Cross-reactive Immunodeterminants on *Streptococcus sanguis* and Collagen

**PREDICTING A STRUCTURAL MOTIF OF PLATELET-INTERACTIVE DOMAINS***

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Cross-reactive immunodeterminants on a fibril-associated surface antigen of *Streptococcus sanguis* and types I and III collagen participate in the induction of aggregation of human platelets. To further understand the basis for this apparent molecular mimicry, type-specific collagen antibodies, anti-KPGEPGPK (an analogue of platelet-interactive domains on collagen) and a panel of KPGEPGPK-like synthetic peptides were used as probes. When collagen or *S. sanguis* cells were pretreated with the anti-collagen antisera, the induction of aggregation of platelet-rich plasma was greatly delayed or abrogated. These anti-collagen antibodies also neutralized KPGEPGPK and purified *S. sanguis* platelet-interactive antigens as inhibitors of *S. sanguis* or collagen-induced aggregation of platelets in plasma. In immunoblot analyses, these anti-collagen antibodies reacted with *S. sanguis* platelet-interactive antigens. Additionally, antisera against the platelet-interactive antigen of *S. sanguis* selectively reacted with undigested type I collagen and with fragments CB3 and CB6 of cyanogen bromide-treated type I collagen. Finally, when platelets were pretreated with synthetic peptides containing specific amino acid substitutions within the KPGEPGPK sequence, the time to onset of platelet-rich plasma aggregation by both agonists was altered. The hierarchical pattern of responses of platelets to these peptides and predictions of the structural changes produced by simulated insertions of each peptide into the CB4 sequence of type III collagen suggested conformational requirements for interactions with platelets. Thus, these data show that cross-reactive immunodeterminants of *S. sanguis* and collagen induce platelet aggregation. The platelet-interactive domains are predicted to be characterized by a structural motif with the consensus sequence X-P-G-E-P/Q-G-P-X.

Proteinaceous fibrils on the surface of cells of *Streptococcus sanguis* induce human platelets in plasma to aggregate in vitro (1–3). Platelet-adhering (Adh) and aggregation-inducing (Agg) phenotypic variants occur across strains (4). The Agg+ phenotype is associated with a platelet-interactive immunodeterminant located in a 23-kDa segment of a 65-kDa cell wall-bound protein fragment (5). This immunodeterminant is also identified on exported and released precursor proteins of 111 and 115 kDa, respectively, from cultured protoplasts (6). When disassembled from the surface fibrils on the cell wall, these proteins or protein fragments do not act as agonists, but as inhibitory hapten for *S. sanguis* and collagen-induced platelet aggregation (2, 7, 8).

Monospecific antibodies against the *S. sanguis* platelet-interactive immunodeterminant (class II antigen) react with collagen of *S. sanguis* and types I or III collagen to inhibit induction of platelet aggregation (7). These antibodies are specifically neutralized by purified platelet-interactive proteins or fragments from *S. sanguis* and a synthetic peptide (KPGEPGPK),1 which also acts as an inhibitory hapten and is patterned from platelet-interactive domains on collagen (7, 8).

To confirm the molecular mimicry of collagen, antibodies against type-specific collagens and anti-KPGEPGPK were reacted with platelet-interactive immunodeterminants of *S. sanguis*. In addition, antibodies specific for the *S. sanguis* antigen were reacted with cyanogen bromide digests of type I collagen. A panel of peptides similar to KPGEPGPK was synthesized with selected amino acid substitutions. The specificity of cross-reactivity was explored using these congener octapeptides as inhibitors of *S. sanguis*-induced platelet aggregation. Structural predictions based upon the hierarchical pattern of responses suggest common structural features of the platelet-interactive domain of the class II antigen of *S. sanguis* and collagen.

**EXPERIMENTAL PROCEDURES**

Preparation of Antibody Probes—Monospecific rabbit anti-types I (titer 1:200, by ELISA), III (titer 1:250), and IV (titer 1:150) collagen were obtained from Chemicon International (B Segundo, CA) and tested to verify specificity (data not shown). Rabbit antiserum against *S. sanguis* I 133-79 whole cells (titer 1:128, by immunodiffusion), 23-kDa fragment of the platelet interactive antigen (titer 1:32), or non-specific hyperimmune serum were prepared in female New Zealand White rabbits as described previously (2, 3). Monospecific antibody (titer 1:8) against KPGEPGPK was prepared as above using a bovine serum albumin conjugate as immunogen and an ovalbumin conjugate for screening. Conjugates were prepared by cross-linking with carbodiimide (9). Briefly, BSA or ovalbumin (30 mg) and KPGEPGPK (15 mg) were dissolved in 1 ml of distilled H2O, stirred, and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide HCl was added slowly. The peptide-carrier conjugate was purified by gel filtration with carbodiimide (9).

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1 The abbreviations used are: KPGEPGPK, NH2-Lys-Pro-Gly-Glu-Pro-Gly-Pro-Lys-COOH; PRP, platelet-rich plasma; ELISA; enzyme-linked immunosorbent assay; BSA, bovine serum albumin; HPLC, high performance liquid chromatography; SDS, sodium dodecyl sulfate; PBS, phosphate-buffered saline.
and confirmed by amino acid analysis. For use in immunoblot analyses, IgG was prepared from rabbit antisera by precipitation with saturated ammonium sulfate (10), followed by ion exchange chromatography (5).

**Synthetic Peptides**—The platelet-active collagen-like octapeptide KPGEPGPK, originally described by Legrand et al. (8), was synthesized by the Microchemical Facility of the University of Minnesota. Purity was confirmed by amino acid analysis and HPLC. A panel of 24 additional congener peptides, with selected amino acid substitutions, was synthesized by the Microchemical Facility on a parallel peptide synthesizer (model 350; Advanced Chem Tech, Louis ville, KY) and purity-confirmed by HPLC.

**Platelet Aggregometry**—Platelet aggregometry was performed as described (1, 2) with freshly isolated citrated human platelet-rich plasma (PRP) from a single healthy medication-free donor according to procedures approved by the Committee on the Use of Human Subjects in Research of the University of Minnesota. Fifty microliters of soluble type I collagen (0.05 mg/ml, final concentration), prepared as described previously (7), or standardized suspensions of cells of S. sanguis (2 × 10^9 cells/ml, final concentration) were added to stirred (1200 rpm), warmed (37 °C) PRP (0.45 ml adjusted to 2 × 10^8 platelets/ml) in a recording aggregometer. The lag time to onset and extent of platelet aggregation were measured.

In some experiments, PRP (3.4 ml) was preincubated for 10 min at 37 °C with 0.5-50 pmol (in 0.05 ml) of purified 115-kDa exported precursor protein (6), purified 65-kDa surface protein fragment (5), its 23-kDa functional segment (5), the collagen-like octapeptide KPGEPGPK (7), or 15 pmol of congener peptides. In other experiments, cells of S. sanguis I 133-79 were pretreated for 10 min at 37 °C with 0.1 ml of rabbit anti-S. sanguis whole cells, anti-platelet-interactive antigen, anti-types I, III, or IV collagen, anti-KPGEPGPK-BSA, or nontarget hyperimmune serum in the presence or absence of purified platelet-interactive antigen or octapeptide. The effects on aggregation of PRP were determined.

**Platelet Adhesion**—The platelet-bacterial adhesion assay was performed as described previously (1, 7). In some experiments, cells of S. sanguis I 133-79 were pretreated for 10 min at 37 °C with rabbit antisera against type-specific collagens.

**Immunoblots**—To demonstrate reaction with anti-collagen antisera, minimal tryptic digest and purified platelet-interactive antigens were prepared from cells of S. sanguis and subjected to electrophoresis on 5-10% gradient SDS-polyacrylamide gels (5, 11). Unstained gels were electroblotted onto nitrocellulose, quenched with hemoglobin, and incubated with specific rabbit antisera (5). In some experiments the antisera had been neutralized with the 23-kDa fragment of the S. sanguis platelet-interactive protein. The binding of rabbit IgG antibodies to specific antigens was detected by incubation with goat anti-rabbit IgG, which was conjugated to alkaline phosphatase and reacted with 5-bromo-4-chloro-3-indoyl phosphate (12).

**Peptide Structure Predictions**—Based on the inhibitory activity of the congeners of KPGEPGPK, a hierarchy of activity was developed. The KPGEPGPK sequence within CB4 of collagen III was then replaced with each congener sequence, and predictions of secondary structures were performed using MELPROT (Biochemistry Department, Melbourne University; Ref. 14), which incorporated the predictive algorithms of Chou and Fasman (15, 16), Hopp and Woods (17), and Pruitt et al. (18), and confirmed by amino acid analysis. For use in immunoblot analyses, IgG was prepared from rabbit antisera by precipitation with saturated ammonium sulfate (10), followed by ion exchange chromatography (5).

**RESULTS**

**Effects on Platelet aggregation**—To establish that collagen and cells of S. sanguis share platelet-interactive immunodeterminants, these agonists were pretreated with antibodies against type-specific collagens. The anti-types I, III, and IV collagen and anti-KPGEPGPK antisera each delayed the onset or abrogated the platelet response to soluble type I collagen or S. sanguis (Table I). When aggregation occurred, the rate and extent were unaffected (data not shown). S. sanguis and collagen retained their ability to induce aggregation of platelets in plasma when pretreated with unrelated hyperimmune rabbit serum or PBS. Pretreatment of cells of S. sanguis with anti-collagen antibodies did not affect adhesion to platelets (data not shown).

**S. sanguis Antigens React with Anti-collagen Antibodies**—Anti-types I and III collagen antisera (0.1 ml) were then pretreated with 0.3 nmol of either the 23-kDa fragment of platelet-interactive antigen or collagen-like octapeptide. When these mixtures were incubated with cells of S. sanguis or collagen, the ability of the anti-collagen antibodies to abrogate platelet aggregation was neutralized (Table II). Non-

| Agonist pretreatment | Lag time with agonists |
|----------------------|------------------------|
|                      | S. sanguis | Collagen |
| None                 | 2.5 ± 0.2 (6) | 2.2 ± 0.3 (6) |
| PBS                  | 2.6 ± 0.2 (6) | 2.4 ± 0.2 (6) |
| Nonspecific antisera | 2.6 ± 0.1 (4) | 2.3 ± 0.1 (4) |
| Anti-type I collagen | <20 (4)       | >20 (4)       |
| Anti-type III collagen| >20 (4)       | 19.7 ± 3.5 (4) |
| Anti-KPGEPGPK-BSA    | >20 (3)       | >20 (3)       |

**Table I**

**Effects on PRP aggregation of S. sanguis and collagen pretreatment with various antisera preparations**

**Effects on PRP aggregation of S. sanguis and collagen pretreatment with various antisera preparations**

| Antigen                  | Lag time with agonist |
|--------------------------|-----------------------|
|                         | S. sanguis | Collagen |
| None                     | 2.5 ± 0.2 (6) | 2.2 ± 0.3 (6) |
| PBS                      | 2.6 ± 0.2 (6) | 2.4 ± 0.2 (6) |
| Nonspecific antisera     | 2.6 ± 0.1 (4) | 2.3 ± 0.1 (4) |
| Anti-type I collagen     | >20 (4)       | >20 (4)       |
| Anti-type III collagen   | >20 (4)       | 19.7 ± 3.5 (4) |
| Anti-KPGEPGPK-BSA        | >20 (3)       | >20 (3)       |

**Table II**

**Interactions between antisera and soluble protein/peptide preparations**

Antibody preparations (0.1 ml) were preincubated as described in the text with 0.3 nmol of KPGEPGPK or the S. sanguis 23-kDa platelet-interactive peptide. 0.05 ml of the mixture was then used to pretreat S. sanguis cells or soluble type I collagen. Values are means ± S.D.; number of experiments in parentheses.
specific hyperimmune rabbit serum was also pretreated with the 23-kDa fragment or octapeptide, incubated with both agonists, and then used to challenge platelets. Platelet aggregation was inhibited, reflecting the unneutralized effect of the peptide inhibitors. Neutralization of 0.1 ml of anti-type I (titer 1:200) and anti-type III (titer 1:250) was maximal with 0.2–0.4 nmol of 23-kDa fragment or collagen-derived KPGEPGPK (Table III).

Anti-collagen antisera were reacted with antigens from cells of S. sanguis in immunoblots (Fig. 1). Anti-types I, III, and IV collagen antisera each reacted (panels 2–4) with the 23- (lane B) and 65 (lane C)-kDa forms of platelet-interactive antigen from purified preparations or in a minimal trypsin digest of whole cells (lane A). As expected, these antisera also reacted (data not shown) with the untrypsinized 115-kDa form of platelet-interactive antigen obtained from protoplast culture fluid (6). Pretreatment of these antisera with the 23-kDa fragment of the platelet-interactive antigen neutralized reactions in immunoblots with the 23- and 65-kDa forms of the S. sanguis antigens (panels 6–8). Similarly, a single precipitin resolved in immunodiffusion reaction between the anti-collagen sera and the 65-kDa antigen and failed to appear when preceded by absorption with the 23 kDa fragment (data not shown). Rabbit anti-octapeptide(-BSA) showed a reaction of identity in immunodiffusion with equimolar concentration of KPGEPGPK-ovalbumin (Fig. 2, well 1), the 23- (well 2) and 65-kDa (well 3) fragments, and the 115-kDa platelet-interactive protein (well 4), but was unreactive with ovalbumin alone (data not shown). Similar molar concentrations of each were also required to inhibit platelet aggregation in response to cells of S. sanguis or collagen (Table IV). Inhibition was apparently specific, since ADP-induced platelet aggregation was unaffected by pretreatment with any of the four platelet-interactive preparations.

**TABLE III**

Neutralization of the platelet-aggregation inhibitory effects of anti-type I and anti-type III collagen antisera by the 23-kDa fragment of KPGEPGPK

| Antisera | Agonist | Neutralization concentration |
|---------|--------|----------------------------|
| Anti-type I<sup>a</sup> | S. sanguis | 0.37 0.25 |
|         | Collagen | 0.38 0.28 |
| Anti-type III<sup>b</sup> | S. sanguis | 0.40 0.28 |
|         | Collagen | 0.39 0.33 |

<sup>a</sup> Nanomol of 23-kDa fragment of S. sanguis platelet-interactive peptides necessary to completely neutralize 0.1 ml of each antisera.

<sup>b</sup> Nanomol of collagen-derived, KPGEPGPK necessary to completely neutralize 0.1 ml of each antiserum.

<sup>c</sup> 0.05 ml of pretreated anti-type I collagen (titer = 1:200, by ELISA).

<sup>d</sup> 0.05 ml of pretreated anti-type III collagen (titer = 1:250, by ELISA).

**Effects of Collagen-like Peptides on Platelet Interactions—**

To learn more about the structural requirements of KPGEPGPK, a panel of similar peptides, with selected amino acid substitutions, were prepared. The S. sanguis-induced platelet aggregation responses were then compared after pretreatment (10 min, 37 °C) of platelets with peptides at concentrations corresponding to the ID₅₀ of the octapeptide (30 nM). As shown in Table V, inhibition was actually enhanced when the amino-terminal residue was altered and the carboxyl 7 residues left intact. Replacing the charged amino acid at residue 4 with a neutral amino acid significantly reduced the inhibitory activity. In addition, substitutions which led to the reduction of the β-turn potential of residues 5 and 7 signifi-

**TABLE IV**

Inhibition of S. sanguis and collagen-induced platelet aggregation by platelet-interactive peptides

|                | ID₅₀ with agonists<sup>a</sup> |
|----------------|-----------------------------|
|                | S. sanguis<sup>₅</sup> | Collagen<sup>₅</sup> |
| S. sanguis platelet-interactive antigens | 115-kDa protein | 15 19 |
|                | 65-kDa protein | 19 21 |
|                | 23-kDa fragment | 20 25 |
|                | KPGEPGPK<sup>₅</sup> | 31 16 |

<sup>a</sup> No inhibition of ADP-induced PRP aggregation.

<sup>b</sup> S. sanguis 133-79 (2 x 10⁶ cells/ml), 0% inhibition = 2.6-min lag time.

<sup>c</sup> Soluble type I collagen (0.05 mg/ml), 0% inhibition = 2.1-min lag time.

<sup>d</sup> Synthetic peptide analogue of the platelet interactive domain from CB4 of type III collagen.
reactive with the platelet-interactive domain on \textit{S. sanguis}. Also cross-reactive, the platelet-interactive fragment \(\alpha_1(III)-CB4\) includes residues 479–486 from which KPGEPGPK was derived (Table VI). Highly conserved sequences were identified in residues 480–485 of bovine \(\alpha_1(I)-CB3\) and in residues 948–953 of bovine \(\alpha_1(I)-CB6\). The 6-amino acid sequence of \(\alpha_1(I)-CB6\) is identical to residues 480–485 of \(\alpha_1(III)-CB4\), except for a glutamine (residue 951) replacing proline (residue 483). More recently, platelet recognition sites have been reported in human types I and III collagen, specifically \(\alpha_1(I)-CB7\) and -CB8 and \(\alpha_1(III)-CB3\) and -CB4 (22).

The structural motif common to these platelet-interactive domains in bovine and human types I and III collagen (21, 22) and \textit{S. sanguis} was modeled by establishing a hierarchy of activity shown by members of a panel of congener peptides. Using MELPROT, each peptide sequence was inserted into \(\alpha_1(III)-CB4\), and the changes in structural predictions were compared with the activity with platelets. These data suggest that the platelet-interactive site requires a negatively charged amino acid surrounded by two regions of amino acids with \(\beta\)-turn potential. This interruption in the \(\alpha\)-helix of collagen may facilitate interactions with platelets. Amino acid substitutions that altered the predicted structure reduced the inhibitory activity of the peptide, a criterion for loss of platelet interactivity. Although the spacing of basic residues (lysine and arginine) was suggested to be a structural requirement for induction of platelet aggregation, this criterion does not apply to all platelet-interactive domains of human collagen (e.g. \(\alpha_1(I)-CB8\)). When the cyanogen bromide fragments of human collagens were surveyed for domains that fit the predictive requirements, several domains were identified that fit our criteria (Table VI). All known platelet aggregation-associated sites do, therefore, fit the predicted structural motif of two regions with \(\beta\)-turn potentials that surround an acidic amino acid within a 7-residue portion of polypeptide. Therefore, the sequence Pro-Gly-Glu-(Pro/Gln)-Gly-Pro within the polypeptide may form the minimal structural motif for the cross-reactive platelet-interactive domains of collagen and \textit{S. sanguis}.

This \textit{S. sanguis} immunodeterminant, which shows immunochemical and functional mimicry for collagen, must have access to a signal-transducing receptor on platelets to induce PRP aggregation. Since glycoprotein Ia-deficient platelets fail to aggregate in response to cells of \textit{S. sanguis} and collagen (24), a common receptor may exist. Soluble and particulate collagens induce platelets to aggregate, but the response pathways differ (7). Although particulate type I collagen induces platelet aggregation within seconds (7, 25, 26), with differences in the dose affecting the rate and extent, soluble collagen and \textit{S. sanguis} show only a dose-dependent effect on lag time to onset of aggregation. Glycoprotein Ia-IIa appears to interact directly with collagen in plasma, contributing to the transduction of signal involved in modulating the lag time (27). Indeed, the lag time to onset of aggregation may reflect the signal transduction mechanism of platelets in the absence of higher avidity adhesion events.

Strong adhesive interactions between glycoprotein Ia-IIa and collagen appear to require \(\text{Mg}^{2+}\) and are demonstrable predominately in the absence of plasma (27). In these conditions, isolated platelet Ia-IIa (\(\alpha_\text{Ib}\beta_3\)) integrin receptor complex selectively binds the \(\alpha_1(I)-CB3\) peptide of collagen (28). When \(\text{Ca}^{2+}\) and additional plasma constituents are present, other collagen domains may bind glycoprotein Ia-IIa. Indeed, the platelet aggregation domain of type III collagen is active in plasma and in the presence of \(\text{Ca}^{2+}\). Since this domain and a related immunodeterminant on type I collagen are cross-
Platelet-interactive domains identified in bovine and human collagens

| Peptide* | Sequence* | Inhibition | Predicted structure* |
|----------|-----------|------------|----------------------|
| A1       | Lys-Pro-Gly-Glu-Pro-Gly-Pro-Lys | 54         | %                    |
| A3       | Lys-Pro-Gly-Asn-Pro-Gly-Pro-Lys | 18         | 2 5 7                |
| A5       | Gln-Pro-Gly-Glu-Pro-Gly-Pro-Lys | 88         | 2 5 7                |
| A7       | Gln-Pro-Gly-Asn-Pro-Gly-Pro-Lys | 18         | 2 5 7                |
| A9       | Leu-Pro-Gly-Glu-Pro-Gly-Pro-Lys | 67         | 2 5 7                |
| A11      | Lys-Pro-Gly-Val-Pro-Gly-Pro-Lys | 17         | 2 5 7                |
| B2       | Leu-Pro-Gly-Val-Pro-Gly-Pro-Lys | 16         | 2 5 7                |
| B4       | Lys-Pro-Gly-Glu-Pro-Ala-Pro-Lys | 1          | 2                    |
| B6       | Lys-Pro-Ala-Pro-Gly-Pro-Lys     | 31         | 5                    |
| B8       | Lys-Pro-Ala-Pro-Glu-Pro-Lys     | 0          | 5                    |
| B10      | Lys-Pro-Gly-Glu-Pro-Trp-Pro-Lys | 0          | 5                    |
| B12      | Lys-Pro-Trp-Pro-Gly-Pro-Lys     | 32         | 5                    |
| C1       | Lys-Pro-Trp-Pro-Glu-Pro-Lys     | 0          | 5                    |
| C3       | Lys-Ser-Gly-Pro-Glu-Pro-Lys     | 34         | 5                    |
| C5       | Lys-Pro-Gly-Glu-Ser-Gly-Pro-Lys | 8          | 2                    |
| C7       | Lys-Pro-Gly-Glu-Pro-Gly-Ser-Lys | 9          | 2                    |
| C9       | Lys-Ser-Gly-Ser-Gly-Pro-Lys     | 8          | 2                    |
| C11      | Lys-Pro-Gly-Ser-Gly-Ser-Lys     | 3          | 2                    |
| D2       | Lys-Ser-Gly-Pro-Glu-Ser-Lys     | 5          | 2                    |
| D4       | Lys-Arg-Gly-Glu-Pro-Gly-Pro-Lys | 34         | 5                    |
| D6       | Lys-Pro-Gly-Glu-Arg-Pro-Lys     | 8          | 2                    |
| D8       | Lys-Pro-Gly-Glu-Pro-Glu-Arg-Lys | 9          | 2                    |
| D10      | Lys-Arg-Gly-Glu-Arg-Pro-Lys     | 6          | 2                    |
| D12      | Lys-Pro-Gly-Glu-Arg-Aryg-Pro-Lys | 1          | 2                    |
| E1       | Lys-Arg-Gly-Glu-Pro-Gly-Aryg-Lys | 1          | 2                    |

* Identification for each peptide.
1 Boldface designates substitution of residue compared with octapeptide sequence A1.
2 As described under "Experimental Procedures."
3 Dash designates a negative charged amino acid residue at position 4, 0 designates a neutral amino acid residue at position 4.
4 Blank spaces designate residues which lost or had reduced predicted $\beta$-turn potential.

Predicted of the structural features of the minimal platelet-interactive sequence

| Peptide* | Sequence* | Inhibition | Predicted structure* |
|----------|-----------|------------|----------------------|
| A1       | Lys-Pro-Gly-Glu-Pro-Gly-Pro-Lys | 54         | %                    |
| A3       | Lys-Pro-Gly-Asn-Pro-Gly-Pro-Lys | 18         | 2 5 7                |
| A5       | Gln-Pro-Gly-Glu-Pro-Gly-Pro-Lys | 88         | 2 5 7                |
| A7       | Gln-Pro-Gly-Asn-Pro-Gly-Pro-Lys | 18         | 2 5 7                |
| A9       | Leu-Pro-Gly-Glu-Pro-Gly-Pro-Lys | 67         | 2 5 7                |
| A11      | Lys-Pro-Gly-Val-Pro-Gly-Pro-Lys | 17         | 2 5 7                |
| B2       | Leu-Pro-Gly-Val-Pro-Gly-Pro-Lys | 16         | 2 5 7                |
| B4       | Lys-Pro-Gly-Glu-Pro-Ala-Pro-Lys | 1          | 2                    |
| B6       | Lys-Pro-Ala-Pro-Gly-Pro-Lys     | 31         | 5                    |
| B8       | Lys-Pro-Ala-Pro-Glu-Pro-Lys     | 0          | 5                    |
| B10      | Lys-Pro-Gly-Glu-Pro-Trp-Pro-Lys | 0          | 5                    |
| B12      | Lys-Pro-Trp-Pro-Gly-Pro-Lys     | 32         | 5                    |
| C1       | Lys-Pro-Trp-Pro-Glu-Pro-Lys     | 0          | 5                    |
| C3       | Lys-Ser-Gly-Pro-Glu-Pro-Lys     | 34         | 5                    |
| C5       | Lys-Pro-Gly-Glu-Ser-Gly-Pro-Lys | 8          | 2                    |
| C7       | Lys-Pro-Gly-Glu-Pro-Gly-Ser-Lys | 9          | 2                    |
| C9       | Lys-Ser-Gly-Ser-Gly-Pro-Lys     | 8          | 2                    |
| C11      | Lys-Pro-Gly-Ser-Gly-Ser-Lys     | 3          | 2                    |
| D2       | Lys-Ser-Gly-Pro-Glu-Ser-Lys     | 5          | 2                    |
| D4       | Lys-Arg-Gly-Glu-Pro-Gly-Pro-Lys | 34         | 5                    |
| D6       | Lys-Pro-Gly-Glu-Arg-Pro-Lys     | 8          | 2                    |
| D8       | Lys-Pro-Gly-Glu-Pro-Glu-Arg-Lys | 9          | 2                    |
| D10      | Lys-Arg-Gly-Glu-Arg-Pro-Lys     | 6          | 2                    |
| D12      | Lys-Pro-Gly-Glu-Arg-Aryg-Pro-Lys | 1          | 2                    |
| E1       | Lys-Arg-Gly-Glu-Pro-Gly-Aryg-Lys | 1          | 2                    |

* Identification for each peptide.
1 Boldface designates substitution of residue compared with octapeptide sequence A1.
2 As described under "Experimental Procedures."
3 Dash designates a negative charged amino acid residue at position 4, 0 designates a neutral amino acid residue at position 4.
4 Blank spaces designate residues which lost or had reduced predicted $\beta$-turn potential.

TABLE V

| Peptide* | Sequence* | Inhibition | Predicted structure* |
|----------|-----------|------------|----------------------|
| A1       | Lys-Pro-Gly-Glu-Pro-Gly-Pro-Lys | 54         | %                    |
| A3       | Lys-Pro-Gly-Asn-Pro-Gly-Pro-Lys | 18         | 2 5 7                |
| A5       | Gln-Pro-Gly-Glu-Pro-Gly-Pro-Lys | 88         | 2 5 7                |
| A7       | Gln-Pro-Gly-Asn-Pro-Gly-Pro-Lys | 18         | 2 5 7                |
| A9       | Leu-Pro-Gly-Glu-Pro-Gly-Pro-Lys | 67         | 2 5 7                |
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| B2       | Leu-Pro-Gly-Val-Pro-Gly-Pro-Lys | 16         | 2 5 7                |
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| B6       | Lys-Pro-Ala-Pro-Gly-Pro-Lys     | 31         | 5                    |
| B8       | Lys-Pro-Ala-Pro-Glu-Pro-Lys     | 0          | 5                    |
| B10      | Lys-Pro-Gly-Glu-Pro-Trp-Pro-Lys | 0          | 5                    |
| B12      | Lys-Pro-Trp-Pro-Gly-Pro-Lys     | 32         | 5                    |
| C1       | Lys-Pro-Trp-Pro-Glu-Pro-Lys     | 0          | 5                    |
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| C5       | Lys-Pro-Gly-Glu-Ser-Gly-Pro-Lys | 8          | 2                    |
| C7       | Lys-Pro-Gly-Glu-Pro-Gly-Ser-Lys | 9          | 2                    |
| C9       | Lys-Ser-Gly-Ser-Gly-Pro-Lys     | 8          | 2                    |
| C11      | Lys-Pro-Gly-Ser-Gly-Ser-Lys     | 3          | 2                    |
| D2       | Lys-Ser-Gly-Pro-Glu-Ser-Lys     | 5          | 2                    |
| D4       | Lys-Arg-Gly-Glu-Pro-Gly-Pro-Lys | 34         | 5                    |
| D6       | Lys-Pro-Gly-Glu-Arg-Pro-Lys     | 8          | 2                    |
| D8       | Lys-Pro-Gly-Glu-Pro-Glu-Arg-Lys | 9          | 2                    |
| D10      | Lys-Arg-Gly-Glu-Arg-Pro-Lys     | 6          | 2                    |
| D12      | Lys-Pro-Gly-Glu-Arg-Aryg-Pro-Lys | 1          | 2                    |
| E1       | Lys-Arg-Gly-Glu-Pro-Gly-Aryg-Lys | 1          | 2                    |

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