Bone sialoprotein (BSP) is an anionic phosphorylated glycoprotein that is expressed almost exclusively in mineralized tissues and has been shown to be a potent nucleator of hydroxyapatite formation. The binding of BSP to collagen is thought to be important for the initiation of bone mineralization and in the adhesion of bone cells to the mineralized matrix. Using a solid phase assay, we have investigated the interaction between BSP and collagen. Initial studies showed that raising the ionic strength, decreasing the pH below 7, or introducing divalent cations diminishes but does not abolish the binding of BSP to collagen, indicating that the interaction is partly electrostatic in nature. Both bone-extracted and recombinant (r)BSP exhibited similar binding affinities, indicating that post-translational modifications are not critical for binding. To identify the collagen-binding domain, recombinant peptides of BSP were studied. Peptide rBSP-(1–100) binds to type I collagen with an affinity similar to that of full-length rBSP, whereas peptides containing the sequences 99–201 or 200–301 do not bind. Further studies showed that rBSP-(1–75) competitively inhibits the binding of rBSP-(1–100), whereas rBSP-(21–100) inhibits binding to a lesser extent, and rBSP-(43–100) does not inhibit binding. These results suggest that the collagen-binding site of rat BSP is within the sequence 21–42, with residues N-terminal of this region likely also involved. This site was confirmed by the demonstration of collagen-binding activity of a synthetic peptide corresponding to residues 19–46. The collagen-binding domain, which is highly conserved among species, is enriched in hydrophobic residues and lacks acidic residues. We conclude that residues 19–46 of BSP represent a novel collagen-binding site.

Bone sialoprotein (BSP) is a highly anionic phosphoprotein normally expressed only in mineralized tissues and at sites of new mineral formation (1, 2). Studies by nuclear magnetic resonance, circular dichroism (CD), and small angle x-ray scattering have concluded that BSP has a flexible conformation with very little, if any, secondary structure (3–6).

BSP has been shown to be involved in cell attachment, cell signaling, hydroxyapatite binding, hydroxyapatite nucleation, and collagen binding (7). It has been proposed that the unstructured, flexible nature of BSP is required for its function as a bridging molecule with multiple binding partners (3). An understanding of the interaction between BSP and collagen is important, as the matrix-mineral relationship in bone is characterized by the presence of hydroxyapatite crystals in the interstitial tissue, which is rich in collagen fibrils and by their preferential orientation parallel to the fibril axes (8–10). To account for this pattern of initial mineral deposition, it is believed that BSP, which has been shown to be a potent nucleator of hydroxyapatite in vitro (5, 11, 12), is closely associated with type I collagen fibrils.

An interaction between BSP and collagen type I has been described previously. BSP, but not osteopontin or osteonectin, is associated with the demineralized and guanidine-extracted collagenous matrix of bone (13, 14), implying a close relationship between BSP and collagen. It has been shown that BSP interacts with reconstituted fibrillar collagen (15, 16) and delays collagen fibrillogenesis in a manner similar to that of decorin (16). In addition, there is some evidence to suggest that BSP localizes to the “hole” region of type I collagen fibrils (17), a site associated with early mineral deposition (8).

However, the mechanism of the BSP-collagen interaction is not known. It has previously been postulated that binding may be because of an electrostatic interaction with the positively charged collagen, as was originally suggested for the phosphophoryn-collagen interaction (18). Studies by Fujisawa and Kuboki (16) have indicated that BSP binds to gelatin (heat-denatured collagen) as well as to fibrillar collagen. This suggests that binding is not dependent on a triple helical or fibrillar collagen structure and is consistent with a generalized electrostatic interaction. Studies by Zhu et al. (19) have indicated that BSP isoforms enriched either in phosphate or sulfate have differing affinities for collagen and mineral crystals. Thus, post-translational modifications of BSP may be relevant in binding to collagen.

Many proteins have been found to bind collagens, and the mechanisms of interaction vary greatly. For example, the von Willebrand factor A3 domain has a rather hydrophobic type III collagen-binding site (20), decorin has multiple binding sites with lysine residues essential for binding (21), phosphophoryn binds two or more collagen molecules by an electrostatic mechanism (22), osteonectin binds collagen in a calcium-dependent manner (23) with polar and non-polar residues contributing to electrostatic and van der Waals interactions, respectively (24), and two acidic clusters in dentin matrix protein 1 drive binding to collagen (25). This variability in the collagen-binding sites of extracellular matrix proteins makes it extremely difficult to predict a collagen-interaction domain by sequence alone. In this study, we have used recombinant and synthetic peptides to...
identify a hydrophobic sequence close to the N terminus as the collagen-binding domain of BSP.

**EXPERIMENTAL PROCEDURES**

**Materials**—Rat tail tendon type I collagen was prepared as described previously (26). BSP was purified from the extracellular matrix of newborn rat bones (27, 28) and biotinylated with EZ-Link™ sulfo-NHS-biotin (Pierce) following the manufacturer's protocol. Rat recombinant BSP (rBSP) and rBSP-(43–101) were expressed in *Escherichia coli* and purified as described previously (5), rBSP-pE1,2D and rBSP-pE1,2A are mutants of rBSP in which the two contiguous poly(Glu) sequences of rBSP are mutated to aspartic acid and alanine residues, respectively (5).

To detect the binding of biotinylated synthetic peptides and bone-synthesized and purified as described previously (30, 31) and included an (NGVFKYRPRYFLYKHAYFYPPLKRFPVQ) of rat BSP were synthesized and purified by nickel-affinity, ion-exchange, and size-exclusion chromatography following established protocols (5). Proteins were analyzed for purity and protein content by SDS-PAGE and amino acid analysis.

**Synthetic Peptides**—Peptides corresponding to residues 19–32 (NGVFKYPYPRFILYK), 33–46 (HAYFPYPPKRFPVQ), or 19–46 (NGVFKYPYPRFILYKHAYFPYPPKRFPVQ) of rat BSP were synthesized and purified as described previously (30, 31) and included an N-terminal biotin label. Peptides were purified by analytical high pressure liquid chromatography to >95% purity, and their identities were confirmed by electrospray ionization mass spectrometry (Micromass Quadrupole II).

**Solid Phase Collagen-binding Assay**—The binding of proteins and peptides to type I collagen was studied by a modification of the method of Calderwood et al. (32). Type I collagen (1 µg/100 µl) in phosphate-buffered saline was incubated overnight at 4 °C in 96-well Maxisorp microtiter plates (Nunc). Wells were blocked with 1% myoglobin for 2 h at 37 °C and then incubated with His<sub>6</sub>-tagged protein or peptide for 3 h at room temperature. The wells were incubated overnight at 4 °C with 1/2000 Penta-His antibody (Qiagen) and subsequently incubated at room temperature for 45 min with 1/4000 horseradish peroxidase-conjugated goat anti-mouse IgG (Sigma). The bound proteins were then detected using 0.4 mg/ml ortho-phenylenediamine (Sigma) in phosphate-citrate buffer, pH 5 (200 mM Na<sub>2</sub>HPO<sub>4</sub>, 100 mM citric acid), and 0.01% H<sub>2</sub>O<sub>2</sub>. The reaction was stopped after 15 min by the addition of 2.5 mM H<sub>2</sub>SO<sub>4</sub>, and the absorbance was read at 492 nm. Duplicate experiments were performed with each concentration in triplicate, and data were fitted to a one-site binding model using GraphPad Prism™.

To detect the binding of biotinylated synthetic peptides and bone-extracted BSP to type I collagen, the bound protein was incubated with 10 µg/ml ExtrAvidin peroxidase (Sigma) in Tris-buffered saline for 15 min at room temperature, wells were washed, and bound biotinylated protein was determined using 0.4 mg/ml ortho-phenylenediamine (Sigma) as above.

**Competition of rBSP Binding to Type I Collagen**—As a measure of specificity of binding, the ability of unlabeled proteins to compete with biotinylated protein was assessed. rBSP and rBSP-(1–100) were biotinylated with EZ-Link™ sulfo-NHS-biotin following the manufacturer's protocol. Rat recombinant protein was incubated with collagen for 3 h as above. Following the collagen incubation, the wells were incubated with 10 µg/ml ExtrAvidin peroxidase in Tris-buffered saline for 15 min at room temperature. The bound protein was then detected using *ortho*-phenylenediamine in phosphate-citrate buffer. The <sup>125</sup>I-labeled rBSP in the absence of competitor was set to 100%, and the percentage of biotinylated rBSP bound with increasing competitor was plotted. The competition assays included 10 nM nonlabeled rBSP incubated with 0–1000 nM of unlabeled rBSP-pe1,2D, rBSP-pE1,2A, 25 nM biotinylated rBSP incubated with 0–1000 nM of unlabeled rBSP-(1–100), rBSP-(99–201), or rBSP-(200–301), 50 nM biotinylated rBSP-(1–100) incubated with 0–1000 nM of unlabeled rBSP-(1–100), rBSP-(1–75), rBSP-(21–100), or rBSP-(43–101), and 50 nM rBSP-(1–100) incubated with 0–1000 nM of synthetic peptides 19–32, 33–46, and 19–46.

**Characterization of the Interaction Between Type I Collagen and BSP**—The above assay was modified to examine the role of electrostatic interactions in the binding of rBSP and rBSP-(1–100) to collagen. The effect of varied pH on binding was examined using 0.1 M phosphate, adjusted to pH 2.5–8.0, as the buffer during the protein incubation and in all washes. Similarly, the effect of ionic strength was investigated by using 25 mM Tris, pH 7.0, containing 0–1000 mM NaCl. The effect of divalent or trivalent cations on collagen binding was examined using increasing concentrations of CaCl<sub>2</sub>, MnCl<sub>2</sub>, or LaCl<sub>3</sub>. The binding of bone-extracted BSP (<sup>1</sup>25I) and rBSP (<sup>1</sup>25I) to type I collagen were fitted to a 1:1 binding model, and K<sub>D</sub> values were determined. The binding of bone-extracted BSP (<sup>1</sup>25I) and rBSP (<sup>1</sup>25I) to type I collagen are shown. Data are presented as mean ± S.E.

**RESULTS**

**Role of Post-translational Modifications in Collagen Binding**—Bone-extracted and recombinant BSP were tested for collagen-binding activity to examine the contributions of the numerous post-translational modifications of BSP to collagen binding. Both the bone-extracted and rBSP demonstrate saturable binding to type I collagen (Fig. 1) and based on a one-site binding model have similar affinities for collagen. Bone-extracted BSP has a K<sub>D</sub> = 12.14 ± 1.10 nM, and rBSP has a K<sub>D</sub> = 14.24 ± 1.27 nM.

**Role of the Poly(Glu) Domains in Collagen Binding**—rBSP, rBSP-pE1,2D and rBSP-pE1,2A were tested for collagen-binding activity to examine the contributions of the contiguous glutamic acid residues to collagen binding. rBSP, rBSP-pe1,2A, and rBSP-pE1,2D were expressed in *E. coli* and were purified following the manufacturer's protocol. The far-UV spectra of rBSP-(1–75) was recorded in quartz cells of 1-mm optical path length using a Jasco-J810 spectropolarimeter between 190 and 260 nm, in 0.5-nm steps. The protein was studied at 0.2 mg/ml in buffers containing 0.1 M phosphate with pH 2.5–8.0 or 25 mM Tris-HCl, pH 7.0, with 0–1000 mM NaCl, as used above. A base line with buffer only was recorded separately and subtracted from each spectrum. All spectra were recorded at room temperature. The molar ellipticity (θ) expressed in degrees cm<sup>2</sup> dmol<sup>−1</sup> was calculated on the basis of mean residue molecular mass. Estimates of protein secondary structure from the CD data were made using the Circular Dichroism Deconvolution by Backpropagation Neural Networks (CDNN) program (33) as well as a calculation from [θ]<sup>200</sup> nm.

**Localization of the Collagen-binding Domain on rBSP**—To locate the collagen-binding domain of rBSP, several peptides were expressed and tested for collagen-binding activity. Both rBSP-(99–201) and rBSP-(200–301) show negligible binding to type I collagen.
collagen (Fig. 3A), which is confirmed by the inability of either peptide to compete with the binding of rBSP to collagen (Fig. 3B). rBSP-(1–100), however, bound to collagen with a $K_D = 5.64 \pm 0.59$ nM, which is an increased affinity compared with the full-length protein ($K_D = 22.85 \pm 3.9$ nM). This strong affinity for collagen is evident by the ability of rBSP-(1–100) to compete with rBSP for binding (Fig. 3B). rBSP-(1–75) shows binding comparable with rBSP-(1–100) with a $K_D = 4.04 \pm 0.59$ nM; however, the binding of rBSP-(21–100) was somewhat lower, with $K_D = 50.26 \pm 9.46$ nM (Fig. 3C). The specificity of these two peptides was demonstrated by their ability to compete for binding with rBSP-(1–100), as shown in Fig. 3D. rBSP-(43–101) was not tested for binding activity; however, it was not able to compete for binding with rBSP-(1–100), which indicates that the binding domain is not within residues 43–101 (Fig. 3D).

Synthetic peptides incorporating residues 19–32, 33–46, and 19–46 were tested for binding activity. Only the longer 19–46 peptide was able to bind to collagen, with a $K_D = 149.9 \pm 10.4$ nM (Fig. 4A), and inhibit binding of rBSP-(1–100) (Fig. 4B). The two smaller peptides, 19–32 and 33–46, showed little binding to collagen and were unable to inhibit the binding of rBSP-(1–100) (Fig. 4).

**Electrostatic Interactions in the Binding of rBSP to Type I Collagen**—No difference in rBSP binding was observed when using Tris or phosphate buffers at physiological pH (data not shown). The binding of rBSP to collagen was reduced by increasing the ionic strength of the buffer (Fig. 5A). Similarly, binding decreased at pH values lower than 7 (Fig. 5B). Increasing concentrations of CaCl$_2$, MnCl$_2$, or LaCl$_3$ caused binding to decrease until physiological strengths were reached, and then binding stayed constant (Fig. 5C). These studies indicate that there is an electrostatic component to the binding of rBSP to collagen. However, 25–45% of the protein still remained bound at high salt or low pH, implying that binding is not entirely electrostatic.

**Effect of Varying pH and Ionic Strength on Conformation of rBSP-(1–75)**—The CD spectrum of rBSP-(1–75) in 0.1 M phosphate buffer pH 7.0 and at physiological ionic strength is shown in Fig. 6. The CD spectrum of this peptide in 25 mM Tris is very similar (data not shown). Secondary structure estimates by the CDNN program estimates that rBSP-(1–75) at pH 7.0 exhibits 5.9% $\alpha$-helix, 36.4% anti-parallel $\beta$-sheet, 3.2% parallel $\beta$-sheet, 20.2% $\beta$-turn, and 34.1% unordered structure. Increasing concentrations of NaCl do not alter these conformations as the spectra are identical (data not shown). Decreasing the pH of the buffer, however, does alter the conformation of the peptide slightly and a shift of the minima to the right is seen (Fig. 6). This right shift is indicative of an increase in $\alpha$-helical content. The $\alpha$-helical content of rBSP-(1–75) is 6.2% at pH 4, 6.9% at pH 3.5, and 7.5% at pH 2.5. The other secondary structure elements are unchanged. A calculation of secondary structure from [*$\Theta_2$](nm) gives different percentages; however, it shows the same trend of increasing $\alpha$-helical content. By this method, the $\alpha$-helical content is 1.9% at pH 7.0 and begins to increase to 2.3% at pH 4.5, 3.0% at pH 4.0, 5.6% at pH 3.5, and 6.2% at pH 2.5.

**DISCUSSION**

Binding of BSP to collagen has been proposed to involve either nonspecific electrostatic interactions (16, 18) or specific interactions (34). It was suggested that the negatively charged post-translational modifications of phosphoproteins may bind electrostatically to the slightly positively charged surface of collagen (18). Here, however, we have demonstrated that both the bone-extracted BSP and the prokaryotically expressed protein are able to interact with type I collagen, and both show comparable affinities and binding capacities for collagen. This suggests that it is not simply a nonspecific interaction between the numerous post-translational modifications of BSP and collagen and that a more specific interaction between the residues of BSP and collagen is involved.

BSP is also enriched in glutamic acid residues, notably two poly(Glu) regions, which may interact with the positively charged collagen. However, synthetic homopolymers of poly(Glu) do not bind to collagen (data not shown) suggesting that the poly(Glu) sequences of BSP may have only a minor role in binding to collagen. Although the poly(Glu) sequences of BSP may not be directly responsible for binding activity, there is the possibility that electrostatic interactions, and therefore these sequences, are involved in the stabilization of binding of BSP to collagen. Electrostatic interactions have been found to be involved in protein-protein interactions in two ways. Nonspecific, long range electrostatic forces can lead to the formation of a low affinity complex, prior to the formation of short range interactions that give the final high affinity complex. If favorable electrostatic interactions exist between the two proteins, a higher reaction rate is achieved (35). It has also been shown that electrostatic interactions can confer specificity on protein-protein interactions without being energetically favorable. Essentially, the energy of electrostatic interactions between charged residues at the binding surface will equal the energetically unfavorable desolvation. The net result is that the electrostatic interactions increase the specificity of binding without affecting the affinity (36, 37).

The involvement of the poly(Glu) sequences within BSP in

**Fig. 2. Role of polyglutamic acid domains in binding.** A, collagen-coated wells were incubated with increasing concentrations of His$_6$-tagged protein, curves were fitted to a 1:1 binding model, and $K_D$ values were determined. Binding of rBSP (●, ——), rBSP-pE1,2D (■, ——), and rBSP-pE1,2A (○, —) to type I collagen are shown. Data are presented as mean ± S.E. B, competition of biotinylated rBSP by unlabeled proteins. 10 nM biotinylated rBSP was incubated simultaneously with 0–1000 mM of unlabeled rBSP-pE1,2D (■, ——), and rBSP-pE1,2A (○, —). Binding is indicated as mean percentage bound ± S.E., where 100% is the absorbance of biotinylated rBSP containing no competitor.
Binding to collagen was studied by testing rBSP-pE1,2D and rBSP-pE1,2A (mutated proteins that have the poly(Glu) sequences mutated to poly(Asp) and poly(Ala), respectively). Both proteins showed saturable binding to collagen and had a slightly higher affinity for collagen than wild-type protein. The increase in affinity seen with rBSP-pE1,2A implies that the poly(Glu) regions are likely not involved in collagen binding and may be decreasing the affinity of rBSP for collagen. It is more likely, however, that a mutation of the poly(Glu) domains to poly(Ala) increases the collagen-binding affinity by increasing the hydrophobicity of the protein. It is also possible that a conformational change in the protein allowed for the higher affinity. rBSP is an unordered, flexible protein (3, 5, 6) that may adopt a more ordered conformation in the presence of a binding partner, i.e. collagen. In this instance, the mutation of the poly(Glu) sequences to poly(Ala) may have induced the protein to fold into a more ordered conformation that was favorable to collagen binding, thereby increasing the rate of association, $k_a$, and accordingly the $K_D$, of the two proteins. Our previous work has shown that mutation of the poly(Glu) domains to poly(Ala) does cause a conformational change in the protein, as indicated by CD studies; however, the exact changes in secondary structure have not been elucidated (5). rBSP-pE1,2D is thought to be structurally similar to rBSP as indicated by these same CD studies, and there may be some small, localized changes in conformation that were not detected by CD, and these may have caused the slightly higher affinity seen with the rBSP-pE1,2D mutant.

To locate the collagen-binding domain within the rBSP sequence, three peptides, each encompassing a third of the pro-
tein, were tested for collagen-binding activity. rBSP-(1–100) bound to collagen with high affinity and competitively inhibited the binding of rBSP, indicating the high specificity of the peptide. rBSP-(99–200) and rBSP-(200–301), however, demonstrated no affinity for collagen, and this was confirmed by their inability to competitively inhibit the binding of rBSP to collagen. The lack of binding by the poly(Glu)-containing rBSP-(99–200) shows once again that the poly(Glu) regions are not responsible for binding.

Two smaller N-terminal peptides were tested for collagen-binding activity, and it was found that rBSP-(1–75) bound to collagen with similar affinity to the rBSP-(1–100) peptide, indicating that the binding domain is within the N-terminal 75 residues of BSP. The rBSP-(21–100) peptide demonstrated saturable binding to collagen but with a lower affinity than rBSP-(1–100). This suggests that the first 21 residues of BSP are somewhat involved in collagen binding but are not entirely necessary to achieve binding. The specificities of the rBSP-(1–75) and rBSP-(21–100) peptides was confirmed by their abilities to competitively inhibit the binding of rBSP-(1–100) to collagen. A final peptide, rBSP-(43–101), was not able to inhibit binding of rBSP-(1–100), giving further insight into the location of the collagen-binding domain. It appears that the collagen-binding domain is located within residues 21–42. However, residues outwith this region may be involved in long range electrostatic interactions.

The synthetic peptide encompassing residues 19–46 was found to bind collagen with relatively high affinity and could inhibit the binding of rBSP-(1–100), which indicates that these residues do indeed contain the collagen-binding domain of BSP. Neither peptide 19–32 nor peptide 33–46 was found to bind collagen, which suggests that amino acids in the overlapping sequence of these peptides are required for binding. The lower affinity of the 19–46 peptide compared with rBSP-(1–100) also suggests that residues outwith this region may be involved in long range electrostatic interactions.

Varying ionic strength has often been conveniently used to test for the presence of electrostatic interactions (38, 39). The bond energy of a salt bridge will depend on the extent of protonation of both groups involved. Thus, a general treatment of electrostatic contributions must effectively describe the pH dependence of electrostatic interactions (40). Therefore, the involvement of electrostatic interactions in the binding of rBSP and rBSP-(1–100) to collagen was examined by increasing the ionic strength or decreasing the pH of the buffer used during binding. Both of these changes caused a considerable decrease in binding to collagen; however, higher than physiological concentrations of salt did not have a significant effect on binding. Although altering the pH of the buffer does cause an increase in α-helical content of the rBSP-(1–75) peptide, it does not seem that conformational changes are solely responsible for the decrease in binding, as no change in conformation is seen with increasing ionic strength. The observed decreases in binding therefore indicate an electrostatic interaction between BSP and collagen. Interestingly, binding to collagen was never completely abolished, even at very low pH or very high salt, so it would appear that electrostatics are only a component of the binding mechanism.

The binding of BSP to collagen is calcium-independent. In fact, addition of calcium, as well as manganese and lanthanum, was found to decrease the amount of protein bound to collagen. In vivo, binding of BSP to collagen will occur in a calcium-rich environment. However, the binding to collagen was not completely abolished by calcium even at the highest concentration studied. Once again, an electrostatic mechanism of binding is evident, as the cations are likely binding weakly to the negatively charged surface of BSP and interfering with the electrostatic interactions involved in collagen binding.

Our results indicate that electrostatic interactions are in-
involved in the binding of BSP to collagen, potentially through long range electrostatic interactions that stabilize the formation of an initial low affinity complex prior to the formation of more specific, short range interactions. What these short range interactions are remains unclear. In general, protein-protein interfaces usually have a higher proportion of charged and polar residues (41), and these heterocomplexes are dominated by hydrophobic-hydrophilic interactions where salt bridges are common (42). Hydrophobic interactions are more frequent in the interior of the monomers and in permanent interactions than in the interior of the transient protein-protein interfaces (41, 42).

The residues involved in the non-electrostatic interactions in BSP are apparently located within residues 19–46, with residues on either side of this region likely involved in electrostatic interactions. There are five positively charged and four negatively charged residues within the first 19 residues of BSP, as well as numerous negatively charged residues downstream of residue 46, which may be involved in these long range electrostatic interactions.

Residues 19–46 (rat, NGVFKYPRPYFLKAYFYPPLK-RFPVQ) are in a region that is very highly conserved. Between the rat, mouse, pig, cow, and human sequences 20 residues are identical, 4 are highly conserved, 2 are conserved, and 1 is non-conserved. Uncharacteristic of the rest of the sequence of BSP is the lack of negatively charged residues within this region. There are positively charged Lys, Arg, and His residues, however, as well as an enrichment of Pro, Tyr, and Phe residues. The conservation of Phe residues has been found to imply the binding of a specific short range interactions. It appears that the specific residues involved in collagen binding are located within residues 19–46, with charged residues both upstream and downstream of this region likely contributing electrostatically to binding. Future studies will aim to identify specific residues within both the BSP and collagen sequences that are involved in binding.

Of note, our recent unpublished data show that rBSP is the lack of negatively charged residues within this region. BSP is the lack of negatively charged residues within this region. There are positively charged Lys, Arg, and His residues, however, as well as an enrichment of Pro, Tyr, and Phe residues. The conservation of Phe residues has been found to imply the binding of a specific short range interactions. It appears that the specific residues involved in collagen binding are located within residues 19–46, with charged residues both upstream and downstream of this region likely contributing electrostatically to binding. Future studies will aim to identify specific residues within both the BSP and collagen sequences that are involved in binding.

Acknowledgments—We thank O. V. Litvinova for the preparation of the synthetic peptides. Circular dichroism experiments were carried out at the University of Western Ontario Biomolecular Interactions and Conformations Facility, which is supported by a Multi-User Equipment and Maintenance Grant from the Canadian Institutes of Health Research.