(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
26 January 2006 (26.01.2006)

PCT

(10) International Publication Number
WO 2006/008266 A2

(51) International Patent Classification:
C07K 7/00 (2006.01) *C07K 1/14* (2006.01)
C07K 14/47 (2006.01) *G01N 33/50* (2006.01)

(21) International Application Number:
PCT/EP2005/053387

(22) International Filing Date: 14 July 2005 (14.07.2005)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

PA 2004 01141	23 July 2004 (23.07.2004)	DK
60/593,000	30 July 2004 (30.07.2004)	US
PCT/DK2004/000810	22 November 2004 (22.11.2004)	DK
05101584.0	2 March 2005 (02.03.2005)	EP

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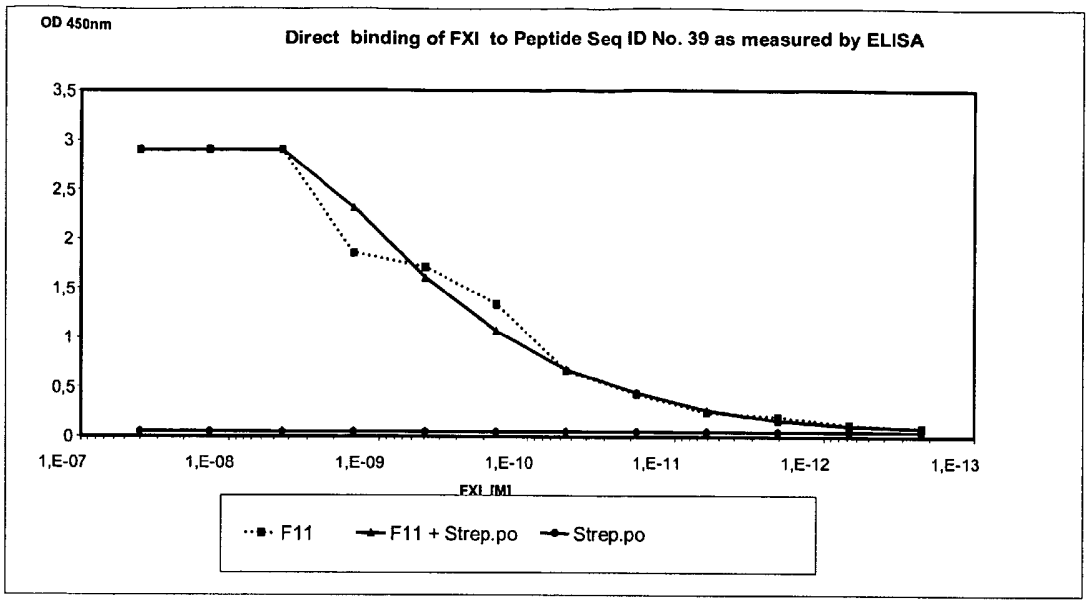
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: FACTOR XI-BINDING PROTEINS



(57) Abstract: Novel peptides that specifically bind human Factor XI and related proteins are provided, as are methods of using such proteins, methods of identifying such proteins, and additional related compounds, compositions, and methods.

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FACTOR XI-BINDING PROTEINS

FIELD OF THE INVENTION

The invention described herein pertains to new proteins that are capable of specifically binding human Factor XI, methods of identifying such Factor XI-binding proteins, and methods of using such Factor XI-binding proteins.

BACKGROUND OF THE INVENTION

Human Factor XI is a serine protease consisting of two identical subunits, each having a molecular mass of about 80 kDa. Human Factor XI (FXI) circulates in plasma as a disulfide-linked homodimer having a molecular mass of ~160 KDa. Plasma FXI is encoded by a 23 kb gene located on chromosome 4 (4q35), 15 exons and 14 introns coding for a mRNA consisting of 2,097 nucleotides, which in turn encodes an amino-terminal signal (leader) peptide of 18 amino acids and the 607 amino acids present in each monomer of the mature protein. Additional isoforms of human FXI also have been described. See, e.g., Fujikawa et al., Biochem. 25:2417 (1986) (see also, Semin Thromb Hemost. 1991 Jan;17(1):55-72; Asakai et al., Biochemistry 26 (23), 7221-7228 (1987); and GenBank Access Nos. P03951, AAA51985, AAC24506, AAN85554, NP_062505, and NP_000119). Platelet-derived FXI (pd-FXI), for example, is described in, e.g., in Hsu et al. (1998), J. Biol. Chem. 273:13787-93. Preparation and characterization of non-human FXI has been described, e.g., by Gailani (1997), Blood 90:1055 (see also US Patent 5,252,217). Factor XI is involved in hemostasis including, for example, formation of stable fibrin clots. Antibodies against Factor XI have been described (see, e.g., US Patent Publication No. 20040146511). However, given its various import biological functions, alternative proteins that specifically bind Factor XI are highly desirable.

BRIEF SUMMARY OF THE INVENTION

The invention described herein provides peptides that are useful in having the ability to specifically bind human Factor XI and proteins related to Factor XI, methods of identifying such Factor XI-binding peptides from peptide libraries, various methods of using such peptides, and additional related compounds, compositions, and methods.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 displays direct binding of FXI to an immobilized exemplary Factor XI-binding peptide-BSA conjugate according to an aspect of the invention. FXI dilution is

plotted on the X-axis and the optical density (OD) at 450nm is plotted on the Y-axis. High OD values indicate stronger binding while low OD values indicate relatively weaker binding.

Figure 2 displays the results of a competition experiment performed using various Factor XI-binding peptides identified by screening for such peptides in peptide libraries.

Figure 3 provides a binding profile as a function of pH in the incubation buffer for an exemplary factor XI-binding peptide according to the invention. The value of the Y-axis is the OD 450 nm value and the pH value of the incubation buffer is displayed on the X-axis. Higher OD values indicate stronger binding.

Figure 4 provides a profile of the purification of human factor XI on a Sepharose column comprising an exemplary Factor XI-binding peptide of the invention. The UV trace shows the proteins and the second curve shows the pH of the buffer.

Figures 5A-5B show chromatogram data for a composition comprising Factor XI purified by HPLC with an exemplary factor XI-binding peptide of the invention. Specifically, Figure 5A shows the HPLC analysis of the sample before purification; Figure 5B shows a chromatograph for Factor XI purified by binding with the exemplary Factor XI-binding peptide.

Figure 6 shows the chemical structure of 19-amino-5-oxo-3,10,13,16-tetraoxa-6-azanonadecanoic acid, which is an exemplary spacer that may be placed between a Factor XI binding protein of the invention and a suitable support (such as a chromatography resin). Other suitable spacers can similarly be used, and compositions comprising such combinations of binding protein, spacer, and support are an exemplary feature of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to novel molecules comprising an amino acid sequence portion that is capable of specifically binding human Factor XI and/or Factor XI-like proteins (collectively, "target proteins"). These novel molecules may be referred to as Factor XI-binding proteins ("FXIBPs").

In one advantageous aspect, the invention provides FXIBPs that exhibit specific binding to Factor XI (which may be abbreviated "FXI") and Factor XI-like proteins but also possess characteristics that allow for release from bound target proteins upon the occurrence of certain conditions that typically are obtainable without damaging the bound Factor XI or Factor XI-like protein, such as release (e.g., elution) from such target proteins during purification procedures when conditions are

modified (e.g., pH adjusted), such as in chromatographic purification of target proteins by binding with one or more support-associated FXIBPs.

Unless otherwise indicated, the term Factor XI (or FXI) herein refers to human Factor XI. Unless otherwise indicated, FXI encompasses proteins produced or obtained by any suitable method (e.g., recombinant expression, chemical synthesis, or purification from human biological samples). Unless otherwise indicated, the term FXI refers to any suitable isoform of FXI. In a particular aspect, the invention provides FXIBPs that are specific or selective for wild-type human plasma FXI (SEQ ID NO:53). In another aspect, the invention provides FXIBPs that also or alternatively are specific and/or selective for platelet-derived FXI (pd-FXI) (SEQ ID NO:54). FXIBPs can bind FXI in any suitable form. FXIBPs may, for example, bind FXI monomers, dimers, or both.

Terms such as "Factor XI-like protein", "FXI-like protein", FXILP, and the like are used herein to refer to proteins that are selected from (a) truncated forms of a human Factor XI protein (i.e., a peptide corresponding to or being a fragment of a human Factor XI protein), (b) proteins comprising the sequence of Factor XI or a fragment of Factor XI and at least one additional amino acid sequence (e.g., a Factor XI fusion protein), (c) a FXI variant, which is a protein comprising an amino acid sequence that is highly similar to the sequence of human Factor XI or a portion thereof, but not identical thereto (due to one or more substitutions, additions, deletions, and/or insertions), or (d) a derivative of any of (a)-(c).

A highly similar protein in the context of this invention refers to a protein comprising a sequence of at least about 10 amino acids, such as at least about 15 amino acids, such as at least about 20, at least about 25, at least about 30 or more amino acids (e.g., at least about 40, at least about 50, at least about 70, at least about 100, at least about 150, at least about 200, or more amino acids) having at least about 45% amino acid sequence identity, and typically at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% identity to a contiguous sequence of human FXI.

Identity in the context of amino acid sequences of the invention can be determined by a Needleman-Wunsch alignment analysis (see Needleman and Wunsch, J. Mol. Biol. (1970) 48:443-453), such as by analysis with ALIGN 2.0 using the BLOSUM50 scoring matrix with an initial gap penalty of -12 and an extension penalty of -2 (see Myers and Miller, CABIOS (1989) 4:11-17 for discussion of the global alignment techniques incorporated in the ALIGN program). A copy of the ALIGN 2.0 program is available through, e.g., the San Diego Supercomputer (SDSC) Biology Workbench. Because Needleman-Wunsch alignment provides an overall or

global identity measurement between two sequences, it should be recognized that target sequences which may be portions or subsequences of larger peptide sequences may be used in a manner analogous to complete sequences or, alternatively, local alignment values can be used to assess relationships between subsequences, as determined by, e.g., a Smith-Waterman alignment (J. Mol. Biol. (1981) 147:195-197), which can be obtained through available programs (other local alignment methods that may be suitable for analyzing identity include programs that apply heuristic local alignment algorithms such as FastA and BLAST programs). Further related methods for assessing identity between sequences are described in, e.g., International Patent Application WO 03/048185.

The term peptide herein refers to a molecule that comprises an amino acid sequence of about 30 amino acids or less in length, such as about 5-25 amino acids, about 10-25 amino acids, about 15-25 amino acids, about 20-25 amino acids, about 5-20 amino acids, about 10-20 amino acids, about 15-20 amino acids, or about 10-15 amino acids. The term polypeptide refers to amino acid polymer compounds that comprise a larger number of amino acid residues than peptides, e.g., a molecule comprising an amino acid sequence of at least about 50 amino acid residues in length. The term protein herein encompasses both peptides and proteins.

A derivative is a protein in which one or more of the amino acid residues of the peptide have been chemically modified (e.g. by alkylation, acylation, ester formation, amide formation, or the like) and/or associated with one or more non-amino acid organic and/or inorganic atomic or molecular substituents (e.g., a polyethylene glycol (PEG) group, a lipophilic substituent (which optionally may be linked to the amino acid sequence of the peptide by a spacer residue or group such as β-alanine, gamma-aminobutyric acid (GABA), L/D-glutamic acid, succinic acid, and the like), a fluorophore, biotin, a radionuclide, etc.) and also or alternatively can comprise non-essential, non-naturally occurring, and/or non-L amino acid residues. Chemically modified amino acid residues include residues modified by alkylation, acylation, ester formation, amide formation, or other similar type of modification. In another aspect, one or more amino acid residues of a derivative can be modified by covalent association with one or more heterologous substituents (e.g., a lipophilic substituent, a PEG moiety, a peptide side chain linked by a suitable organic moiety linker, etc.). The second type of derivative can separately be described as a conjugate. Non-limiting examples of unusual and modified amino acids that may make up part of a derivative include glycosylated amino acids, sulfated amino acids, prenlyated (e.g., farnesylated, geranylgeranylated) amino acids, acetylated amino acids, acylated amino acids, PEGylated amino acids, biotinylated amino acids,

carboxylated amino acids, phosphorylated amino acids, and the like. References adequate to guide one of skill in the modification of amino acids are replete throughout the literature. Exemplary protocols are found in, e.g., Walker (1998) PROTEIN PROTOCOLS ON CD-ROM Humana Press, Towata, NJ.

FXIBPs can have any suitable size and composition. In one aspect, the invention provides FXIBPs in the form of single-chain peptides of about 1-15 amino acid residues, such as about 1-12 amino acid residues, such as about 8-12 residues, for example 10-12 residue in length.

FXIBPs can be characterized as comprising at least one amino acid sequence that contributes to specific binding of at least one FXILP or FXI with sufficient affinity to permit detection of a resultant FXIBP:FXILP or FXIBP:FXI complex when the FXIBP is mixed with a composition comprising a FXILP or FXI, and commonly with an affinity sufficient for the FXIBP to retain bound FXILP or FXI until chemical conditions significantly change (e.g., pH) so as to overcome the binding interaction. It may be possible that a FXIBP will bind to two or more types of FXI, human FXI and non-human FXI proteins, two or more FXILPs, a human FXI and a FXILP, or other combinations of target proteins. Such cross-reactive FXILPs are another feature of the invention. In one aspect, the invention provides FXILPs that are selective for a human FXI, such as a polypeptide consisting or consisting essentially of SEQ ID NO:53 or SEQ ID NO:54.

Terms like "specific" and "specificity" herein refer to the ability of a FXIBP to bind one or more target proteins while only having relatively little or no detectable reactivity with other biological molecules typically presented in the context of a FXI, such as other plasma biomolecules and/or other coagulation factors. A FXIBP can be described as "selective" for a particular target protein if it shows greater affinity for the target than one or more other molecules, as determined by, e.g., a competition assay involving the molecules of interest (e.g., a first human FXI, a second human FXI, and a FXIBP).

In general, FXIBPs are able to detectably bind to FXI and/or FXILP under a range of determinable (and typically predetermined) conditions; typically under conditions that are similarly to physiological conditions (e.g., pH of about 6-8) and for any suitable period (e.g., for at least about 0.5 hour, at least about 1 hour, at least about 2 hours, at least about 3 hours, at least about 4 hours, at least about 5 hours, at least about 6 hours, at least about 10 hours, at least about 18 hours, at least about 1 day, at least about 2 days, at least about 1 week, etc.). Detection of FXIBP:FXI or FXIBP:FXILP binding can be made by any suitable technique. Examples of suitable techniques include ELISA assays as exemplified in the Experimental Section,

although other methods known in the art could similarly be applied (e.g., mass spectrometry analysis; spectrophotography analysis; weight/density differential centrifugation or electrophoresis; etc.).

In one aspect, the invention relates to FXIBPs that have an affinity for FXI that is reflected by a relative affinity of at least about 1%, at least about 5%, at least about 10%, or at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 50%, at least about 60%, at least about 65%, at least about 75%, or at least about 80% of the affinity of a FXIBP consisting or consisting essentially of SEQ ID NO:39 for FXI at about 1 x 10^{-4} M FXI to about 1 x 10^{-11} M FXI, e.g., about 1 x 10^{-5} M to about 1 x 10^{-10} M FXI (for example, about 1 x 10^{-5} M, 1 x 10^{-6} M, 1 x 10^{-7} M, 1 x 10^{-8} M, 1 x 10^{-9} M, 1 x 10^{-10} M, or 1 x 10^{-5} to 1 x 10^{-9} M, 1 x 10^{-5} to 1 x 10^{-8} M, 1 x 10^{-5} to 1 x 10^{-7} M, 1 x 10^{-6} M to 1 x 10^{-9} M, or 1 x 10^{-6} M to 1 x 10^{-8} M). In another aspect, the invention provides FXIBPs that have an affinity that is at least about as great for FXI as a FXIBP consisting or consisting essentially of SEQ ID NO:39, or will have an affinity that substantially similar to the affinity of such a SEQ ID NO:39 peptide for FXI (e.g., at least about 90-110% of the affinity of such a peptide for FXI). Such a relative affinity can be determined by, e.g., competition ELISA assays, as exemplified in the Experimental Section.

In another aspect, the invention provides FXIBPs that bind to FXI, a FXILP, or both with an EC_{50} of about 100 µM or less, such as about 80 µM or less, such as about 60 µM or less, such as about 50 µM or less, such as about 40 µM or less, such as about 25 µM or less, such as, for example, about 10 µM or less. The EC_{50} can be determined by way of an ELISA, as exemplified in the Experimental Section herein, or by any other suitable method. In a particular aspect, the invention provides FXIBPs that bind to FXI, a FXILP, or both with an EC_{50} of less than about 10 µM, such as about 5 µM or less, such as about 2 µM or less. In one aspect, the invention provides FXIBPs that have an EC_{50} of about 1 µM or less. For example, the invention provides FXIBPs that have an EC_{50} in connection with binding of FXI that is comparable to that of an FXIBP consisting of SEQ ID NO:39 (i.e., about 1 µM). In another aspect, the invention provides FXIBPs that bind to FXI, a FXILP, or both with a substantially lower EC_{50} than about 1 µM, such as about 0.5 µM or less, such as about 0.3 µM or less, such as about 0.2 µM or less, such as about 0.1 µM or less.

FXIBPs provided by the invention can be characterized as comprising an amino acid sequence according to one or more sequences or formulas (i.e., patterns or motifs) provided herein that contribute to FXI and/or FXILP binding.

In one aspect, the invention provides FXIBPs that comprise an amino acid sequence according to the formula $Xaa_6\,Xaa_7\,Xaa_8\,Xaa_9\,Xaa_{10}$, wherein Xaa_6 represents a cycloalkenyl-associated amino acid residue; Xaa_7 represents any suitable amino acid residue; Xaa_8 represents a small, polar, and flexible amino acid residue; Xaa_9 represents an aromatic residue or an aliphatic amino acid residue; and Xaa_{10} represents a small flexible amino acid residue. The following table classifies the twenty typical naturally occurring amino acid residues based on these and other functional characteristics that define various formulas used herein and can be used with reference to the production of suitable FXIBP sequence variants.

Table 1 – Physiochemical/Functional Classifications of Amino Acid Residues

Alcohol group-containing residues	S and T
Aliphatic residues	I, L, V, and M
Cycloalkenyl-associated residues	F, H, W, and Y
Aromatic residues	F, W, and Y
Hydrophobic residues	A, C, F, G, H, I, L, M, R, T, V, W, and Y
Negatively charged residues	D and E
Polar residues	C, D, E, H, K, N, Q, R, S, and T
Small residues	A, C, D, G, N, P, S, T, and V
Very small residues	A, G, and S
Residues involved in turn formation	A, C, D, E, G, H, K, N, Q, R, S, P, and T
Flexible residues	E, Q, T, K, S, G, P, D, E, and R
Acidic residues	A and E
Basic residues	K, R, and H
Hydrophilic uncharged residues	S, T, N, and Q
Aliphatic uncharged residues	G, A, V, L and I
Non-polar uncharged residues	C, M, and P

Unusual amino acid residues having similar physiochemical characteristics to any one of the classes described above may be used in place of such typical residues. In one aspect, the invention provides FXIBPs that are characterized in only comprising such typical residues. However, in another aspect, the invention provides FXIBPs wherein one or more residues are unusual amino acid residues. A number of

exemplary unusual and modified amino acid residues that may be incorporated into FXIBPs of this aspect are provided in the following table:

Names of and Abbreviations for Exemplary Modified/Unusual Amino Acid Residues

Amino Acid	**Abbr.**	**Amino Acid**	**Abbr,**
3-Aminoisobutyric acid	Baib	N-Methylisoleucine	Melle
2-Aminopimelic acid	Apm	6-N-Methyllysine	MeLys
2,4-Diaminobutyric acid	Dbu	N-Methylvaline	MeVal
Desmosine	Des	Norvaline	Nva
2-2'-Diaminopimelic Acid	Dpm	Norleucine	Nle
2,3-Diaminopropionic acid	Dpr	Ornithine	Orn
N-Ethylglycine	EtGly		

For production of sequence variants, alternative and more conservative substitutions groupings known in the art may be considered. For example, examples of conservative substitution groupings include: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine. Additional groups of amino acids can also be formulated using the principles described in, e.g., Creighton (1984) PROTEINS: STRUCTURE AND MOLECULAR PROPERTIES (2d Ed. 1993), W.H. Freeman and Company.

Other considerations in producing variants include hydrophobicity/hydrophilic characteristics of the residues, size of the residues, rigidity/flexibility of the residues, etc., that are involved in a substitution. Hydrophilicity considerations can be based on the Kyte-Doolittle method (see, e.g., US Patent 4,554,1010). The typical amino acid residues have the following hydrophilicity values: arginine (+3.0); lysine (+3.0); aspartate (+3.0 +/- 1); serine (+3.0); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 +/- 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleuicine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). Typically, substitutions of residues having hydrophilicity values within about +/-2, more typically within about +/-1, and even more typically +/- 0.5 of the score of the substituted residue will be suitable.

Methods for assessing similarity of peptides in terms of conservative substitutions, hydropathic properties, and similar considerations, and additional relevant related principles are described in e.g., International Patent Applications WO 03/048185, WO 03/070747, WO 03/089616, and WO 03/027246.

In some instances it can be useful to further characterize substitutions based on two or more of such features (e.g., substitution with a "small polar" residue, such as a Thr residue, can represent a highly conservative substitution in an appropriate context). Thus, for example, small, polar, and flexible residues selected from the typical naturally occurring amino acid residues shown above include Asp and Ser. As previously indicated in one aspect, typical residues having any one or combination of physiochemical/functional properties may be substituted with unusual or modified residues that may have similar physiochemical/functional properties. In another aspect, non-amino acid residue chemical moieties having similar functional/physiochemical properties (as would be recognized in the art) are used as amino acid residue surrogates.

In one aspect, the invention provides FXIBPs that are free from additional functional sequences or domains that can induce a non-immune cellular response in a mammal, such as in a human.

Typical FXIBPs can be characterized in comprising a sequence according to the formula $Xaa_6\,Xaa_7\,Xaa_8\,Xaa_9\,Xaa_{10}$, wherein Xaa_6 represents a cycloalkenyl-associated amino residue (e.g., Phe, His, Trp, or Tyr), Xaa_7 represents any suitable amino acid residue; Xaa_8 represents a small, polar, and flexible residue (Asp, Ser, or Thr); Xaa_9 represents an aromatic residue (e.g., Phe, Trp, or Tyr) or an aliphatic residue (e.g., Ile, Leu, or Val); and Xaa_{10} represents a small flexible residue (e.g., Pro, Asp, Ser, Gly, or Thr) (Formula I).

In general herein, the use of a previously defined reference for a variable amino acid residue position in a formula (e.g., Xaa_6), after its introduction, should be construed the same as initially introduced, unless otherwise indicated. For example, subsequent references to Xaa_6 herein should be interpreted as referring to a cycloalkenyl-associated amino acid residue, unless otherwise stated.

In one aspect, the invention provides FXIBPs comprising a sequence according to Formula I, wherein Xaa_8 is Asp (Formula II).

In a more particular aspect, the invention provides FXIBPs comprising a sequence according to Formula II, wherein Xaa_9 is Phe (Formula III).

In yet a further particular aspect, the invention provides FXIBPs comprising an amino acid sequence according to Formula III wherein Xaa_{10} is Pro (Formula IV).

In still another aspect, the invention provides FXIBPs comprising an amino acid sequence according to Formula III wherein (a) Xaa_8 is Asp and either Xaa_9 is Phe or Xaa_{10} is Pro or (b) Xaa_9 is Phe and Xaa_{10} is Pro.

In another aspect, the invention provides FXIBPs that comprise an amino acid sequence according to the formula $Xaa_4\,Xaa_5\,Xaa_6\,Xaa_7\,Xaa_8\,Xaa_9\,Xaa_{10}$, wherein

Xaa_4 is residue involved in turn formation (e.g., His, Ala, Asn, Lys, Thr, Pro, Ser, Arg, Gln, Gly, Glu, or Cys) and Xaa_5 is any suitable amino acid residue (Formula V).

A suitable amino acid residue in the context of FXIBPs provided herein refers to any residue that permits the FXIBP to specifically and/or selectively bind to at least one FXILP or FXI with sufficient affinity to permit detection and preferably to permit isolation of the FXIBP:FXI/FXILP complex. In one aspect, one or more suitable amino acid residues can be replaced with non-amino acid moieties that act as sufficient spacers for the amino acid residues that it/they connect. Typically, a suitable amino acid residue is an L-amino acid residue, selected from the twenty typical and naturally occurring L-amino acid residues.

In one particular aspect, the invention provides FXIBPs that comprise a sequence according to Formula V, wherein Xaa_4 is a His, Thr, Ala, Asn, Phe, or Lys residue (Formula Va). In yet another exemplary aspect, the invention provides FXIBPs comprising a sequence according to Formula V, wherein Xaa_4 is an aromatic residue (e.g., Phe, Tyr, or Trp) or a positively charged residue (e.g., His, Lys, or Arg) (Formula Vb). In one exemplary aspect, the invention provides FXIBPs wherein Xaa_4 is a cycloalkenyl-associated residue (e.g., His, Phe, Trp, or Arg).

In a further aspect, the invention provides FXIBPs that comprise an amino acid sequence according to Formula V, Formula Va, or Formula Vb, wherein Xaa_5 is an Arg, Thr, Lys, Ile, Ala, His, Gln, Val, Gly, or Pro residue (Formula Vc). In a further and more particular aspect, the invention provides FXIBPs that comprise a sequence according to Formula V, Va, or Vb wherein Xaa_5 is an Arg, Thr, Lys, Ile, or Ala residue (Formula Vd). Thus, for example, in one aspect, the invention provides FXIBPs that comprise a Formula V sequence wherein Xaa_5 is an Arg residue.

In an additional aspect, the invention provides FXIBPs that comprise an amino acid sequence according to the formula $Xaa_4\,Xaa_5\,Xaa_6\,Xaa_7$ Asp Phe Pro (Formula VI).

In a further particular aspect, the invention provides FXIBPs that comprise an amino acid sequence according to Formula VI, but wherein Xaa_4 is a His residue.

In an additional aspect, the invention provides FXIBPs that comprise an amino acid sequence according to the formula $Xaa_1\,Xaa_2\,Xaa_3\,Xaa_4\,Xaa_5\,Xaa_6\,Xaa_7\,Xaa_8\,Xaa_9\,Xaa_{10}$, wherein Xaa_1 represents a cycloalkenyl-associated residue, an aliphatic residue (e.g., Ile, Leu, or Val), or a flexible polar residue (e.g., Glu, Gln, Thr, Lys, Ser, Asp, or Arg), Xaa_2 is any suitable residue, and Xaa_3 is any suitable residue (Formula VII). One feature of the invention is embodied in FXIBPs that comprise an amino acid sequence according to Formula VII wherein Xaa_1 is a cycloalkenyl-associated residue, an aliphatic residue, an otherwise positively charged residue (Lys

or Arg), or a Gln residue (Formula VIIa). In another aspect, the invention provides FXIBPs comprising a Formula VII or Formula VIIa sequence wherein Xaa_2 is a Pro residue, a cycloalkenyl-associated residue, or an aliphatic residue. In a further exemplary aspect, the invention provides FXIBPs comprising a Formula VII or Formula VIIa sequence wherein Xaa_2 is selected from Pro, Phe, Leu, and Val (Formula VIIb and Formula VIIc, respectively).

In one aspect, the invention provides FXIBPs that comprise a Formula VII, Formula VIIa, Formula VIIb, or Formula VIIc, wherein (a) Xaa_8 is Asp and Xaa_9 is Phe or Xaa_{10} is Pro, (b) Xaa_9 is Phe and Xaa_{10} is Pro, or (c) one of the following - Xaa_8 is Asp, Xaa_9 is Phe, and Xaa_{10} is Pro.

Another particular exemplary aspect of the invention is embodied in FXIBPs that comprise a sequence according to the formula Xaa_1 Xaa_2 Xaa_3X_4 Xaa_5 Xaa_6 Xaa_7 Asp Phe Pro (Formula VIII). In a further exemplary aspect, the invention provides FXIBPs that comprise a Formula VIII sequence wherein Xaa_4 is a His residue (Formula IX).

In yet another aspect, the invention provides FXIBPs that are characterized in comprising a sequence of at least six consecutive amino acid residues that has at least about 75% amino acid sequence identity to one or more of SEQ ID NOs:1-52. In more particular aspects, the invention provides FXIBPs that are characterized in comprising a sequence of at least six consecutive amino acid residues that has at least about 85% amino acid sequence identity to one or more of SEQ ID NOs:1-52. In one aspect, the invention provides FXIBPs that are characterized as variants of one or more of SEQ ID NOs:1-52 and that exhibit significant competition with one or more SEQ ID NOs:1-52 for binding to a human FXI (e.g., reduces the binding of the reference peptide for FXI by at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 65%, at least about 75%, etc.). In a particular aspect, the invention provides FXIBPs that comprise one or more of SEQ ID NOs:1-52 (e.g., FXIBPs that comprise a sequence of one of the peptides of one or two of the three libraries described in the Experimental Section below). In a further aspect, the invention provides FXIBPs that consist or consist essentially of one or more of SEQ ID NOs:1-52. Alternatively, FXIBPs can be characterized as comprising a sequence that consists essentially of one or more sequences according to one or more of the formulas described herein or according to one or more of SEQ ID NOs:1-52. In this respect, the basic and novel properties of such sequences include the ability to detectably bind and retain FXI or at least one FXILP under determinable conditions, and desirably bind and retain FXI or at least one FXILP in a manner such that the bound FXI or FXILP(s) can be detected in a standard analytical assay (e.g.,

an ELISA, blotting assay, etc.) or can be used to substantially purify or isolate the bound FXI or FXILP(s).

FXIBPs provided by the invention include FXIBPs that desirably are capable of binding FXI or at least one FXILP with an affinity that is at least about as great as an FXIBP that comprises, consists essentially, or consists of SEQ ID NO:25. In one respect, the invention provides FXIBPs that bind to FXI or at least one FXILP with an affinity that is at least about 3 fold, at least about 5 fold, at least about 10 fold, at least about 20 fold, at least about 25 fold, at least about 50 fold, at least about 75 fold, at least about 100 fold, or more of the affinity exhibited by the SEQ ID NO:25 protein for FXI or FXILP(s). Such determinations can be made by, e.g., performing a competition assay as described in the Experimental Section.

FXIBPs can be characterized as being from natural origins (e.g., obtained by screening a composition of naturally occurring proteins), but typically can be characterized as peptides that have no corresponding wild-type protein counterpart.

In one aspect, FXIBPs can be characterized as having a pH-sensitive affinity for FXI or a FXILP. In a specific exemplary aspect, the invention provides FXIBPs that exhibit significant less binding to a target protein at a pH of less than about 6, intermediate binding at a pH of about 6-8, and optimal binding at a pH of about 8 or more. An example of such a binding profile between an FXIBP and FXI is provided by Fig. 3.

As suggested by portions of the preceding description of various aspects of the invention, unless otherwise stated or clearly contradicted by context it should be understood that FXIBPs can be in any suitable form. Thus, for example, FXIBPs can be in the form of linear peptides, multimeric peptides, cyclic single-chain peptides, such as peptides that are rendered cyclic by inclusion of a disulphide bridge, a thioether moiety, or a lactam ring, etc. Protein modification and engineering techniques that can be applied to proteins having the characteristics described herein, such as peptides comprising a sequence specifically disclosed herein or falling within one of the various formulas provided herein, are well known in the art.

FXIBPs are useful in a variety of contexts and the invention provides a number of new and useful experimental, diagnostic, and production processes related to FXIBPs. For example, particular features of the invention include, e.g., isolating or purifying FXILPs or FXI, from compositions containing such proteins, comprising contacting such a composition with one or more FXIBPs under conditions suitable for formation of a FXILP:FXIBP or FXI:FXIBP complex and subjecting the composition or related composition to procedures that isolate any thus formed FXILP:FXIBP or FXI:FXIBP complexes. In a particular exemplary aspect, the

invention relates to methods of identifying FXIBPs in a composition, for example in a synthetic peptide bead library. Other solutions from which FXILPs or FXI may be isolated and purified from include, but are limited to, blood, blood fractions, and recombinant cell culture supernatants containing FXI or FXILP(s) produced and secreted by recombinant host cells.

A further feature of the invention is in the detection of FXILPs or FXI in a composition (e.g., an *in vitro* cell medium, a biological sample, or a particular tissue of a mammalian subject, such as a human patient) or the evaluation of the absence of such proteins from compositions by contacting the composition with a sufficient amount of one or more FXIBPs under conditions suitable for FXILP:FXIBP or FXI:FXIBP binding and detecting whether such binding has occurred.

Additional uses of FXIBPs can be found in binding FXILPs or FXI for X-ray crystallography. Crystals suitable for X-ray structure determination may be obtained in better quality when the peptide is co-crystallized with a FXIBP of the invention. The crystallization of FXILPs with FXIBPs is thus another aspect of the invention, as is a method of determining the X-ray structure or other type of crystal structure of a FXILP by subjecting such a method to X-ray or similar crystal structure analysis. Various suitable methods of analyzing crystal structures are known in the art.

In another aspect, the invention provides methods for identifying and isolating factor XI and factor XI-like proteins via display technologies, such as phage display technology and ribosome display technology. Various display technologies, including numerous phage display methods, are known. In one general method, nucleic acid sequences encoding FXIBPs, suspected FXIBPs, or random peptides that may comprise FXIBPs, can be introduced to phage genome or other appropriate genome and displayed on the surface of the resultant particles (e.g., phage particles) and thereafter screened with one or more FXILPs or FXI. In a similar sense, compositions comprising FXI or one or more FXILPs (e.g., chromatography column materials) can be used to screen for FXIBPs.

In an additional aspect, an amino acid sequence according to one of the foregoing formulas, SEQ ID NOs:1-52, or a sequence highly similar to one or more of SEQ ID NOs:1-52 (a sequence having at least about 75% identity thereto, such as at least about 85% identity thereto) can be engineered into any given protein or peptide sequence giving this protein the ability to bind (interact) *in vitro* and/or *in vivo* to FXI and/or one or more FXILPs. This also includes, e.g., the engineering of such sequences into FXI itself, thus obtaining complexes, homo dimers, or higher aggregates of FXI (complexes of FXILPs can be obtained by similar inclusion of FXIBP sequences). Unless otherwise stated, such sequences may be located N-

terminal, C-terminal, or elsewhere in a protein of interest. In one aspect, the sequence is located internally. In another aspect, the sequence is located towards the C-terminus of the protein. In another aspect, the sequence is located at or within a few residues of the C-terminus of the protein.

In another exemplary aspect, FXIBPs can be used for diagnostic purposes, e.g., as a component of a biosensor for the measurement of the *in vitro* and/or *in vivo* level (amount and/or activity) of FXI and FXI-like proteins.

In another aspect, FXIBPs can be used to modulate the activity of FXI or FXI-associated cells by binding thereto. In another aspect, FXIBP sequences or sequences according to the various formulas provided herein can be used to target proteins or chemical entities to FXI-associated tissues, cells, etc., for diagnostic or therapeutic processes. Thus, the invention in one sense provides compositions comprising one or more FXIBPs and in a more particular aspect provides compositions comprising FXIBPs with other pharmaceutical carriers and/or diagnostic reagents. FXIBPs may be used to, for example, quantify or localize target proteins in a biological sample or tissue in an organism (e.g., FXIBP conjugates could be used for visualizing FXI-associated tissues).

In another aspect, the invention provides compositions and compounds comprising at least one nucleic acid molecule comprising a sequence encoding at least one sequence coding for production of a FXIBP in a competent host cell (whether *in vitro* – e.g., in a unicellular organism or suitable cell line - or *in vivo*). In a further aspect, the invention provides a recombinant vector (e.g., a plasmid, linear expression element, viral vector, or other vector) comprising such a FXIBP-encoding nucleic acid molecule. In yet a further aspect, the invention provides a host cell comprising one or more of such a nucleic acid molecule and/or such a vector. FXIBPs can be used to target other biological molecules, such as cells, vectors, etc., as well as non-biological molecules for delivery to FXI-associated tissues. The invention also provides a method of producing FXIBPs comprising expressing such nucleic acid molecules in suitable hosts or host cells. In another aspect, the invention provides a method of producing antibodies against an FXIBP comprising contacting antibody producing cells with an FXIBP under conditions suitable for producing antibodies thereto. Various known methods can be used to obtain monoclonal antibodies against FXIBPs.

EXPERIMENTAL SECTION

The following experimental methods and data are presented here to further illustrate aspects of the invention but, of course, should not be construed as in any way limiting its scope.

These experiments describe an exemplary method for identifying FXIBPs and provide particular examples of FXIBPs that may be used in various described aspects of the invention.

Synthesis and format of libraries

Peptide bead libraries were synthesized using Fmoc solid phase peptide synthesis on Tentagel resin bead from Rapp Polymere (Germany). Three different peptide bead libraries were used in the screening. They are named BL121, BL122 and BL123.

The format of the library BL121 is:

O_1-O_2-O_3-O_4-O_5-O_6-O_7-O_8-O_9-O_{10}-O_{11}-O_{12}-O_{13}-O_{14}-Tentagel resin, where On represents an L-amino acid; n=1,2; 11,12 can be any DNA encoded L-amino acid except Trp, Phe, Tyr, Leu, Ile, Lys, methionine and cysteine; n=4,5 and 7,8 and 10,11 and 13,14 can be any DNA encoded L-amino acid except Trp, Phe, Tyr, Leu, Ile, Lys , Methionine and cysteine or it could be a deletion (no amino acid present); and n=3,6,9,12 can be Phe, Trp, Tyr, Leu

The format of the library BL122 is:

O_1-O_2-O_3-O_4-O_5-O_6-O_7-O_8-O_9-O_{10}-O_{11}-O_{12}-Tentagel resin, where On is a L-amino acid and n=1 -12 can be any DNA encoded L-amino acid except methionine and cysteine

The format of the library BL124 is:

O_1-O_2-O_3-O_4-O_5-O_6-O_7-Asp-Phe-Pro-O_8-O_9-O_{10}-O_{11}- Tentagel resin, where On is a L-amino acid and n=1 -11 can be any DNA encoded L-amino acid except methionine and cysteine

Each of the libraries comprise about 1 million random peptides.

General synthesis protocol

Tentagel resin from rapp Polymere (Tubingen, Germany) was used for all libraries. Fmoc-amino acids were purchased from Novabiochem or Advanced Chemtech. Synthesis was started by weighing 1 – 3 gram of resin in a 30 ml syringe swelled in N-methylpyrrolidon (NMP). The resin was then divided into n portions, where n is number of different amino acids used in the library synthesis. For libraries BL122, BL123 and Bl124 the n number is 18.

One ml of Fmoc-amino acid solution (0.5M) in a mixture of 0.15M HOAt and 0.35 M HOBt in NMP was added to each resin portion. Then, 80 ul of diisopropylcarbodiimid (DIC) was added to the mixture. The reaction was allowed to proceed for 1 – 16 hours. The resin portions were then combined and washed in NMP and thereafter 25% piperidine was added in order to remove the Fmoc-group followed by NMP washing. The resin was then ready for at new coupling reaction after dividing into n portions. This procedure was repeated until peptides having the desired length were obtained. At three positions in the synthesis of library BL124, no split procedure was performed – only addition of a single Fmoc-amino acid - to give the motif Asp-Phe-Pro (DFP).

After synthesis, the resin was washed in DCM then deprotected with 92% TFA + 5% triisopropylsilane + 3% thioanisol for 2 hours in order to removed the side chain protecting groups. Then the resin was in DCM and then washed in NMP. Finally, the resin was washed in water and was ready for screening against biotinylated FXI.

Screening the peptide bead libraries

Recombinant factor XI was purchased from Heamatologic Technologies and biotinylated according to standard laboratory protocols. Five µl of biotinylated factor XI (concentration 1.2 uM) and 1 ul streptavidin-alkaline phosphatase (concentration 1 mg/ml, Sigma) was added to three synthetic peptide bead libraries, BL121, BL122 and BL124, respectively, and allowed to incubate for about 2-3 hours. The incubation buffer was 15mM TRIS-HCl, pH=7,4, 0,15M NaCl, 0,5% bovine Serum Albumin (BSA) and 0,05% Tween20.

After washing with washing buffer (M TRIS-HCl, pH=7,4; 0.15M NaCl; and 0,05% Tween20), 5-bromo4-chloro-3-indolyl phosphate (BCIP) from Sigma and nitro blue tetrazolium (NBT) from Sigma were added in color buffer (50mM TRIS-HCl pH = 8,8, 0,15M NaCl, 0,05% Tween20 and 15 mM $MgCl_2$) and coloration was allowed to proceed 30 min – 1,5 h. Only stained blue beads were removed for sequencing

Sequence determination

Active blue beads were removed from the library and sequenced by the Edman sequencer (Procise, Applied Biosystems).

Direct Binding Analysis by ELISA

In order to verify that the peptides identified binds to FXI, an ELISA assay was developed and employed. As an exemplification, an FXIBP peptide having a sequence comprising SEQ ID NO:39 was made with a C-terminal cysteine and a spacer arm between the peptide and cysteine. The peptide was then conjugated to maleiimidoactivated BSA using the cysteine residue as an anchor point. The peptide-BSA conjugate was immobilised in microtiter wells and biotinylated FXI was added in a serial dilution either alone or with streptavidin-peroxidase conjugate. As a control, streptavidin-peroxidase was added in a serial dilution. After incubation and washing, streptavidin-peroxidase diluted 1:5000 was added to all wells. Binding could be found for FXI either alone or when co-incubated with streptavidin-peroxidase. No binding of streptavidin-peroxidase alone could be established.

Competition experiments

Competition experiments were performed to test the relative affinities of the peptides found in two different libraries - BL122 and BL124. BSA-FXIBP Peptide SEQ ID NO:39 was immobilised in maxisorp wells as described above. Serial dilutions of FXIBP peptides having sequences consisting of SEQ ID NOs:39 and 41 (from BL124) and FXIBP peptides having sequences consisting of SEQ ID NOs:25 and 19 (from Bl122) were used to assess competition. All of the peptides were able to displace binding of FXI, but with varying affinity (see Fig. 2). FXIBPs consisting of SEQ ID NOs:41 and 39 (from BL124) bound stronger to FXI than peptides from the first library BL122. For example, the FXIBP consisting of SEQ ID NO:39 bound about 100 fold stronger than the FXIBP consisting of SEQ ID NO:25.

Analysis of elution conditions

A pH binding profile was made in order to find conditions under which an FXIBP peptide will dissociate from FXI and FXILPs.

A direct binding ELISA with an immobilised FXIBP peptide (SEQ ID NO:39-BSA conjugate) was made, but with varying the pH of the incubation buffer. A series of binding studies was performed from pH = 3 to pH = 10. As can be seen from Fig. 3, the binding between FXI and the FXIBP is strongly depended on the pH of the

buffer. At pH < 6 no binding could be seen, intermediate binding in the interval 6-8, and optimal binding at pH > 8.

Such assays can be used to identify and/or produce FXIBPs having similar profiles (i.e., that bind strongly to FXI or a FXILP at a pH of about 8 or more; bind intermediately to FXI or the FXILP at a pH of about 6-8; and do not bind at pH of about 6 or less). Such FXIBPs can be advantageously used to bind FXI and/or a FXILP under certain conditions, while eluting at other conditions. Such peptides are another advantageous feature of the invention.

A purification study of immobilised peptide on matrix

A purification study was performed with an immobilised FXIBP peptide, YPRHIYPDFPTDTT-Atan-K, where Atan is 19-amino-5-oxo-3,10,13,16-tetraoxa-6-azanonadecanoic acid and functions as a spacer between resin and peptide (see Fig. 6, attached to a matrix (other suitable spacers could similarly be used). The peptide was attached to Sepharose NHS resin from Amersham (2 ml) and used in an experiment with FIX.

The FXI fraction was applied to the column in buffer (90mM K_2HPO_4 + 10mM KH_2PO_4 mixture) at pH 8.0 and elution was done by changing pH. This pH gradient was obtained by eluting with an elution buffer comprising 100mM NaAc pH 4,6 (dashed line in Fig. 4). The UV trace (proteins) is shown in solid line.

Two samples were removed for HPLC analysis: a sample of the unpurified FXI fraction, and the purified FXI fraction (between 33 - 40 ml). The HPLC analysis of these three fractions is shown in Fig. 5.

By applying methods such as those described above, a number of FXIBPs were identified in libraries BL121, BL122 and BL123. These FXIBPs are presented here.

Sequences of FXIBPs found in library BL121:

SEQ ID NO:01:	VDWQWSRFDDFPS
SEQ ID NO:02:	HPWFDDFPHLFQ
SEQ ID NO:03:	SRWPWSVFPDFPD
SEQ ID NO:04:	DVWDYVVFDDFPS
SEQ ID NO:05:	QRWVPYDDFPSLRS
SEQ ID NO:06:	RHFHVFPDFPFVH
SEQ ID NO:07:	HHFPPFSHFPDLPQ
SEQ ID NO:08:	RRLPLSRLPDFP
SEQ ID NO:09:	HPFFRGYPDFPD

SEQ ID NO:10: HPWHLVYPDFPS
SEQ ID NO:11: HDWLVRWPDFPS
SEQ ID NO:12: SHFWRQWPDFSD
SEQ ID NO:13: PQLRWHDFPDFGS
SEQ ID NO:14: VVWRHWQDFDQFVV

Sequences from library BL122

SEQ ID NO:15: YKWIHHDDFPLV
SEQ ID NO:16: FDRKRVHPDFPH
SEQ ID NO:17: DVWDYVVFDDFPS
SEQ ID NO:18: QQPIQRFPDFP
SEQ ID NO:19: QAIFTRFPDFPN
SEQ ID NO:20: EWFPDFPEGSDG
SEQ ID NO:21: HTHAFPDFPPH
SEQ ID NO:22: LVKGFPDFPNHN
SEQ ID NO:23: GPFPYAYEDFPE
SEQ ID NO:24: FYLKTRYYDFPE
SEQ ID NO:25: FQARHTIGDFPA
SEQ ID NO:26: RIKDFPSDSNTV
SEQ ID NO:27: IWESHKVIEDFP
SEQ ID NO:28: QWFSVSRYQDFD
SEQ ID NO:29: QKDFHWRILPDF
SEQ ID NO:30: KIVKFPHTFPDL
SEQ ID NO:31: HLYDFDLDNEY
SEQ ID NO:32: KTILGDVDFDI
SEQ ID NO:33: RQLHPFHHFHG
SEQ ID NO:34: RSWLRYGYGH
SEQ ID NO:35: FNWNNVDEYYDW
SEQ ID NO:36: DQWDWEDYDEAW
SEQ ID NO:37: YDIYDDYEIWA

Sequences of FXIBPs found in BL124:

SEQ ID NO:38: YPKHIYADFPSTRL
SEQ ID NO:39: YPRHIYPDFPTDTT
SEQ ID NO:40: YLKHAWPDFPKLQQ
SEQ ID NO:41: YVRHRFEDFPTALP
SEQ ID NO:42: FPWHKYEDFPSPRT

SEQ ID NO:43:	QPAHRYPDFPRNNH
SEQ ID NO:44:	LPKTRFLDFPHVSF
SEQ ID NO:45:	LPPARYPDFPAAKK
SEQ ID NO:46:	IPKNRFSDFPDAQG
SEQ ID NO:47:	LPSFRFPDFPATKT
SEQ ID NO:48:	RVLNRYPDFPTTNQ
SEQ ID NO:49:	FFKKTYADFPTSQT
SEQ ID NO:50:	IFKKTYEDFPRFVY
SEQ ID NO:51:	VLHNKYDDFPRVKK
SEQ ID NO:52:	KVKHRFNDFPVWGN

All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference in their entirety and to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein (to the maximum extent permitted by law), regardless of any separately provided incorporation of particular documents made elsewhere herein.

The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context.

Unless otherwise stated, all exact values provided herein are representative of corresponding approximate values (e.g., all exact exemplary values provided with respect to a particular factor or measurement can be considered to also provide a corresponding approximate measurement, modified by "about," where appropriate).

The description herein of any aspect or embodiment of the invention using terms such as "comprising", "having," "including," or "containing" with reference to an element or elements is intended to provide support for a similar aspect or embodiment of the invention that "consists of", "consists essentially of", or "substantially comprises" that particular element or elements, unless otherwise stated or clearly contradicted by context (e.g., a composition described herein as comprising a particular element should be understood as also describing a composition consisting of that element, unless otherwise stated or clearly contradicted by context).

All headings and sub-headings are used herein for convenience only and should not be construed as limiting the invention in any way.

The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

The citation and incorporation of patent documents herein is done for convenience only and does not reflect any view of the validity, patentability, and/or enforceability of such patent documents.

CLAIMS

1. A protein that detectably binds human factor XI (FXI), at least one FXI-like protein (FXILP), or both and comprises an amino acid sequence according to the formula $Xaa_6\ Xaa_7\ Xaa_8\ Xaa_9\ Xaa_{10}$, wherein Xaa_6 represents a cycloalkenyl-associated amino acid residue; Xaa_7 represents any suitable amino acid residue; Xaa_8 represents a small, polar, and flexible amino acid residue; Xaa_9 represents an aromatic residue or an aliphatic amino acid residue; and Xaa_{10} represents a small flexible amino acid residue.

2. The protein of claim 1, wherein Xaa_8 represents an Asp residue.

3. The protein of claim 1 or claim 2, wherein Xaa_9 represents a Phe, Tyr, or Leu residue.

4. The protein of claim 3, wherein Xaa_9 represents a Phe residue.

5. The protein of any one of claims 1-4, wherein Xaa_{10} represents a Pro or Asp residue.

6. The protein of claim 5, wherein Xaa_{10} represents a Pro residue.

7. The protein of claim 1, wherein the protein comprises a sequence according to the formula $Xaa_6\ Xaa_7$ Asp Phe Xaa_8.

8. The protein of claim 7, wherein the protein comprises a sequence according to the formula $Xaa_6\ Xaa_7$ Asp Phe Pro.

9. The protein of any one of claims 1-8, wherein the protein comprises a sequence according to the formula $Xaa_4\ Xaa_5\ Xaa_6\ Xaa_7\ Xaa_8\ Xaa_9\ Xaa_{10}$, wherein Xaa_4 is residue involved in turn formation and Xaa_5 is any suitable amino acid residue.

10. The protein of claim 9, wherein Xaa_5 is an Arg, Thr, Lys, Ile, Ala, His, Gln, Val, Gly, or Pro residue.

11. The protein of claim 10, wherein Xaa_5 is an Arg, Thr, Lys, Ile, or Ala residue.

12. The protein of any one of claims 9-11, wherein Xaa_4 is a His, Ala, Asn, Lys, Thr, Pro, Ser, Arg, Gln, Gly, or Glu residue.

13. The protein of claim 12, wherein Xaa_4 is a His, Lys, Arg, Phe, Tyr, or Trp residue.

14. The protein of claim 13, wherein Xaa_4 is a His residue.

15. The protein of any one of claims 1-14, wherein the protein comprises a sequence according to the formula $Xaa_1\,Xaa_2\,Xaa_3\,Xaa_4\,Xaa_5\,Xaa_6\,Xaa_7\,Xaa_8\,Xaa_9\,Xaa_{10}$, wherein Xaa_1 represents a cycloalkenyl-associated residue, an aliphatic residue, or a flexible polar residue, Xaa_2 is any suitable residue, and Xaa_3 is any suitable residue.

16. The protein of claim 15, wherein Xaa_2 is a Pro residue, a cycloalkenyl-associated residue, or an aliphatic residue.

17. The protein of claim 16, wherein Xaa_2 is a Pro, Phe, Leu, or Val residue.

18. The protein of any one of claims 15-17, wherein Xaa_1 is an aromatic residue.

19. The protein of any one of claims 1-18, wherein the protein is a single chain peptide of about 5-30 amino acid residues in length.

20. The protein of any one of claims 1-18, wherein the protein is a fusion protein.

21. The protein of any one of claims 1-20, wherein the protein binds FXI, at least one FXILP, or a combination thereof, in an affinity chromatography column, at a pH of greater than about 6, but releases the bound FXI and/or FXILP(s) at a pH of about 6 or less.

22. The protein of any one of claims 1-21, wherein the protein binds human FXI.

23. The protein of claim 22, wherein the protein has an EC_{50} of about 100 µg or less in a FXI ELISA.

24. The protein of claim 23, wherein the protein has an EC_{50} of about 10 µg or less in a FXI ELISA.

25. A method of purifying FXI, at least one FXILP, or a combination thereof from a composition comprising contacting the composition with one or more proteins according to any one of claims 1-24 under conditions suitable for formation of complexes of the one or more proteins and the FXI and/or FXILP(s) in the composition and at least substantially isolating a fraction of the composition comprising the complexes so as to obtain a composition enriched for such complexes.

26. The method of claim 25, wherein the method comprises separating complexes in the enriched composition and enriching the composition for the FXI or FXILP(s) bound by the protein(s).

27. The method of claim 25 or claims 26, wherein the composition comprises FXI.

28. A method of determining whether FXI or a FXILP is present in a composition comprising contacting the composition with a protein according to any one of claims 1-24 under conditions suitable for formation of a complex between the protein and FXI, a FXILP, or both, and evaluating whether such a complex is formed.

29. The method of claim 28, wherein the method is applied to determine whether FXI is present in a composition.

30. The method of claim 28 or claim 29, wherein the method is performed *in vivo*.

31. The method of claim 28 or claim 29, wherein the method is performed on a biological sample taken from a mammalian subject.

FIGURE 1

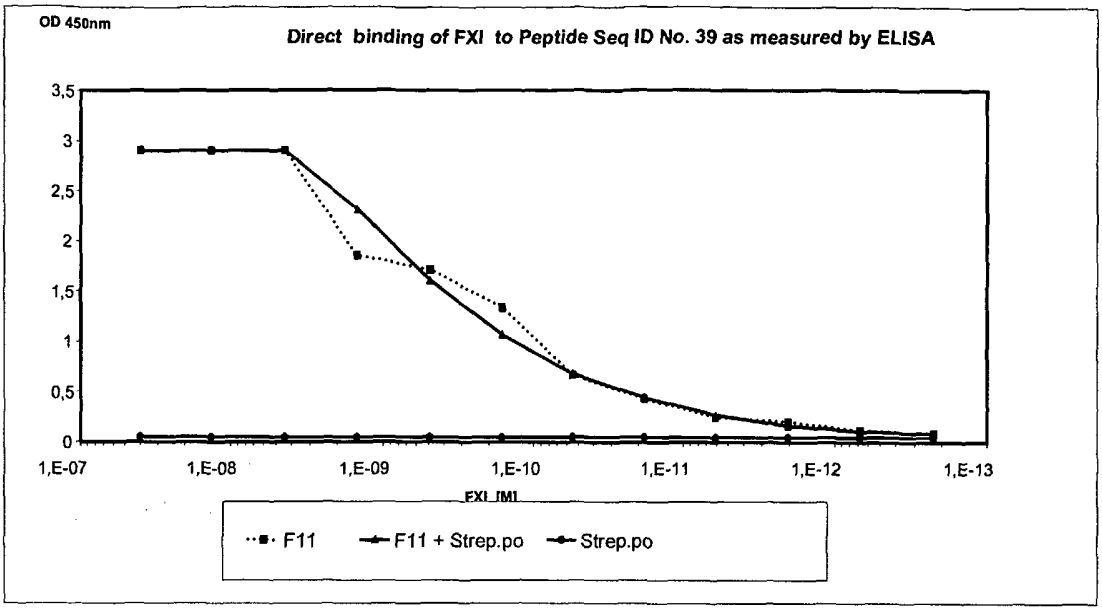
OD 450nm
Direct binding of FXI to Peptide Seq ID No. 39 as measured by ELISA
3,5
3
2,5
2
1,5
1
0,5
0
1,E-07
1,E-08
1,E-09
1,E-10
1,E-11
1,E-12
1,E-13
FXI [M]
F11
F11 + Strep.po
Strep.po

FIGURE 2

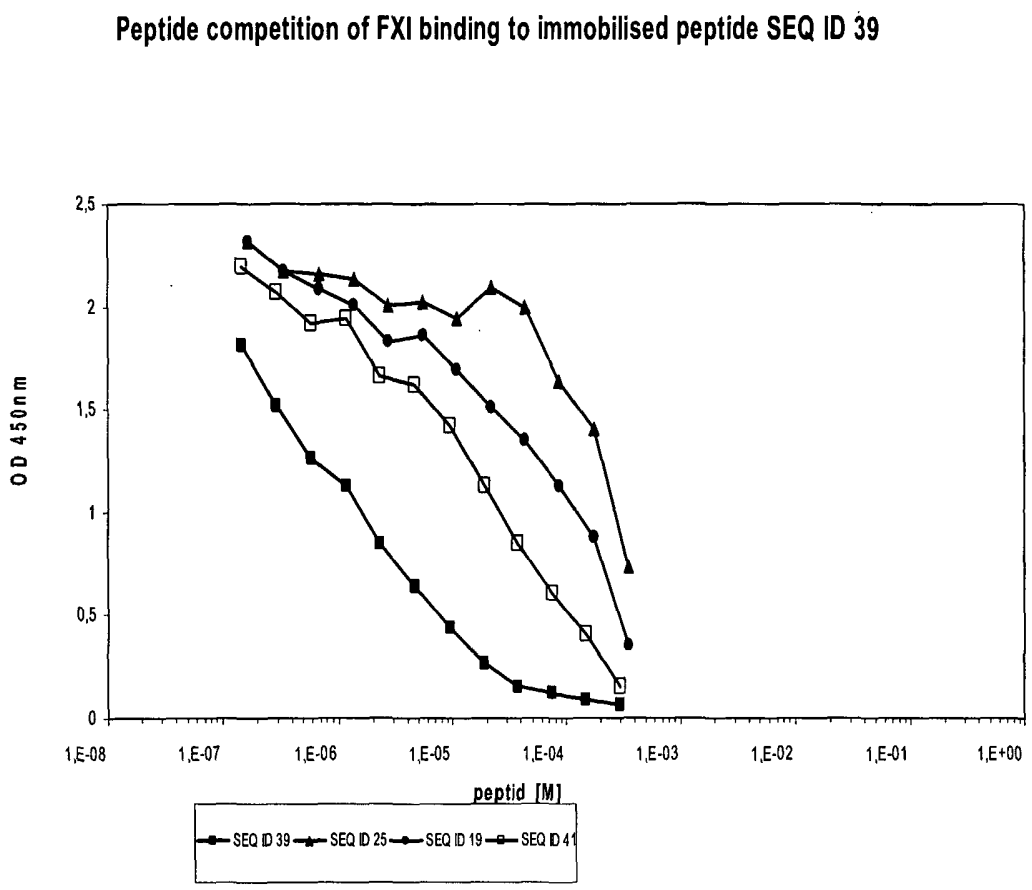
Peptide competition of FXI binding to immobilised peptide SEQ ID 39
OD 450nm
2,5
2
1,5
1
0,5
0
1,E-08
1,E-07
1,E-06
1,E-05
1,E-04
1,E-03
1,E-02
1,E-01
1,E+00
peptid [M]
SEQ ID 39
SEQ ID 25
SEQ ID 19
SEQ ID 41

FIGURE 3

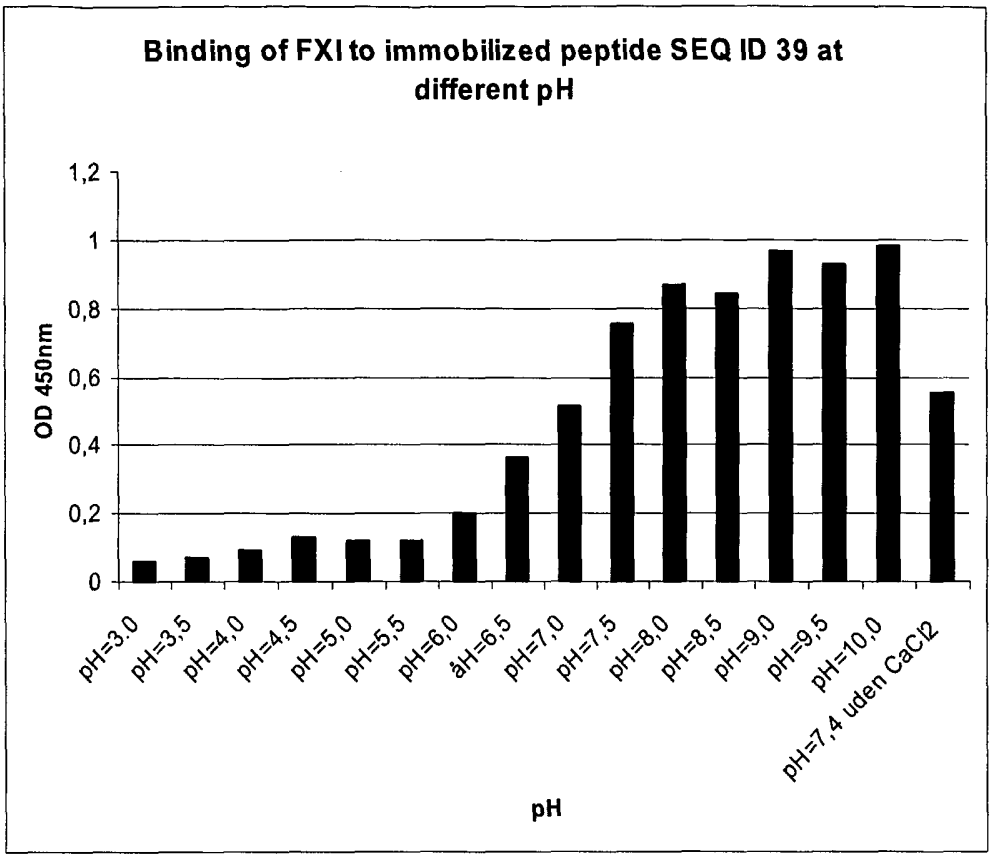
Binding of FXI to immobilized peptide SEQ ID 39 at different pH
OD 450nm
1,2
1
0,8
0,6
0,4
0,2
0
pH=3,0
pH=3,5
pH=4,0
pH=4,5
pH=5,0
pH=5,5
pH=6,0
åH=6,5
pH=7,0
pH=7,5
pH=8,0
pH=8,5
pH=9,0
pH=9,5
pH=10,0
pH=7,4 uden CaCl2
pH

FIGURE 4

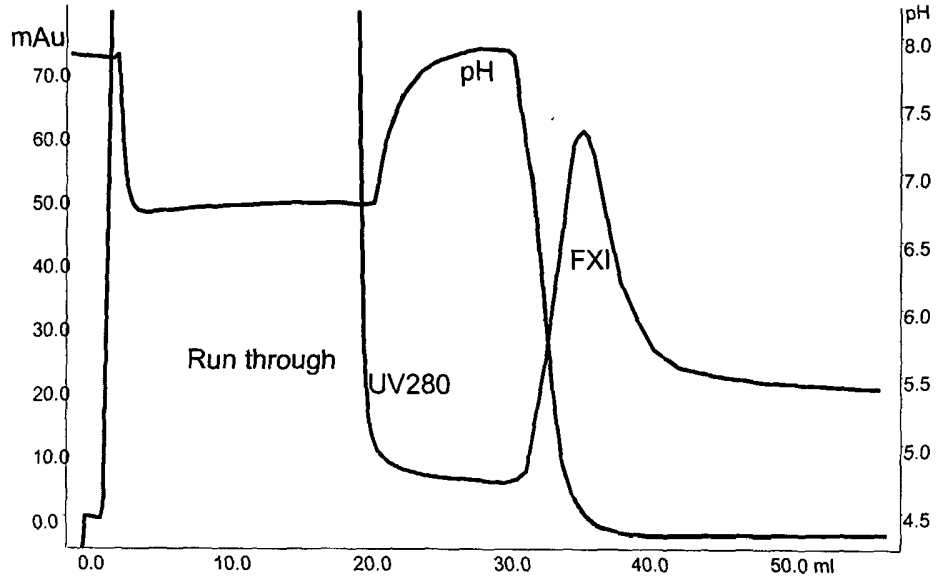
mAu
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
pH
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
pH
FXI
Run through
UV280
0.0
10.0
20.0
30.0
40.0
50.0 ml

FIGURE 5

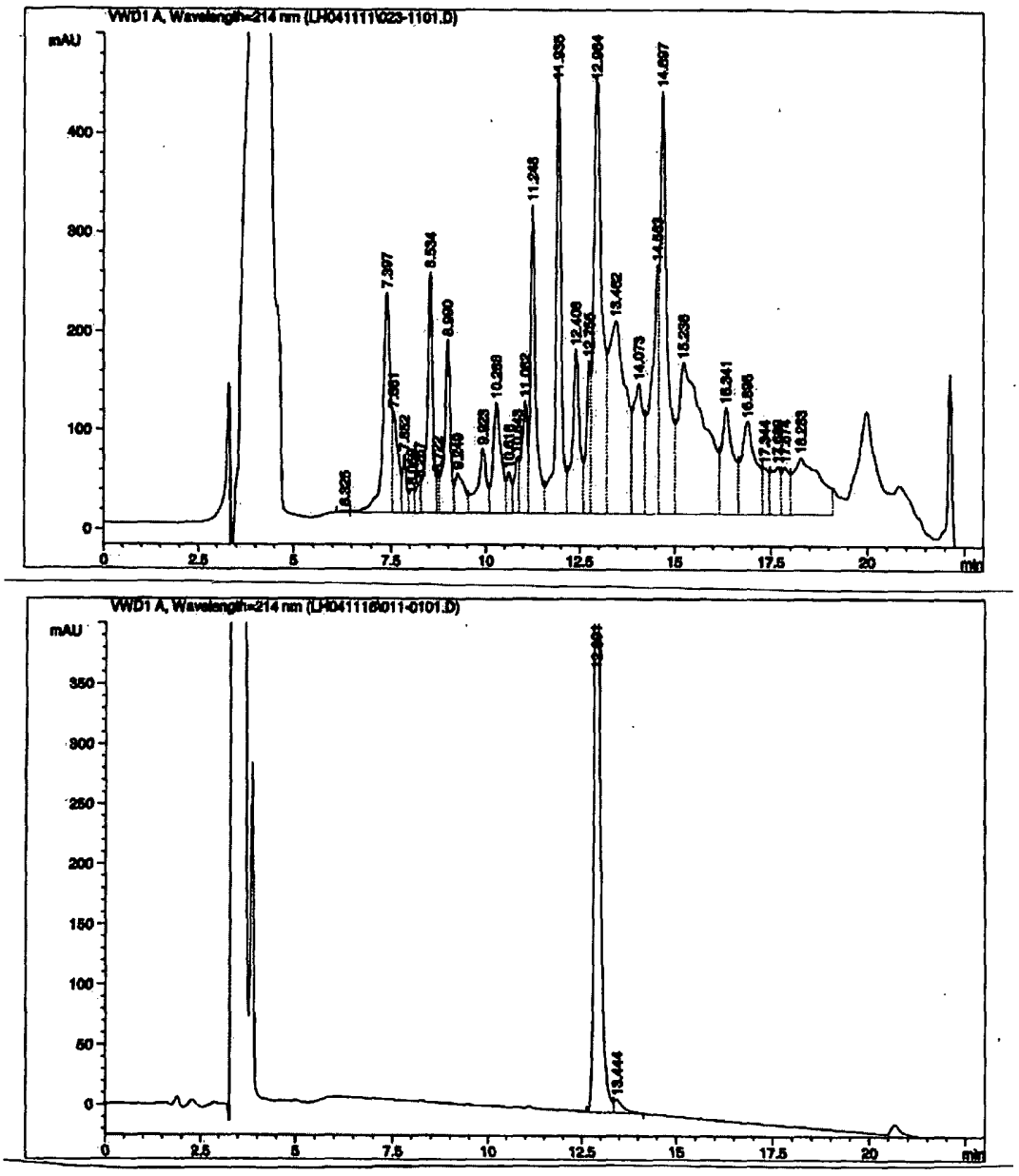
VWD1 A, Wavelength=214 nm (LH041111\023-1101.D)
mAU
7.397
8.534
11.248
11.935
12.984
14.697
VWD1 A, Wavelength=214 nm (LH041116\011-0101.D)
mAU
13.444
min

FIGURE 6