeptides can be non-naturally occurring, and they can be produced synthetically or recombinantly, as noted above, thereby avoiding potential contamination of transmissible diseases that can arise during isolation of a protein from a natural animal source, such as in the case of aprotinin, which is isolated from bovine lung. Increasingly important to administrative and public acceptance of a treatment or pharmaceutical composition comprising a polypeptide is the avoidance of possible contamination with and transmission to human patients of various pathological agents. Of particular interest for the safety of proteins isolated from a bovine tissue is the elimination of the possible risk of exposure to viral mediated diseases, bacterial mediated diseases, and, especially, transmissible bovine spongiform encephalopathies.

As variants of the Kunitz domain 1 of the human LACI protein, fewer side effects are expected from administering the KI polypeptides to patients than for aprotinin, which is a bovine protein that is documented to cause anaphylactic and anaphylactoid responses in patients, especially in repeat administrations, such as second time CABG procedures. Additionally, the highly specific binding of the KI polypeptides described herein to kallikrein will effectively limit or eliminate the thrombotic tendencies observed with aprotinin, and reduce the problems observed with graft patency following CABG procedures.

The invention will be further described with reference to the following non-limiting examples. The teachings of all the patents, patent applications and all other publications and websites cited herein are incorporated by reference in their entirety.

EXAMPLE 1

A Representative KI Polypeptide

A non-naturally occurring, KI polypeptide useful in the compositions and methods of the invention was identified as a kallikrein binding polypeptide displayed on a recombinant plasmid from a plasmid library. PEP-1 has the following amino acid sequence: Glu Ala Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Asn Ile Phe Thr Arg Gln Cys Gru Gnu Phe Ile Tyr Gly Cys Gru Gnu Gly Asn Gnu Asn Arg Phe Glu Ser Leu Gru Cys Lys Met Cys Thr Arg Asp (SEQ ID NO:2). The molecular weight of PEP-1 is 7,054 Daltons.

The nucleotide sequence (SEQ ID NO:3) encoding the PEP-1 amino acid sequence (SEQ ID NO:2), was derived from a peptide that was isolated and sequenced by standard methods determined from the recombinant plasmid DNA. PEP-1 was produced in amounts useful for further characterization as a recombinant protein in His4.sup.- phenotype host cells of yeast strain Pichia pastoris.

EXAMPLE 2

Construction of a Recombinant Plasmid to Express KI Polypeptides

The initial plasmid, pHIL-D2, is ampicillin resistant and contains a wild-type allele of His4 from P. pastoris. The final DNA sequence comprising the coding sequence for the mat.alpha. Prepro-PEP-1 fusion protein in the recombinant expression plasmid pPIC-K503 is shown in FIG. 2. The DNA sequence of pHIL-D2 was modified to produce pPIC-K503, as follows:

1. The BstBI site in the 3’ AOX1 region of pHIL-D2, located downstream of the His4 gene, was removed by partial restriction digestion, fill-in, and ligation, altering the sequence from TTGGA to TTCCGAA (SEQ ID NO:23) to TTCGAA (SEQ ID NO:24). This modification was made to facilitate and direct the cloning of the expression cassette into the plasmid.

2. The AatII site bearing the bla gene located downstream of His4 was removed by restriction digestion, fill-in, and ligation modifying the sequence from GACGTC to GAGCTC (SEQ ID NO:25) to GAGCTC (SEQ ID NO:26). This modification was made to facilitate the cloning of expression cassettes having AatII sites into the plasmid. The DNA encoding PEP-1 was synthesized based on the nucleotide sequence from the original kallikrein-binding domain phage and consisted of 450 base pairs (bp). The final DNA sequence of the insert in the pHIL-D2 plasmid is flanked by a 5’ AOX1 sequence and a 3’ AOX1 sequence (portions of which are shown in FIG. 2) and encode a fusion protein comprising the mat.alpha. prepro signal peptide of S. cerevisiae fused to the structural coding sequence for the PEP-1 KI polypeptide. The signal peptide was added to facilitate the secretion of PEP-1
from the yeast host cells. The oligonucleotides to form the insert were synthesized and obtained commercially (Genesis Labs, The Woodlands, Tex.), and the insert was generated by polymerise chain reaction (PCR). The linked synthetic DNA encoding the malt alpha, prepro/PEP-1 fusion protein was then incorporated by ligation into the modified pHIL-D2 plasmid between the BstBI and EcoRI sites.

[0068] The ligation products were used to transform Escherichia coli strain XL1 Blue. A PCR assay was used to screen E. coli transformants for the desired plasmid construct. DNA from cell extracts was amplified by PCR using primers containing the 5' AOX1 and 3' AOX1 sequences (see above and Fig. 2). PCR products of the correct number of base pairs were sequenced. In addition, approximately 20-50 bp on either side of the cloning sites were sequenced, and the predicted sequence was obtained. The final DNA sequence of the insert in the pHIL-D2 plasmid (to yield plasmid pPIC-K503) is shown in Fig. 2 along with portions of flanking 5' and 3' AOX1 sequences and corresponding amino acid sequence of the fusion protein comprising the malt alpha, prepro signal peptide of S. cerevisiae fused to the structural coding sequence for the PEP-1 K1 polypeptide. A transformant with the desired expression plasmid construct, plasmid pPIC-K503, was selected for preparing yeast cell lines for routine production of PEP-1.

EXAMPLE 3

Manufacture of PEP-1 From Recombinant Yeast Cell Line

[0069] Spheroplasts of P. pastoris GS115 having the His4, sup-phenotype were transformed with the expression plasmid pPIC-K503 (above) following linearization of the plasmid at the SaeI site and homologous recombination of the plasmid DNA into the host 5' AOX1 locus. The phenotype of the production strain is His4 sup+. The entire plasmid was inserted into the 5' AOX1 genomic sequence of the yeast.

[0070] Isolates from the transformation were screened for growth in the absence of exogenous histidine with methanol as the sole carbon source. Greater than 95% of the transformants retained the wild-type ability to grow with methanol as the sole carbon source, thereby demonstrating that the plasmid had integrated into the host genome. Selected colonies were cloned. Small culture expression studies were performed to identify clones secreting the highest levels of active PEP-1 into the culture medium. PEP-1 secretion levels in clarified culture supernatant solutions were quantified for PEP-1 levels by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and evaluated for kallikrein inhibition. A yeast clone was selected for PEP-1 production based on its high level of PEP-1 expression among cultures sampled.

[0071] Muster and working cell banks of P. pastoris producing PEP-1 were prepared commercially (MDS Pharma Services, Bothell, Wash.). A standard production of PEP-1 in yeast comprised three steps as follows: (1) preparation of the seed culture, (2) fermentation, and (3) recovery of the culture.

[0072] The seed culture step consisted of the inoculation of six flasks (300 mL each) containing sterile inoculum broth (yeast nitrogen base, potassium phosphate, and glycerol, pH 5) with the contents of a single vial of a working cell bank of P. pastoris producing PEP-1. Flasks were inoculated in an orbital shaker (300 rpm) for approximately 13 hours at 30.degree. C. 21.2 degree. C.

[0073] Fermentations were performed in a closed 100 liter Braun fermenter filled with sterile broth. Each fermentation was initiated with the transfer of the contents of the six seed culture flasks to the fermenter. After approximately 24 hours, the glycerol in the fermenter became exhausted and additional glycerol was added for approximately 8 additional hours.

[0074] A mixed feed phase, which lasted approximately 83 hours, was then initiated by the addition of a glycerol and methanol feed. At the end of this time, the fermentation was terminated, and the fermenter contents were diluted with purified water. The purification and processing of PEP-1 consisted of five steps as follows: (1) expanded bed chromatography, (2) cation exchange chromatography, (3) hydrophobic interaction chromatography (HIC), (4) ultrafiltration and diafiltration, and (5) final filtration and packaging.

[0075] The initial purification step consisted of expanded bed chromatography. The diluted fermenter culture was applied to the equilibrated column packed with Streamline SP resin (Amersham Pharmacia Streamline 200 chromatography column, Amersham Pharmacia, Piscataway, N.J.). The column was then washed (50 mM acetic acid, pH 3-0.3) in an up-flow mode to flush the yeast cells from the expanded bed. The top adaptor was raised above the expanded bed enhance washing. The flow was stopped and the bed was allowed to settle. The adaptor was moved down so that it was slightly above the settled bed. The direction of the flow was reversed. The effluent was collected. Washing was continued in a downward mode using 50 mM sodium acetate, pH 4.0. The effluent was collected. PEP-1 was eluted from the column using 50 mM sodium acetate, pH 6.0. The eluate was collected in a 50 liter container. The eluate was then filtered through a 0.22 mu. filter into a clean container located in the purification site. Additional samples were collected for the determination of PEP-1 concentration. A cation exchange chromatography step was then performed using the filtered eluate from the expanded bed column. PEP-1 was eluted from the column using 15 mM trisodium citrate, pH 6.2.

[0076] Additional proteins were removed from the PEP-1 preparation by hydrophobic interaction chromatography (HIC). Prior to HIC, the eluate from the cation exchange column was diluted with ammonium sulfate. The eluate was applied to the column, and the PEP-1 was eluted using ammonium sulfate (0.572 M) in potassium phosphate (100 mM), pH 7.0. The eluate was collected in fractions based on A280 values. All fractions were collected into sterile, pre-weighed PETG bottles.

[0077] Selected fractions were pooled into a clean container. The pool was concentrated by ultrafiltration. The concentrated PEP-1 preparation was immediately diafiltered against ten volumes of PBS, pH 7.0.

[0078] A final filtration step was performed prior to packaging in order to minimize the bioburden in the bulk PEP-1. The bulk solution was filtered through a 0.22 mu. filter and collected into a sterile, pre-weighed PETG bottle. A sample was removed for lot release testing. The remainder of the bulk was dispensed aseptically into sterile PETG bottles and stored at -20.degree. C.
EXAMPLE 4

Kallikrein Inhibition Assay

A kinetic test was used to measure inhibitory activity of KI polypeptides, such as PEP-1. The kinetic assay measures fluorescence following kallikrein-mediated cleavage of a substrate, prolylphenylalaninearginyl amino methyl coumarin. A known amount of kallikrein was incubated with a serially diluted KI polypeptide reference standard or serially diluted KI polypeptide test samples, in a suitable reaction buffer on a microtiter plate. Each sample was run in triplicate.

The substrate solution was added, and the plate read immediately using an excitation wavelength of 360 nm and an emission wavelength of 460 nm. At least two each of the reference standard and sample curves were required to have an R-squared value of 0.95 to be considered valid.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.
FEATURE:
NAME/KEY: VARIANT
LOCATION: (31)...(31)
OTHER INFORMATION: Xaa = Glu, Asp, Gln, Asn, Ser, Ala, Val, Leu, Ile or Thr

FEATURE:
NAME/KEY: VARIANT
LOCATION: (32)...(32)
OTHER INFORMATION: Xaa = Glu, Gln, Asp, Asn, Pro, Thr, Leu, Ser, Ala, Gly or Val

FEATURE:
NAME/KEY: VARIANT
LOCATION: (34)...(34)
OTHER INFORMATION: Xaa = Thr, Ile, Ser, Val, Ala, Asn, Gly or Leu

FEATURE:
NAME/KEY: VARIANT
LOCATION: (35)...(35)
OTHER INFORMATION: Xaa = Tyr, Trp or Phe

FEATURE:
NAME/KEY: VARIANT
LOCATION: (39)...(39)
OTHER INFORMATION: Xaa = Glu, Gly, Ala, Ser or Asp

SEQUENCE: 1

Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Gly Xaa Cys Xaa Xaa
1  5  10  15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa
20  25  30

Phe Xaa Xaa Gly Gly Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa
35  40  45

Xaa Xaa Cys Xaa Xaa Xaa Cys Xaa Xaa Xaa
50  55

SEQUENCE: 2

Glu Ala Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys
1  5  10  15

Arg Ala Ala His Pro Arg Trp Phe Asn Ile Phe Thr Arg Gln Cys
20  25  30

Glu Phe Ile Tyr Gly Gly Cys Glu Gly Asn Glu Asn Arg Phe Glu
35  40  45

Ser Leu Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50  55  60

SEQUENCE: 3

gaggtctagc actcttttctg tgttttcaag gtagcgacgc gtcgtgcaga getgctcacc
30

cagatgtgct tctcaacatc tctacgctc aatgcaagga gttcatctac ggtggttgtg
120

agggtacca aacagagtc gtagttctag aggagtgtga gaagatgtgt actagagac
179

SEQUENCE: 4
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide

<400> SEQUENCE: 4

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Lys Ala
1   5   10   15
Asn His Leu Arg Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu
20  25   30
Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu
35  40  45
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50  55

<210> SEQ ID NO 5
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide

<400> SEQUENCE: 5

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Lys Ala
1   5   10   15
Asn His Gln Arg Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu
20  25   30
Phe Thr Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu
35  40  45
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50  55

<210> SEQ ID NO 6
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide

<400> SEQUENCE: 6

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Lys Ala
1   5   10   15
Asn His Gln Arg Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu
20  25   30
Phe Thr Tyr Gly Gly Cys Ala Asp Asp Gly His Cys Lys Ala
35  40  45
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50  55

<210> SEQ ID NO 7
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide

<400> SEQUENCE: 7

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Lys Ala
1   5   10   15
Ser Leu Pro Arg Phe Phe Phe Asn Ile Phe Thr Arg Glu Gln Cys Glu Glu
20 25 30
Phe Ile Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu
35 40 46
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50 55

<210> SEQ ID NO 8
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide

<400> SEQUENCE: 8
Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Lys Ala
1 5 10 15
Asn His Gin Arg Phe Phe Asn Ile Phe Thr Arg Glu Gln Cys Glu Glu
20 25 30
Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu
35 40 45
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50 55

<210> SEQ ID NO 9
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide

<400> SEQUENCE: 9
Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly His Cys Lys Gly
1 5 10 15
Ala His Leu Arg Phe Phe Asn Ile Phe Thr Arg Glu Gln Cys Glu Glu
20 25 30
Phe Ile Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu
35 40 45
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50 55

<210> SEQ ID NO 10
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide

<400> SEQUENCE: 10
Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Arg Cys Lys Gly
1 5 10 15
Ala His Leu Arg Phe Phe Asn Ile Phe Thr Arg Glu Gln Cys Glu Glu
20 25 30
Phe Ile Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu
35 40 45
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50 55
<210> SEQ ID NO 11
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide

<400> SEQUENCE: 11
Met His Ser Phe Cys Ala Phe Lys Ala Asp Gly Gly Arg Cys Arg Gly
1  5    10  15
Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu
20 25  30
Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu
35 40  45
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50  55

<210> SEQ ID NO 12
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide

<400> SEQUENCE: 12
Met His Ser Phe Cys Ala Phe Lys Ala Asp Gly Pro Cys Arg Ala
1  5    10  15
Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu
20 25  30
Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu
35 40  45
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50  55

<210> SEQ ID NO 13
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide

<400> SEQUENCE: 13
Met His Ser Phe Cys Ala Phe Lys Ala Asp Val Gly Arg Cys Arg Gly
1  5    10  15
Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu
20 25  30
Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu
35 40  45
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50  55

<210> SEQ ID NO 14
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide

<400> SEQUENCE: 14
Met His Ser Phe Cys Ala Phe Lys Ala Asp Val Gly Arg Cys Arg Gly
1  5  10  15

Ala Gln Pro Arg Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu
20  25  30

Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu
35  40  45

Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50  55

-Oct 23, 2008-

<210> SEQ ID NO 15
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide

<400> SEQUENCE: 15

Met His Ser Phe Cys Ala Phe Lys Ala Asp Gly Ser Cys Arg Ala
1  5  10  15

Ala His Leu Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu
20  25  30

Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu
35  40  45

Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50  55

<210> SEQ ID NO 16
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide

<400> SEQUENCE: 16

Met His Ser Phe Cys Ala Phe Lys Ala Glu Gly Gly Ser Cys Arg Ala
1  5  10  15

Ala His Gln Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu
20  25  30

Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu
35  40  45

Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50  55

<210> SEQ ID NO 17
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide

<400> SEQUENCE: 17

Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Arg Gly
1  5  10  15

Ala His Leu Arg Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu
20  25  30

Phe Ser Tyr Gly Gly Cys Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu
35  40  45

Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
isolated binding peptide

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<210> SEQ ID NO 18
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide

<400> SEQUENCE: 18
Met His Ser Phe Cys Ala Phe Lys Ala Asp Arg Gly His Cys Arg Gly
1  5  10  15
Ala Leu Pro Arg Trp Phe Asn Ile Phe Thr Arg Glu Cys Glu Glu
20 25 30
Phe Ser Tyr Gly Gly Cys Gly Gly Asn Glu Asn Arg Phe Glu Ser Leu
35 40 45
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50 55

<210> SEQ ID NO 19
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide

<400> SEQUENCE: 19
Met His Ser Phe Cys Ala Phe Lys Ala Asp Ser Gly Asn Cys Arg Gly
1  5  10  15
Asn Leu Pro Arg Phe Asn Ile Phe Thr Arg Glu Cys Glu Glu
20 25 30
Phe Ser Tyr Gly Gly Cys Gly Gly Asn Glu Asn Arg Phe Glu Ser Leu
35 40 45
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50 55

<210> SEQ ID NO 20
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide

<400> SEQUENCE: 20
Met His Ser Phe Cys Ala Phe Lys Ala Asp Ser Gly Arg Cys Arg Gly
1  5  10  15
Asn His Glu Arg Phe Asn Ile Phe Thr Arg Glu Cys Glu Glu
20 25 30
Phe Ser Tyr Gly Gly Cys Gly Gly Asn Glu Asn Arg Phe Glu Ser Leu
35 40 45
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50 55

<210> SEQ ID NO 21
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide

<400> SEQUENCE: 21
Asp Asp Gly His Phe Thr Arg Gln Gln Asn Arg Phe Asp
45
Glu Glu Cys Arg Gly 30 Cys Glu Glu 40 Glu Ser Lys Leu.
Cys Arg Gly 15 Cys Glu Glu 30 Glu Ser Lys Leu.
Cys Arg Gly 15 Cys Glu Glu 30 Glu Ser Lys Leu.

Oct. 23, 2008
Met His Ser Phe Cys Ala Phe Lys Ala Asp Gly Gly Arg Cys Arg Ala
1      5     10     15
Ile Gln Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu
20      25     30
Phe Ser Tyr Gly Gly Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu
35      40     45
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50      55

<210> SEQ ID NO 22
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Isolated Binding Peptide

<400> SEQUENCE: 22
Met His Ser Phe Cys Ala Phe Lys Ala Asp Gly Gly Arg Cys Arg Gly
1      5     10     15
Ala His Pro Arg Trp Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu
20      25     30
Phe Ser Tyr Gly Gly Gly Gly Asn Gln Asn Arg Phe Glu Ser Leu
35      40     45
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
50      55

<210> SEQ ID NO 23
<211> LENGTH: 6
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Modified Cloning Site

<400> SEQUENCE: 23
ttcgaa

<210> SEQ ID NO 24
<211> LENGTH: 8
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Modified Cloning Site

<400> SEQUENCE: 24
ttcgcaaa

<210> SEQ ID NO 25
<211> LENGTH: 6
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Modified Cloning Site

<400> SEQUENCE: 25
gacgctc

<210> SEQ ID NO 26
<211> LENGTH: 10
<212> TYPE: DNA
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Modified Cloning Site

<400> SEQUENCE: 26

gacgtaagtc

<210> SEQ ID NO 27
<211> LENGTH: 548
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Nucleotide Sequence of Fusion Protein

<400> SEQUENCE: 27

cyacttttac cgacacttg aagagatcga aaacaacta attatcga aacagagat
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tccatctct ttctcactg gttttgtcgc ctgctctct tcgggggctttg getocagta
120
acacacactc tgaaacagcg acctctcaca ttctctctga ggcgttctat ggtttacttg
180
actggaagg tgacgctgac gtcgctgttt tgcgctctct taacctctact aacaagrrgt
240
tgtctgctat caacactacc atcctctctca tcgtctctaa ggcaggaggt gttctctctg
300
agaagaagg ggcgatgacg tcgctctcgg tcgactctgg gatggcggct gctggcggag
360
catctcacc aagatggttc ttcacactct ttcgctctca atcggaggag ttcctctcag
420
gtctgctgtga ggttaacaa aacagatcc agctctctga gsggtgtaag aagatgtgta
480
cggtagaca ctgacagaggac gcgtctcttc tcaagttcag ttcgctctttt tcgggctcct
540
acgagaag
548

<210> SEQ ID NO 28
<211> LENGTH: 145
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Fusion Protein

<400> SEQUENCE: 28

Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser 1 5 10 15
Ala Leu Ala Ala Pro Val Asn Thr Thr Thr Glu Asp Glu Thr Ala Gln 20 25
Ile Pro Ala Glu Ala Val Ile Gly Tyr Ser Asp Leu Glu Gly Asp Phe 30 35 40
Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu Leu 45 50 55 60
Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val 65 70 75 80
Ser Leu Glu Lys Arg Glu Ala Met His Ser Phe Cys Ala Phe Lys Ala 85 90 95
Asp Asp Gly Pro Cys Arg Ala Ala His Pro Arg Trp Phe Phe Asn Ile 100 105 110
Phe Thr Arg Gln Cys Glu Glu Phe Ile Tyr Gly Cys Glu Gly Asn 115 120 125
Gln Asn Arg Phe Glu Ser Leu Glu Cys Lys Lys Met Cys Thr Arg 130 135 140
Asp
<210> SEQ ID NO 29
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: BPTI Sequence

<400> SEQUENCE: 29

Arg Pro Asp Phe Cys Leu Glu Pro Pro Tyr Thr Gly Pro Cys Lys Ala
1 5 10 15
Arg Ile Ile Arg Tyr Phe Tyr Asn Ala Lys Ala Gly Leu Cys Gin Thr
20 25 30
Phe Val Tyr Gly Gly Cys Arg Ala Lys Arg Asn Phe Lys Ser Ala
35 40 45
Glu Asp Cys Met Arg Thr Cys Gly Gly Ala
50 55

<210> SEQ ID NO 30
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ITI-D1 Sequence

<400> SEQUENCE: 30

Lys Glu Asp Ser Cys Gln Leu Gly Tyr Ser Ala Gly Pro Cys Met Gly
1 5 10 15
Met Thr Ser Arg Tyr Phe Tyr Asn Ala Lys Ala Cys Glu Thr
20 25 30
Phe Glu Tyr Gly Gly Cys Met Gly Asn Gly Asn Phe Val Thr Glu
35 40 45
Lys Glu Cys Leu Gln Thr Cys Arg Thr Val
50 55

<210> SEQ ID NO 31
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ITI-D2 Sequence

<400> SEQUENCE: 31

Thr Val Ala Ala Cys Asn Leu Pro Ile Val Arg Gly Pro Cys Arg Ala
1 5 10 15
Phe Ile Gln Leu Trp Ala Phe Asp Ala Val Lys Gly Lys Cys Val Leu
20 25 30
Phe Pro Tyr Gly Gly Cys Gln Gly Asn Gly Asn Lys Phe Tyr Ser Glu
35 40 45
Lys Glu Cys Arg Glu Tyr Cys Gly Val Pro
50 55

<210> SEQ ID NO 32
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: LACI-D1 Sequence
<400> SEQUENCE: 32
Met His Ser Phe Cys Ala Phe Lys Ala Asp Asp Gly Pro Cys Lys Ala
1  2  3  4  5  6  7  8  9  10  11  12
Ile Met Lys Arg Phe Phe Phe Asn Ile Phe Thr Arg Gln Cys Glu Glu
13 14 15 16 17 18 19 20 21 22 23 24
Phe Ile Tyr Gly Gly Cys Glu Gly Asn Glu Asn Arg Phe Glu Ser Leu
25 26 27 28 29 30 31 32 33 34 35 36
Glu Glu Cys Lys Lys Met Cys Thr Arg Asp
37 38 39 40 41 42 43 44 45 46 47 48

<210> SEQ ID NO 33
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: LACI-D2 Sequence

<400> SEQUENCE: 33
Lys Pro Asp Phe Cys Phe Leu Glu Asp Pro Gly Ile Cys Arg Gly
1  2  3  4  5  6  7  8  9 10  11  12
Tyr Ile Thr Arg Tyr Phe Tyr Asn Asn Thr Lys Gln Cys Glu Arg
13 14 15 16 17 18 19 20 21 22 23 24
Phe Lys Tyr Gly Gly Cys Leu Gly Asn Met Asn Phe Glu Thr Leu
25 26 27 28 29 30 31 32 33 34 35 36
Glu Glu Cys Lys Asn Ile Cys Glu Asp Gly
37 38 39 40 41 42 43 44 45 46 47 48

<210> SEQ ID NO 34
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: LACI-D3 Sequence

<400> SEQUENCE: 34
Gly Pro Ser Trp Cys Leu Thr Pro Ala Asp Arg Gly Leu Cys Arg Ala
1  2  3  4  5  6  7  8  9 10  11  12
Asn Glu Asn Arg Phe Tyr Tyr Asn Ser Val Ile Gln Lys Cys Arg Pro
13 14 15 16 17 18 19 20 21 22 23 24
Phe Lys Tyr Ser Gly Cys Gly Asn Glu Asn Phe Thr Ser Lys
25 26 27 28 29 30 31 32 33 34 35 36
Gln Glu Cys Leu Arg Ala Cys Lys Lys Gly
37 38 39 40 41 42 43 44 45 46 47 48

<210> SEQ ID NO 35
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: HKI B9 Sequence

<400> SEQUENCE: 35
Leu Pro Asn Val Cys Ala Phe Pro Met Glu Lys Gly Pro Cys Gln Thr
1  2  3  4  5  6  7  8  9 10  11  12
Tyr Met Thr Arg Trp Phe Phe Asn Phe Glu Thr Gly Glu Cys Glu Leu
13 14 15 16 17 18 19 20 21 22 23 24
Phe Ala Tyr Gly Gly Cys Gly Gly Asn Ser Asn Asn Phe Leu Arg Lys
25 26 27 28 29 30 31 32 33 34 35 36

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Glu Lys Cys Glu Lys Phe Cys Lys Phe Thr
50 55

<210> SEQ ID NO 36
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: C alpha 3 Sequence
<400> SEQUENCE: 36
Glu Thr Asp Ile Cys Lys Leu Pro Lys Asp Glu Gly Thr Cys Arg Asp
1 5 10 15
Phe Ile Leu Lys Trp Tyr Asp Pro Asn Thr Lys Ser Cys Ala Arg
20 25 30
Phe Trp Tyr Gly Gly Cys Gly Gly Asn Glu Asn Lys Phe Gly Ser Glu
35 40 45
Lys Glu Cys Glu Lys Val Cys Ala Pro Val
50 55

<210> SEQ ID NO 37
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: TFPI-2 D1 Sequence
<400> SEQUENCE: 37
Ann Ala Glu Ile Cys Leu Leu Pro Leu Asp Tyr Gly Pro Cys Arg Ala
1 5 10 15
Leu Leu Leu Arg Tyr Tyr Asp Arg Tyr Thr Glu Ser Cys Arg Glu
20 25 30
Phe Leu Tyr Gly Gly Cys Glu Gly Asn Ala Asn Phe Tyr Thr Trp
35 40 45
Glu Ala Cys Asp Asp Ala Cys Trp Arg Ile
50 55

<210> SEQ ID NO 38
<211> LENGTH: 61
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: TFPI-2 D2 Sequence
<400> SEQUENCE: 38
Val Pro Lys Val Cys Arg Leu Glu Val Ser Val Asp Asp Glu Cys Glu
1 5 10 15
Gly Ser Thr Glu Lys Tyr Phe Phe Asn Leu Ser Ser Met Thr Cys Glu
20 25 30
Lys Phe Phe Ser Gly Gly Cys His Arg Asn Arg Ile Glu Asn Arg Phe
35 40 45
Pro Asp Glu Ala Thr Cys Met Gly Phe Cys Ala Pro Lys
50 55 60

<210> SEQ ID NO 39
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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Phe Val Tyr Gly Gly Cys Met Gly Asn Gly Asn Asn Phe Val Thr Glu 35 40 45
Lys Asp Cys Leu Gln Thr Cys Arg Gly Ala 50 55

<210> SEQ ID NO 43
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: MUTT26A Sequence

<400> SEQUENCE: 43
Arg Pro Asp Phe Cys Gln Leu Gly Tyr Ser Ala Gly Pro Cys Val Ala 1 5 10 15
Met Phe Pro Arg Tyr Phe Tyr Asn Gly Ala Ser Met Ala Cys Gln Thr 20 25 30
Phe Val Tyr Gly Gly Cys Met Gly Asn Gly Asn Asn Phe Val Thr Glu 35 40 45
Lys Asp Cys Leu Gln Thr Cys Arg Gly Ala 50 55

<210> SEQ ID NO 44
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: MUTQ6 Sequence

<400> SEQUENCE: 44
Arg Pro Asp Phe Cys Gln Leu Gly Tyr Ser Ala Gly Pro Cys Val Ala 1 5 10 15
Met Phe Pro Arg Tyr Phe Tyr Asn Gly Ala Ser Met Ala Cys Gln Thr 20 25 30
Phe Val Tyr Gly Gly Cys Met Gly Asn Gly Asn Asn Phe Val Thr Glu 35 40 45
Lys Asp Cys Leu Gln Thr Cys Arg Gly Ala 50 55

<210> SEQ ID NO 45
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: MUT1619 Sequence

<400> SEQUENCE: 45
Arg Pro Asp Phe Cys Gln Leu Gly Tyr Ser Ala Gly Pro Cys Val Gly 1 5 10 15
Met Phe Ser Arg Tyr Phe Tyr Asn Gly Thr Ser Met Ala Cys Gln Thr 20 25 30
Phe Val Tyr Gly Gly Cys Met Gly Asn Gly Asn Asn Phe Val Thr Glu 35 40 45
Lys Asp Cys Leu Gln Thr Cys Arg Gly Ala 50 55

<210> SEQ ID NO 46
<211> LENGTH: 62
<212> TYPE: PRT
Tyr Gly Gly Cys Glu Gly Asn Gly Asn Lys Phe Tyr Ser Glu Lys Glu

Cys Arg Glu Tyr Cys Gly Val Pro

<210> SEQ ID NO 50
<211> LENGTH: 60
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DPI14 KR Sequence

<400> SEQUENCE: 50
Glu Ala Val Arg Glu Val Cys Ser Glu Gln Ala Glu Thr Gly Pro Cys
1  5   10  15
Ile Ala Phe Phe Pro Arg Trp Tyr Phe Asp Val Thr Glu Gly Lys Cys
20  25  30
Ala Pro Phe Phe Tyr Gly Gly Cys Gly Gly Asn Arg Asn Asp Phe Arg
35  40  45
Thr Glu Glu Tyr Cys Met Ala Val Cys Gly Ser Ala
50  55  60

<210> SEQ ID NO 51
<211> LENGTH: 60
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DPI14 KR Sequence

<400> SEQUENCE: 51
Glu Ala Asn Ala Glu Ile Cys Leu Leu Pro Leu Asp Tyr Gly Pro Cys
1  5   10  15
Ile Ala Phe Phe Pro Arg Tyr Tyr Tyr Asp Arg Tyr Thr Gln Ser Cys
20  25  30
Arg Gln Phe Leu Tyr Gly Gly Cys Glu Gly Asn Ala Asn Asp Phe Tyr
35  40  45
Thr Trp Glu Ala Cys Asp Asp Ala Cys Trp Arg Ile
50  55  60

<210> SEQ ID NO 52
<211> LENGTH: 60
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: DPI14 KR Sequence

<400> SEQUENCE: 52
Glu Ala Lys Pro Asp Phe Cys Phe Leu Glu Glu Asp Pro Gly Ile Cys
1  5   10  15
Ile Gly Phe Phe Pro Arg Tyr Phe Tyr Asn Asn Gln Ala Lys Gln Cys
20  25  30
Glu Arg Phe Val Tyr Gly Gly Cys Leu Gly Asn Met Asn Asp Phe Glu
35  40  45
Thr Leu Glu Glu Cys Lys Asn Ile Cys Glu Asp Gly
50  55  60

<210> SEQ ID NO 53
US 2008/0260752 A1

Glu Ala Glu Thr Asp Ile Cys Lys Leu Pro Lys Asp Glu Gly Thr Cys
1  5    10    15

Ile Ala Phe Phe Pro Arg Trp Tyr Asp Pro Asn Thr Lys Ser Cys
20  25

Ala Arg Phe Val Tyr Gly Gly Cys Gly Gly Asn Glu Asn Lys Phe Gly
30  40  45

Ser Glu Cys Glu Lys Thr Ala Pro Val
50  55  60

<211>  SEQ ID NO 54
<212>  LENGTH: 58
<213>  TYPE: PRT
<213>  ORGANISM: Artificial Sequence
<220>  FEATURE:
<223>  OTHER INFORMATION: Variant
<220>  FEATURE:
<221>  NAME/KEY: VARIANT
<222>  LOCATION: 10
<223>  OTHER INFORMATION: Xaa = Asp or Glu
<220>  FEATURE:
<221>  NAME/KEY: VARIANT
<222>  LOCATION: 11
<223>  OTHER INFORMATION: Xaa = Asp, Gly, Ser, Val, Asn, Ile, Ala or Thr
<220>  FEATURE:
<221>  NAME/KEY: VARIANT
<222>  LOCATION: 13
<223>  OTHER INFORMATION: Xaa = Arg, His, Pro, Asn, Ser, Thr, Ala, Gly, Lys or Gln
<220>  FEATURE:
<221>  NAME/KEY: VARIANT
<222>  LOCATION: 15
<223>  OTHER INFORMATION: Xaa = Arg, Ala, Ser, Gly, Met, Asn or Gln
<220>  FEATURE:
<221>  NAME/KEY: VARIANT
<222>  LOCATION: 16
<223>  OTHER INFORMATION: Xaa = Ala, Gly, Ser, Asp or Asn
<220>  FEATURE:
<221>  NAME/KEY: VARIANT
<222>  LOCATION: 17
<223>  OTHER INFORMATION: Xaa = Ala, Asn, Ser, Ile, Gly, Val, Gln or Thr
<220>  FEATURE:
<221>  NAME/KEY: VARIANT
<222>  LOCATION: 19
<223>  OTHER INFORMATION: Xaa = His, Leu, Gln or Ala
<220>  FEATURE:
<221>  NAME/KEY: VARIANT
<222>  LOCATION: 19
<223>  OTHER INFORMATION: Xaa = His, Leu, Gln or Ala
<220>  FEATURE:
<221>  NAME/KEY: VARIANT
<222>  LOCATION: 21
<223>  OTHER INFORMATION: Xaa = Trp, Phe, Tyr, His or Ile
<220>  FEATURE:
<221>  NAME/KEY: VARIANT
<222>  LOCATION: 31
<223>  OTHER INFORMATION: Xaa = Glu, Asp, Gln, Asn, Ser, Ala, Val, Leu, Ile or Thr
<220>  FEATURE:
<221>  NAME/KEY: VARIANT
<222>  LOCATION: 32
<223>  OTHER INFORMATION: Xaa = Glu, Gln, Asp, Asn, Pro, Thr, Leu, Ser, Ala, Gly or Val
<220>  FEATURE:
<221>  NAME/KEY: VARIANT
A method for treating bone trauma in a patient, the method comprising:

Administering to the patient a composition comprising a kallikrein inhibitor antibody, in an amount effective for treating bone trauma in a patient.

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