Previously we defined a binding site for high molecular weight kininogen (HK) in the A1 domain of factor XI (FXI). Since thrombin can activate FXI and HK inhibits the activation of FXI by thrombin, we have identified a thrombin binding site in FXI. Both the recombinant A1 domain (Glu₁–Ser⁹⁰) and a synthetic peptide (Phe₅⁶–Ser⁹⁰) containing the HK binding site inhibited FXI activation by thrombin. Both a monoclonal antibody, SF7, recognizing the A1 domain, and the rA1 domain were shown to be competitive inhibitors of thrombin-catalyzed FXI activation. The peptides Ala₄₅–Arg₅₄ and Val₉₉–Arg₇₀ acted synergistically to inhibit FXI activation by thrombin. Mutant rA1 domain constructs (Val₆₄ → Ala and Ile₇₇ → Ala), which do not inhibit FXI binding to HK, retain full capacity to inhibit FXI activation by thrombin. The peptide Ala₄₅–Arg₅₄ inhibited thrombin-catalyzed FXI activation, whereas it had no effect on FXI binding to HK. In contrast, the peptide Asn₇²–Leu₈₃ (which inhibited FXI binding to HK) did not inhibit FXI activation by thrombin. Thus, a thrombin binding site exists in the A1 domain of FXI spanning residues Ala₄₅–Arg₅₄ that is contiguous with but separate and distinct from the HK binding site. These sites may regulate which ligand is bound to FXI and through which pathway FXI is activated.

Factor XI (FXI) is a homodimeric plasma glycoprotein that circulates as a complex with its cofactor high molecular weight kininogen (HK) (1, 2) and is proteolytically activated on negatively charged surfaces by FXIa to give rise to FXIa (3–10). The mechanism, involving interactions of FXII, prekallikrein (PK), and HK, by which contact activation is initiated and its significance in vivo have yet to be established, since individuals congenitally deficient in any one of these contact factors (FXII, HK, and PK) do not experience abnormal bleeding, suggesting that these proteins are not required for coagulation in vivo (11, 12). In contrast, a deficiency of FXI can result in excessive bleeding after trauma or surgery (13, 14). These observations suggest that FXI may be activated in vivo by a protease(s) other than FXIa.

The ability of thrombin, an enzyme generated late in the coagulation cascade, to activate FXI has been demonstrated (15, 16). The site at which FXI is cleaved by thrombin is identical to that cleaved by FXIIa (16, 17). Determination of the kinetic parameters of FXI activation by thrombin and FXIIa indicate that at a physiological concentration of FXI, in the presence of dextran sulfate, thrombin would be the more potent activator (16). Although FXI is readily activated by thrombin in a purified system with dextran sulfate present, the reaction may not proceed as readily in plasma (15, 16, 18), since although HK promotes the FXIIa-mediated reaction it inhibits thrombin-catalyzed activation of FXI (15, 16, 18). These observations raise the following two related questions. Is thrombin a physiological activator of FXI in plasma? What is the mechanism by which HK can inhibit thrombin-catalyzed FXI activation?

The present study was undertaken to determine the sequence of amino acids in FXI that mediate its interaction with thrombin. Clarification of the mechanism of interaction of these two proteins might also help to elucidate the physiological importance of thrombin-catalyzed FXI activation. Four tandem repeat sequences (designated A1, A2, A3, and A4 or Apple domains) are present in the heavy chain of FXI (7). We have previously reported evidence for the presence of an HK binding site in the A1 domain (19, 20), a binding site for FXIIa in the A4 domain (21), a substrate binding site for FIX in the A2 domain (22), and a specific binding site for platelets in the A3 domain (23). Evidence for a binding site in the A1 domain of FXI that is important for interaction with thrombin is reported in the present study.

MATERIALS AND METHODS

Purification of Proteins—FXI (250 units/mg of protein) was purified from human plasma by immunoaffinity chromatography (24). The recombinant A1 (rA1) domain (Glu₁–Ser⁹⁰) and the rA2 domain (Ser⁹₀–Ala₁₃₁) were prepared in Escherichia coli using the QIAexpress pQE-9 expression vector (Qiagen Inc., Chatsworth, CA). PK was purified as described (25). FXI was assayed by minor modifications (26) of the kaolin-activated partial thromboplastin time (27). Human α-thrombin (2,800 NIH units/mg) was purchased from Enzyme Research Laboratories (South Bend, IN). All purified proteins appeared homogeneous by SDS-polyacrylamide gel electrophoresis.

Peptide Synthesis—Peptides were synthesized on an Applied Biosystems 430A peptide synthesizer by a modification (29) of the procedure described by Kent and Clark-Lewis (28). The sequences of the synthetic peptides utilized in this study are given in Table I. All the peptides utilized in this work were rationally designed, conformationally constrained synthetic peptides based upon a previously published (20) molecular model for the A1 domain of FXI. Each peptide was separately modeled using energy minimization calculations (20) that confirmed that the modeled peptides assumed a conformation similar to that of the A1 domain.

Refolding and Reduction and Alkylation of Peptides—A previously published method (24) was used to refold peptides containing cysteine residues. Alternatively, peptides were reduced with dithiothreitol and alkylated with iodoacetamide as described previously (24).

High Performance Liquid Chromatography (HPLC)—The HPLC system employed was from Waters (Waters 600 Gradient Module, model
The numbers indicate the length of each peptide as reported by Fujikawa et al (7). A gap was inserted in the A1 domain at residue 72 to maintain maximal alignment of residues.

Designates a residue in which cysteine replaced one or more amino acid(s) in the normal factor XI sequence.

designates a peptide in which cysteine replaced one or more amino acid(s) in the normal factor XI sequence.

designates a peptide in which alanine replaced one or more amino acid(s) in the normal factor XI sequence.

Effect of Peptides on the Rate of Activation of Factor XI by Thrombin—

To determine whether the A1 domain peptides (Glu1–Ser90, Phe56–Ser86, Ala45–Arg54) inhibit the activity of thrombin in the conversion of fibrinogen to fibrin, an established procedure (31) was employed in which 0.1 ml of human fibrinogen (Sigma), 1.5 mg/ml was diluted in 0.8 ml of 0.05M Tris-HCl, 0.1M NaCl, pH 7.4, to which was added 0.1 ml of a1-thrombin for 5 min at 37 °C, and the mixture was assayed using the chromogenic substrate S-2238 (H-D-Phe-pip-Arg-pNA, Kabi Vitrum, Stockholm, Sweden) at concentrations of 3 × 10−8 M. The results were the same after reduction and alkylation of these same peptides. All reduced and alkylated or refolded peptides were homogeneous preparations consisting of less than 0.02 mol of free SH/mol of peptide, which further verifies that these refolded peptides were homogenously prepared containing the HK binding site (19, 20) also inhibited thrombin-catalyzed FXI activation (IC50 5 × 10−9 M). In contrast, neither the rA2 domain (Ser90–Ala181, containing a sub-
strate binding site for FXI (Ref. 22 and Fig. 2) nor synthetic peptides representing sequences in the A3 domain (Asn235–Arg266, a platelet receptor binding site; Ref. 23) and A4 domain (Glu1–Ser90, an FXIIa binding site; Ref. 21) were able to inhibit the activation of FXI by thrombin (Fig. 1B). The rA1 domain peptide and other A1-derived peptides (including Phe56–Ser86 and Ala45–Arg54) were examined for their capacity to inhibit the amidolytic activity of thrombin and the thrombin-catalyzed conversion of fibrinogen to fibrin (see "Materials and Methods"). No thrombin-inhibitory effects were observed at peptide concentrations up to 1 mM (data not shown), indicating that the inhibitory effects of these peptides are specific for thrombin-catalyzed FXI activation. Thus, these experiments reveal that a binding site for thrombin exists in the A1 domain of FXI, possibly near the binding site for HK in the A1 domain (Phe56–Ser86).

Previously we have identified specific amino acid residues within the A1 domain involved in binding HK (19, 20, 37). Utilizing mutational analysis we have determined that the binding of FXI to HK is mediated at least in part by Val64 and Ile77 in the A1 domain of FXI (37). Therefore, we examined the effects of mutations of these two residues on the capacity of the rA1 domain to inhibit thrombin-catalyzed FXI activation. We found that mutant rA1 domain constructs (Val64→Ala and Ile77→Ala), which have lost the capacity to inhibit FXI binding to HK (37), retain the full capacity of the rA1 domain (Glu1–Ser90) to inhibit thrombin-catalyzed FXI activation (Fig. 2). Therefore, the binding sites for HK and thrombin in the A1 domain, although contiguous, are apparently separate and distinct. Another experiment that supports this conclusion is that after reduction and alkylation, the rA1 domain (Glu1–Ser90) retains the capacity to inhibit FXI binding to HK (IC50=10−6 M) (19, 20, 37), whereas it is unable to inhibit thrombin-catalyzed FXI activation (data not shown).

Effects of Conformationally Constrained, A1-derived Peptides on the Activation of Factor XI by Thrombin—Detailed structural information is not available for the A1 domain or any other part of the FXI molecule. However, we have constructed a molecular model of this region (20, 37), which may or may not ultimately be found to accurately portray the true structure of the FXI A1 domain once it is determined from x-ray crystallography or nuclear magnetic resonance studies. Thus, the model was used as a guide to generate hypotheses about the structure of the A1 domain to be tested in functional studies. Using this hypothetical model, we have made testable predictions about the structure and function of the HK binding site in the A1 domain (20, 37). Our experiments revealed that the sequence of amino acids, Val59, Lys83 within the A1 domain of FXI, contains two antiparallel β-strands connected by β-turns that comprise a continuous surface utilized for the binding of HK (20). Since the rA1 domain peptide (Glu1–Ser90) was more effective in inhibiting FXI activation by thrombin than the peptide (Phe56–Ser86) by 2 orders of magnitude, we suspected that the amino acid sequence Glu1–Trp55 might contain residues important in binding thrombin. Therefore, we examined a molecular model of the A1 domain that predicts the presence of three stem-loop structures (antiparallel β-strands connected by β-turns) defined by amino acid residues Ala45–Arg54, Val59–Arg70, and Asn72–Lys83 (19, 20). We therefore prepared conformationally constrained cyclic peptides comprised...
ing these peptide loop structures to determine whether they might assume a conformation that comprises a thrombin binding site. These peptides were identical to those tested to delineate the HK binding surface (19, 20, 37). Cysteine residues were introduced at the amino and carboxyl terminus of each peptide so that the resulting disulfide bond might stabilize the loop-like structure (19, 20). The peptide designated Ala45–Arg54(C) in which cysteines were substituted at positions 49 and 53 (see Table 1) had no effect (refolded or reduced and alkylated) on FXI binding to HK at concentrations up to 10 mM (20). By comparison, this peptide (Ala45–Arg54) when properly folded inhibited thrombin-catalyzed activation of FXI with an IC50 value of $2 \times 10^{-5} \, M$. This peptide was 1 order of magnitude more effective than peptide F56–S86 (IC50 = $1 \times 10^{-4} \, M$), which indicates that most of the binding energy for thrombin resides within this amino acid sequence (Ala45–Arg54) of FXI (Fig. 3A). The ability of Val59–Arg70 (refolded) to inhibit thrombin-catalyzed FXI activation (Fig. 3B) was identical to that of Phe56–Ser86 (Fig. 3A) with an IC50 of $10^{-4} \, M$. However, Asp72–Lys93 (both refolded and reduced and alkylated) had no effect on thrombin-catalyzed activation of FXI (data not shown), whereas this peptide was a potent inhibitor of HK binding to FXI (IC50 = $1 \times 10^{-4} \, M$; Refs. 20 and 37). These results strongly suggest that Ala45–Arg54 and Val59–Arg70 form two stem-loop structures consisting of antiparallel $\beta$-strands connected by $\beta$-turns, which together form the thrombin binding site. The experiments in Fig. 3 demonstrate synergism in the effects of both peptides together compared with either one alone, since when the two peptides were added together their effect was greater than a simple additive effect, i.e. when used in combination at equimolar concentrations these two peptides were 1 order of magnitude more effective than either one alone in inhibiting thrombin-catalyzed FXI activation.

Prekallikrein, a protein with 58% sequence identity to FXI, also binds HK in the A1 domain within the homologous amino acid sequence Phe56–Gly86 (38). This stretch of amino acids displays 65% homology with a comparable sequence in FXI. Therefore, we tested the PK Phe56–Gly86 peptide for its ability to inhibit thrombin-catalyzed FXI activation. Unlike the FXI Phe56–Ser86 peptide, the PK Phe56–Gly86 peptide did not inhibit thrombin-catalyzed FXI activation (Fig. 3A). It has been reported that this sequence of amino acids in PK (Phe56–Gly86) binds HK (38) as does the homologous sequence of FXI (19, 20). Thus, the amino acid sequences involved in FXI and PK interaction with HK are not involved in binding thrombin.

Fine Mapping of the Thrombin Binding Site in Factor XI—In order to gain information about which specific amino acids comprise the thrombin binding site we prepared synthetic peptides with amino acid substitutions, determined by examining our molecular model for residues that project their side chains into a predicted contact surface (37). One such candidate in FXI was a glutamic acid residue at position 66 (37), which was therefore changed to an alanine in the conformationally constrained synthetic peptide Val59–Arg70. This altered peptide failed to inhibit thrombin-catalyzed FXI activation, whereas the native peptide with a glutamic acid present at position 66 (Val59–Arg70) inhibited thrombin-catalyzed FXI activation with an IC50 of $1 \times 10^{-4} \, M$ (Fig. 3B). However, Glu56 is apparently not involved in binding HK since Val59–Arg70 (Glu56 → Ala) had inhibitory activity equal to that of the native peptide in FXI binding to HK (37). When the linear peptide Ala45–Arg54 (Fig. 3C) was compared with the conformationally constrained cyclic peptide Ala45–Arg54(C) (Fig. 3B) they both inhibited thrombin-catalyzed FXI activation with the same IC50 (2 × $10^{-5} \, M$; see Table I), indicating 1) that Ser49 and Thr53 (which were substituted with cysteines in the cyclic peptide) are not part of the contact surface and 2) that cyclization and conformational constraint are not required for inhibitory activity of this particular peptide. However, when alanine was substituted for Asp51 in peptide Ala45–Arg54(C) the altered peptide (IC50 = $1.5 \times 10^{-4} \, M$) was only one-tenth as effective as the native peptide (IC50 = $2.0 \times 10^{-5} \, M$) in inhibiting thrombin-catalyzed FXI activation. Therefore, Asp51 may be an important constituent of the thrombin binding site.
the altered peptide compared with $2 \times 10^{-5}$ M for the native peptide (Fig. 3C and Table II). Thus, these results indicate that the side chain of Asp$^{51}$ might be directly involved in binding thrombin, whereas Glu$^{50}$ might also make a minor contribution to thrombin binding. When the amino acids comprising part of the putative thrombin binding surface of Ala$^{45}$-Arg$^{54}$, consisting of amino acids Glu$^{50}$-Asp$^{51}$-Pro$^{52}$, were tested as a conformationally constrained cyclic peptide, Ser$^{49}$-Thr$^{53}$, for its thrombin-catalyzed activation of factor XI, a putative substrate (FXI) binding site.

*DISCUSSION*

It is possible to activate FXI in the absence of contact proteins in the presence of the serine protease thrombin (15, 16, 18). Consequently, several laboratories have attempted to determine the physiological conditions required for the activation of FXI by thrombin or other proteases (16, 18, 39). Although FXI is activated by thrombin in a purified system, it is suspected that this reaction may not proceed in plasma (18, 39). It is well known that HK enhances FXIa-mediated activation of FXI in vitro (1–10). However, HK (500 nM) inhibits thrombin-mediated activation of FXI (15, 16, 18, 39). Thus, thrombin may not be a suitable activator of FXI in plasma. To understand the physiological importance of thrombin-mediated FXI activation,
and 4); 2) the rA1 domain peptide (Glu1–Ser90) inhibited the thrombin binding site within thrombin (Fig. 3). The evidence supporting this conclusion is as follows: 1) a monoclonal antibody (5F7) that binds to the A1 domain of FXI (19, 20, 36) can completely block thrombin-catalyzed FXI activation with a Ki of 5 × 10–6 M (close to the Kd for 5F7 binding to FXI; see Ref. 36 and Figs. 1 and 4); 2) the rA1 domain peptide (Glu45, Ser90) inhibited the activation of FXI by thrombin with a Ki of 5 × 10–6 M (Fig. 1 and 4); 3) a molecular model of the A1 domain (Fig. 5) predicts the presence of three peptide loop structures, Ala45–Arg54, Val59–Arg70, and Asn72–Lys83, that form a solvent-exposed surface (37); 4) based on this model, conformationally constrained peptides were synthesized, two of which (Ala45–Arg54, Val59–Arg70) act synergistically to inhibit thrombin-catalyzed FXI activation (Fig. 3); and, 5) Lineweaver-Burk plots of the activation of FXI by thrombin in the presence of either the monoclonal antibody 5F7 or the rA1 domain peptide yielded patterns consistent with a classical competitive inhibition (Fig. 4).

We have previously characterized a binding site for HK in the A1 domain of FXI (19, 20, 36, 37). To perform fine mapping of this site we prepared conformationally constrained synthetic peptides and rA1 domain constructs (20, 37). To identify specific amino acid residues involved in HK binding, conformationally constrained peptides were synthesized containing conservative amino acid replacements at residues suspected to contain side chains involved in binding including Val64 → Ala, Glu66 → A, Arg73 → Ala, and Ile77 → Ala (37). Because abnormal results were obtained with two of these peptides, Val64 → Ala and Ile77 Ala, which failed to compete normally with FXI for binding HK, we prepared two mutant rA1 domains (Val64 → Ala and Ile77 Ala) both of which exhibited diminished capacity to inhibit FXI binding to HK (37). Since the thrombin binding site was localized to the A1 domain and found to contain amino acid sequence overlapping the HK binding site (Fig. 1), we attempted to identify specific amino acid residues in the A1 domain that might bind thrombin. Our results are consistent with the following conclusions: 1) Val64 and Ile77, which are important as contact sites for HK (37), do not participate in the interaction of the A1 domain with thrombin (Fig. 2); 2) Glu66 and Asp51, which are not important as contact sites for binding HK (37), are both apparently important residues for binding thrombin (Fig. 3); and 3) another important difference between the HK and thrombin binding sites in the A1 domain is that reduction and alkylation of the A1 domain virtually destroys the thrombin binding site while leaving the HK binding site intact (37).

We also examined the plasma protein PK, which shares a high degree (58%) of sequence identity with FXI (7), to determine whether homologous amino acid sequences can also inhibit thrombin-catalyzed FXI activation. Unlike the FXI Phe66, Ser86 peptide, the PK Phe66, Gly86 peptide did not inhibit thrombin-catalyzed FXI activation. A glutamic acid is replaced by a glycine at position 66 in PK, and this amino acid substitution Glu66 → Ala66 in FXI Val59–Arg70 rendered this peptide inactive (Fig. 3). It is possible that this and other amino acid replacements in PK render PK Phe66, Gly86 unable to inhibit thrombin-catalyzed FXI activation. It has also been established that PK, like FXI, also binds HK in the A1 domain within the homologous amino acid residues Phe66, Gly86 (38). This stretch of amino acids is 65% identical to the comparable sequence in FXI (Fig. 6). Moreover, a peptide with the amino acid sequence Pro65-Lys64 of PK had no effect on thrombin-catalyzed FXI activation, unlike Ala65–Arg64 of FXI, which was the most effective inhibitor (Fig. 3 and Table II). These two regions of FXI and PK have only 18% identity (Fig. 6), and it seems reasonable that PK does not contain a binding site for thrombin since thrombin does not activate PK (40). We have also attempted to fine map the important contact sites of peptide Ala45–Arg53 (C) that interacts with thrombin. Apparently, Asp51 is important in this interaction. However, amino acid residues in the first half of the peptide Ala45–Pro48 also appear to be important in its interaction with thrombin (Fig. 3).
The data presented in this paper support the conclusion that the thrombin and HK binding sites in the A1 domain, while contiguous, are separate and distinct. However, these two binding sites appear to overlap since they share a common sequence of amino acids (Val59–Arg70). The relationship between the putative thrombin and HK binding sites is depicted in the molecular model shown in Fig. 5. The model and our results predict that if HK is bound to the A1 domain, thrombin-mediated activation of FXI would be blocked and FXIIa-mediated activation of FXI would be favored. The reverse may also occur, i.e. the binding of thrombin to the A1 domain should prevent HK binding and FXIIa-mediated activation of FXI. Therefore, these two contiguous partially overlapping sites could constitute a point of regulation to determine by which pathway (contact activation versus feedback activation) and by which protease (FXIIa or thrombin) FXI might be activated.

Acknowledgments—We are grateful to Patricia Pileggi for assistance in manuscript preparation.

REFERENCES
1. Davie, E. W., Fujikawa, K., Kurachi, K., and Kisiel, W. (1979) Adv. Enzymol. Relat. Areas Mol. Biol. 48, 277–318
2. Thompson, R. E., Mandle, R., and Kaplan, A. P. (1977) J. Clin. Invest. 60, 1376–1380
3. Griffin, J. H., and Cochrane, C. G. (1976) J. Biol. Chem. 251, 1330–1338
4. Ohkubo, I., Fujikawa, K., and Kurachi, K. (1982) Fed. Proc. Am. Soc. Exp. Biol. 41, 656 (abstr.)
5. Fujikawa, K., Chung, D. W., Hendrickson, L., and Davie, E. W. (1986) Biochemistry 25, 2417–2424
6. McMullen, B. A., Fujikawa, K., and Davie, E. W. (1991) Biochemistry 30, 2056–2060
7. van der Graaf, F., Greengard, J. S., Bouna, B. N., Kerbiriou, D. M., and Griffin, J. H. (1983) J. Biol. Chem. 258, 9669–9675
8. Kurachi, K., and Davie, E. W. (1977) Biochemistry 16, 5831–5839
9. Reu, S. D., Cochrane, C. G., and Griffin, J. H. (1977) J. Clin. Invest. 59, 1167–1175
10. Schmaier, A. H., Silverberg, M., Kaplan, A. P., and Colman, R. W. (1987) in Hemostasis and Thrombosis, 2nd Ed. (Colman, R. W., Hirsh, J., Marder, V. J., and Salzman, E. W., eds) pp. 18–38, Lippincott, Philadelphia, PA
11. Revak, S. D., Cochrane, C. G., and Griffin, J. H. (1977) Adv. Enzymol. Relat. Areas Mol. Biol. 48, 656 (abstr.)
12. Schmaier, A. H., Silverberg, M., Kaplan, A. P., and Colman, R. W. (1987) in Hemostasis and Thrombosis, 2nd Ed. (Colman, R. W., Hirsh, J., Marder, V. J., and Salzman, E. W., eds) pp. 148–161, Lippincott, Philadelphia, PA
13. Ragni, M. V., Sinha, D., Seaman, F., Lewis, J. H., Spero, J. A., and Walsh, P. N. (1993) Sem. Thromb. Hemostasis 19, 396–404
14. Bolton-Maggs, P. H. B., Young Wan-Yin, R., McCraw, A., Slack, J., and Kernoff, P. B. A. (1988) Br. J. Haematol. 69, 521–528
15. Naito, K., and Fujikawa, K. (1991) J. Biol. Chem. 266, 7353–7358
16. Galilani, D., and Breeze, G. J. (1991) Science 253, 909–912
17. Mann, K. G., and Lundblad, R. L. (1987) in Hemostasis and Thrombosis, 2nd Ed. (Colman, R. W., Hirsh, J., Marder, V. J., and Salzman, E. W., eds) pp. 1167–1175, Lippincott, Philadelphia, PA
18. Scott, C. F., and Colman, R. W. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 11189–11193
19. Baglia, F. A., Jameson, B. A., and Walsh, P. N. (1990) J. Biol. Chem. 265, 4149–4154
20. Baglia, F. A., Jameson, B. A., and Walsh, P. N. (1992) J. Biol. Chem. 267, 4247–4252
21. Baglia, F. A., Jameson, B. A., and Walsh, P. N. (1993) J. Biol. Chem. 268, 3838–3844
22. Baglia, F. A., Jameson, B. A., and Walsh, P. N. (1991) J. Biol. Chem. 266, 24190–24197
23. Baglia, F. A., Jameson, B. A., and Walsh, P. N. (1995) J. Biol. Chem. 270, 6734–6740
24. Sinha, D., Kothy, A., Seaman, F. S., and Walsh, P. N. (1985) J. Biol. Chem. 260, 10714–10719
25. Page, J. D., and Colman, R. W. (1991) J. Biol. Chem. 266, 8143–8148
26. Scott, C. F., Sinha, D., Seaman, F. S., Walsh, P. N., and Colman, R. W. (1984) Blood 63, 42–50
27. Proctor, R. R., and Rapaport, S. I. (1961) Ann. J. Clin. Pathol. 36, 212–219
28. Kent, S. B. H., and Clark-Lewis, I. (1985) Synthetic Peptides in Biology and Medicine, Elsevier Science Publishers, Amsterdam
29. Walsh, P. N., Baglia, F. A., and Jameson, B. A. (1993) Methods Enzymol. 222, 65–96
30. Hare, A. S. F. A. (1972) Methods Enzymol. 25, 457–464
31. Budzynski, A. Z., Olewa, S. A., and Brizuela, B. S. (1979) Biochim. Biophys. Acta 584, 284–287
32. Segal, J. H. (1974) Enzyme Kinetics, Wiley-Interscience, New York, NY
33. Sinha, D., Seaman, F. S., and Walsh, P. N. (1987) Biochemistry 26, 3768–3775
34. Walsh, P. N., Bradford, H., Sinha, D., Piperno, J. R., and Tuszyński, G. P. (1984) J. Clin. Invest. 73, 1392–1399
35. Cha, S. (1975) Biochem. Pharmacol. 24, 2177–2185
36. Baglia, F. A., Sinha, D., and Walsh, P. N. (1989) Blood 74, 244–251
37. Seaman, F. S., Baglia, F. A., Gurr, J. A., Jameson, B. A., and Walsh, P. N. (1994) Biochem. J. 304, 715–721
38. Heredal, H., Jähnichen-Dent, W., Abd Alia, S. A., Hock, J., Bouna, B., and Muller-Esterl, W. (1993) J. Biol. Chem. 268, 14527–14535
39. Brunnee, T., LaPorta, C., Reddigari, S. R., Salerno, V. M., Kaplan, A. P., and Silverberg, M. (1993) Blood 81, 580–586
40. Galilani, D., and Breeze, S. W. (1993) Sem. Thromb. Hemostasis 19, 396–404
A Binding Site for Thrombin in the Apple 1 Domain of Factor XI
Frank A. Baglia and Peter N. Walsh

J. Biol. Chem. 1996, 271:3652-3658.
doi: 10.1074/jbc.271.7.3652

Access the most updated version of this article at http://www.jbc.org/content/271/7/3652

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 36 references, 19 of which can be accessed free at http://www.jbc.org/content/271/7/3652.full.html#ref-list-1