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(54) Title: A POLYPEPTIDES INHIBITING BINDING BETWEEN BONE SIALOPROTEIN ANS STAPHYLOCOCCAL CELLS

(57) Abstract

A polypeptide of bone sialoprotein selected from an amino acid sequence within position 42 to position 85 in the amino acid sequence set forth in SEQ ID NO: 10 and comprising the amino acid sequence LKRF, said polypeptide having the ability to inhibit the binding of bone sialoprotein to staphylococcal cells, or functional equivalents comprising the sequence LKRF. The invention also comprises the polypeptide for use as a medicament and use of the polypeptide for the manufacture of a medicament for inhibiting staphylococcal adhesion to tissues or implants. Further a method of inhibiting staphylococcal adhesion to tissues or implants of a mammal, including man, is included.

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A POLYPEPTIDES INHIBITING BINDING BETWEEN BONE SIALOPROTEIN ANS STAPHYLOCOCCAL CELLS

Technical field of the invention

The present invention relates to polypeptides of bone sialoprotein having the ability to bind to staphylococcal cells. The invention furter enclose such a polypeptide for use as a medicament and use of the polypeptide for the manufacture of a medicament for inhibiting staphylococcal adhesion to tissues.

Background of the invention

Bone matrix mostly contains hydroxyapatite, whereas the organic matrix is dominated by collagen type I. Bone sialoprotein (BSP), which is found only in bone and dentin (1,2), is one of the noncollagenous proteins of bone tissue. Using immunogold staining techniques this protein has been shown to be present mostly in the osteoid (3), the new forming bone close to the cartilage. BSP has a molecular mass of 59 kDa with a high content of O-glycosidically linked sialic acid-rich oligosaccharides. The protein has been cloned and sequenced (4), and the molecular mass of the protein backbone is 33.6 kDa. The C-terminal part of the molecule contains an RGD-sequence with eukaryotic cell binding properties (5). Furthermore, BSP contains extended sequences of acidic amino acid residues in the form of one stretch of 10 residues and several shorter sequences of glutamic acid (4).

BSP has been shown to be selectively bound by staphylococcal cells isolated from patients suffering from osteomyelitis and/or septic arthritis (6); this interaction takes place in both the coagulase positive and coagulase negative staphylococci (6,7) and is specifically inhibited by the recombinant BSP core protein (8). Staphylococcal cells have been found to bind BSP with a dissocia-

tion constant of about 10 nM (8). BSP is cleaved by CNBr into two fragments of similar size. The aminoterminal part of the BSP molecule efficiently inhibits the binding of native BSP to staphylococcal cells (8), whereas the RGD-containing carboxyterminal part of the molecule lacks affinity for staphylococcal cells. Thus, the BSP molecule could anchor the staphylococci to the bone tissue, either by connecting the bacteria to the cells or to the hydroxyapatite of bone tissue. Young persons have been found to be more prone to hematogenously spread osteomyelitis and arthritis (9). Because the newly forming bone, the osteoid, is particularly rich in BSP, it is intriguing to speculate that the binding of BSP by the staphylococcal cells may be of importance in the specific localization of these bacteria in bone tissue and joints. A cell surface protein, responsible for the specific interaction with BSP, has been isolated from *Staphylococcus.aureus* cells, and has a M_r of 97000 (10). A collagen adhesin on staphylococcal cell walls has been shown to be of importance in the development of septic arthritis in an experimental model for such infections in mice (11). BSP-binding capacity was found in all staphylococci producing septic arthritis in this same model (12), thus indicating the possible significance of this interaction in the development of the disease.

It is accordingly an object of the present invention to determine more specifically where the staphylococcal binding sequence of the BSP molecule is located and also where the inhibitory activity of the molecule is located.

It is a further object of the invention to present an alternative therapy to antibiotics for treatment of staphylococcal adhesion to tissues.

It is further an object of the invention to present an alternative therapy for preventing osteomyelitis and/or septic arthritis.

Summary of the invention

The objects of the invention are obtained by the polypeptide as claimed in the claims. According to the invention a polypeptide of bone sialoprotein is obtained. The polypeptide is selected from an amino acid sequence within position 42 to position 85 in the amino acid sequence set forth in SEQ ID NO: 10 and comprising the amino acid sequence LKRF, said polypeptide having the ability to inhibit the binding of bone sialoprotein to staphylococcal cells, or functional equivalents comprising the sequence LKRF.

Specific embodiments of the invention comprises SEQ ID NO: 12, 13, 14 and 18 as defined in appended claims 4 - 7. Especially said polypeptide has the ability to bind to staphylococcal cells.

Further, the invention provides for a polypeptide as mentioned above for use as a medicament.

Another aspect of the invention is use of said polypeptide for the manufacture of a medicament for inhibiting staphylococcal adhesion to tissues or implants, especially for inhibiting osteomyelitis and/or septic arthritis.

A method of inhibiting staphylococcal adhesion to tissues or implants of a mammal, including man, especially for inhibiting osteomyelitis and/or septic arthritis, is also presented.

Brief description of the appended figures

Fig. 1. Binding of ^{125}I-labeled BSP to *S. aureus* O24 in the presence of potential inhibitors. Binding in buffer alone was set at 100 %, and the inhibitory capacity of added fusion-proteins was related to binding in buffer alone. The data are shown for fibronectin (▲) ,

BSP (◇), and SEQ ID NO: 1 -6 [(<); representing fusion proteins SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6 which all show similar inhibition, SEQ ID NO: 7 (■), and SEQ ID NO: 8 (◆) the different fusion proteins after 3´ deletions of BSP cDNA. Inhibitors were added to concentrations representing 10 - 1000-fold molar excess, compared to ^{125}I-BSP.

Fig. 2. Binding of ^{125}I-BSP to *S. aureus* O24 cells in the presence of potential inhibitors.

Binding in buffer alone was set at 100 % and the 5´ deletions of BSP cDNA used were denoted SEQ ID NO: 9 (□), SEQ ID NO: 10(O) and SEQ ID NO: 11(■). The inhibitory activity of the contruct SEQ ID NO: 12 (Δ) is also shown. Highest concentration of inhibitors used were about 20-40 uG/mL.

Fig. 3. Inhibition of binding of ^{125}I-BSP to *S. aureus* O24 cells by synthetic peptides

The peptides YFYPPLKRFPVQGG, SEQ ID NO: 13 (■), LKRFPVQGG, SEQ ID NO: 14 (◆), GGQVPFRKL (O), KQRFGLGPV (Δ) KRFPVQGG, SEQ ID NO: 15 (⊞), RFPVQGG, SEQ ID NO: 16 (◇), FPVQGG, SEQ ID NO: 17 (●) and LKRF, SEQ ID NO: 18 (▼) were added to the incubation mixture at indicated concentrations and incubated for 90 min. Binding is expressed as % of ^{125}I-BSP bound in the presence of buffer alone.

Brief description in connection to the sequence listing

Sequence of BSP fusion proteins and synthetic peptides. The 3´-deletions SEQ ID NO 1 - 8, 5´-deletions SEQ ID NO 9 -11 and SEQ ID NO 12 were produced in bacteria as

GST-fusionproteins. Amino acid residues are numbered from the initiation Met residue [4].

Detailed description of the invention

Bone sialoprotein is a glycoprotein of the bone and dentin extracellular matrix. This protein consists of 320 amino acid residues of which 25% are glutamic and aspartic acid residues. Sialic acid, containing oligosaccharides and tyrosine sulfate residues, supply additional polyanionic properties. Staphylococcal cells, isolated from patients suffering from infection of bone tissue, bind the bone-derived sialoprotein - an interaction which is specifically inhibited by the recombinant bone sialoprotein core protein. It has previously been shown that the 150 aminoterminal amino acid residues of bone sialoprotein are responsible for the binding to staphylococcal cells. By using recombinant deleted variants of bone sialoprotein and synthetic peptides, it was surprisingly found that the staphylococcal binding site is localized to less than 10 residues in the aminoterminal part of the protein.

EXPERIMENTAL PROCEDURES

Chemicals.

Bovine serum albumin fraction V (BSA), Ovalbumin grade V, Ampicillin, and glutathione were purchased from Sigma Chemical Co. (ST.Louis, MO., USA). Tryptone soy broth, yeast extract, tryptone, and agar base were purchased from Oxoid Ltd. (Basingstoke, UK). Glutathione-Sepharose and Percoll were purchased from Pharmacia Biotech. (Uppsala, Sweden). IPTG (isopropyl β-thiogalactoside) was purchased from Eastman Kodak Co. (New Haven, CT., USA). Bovine bone sialoprotein was purified as previously described (1). ^{35}S-Pro-mix™ was purchased from Amersham (UK). Iodobeads were purchased from

Pierce Chemical Co. (Rockford, Ill., USA), and ^{125}I was purchased from Amersham (Canada). BSP was radiolabeled to a specific activity of 2-4 $x10^7$ cpm/ug.

Bacterial strains.

Staphylococcus aureus, (strain 024), was originally isolated from a patient with acute ostoemyelitis (9), and the *Staphylococcus epidermidis* strain 7686, isolated from a peritoneal catheter, was kindly donated by G.Pulverer, University of Cologne (Germany). The staphylococci were stored at -70°C, subcultured on bloodagar, and grown in Trypticase Soy Broth prior to experiments. The staphylococci were then washed twice in cold PBS and suspended in buffer (137 mM NaCl, 5 mM KCl, 0.7 mM $MgSO_4$, 1.2 mM $CaCl_2$, 10 mM Hepes, pH 7.4, 0.1% ovalbumin), at a concentration of 10^{10} bacteria/ml.

Construction of BSP-glutathione *thio* transferase (GST) fusion proteins and their expression in bacteria.

BSP-GST fusion proteins were produced in bacteria transformed with constructs encoding 3´- and 5´-deleted variants of BSP linked to GST. An Xmn I / Hinc II fragment of BSP (nt. 138-613 in ref.4) was ligated to pBluescript KS II (Stratagene, La Jolla, USA) cleaved with Sma I / Hinc II. 3´-deletions were made by Bal 31 digestions of Hinc II cleaved BSP / pBluescript KS II vector (13). After exonuclease digestions, 3´-deleted BSP cDNA´s were excised with Bam HI and ligated to Bam HI / Sma I cleaved pGEX-3X (Kabi-Pharmacia, Uppsala, Sweden). The extent of deletions was determined by DNA sequence analysis (4).

5´-deletions were made by PCR amplification using BSP / pBluescript KS II as DNA template and the reverse T3 primer (ATTAACCCTCACTAAAG). The forward primers were: SEQ ID NO: 9 (GGGATCCAGGATTCTGAAGAAAACGGG), SEQ ID NO: 10

(GGGATCCGCTACTTTCTTTATAAGCAC) and SEQ ID NO: 11 (GGGATCCTTCCAGTCCAGGGAGGCAGC). The SEQ ID NO: 12 construct was amplified using the SEQ ID NO: 10-primer and SEQ ID NO: 11 rev (GGAATTCTGCCTCCCTGGACTGGAAAC). PCR amplifications were performed in a thermal cycler using Taq DNA polymerase (Boehringer Mannheim, Germany) - 95°C for 30 sec., 55°C for 45 sec., and 72°C for 120 sec., thirty cycles in total. The PCR products were subjected to DNA sequence analysis to confirm their correct sequence, digested with Bam HI / Hinc II, and ligated to Bam HI / Sma I cleaved pGEX-3X. The constructs were used to transfect *E. coli* UT 5600 bacteria (Kabi-Pharmacia, Uppsala, Sweden). Overnight cultures of bacteria were diluted 1:100, grown for 2 hours, and then induced by adding IPTG (0.1mM). Bacteria were harvested 7 hours later, and after washing twice in PBS the bacteria were lysed by mild sonication and then centrifuged. The supernatant was passed over a glutathione-Sepharose column and, subsequently, bound material was eluted with glutathione (14). The eluted material was dialyzed prior to use. The BSP-GST fusion proteins were analyzed by SDS-PAGE to evaluate the differences in size of the fusion peptides. The fusion proteins migrated in SDS-PAGE as single components, with the expected differences in size (results not shown).

These fusion proteins were then used either as potential inhibitors of the binding of native, ^{125}I-labeled BSP to staphylococcal cells, or labeled with ^{35}S for direct binding to the cells.

^{35}S-labeling of fusion protein.

When labeled fusion protein was to be used, *E. coli* bacteria were cultured overnight in 2xYT medium, centrifuged and resuspended in M9 medium. After three hours, ^{35}S-Pro-mix™ was added to a final concentration of 20

uCi/mL, as well as IPTG (at 0.1 mM); bacteria were then allowed to grow for another eight hours. Metabolically labeled fusion protein was then purified as described above, and the radioactivity associated with the fusion protein was determined.

Peptide synthesis.

Peptides were synthesized in an Applied Biosystems 430 A peptide synthesizer, using fluoren-9-ylmethoxycarbonyl (Fmoc) chemistry and activation with 2-([^{1}H]benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU). After cleavage from the resin, peptides were purified by reverse phase chromatography on a Vydac 218YTP1022 column eluted with a gradient of acetonitril in 0.1 % trifluoroacetic acid/water. Fractions were collected after analysis by plasma desorption mass spectrometry and were then freeze-dried.

Binding assays.

Binding of ^{125}I-labeled BSP to *Staphylococcus aureus*.

Staphylococcus aureus, (strain O24), was grown overnight at 37°C in Trypticase Soy broth, washed twice in PBS, and suspended in buffer at a concentration of 10^{10} bacteria/ml. Staphylococci were mixed with ^{125}I-labeled BSP, and buffer or dilutions of the potential inhibitor, at concentrations ranging from 0.2-40 ug/ml - corresponding to a molar excess of about 10-1000 compared to ^{125}I-BSP - in buffer containing 0.1% ovalbumin, as described earlier (9). After 90 minutes of incubation end-over-end at 4°C, 100 ul of the mixture was layered on a Percoll gradient, centrifuged, and the supernatant discarded. Radioactivity associated with the pellet was compared with the amount of radioactivity in 100 ul of incubation mixture. Inhibition of the binding of ^{125}I-labeled BSP by the fusion proteins, as well as by synthetic peptides, was compared with the binding of BSP without inhibitor.

Binding of ^{35}S-labeled fusion proteins to staphylococcal cells.

Staphylococcus aureus cells were grown and harvested as described above, and suspended in buffer at a concentration of 10^{10} bacteria/ml. ^{35}S-labeled fusion protein was added at concentrations of 2-8 $x10^4$ cpm/10^9 bacteria and 2-8 $x10^5$ cpm/ 10^9 bacteria, and incubated as described above. Radioactivity associated with bacteria was measured in a b-counter and compared with the total amount of radioactivity added to the staphylococcal suspension.

RESULTS

Inhibition of BSP binding by BSP-GST constructs.

The BSP-GST fusion constructs (Sequence listing) were used to inhibit the binding of ^{125}I-BSP to staphylococcal cells. The 3´-deletion constructs SEQ ID NO: 1-6 (in Sequence listing), inhibited the binding of ^{125}I-BSP to staphylococcal cells with similar concentration dependence, whereas the 3´-deletion constructs SEQ ID NO: 7 and 8 did not affect the binding (Fig. 1). Extracts of GST from *E. coli* UT 5600 transformed with expression plasmid without insert did not inhibit the binding of ^{125}I-BSP to staphylococcal cells (data not shown). These results suggest that the sequence between Thr50 and Phe59 is essential for staphylococcal binding; the location of this binding site was verified by using BSP constructs deleted from the N-terminus denoted SEQ ID NO: 9-11. However, whereas constructs SEQ ID NO: 9 and 10 inhibited the binding of ^{125}I-BSP to bacteria, construct SEQ ID NO: 11 did not show any inhibitory activity (Fig. 2). The latter results indicate that the binding site is located between Arg42 and Phe59. Based on these results a fusion protein, SEQ ID NO: 12 was constructed which included amino acid residues Arg42-Gly64. In agreement with the results ob-

tained with 3′- and 5′-deletion constructs, the SEQ ID NO: 12 fusion protein inhibited the binding of ^{125}I-labeled BSP to *Staphylococcus aureus* (Fig. 2). In addition, multifunctional binding protein(s) have been found in lysates of staphylococcal cells (see below, ref. 28); such proteins could influence the inhibitory activity of fusion proteins in the present study, and explain inhibitory activities only up to about 50%.

Binding of ^{35}S-labeled fusion proteins to *Staphylococcus aureus*..

In addition to studying the inhibition of ^{125}I-BSP binding by fusion proteins we also investigated the direct binding of metabolically radiolabeled BSP-GST fusion proteins to staphylococcal cells. ^{35}S-methionine/cystein labeled fusion proteins SEQ ID NO: 5 and SEQ ID NO: 6, as well as SEQ ID NO: 9 and SEQ ID NO: 10, bound to bacteria whereas SEQ ID NO: 7 and SEQ ID NO: 11 did not show any significant binding capacity (Table 1). Furthermore, metabolically radiolabelled SEQ ID NO: 12 fusion protein bound to the staphylococcal cells (Table I). These results correlate with the results from the experiments where the BSP-GST fusion proteins were used as inhibitors of ^{125}I-BSP-binding to staphylococcal cells (Figs. 1 and 2). Therefore it was concluded that a stretch of 10-18 amino acid residues in the BSP molecule appeared to be involved in the binding to staphylococcal cells. In the inhibition assays the BSP-GST fusion proteins may have undesirable effects on the ^{125}I-BSP ligand. Possibly, interactions between BSP-GST fusion proteins and ^{125}I-BSP may cause conformational changes which lead to inhibition of ^{125}I-BSP binding. However, the direct binding of BSP-GST fusion proteins to staphylococcal cells strongly suggests that the fusion proteins exert their inhibitory ac-

tivity by competing with the BSP binding site on the staphylococcal cells.

Inhibition of BSP-binding by synthetic peptides.

To further characterize the binding specificity of BSP peptides were synthesized which encompassed amino acid residues Tyr51-Gly64 (Sequence listing). It was established that a synthetic peptide YFYPPLKRFPVQGG, SEQ ID NO: 13, corresponding to Tyr51-Gly64 in BSP, as well as a shorter peptide LKRFPVQGG (Leu56-Gly64) SEQ ID NO: 14, inhibited the binding of ^{125}I-BSP to staphylococcal cells (Fig. 3). It was found that synthetic peptides containing the same amino acids in reverse order GGQVPFRKL showed some inhibitory activity, whereas a randomly scrambled sequence of the same amino acids, KQRFGLGPV, did not significantly inhibit binding of ^{125}I-BSP to staphylococcal cells (Fig. 3). By deleting one amino acid at a time from the NH_2-terminal end of the LKRFPVQGG-peptide, SEQ ID NO: 15-17, it was found that the inhibitory activity depended on the presence of the LKR-sequence. Thus, a peptide containing only the LKRF-sequence, SEQ ID NO: 18, inhibited binding of ^{125}I-BSP to staphylococcal cells (Fig. 3), whereas the fibronectin peptide, GRGDS, used as control, did not inhibit the binding (data not shown). When inhibitory activity at a certain concentration (50 µg/ml) for all peptides used was compared, it was found that the two peptides comprising the longest and most accurate order sequences - YFYPPLKRFPVQGG, SEQ ID NO: 13 and LKRFPVQGG SEQ ID NO: 14, showed the strongest inhibition (60-70%) of ^{125}I-labeled BSP-binding. The reverse peptide - GGQVPFRKL - as well as the two shorter peptides, KRFPVQGG, SEQ ID NO: 15 and RFPVQGG, SEQ ID NO: 16 and the shortest peptide used in the study, LKRF, SEQ ID NO: 18, showed similar inhibi-

tory activity (32%), whereas the randomly scrambled peptide only inhibited BSP-binding by 15% (Fig. 3). The peptide FPVQGG, SEQ ID NO: 17, did not inhibit the BSP binding at all (Fig. 3).

Table I

Binding of ^{35}S-labeled fusion proteins to *S. aureus* O24 cells.

(A) Radioactivity associated with bacteria was compared to total radioactivity (2-8 x10^5 c.p.m.) added to the samples, and expressed as % binding. (B) shows results with a tenfold dilution of the labeled fusion protein added. (For details, see Experimental Procedures.)

	^{35}S-fusion protein bound (%)	
	A	B
BSP	40.9 + 4.7	38.2 + 4.8
SEQ ID NO: 5	47.9 + 4.6	47.6 + 4.8
SEQ ID NO: 6	32.8 + 5.3	30.9 + 5.8
SEQ ID NO: 7	6.3 + 2.0	5.2 + 1.9
SEQ ID NO: 9	27.6 + 6.3	24.1 + 5.2
SEQ ID NO: 10	46.9 + 3.8	44.7 + 3.6
SEQ ID NO: 11	4.1 + 1.8	4.8 + 1.6
SEQ ID NO: 12	42.8 + 9.1	39.9 + 7.3

CONCLUSION

Bone sialoprotein is a bone glycoprotein with a high content of aspartic and glutamic acids constituting 25% of all residues. Such acidic amino acids may be involved in non-specific binding to basic components of bacteria. Staphylococci have earlier been shown to bind to the ami-

noterminal half of BSP which contains stretches of acidic amino acid residues [1,10,15]; however, the binding of radiolabeled BSP is not inhibited by osteopontin [10], another glycoprotein of bone tissue which also contains stretches of exclusively acidic amino acid residues. It has now been determined that the staphylococcal binding site in BSP does not include any acidic amino acid residues. The fusion protein SEQ ID NO: 6 (Phe22-Phe59) does inhibit the binding of BSP and binds to staphylococcal cells. On the other hand, the construct SEQ ID NO: 7 (Phe22-Ala49) does not bind to, nor inhibits the binding of BSP to bacteria. These results show that a sequence of ten amino acid residues, located between Thr50 and Phe59, is essential for the binding of BSP to staphylococcal cells; the GST-fusion proteins deleted from the N-terminus locate the binding site to this region in the BSP molecule. The fusion protein SEQ ID NO: 10 containing BSP-sequences between Arg42 and Glu85 inhibits the binding of BSP and binds to staphylococcal cells, whereas the contruct SEQ ID NO: 11 (Pro60-Glu85) neither binds to bacteria nor inhibits this binding. The GST-fusion protein SEQ ID NO: 12 (Arg42-Gly64) binds to, and inhibits the binding of BSP to staphylococcal cells. The results from the inhibition studies, as well as the direct binding studies with fusion proteins, strongly suggest that the fusion proteins exert their effect by competing with ^{125}I-BSP for the same binding site on the staphylococcal cells. Using synthetic peptides, the finding that a sequence including LKRFPVQGG (Leu56-Gly64) inhibited the binding of BSP to staphylococcal cells was confirmed. The inhibitory effect of the reverse sequence peptide may indicate that the distances between charged and hydrophobic residues play a role for the inhibitory activity, supported also by the finding that a randomly scrambled peptide did not show any significant inhibitory activity.

The short peptide LKRF also inhibited the binding of BSP to staphylococci, though somewhat less efficiently than the longer peptides, including the LKR-sequence; the unrelated fibronectin peptide GRGDS did not inhibit binding. These results show that the binding of BSP to the recently identified 97 kDa staphylococcal cell-surface receptor (10) involves a stretch of about 10 amino acid residues, with basic and hydrophobic amino acids, and depends on the presence of an LKR-sequence. The staphylococcal cells do appear to recognize a short stretch of amino acid residues in BSP but do not seem to depend on the secondary structure determined by surrounding sequences. A comparison of the peptide YFYPPLKRFPVQGG, derived from the rat BSP sequence, with sequences in the Swiss protein sequence bank, did not reveal any significant homologies with other proteins, except with pig and human BSP. The human (YFYPHLKRFPVQGS) and pig (YFYPPLKRFPVQSS) sequences are almost identical to the rat sequence within the motif LKRFPVQGG, differing only in the last amino acid (G-S) in human BSP, and the two last amino acids (G-S and G-S) in the pig sequence. The human sequence also contains a His residue in position five of the peptide sequence, instead of a Pro residue in the rat and pig sequences.

The binding of staphylococcal cells to fibronectin (16-25) and collagen (26-27) has been studied to some extent. One main binding site for *S. aureus* cells in fibronectin is located in a 27-30 kDa fragment present in the *N*-terminal part of the molecule. This site is distinct from the collagen, as well as from the eukaryotic cell binding sites (16-19), and is composed of five type I repeats rich in intramolecular disulfide bonds. A second fibronectin staphylococcal binding domain has been localized to a 120 kDa *C*-terminal fragment (22,23). This

binding site, which has been shown to involve type I repeats, is found only in one of the fibronectin chains, and is dependent on the intrachain disulfide bond located in the region between the heparin and gelatin binding domains (23). It has been shown that staphylococci recognize not only several types of collagens but also isolated collagen type I α-chains and peptides generated by CNBr cleavage of α-chains (26,27). Synthetic peptides $(Pro\text{-}Gly\text{-}Pro)_n$, mimicking the structure of collagen, inhibit the binding of collagen to staphylococcal cells (27), whereas $(Pro\text{-}Ala\text{-}Gly)_n$ and polyproline are not inhibitors of collagen binding to staphylococci (27). In protein-overlay experiments, staphylococcal cell lysates from different strains have been shown to contain cell-surface proteins with somewhat different M_r:s but with similar binding properties, i.e., showing affinity for a variety of different extracellular matrix components [14]. Not only vitronectin and thrombospondin, but also fibrinogen and fibronectin, interact with these proteins and to a lesser extent, BSP and collagen (28). It was possible to block this type of BSP interaction by ovalbumin in protein overlay experiments; however, the interaction between the purified 97 kDa BSP-binding protein and BSP was not affected by the addition of ovalbumin but was specifically inhibited by the addition of unlabeled BSP [10]. This type of multifunctional binding protein could influence the inhibitory activity of fusion proteins and peptides in the present study, and explain inhibitory activities in the order of 50%.

It is believed that, in order to increase their virulence, staphylococci have apparently evolved cell surface receptors which recognize short stretches of amino acid residues in extra cellular matrix proteins. Staphylococcal resistance to antibiotics is an increasing

clinical problem, and alternative methods for prevention and treatment of such infections are vital. The use of peptides to inhibit staphylococcal adhesion to tissues, or to a foreign implanted material, should be considered and tested as a new therapeutic method.

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Claims

1. A polypeptide of bone sialoprotein selected from an amino acid sequence within position 42 to position 85 in the amino acid sequence set forth in SEQ ID NO: 10 and comprising the amino acid sequence LKRF, said polypeptide having the ability to inhibit the binding of bone sialoprotein to staphylococcal cells, or functional equivalents comprising the sequence LKRF.

2. A polypeptide of bone sialoprotein selected from an amino acid sequence within position 42 to position 85 in the amino acid sequence set forth in SEQ ID NO: 10 and comprising the amino acid sequence LKRF, said polypeptide having the ability to inhibit the binding of bone sialoprotein to staphylococcal cells.

3. A polypeptide according to claim 1 or 2, characterized in that the polypeptide has the amino acid sequence from position 42 to position 85.

4. A polypeptide according to claim 1 or 2, characterized in that the polypeptide has the amino acid sequence set forth in SEQ ID NO: 12.

5. A polypeptide according to claim 1 or 2, characterized in that the polypeptide has the amino acid sequence set forth in SEQ ID NO: 13.

6. A polypeptide a according to claim 1 or 2, characterized in that the polypeptide has the amino acid sequence set forth in SEQ ID NO: 14.

7. A polypeptide a according to claim 1 or 2, characterized in that the polypeptide has the amino acid sequence set forth in SEQ ID NO: 18.

8. A polypeptide according to any of the claims 1-7, characterized in that said polypeptide has the ability to bind to staphylococcal cells.

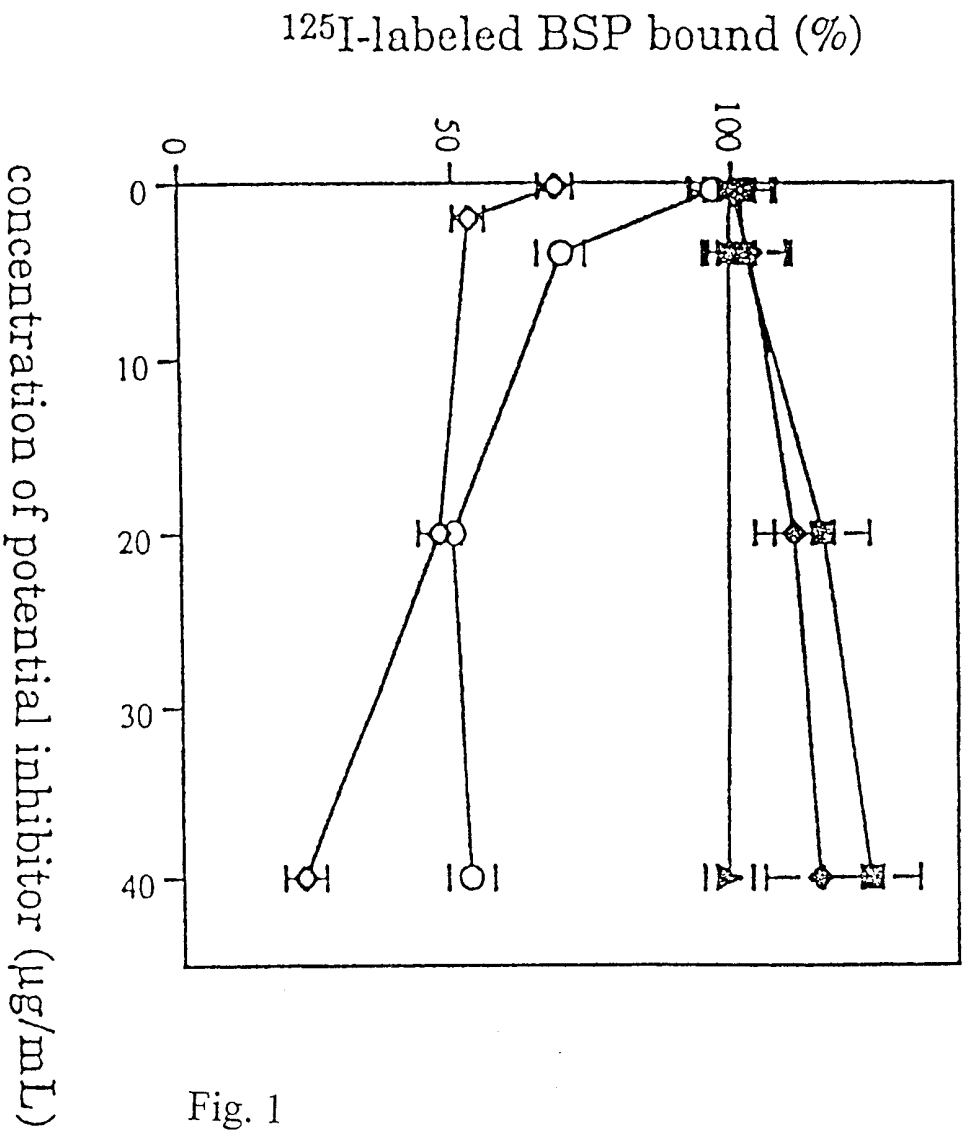
9. A polypeptide according to any of the claims 1-8 for use as a medicament.

10. Use of a polypeptide according to any of the claims 1-8 for the manufacture of a medicament for inhibiting staphylococcal adhesion to tissues or implants.

11. Use according to claim 10 for inhibiting osteomyelitis and/or septic arthritis.

12. A method of inhibiting staphylococcal adhesion to tissues or implants of a mammal, including man, characterized by administering to a mammmal, in need of such control, an amount of a polypeptide according to any of the claims 1-7 effective for said inhibiting.

13. A method according to claim 12 for inhibiting osteomyelitis and/or septic arthritis.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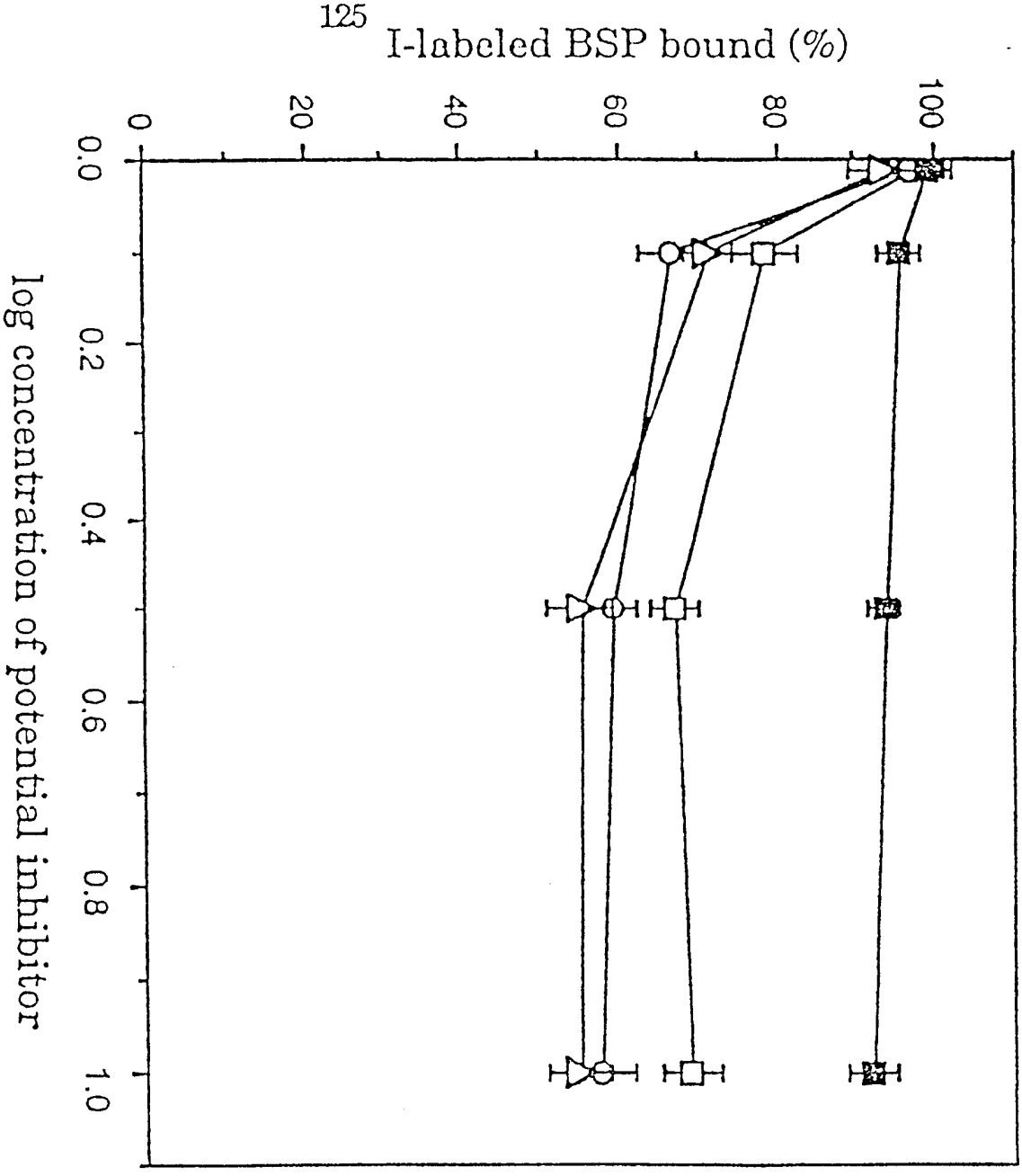
50
100
0
10
20
30
40
concentration of potential inhibitor (μg/mL)

Fig. 1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100
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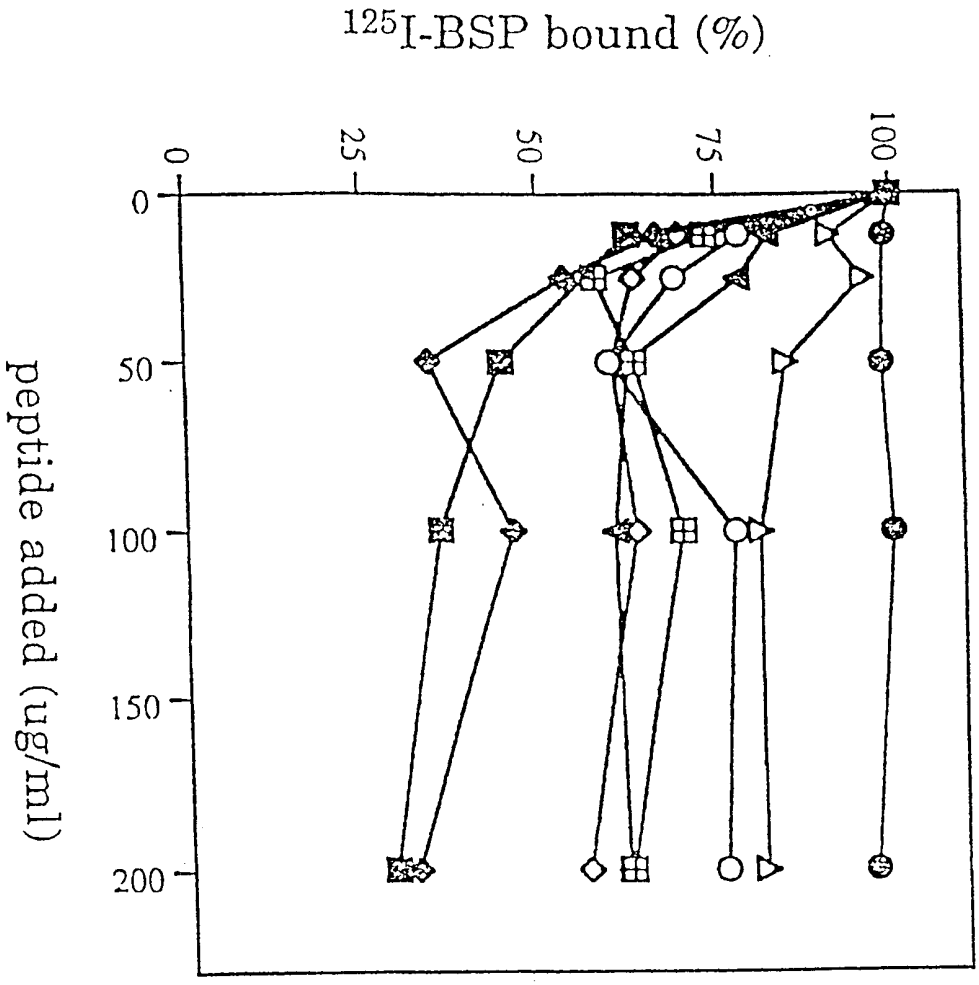
0.2
0.4
0.6
0.8
1.0
log concentration of potential inhibitor

Fig. 2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50
75
100
0
50
100
150
200
peptide added (ug/ml)

Fig. 3

SEQUENCE LISTING

<110> Ryden,. Cecilia
Oldberg, Åke

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<150> SE 9703614-9
<151> 1997-09-25

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Glu Asn Gly Asp Gly Asp Ser Ser Glu Glu Glu Gly Glu Glu Glu Glu
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Thr Ser Asn Glu Glu Glu Asn Asn Glu Asp Ser Glu Gly Asn Glu Asp
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Gln Glu Ala Glu Ala Glu Asn Ala Thr Leu Ser Gly Val Thr Ala Ser
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Thr Ser Asn Glu Glu Glu Asn Asn Glu Asp Ser Glu Gly Asn Glu Asp
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Thr Ser Asn Glu Glu Glu Asn Asn Glu Asp Ser Glu Gly Asn Glu Asp
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/01708

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/78, C07K 14/435, A61K 38/17
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, A61K

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

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Eur. J. Biochem., Volume 184, 1989, Cecilia Rydén et al, "Specific binding of bone sialoprotein to Staphylococcus aureus isolated from patients with osteomyelitis", page 331 - page 336, page 334, column 2, lines 1-4, page 335, column 1, lines 45-49 --	1-11
P,X	Biochem. J., Volume 327, 1997, Cecilia Rydén et al, "Staphylococcus aureus causing osteomyelitis to a nonapeptide sequence in bone sialoprotein", page 825 - page 829, the whole document --	1-11

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 January 1999	22 -01- 1999
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Patrick Andersson Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/01708

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

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

1. [X] Claims Nos.: 12-13
because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

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

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

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

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

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

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

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

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

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.