MerR is complexed with mercuric ions, in the presence of low concentrations of buffer thiols (Shewchuk et al., 1989). Of these two cysteines, the significant ligand is presumably C117 since this residue is evolutionarily conserved and C115 is not (T. Misra and I. Mahler, personal communications). Thus, C117 and C126 are still both candidates for specific Hg(II) ligands.

Taken together, these observations suggest that specific, high-affinity mercuric ion binding by MerR is complex and at least three of the four cysteine residues present in each monomer contribute to transcriptional activation (an a^+ phenotype). The requirement for multiple cysteine residues may reflect sequential interaction with Hg(II) or may be due to adoption of a tri- or tetracoordinate geometry. Site-directed mutagenesis experiments using cysteine → alanine changes may clarify these possibilities (in progress). The availability of cysteine to alanine mutants will rule out specific perturbation of structure due to cysteine to tyrosine changes and may allow specific assignment of [β-^{13}C]cysteine resonances for [^{13}C]cysteine-enriched NMR contact shift analyses.

Genetic and biochemical analysis of the MerR protein suggests that mercuric ion binding is mediated by the cysteine residues clustered near the C-terminus and DNA binding is mediated by a helix–turn–helix motif near the N-terminus. These two activities are genetically separable, but we have been unable to demonstrate a two-domain structure using classical proteolysis techniques [see Shewchuk et al. (1989)]. This suggests that the activation of transcription by MerR may require only subtle changes in gross protein structure. The mode of action of this metal-responsive genetic switch appears distinct from previously studied regulatory proteins that use ligand binding to directly alter DNA recognition (Garges & Adhya, 1988; Zhang et al., 1987).

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Determination of Amino- and Carboxyl-Terminal Sequences of Guinea Pig Liver Transglutaminase: Evidence for Amino-Terminal Processing†

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ABSTRACT: Transglutaminases (EC 2.3.2.13) catalyze the formation of ϵ-(γ-glutamyl)lysine cross-links and the substitution of a variety of primary amines for the γ-carboxamide groups of protein-bound glutaminyl residues. These enzymes are involved in many biological phenomena. In this study, the amino- and carboxyl-terminal sequences of guinea pig liver transglutaminase were identified by sequence analysis to determine whether this enzyme is processed posttranslationally at its terminal regions. Two peptides, believed to contain the amino-terminal sequences of transglutaminase, were isolated from the Pronase digest of the enzyme protein with SP-Sephadex C-25 column chromatography and reverse-phase HPLC. Analyses (amino acid analysis, sequencing after the treatment with an acylamino-acid-releasing enzyme, and fast atom bombardment mass spectrometry) of these peptides indicated that the amino-terminal structure of this enzyme is acetylAla-Glu-Asp-Leu-Ile-Leu-Glu. The candidate for the carboxyl-terminal peptide in the trypsin digest of enzyme was isolated from the unadsorbed fraction of affinity chromatography with anhydrotrypsin agarose gel. The peptide was found to be Asn-Val-Ile-Ile-Gly-Pro-Ala. Both the terminal sequences were completely consistent with those predicted from the cDNA sequence [Ikura, K., Nasu, T., Yokota, H., Tsuchiya, Y., Sasaki, R., & Chiba, H. (1988) *Biochemistry 27*, 2898–2905]. These results indicated that the amino-terminal processing occurred after or in the course of translation of this enzyme, namely, removal of the initiator methionine and a subsequent acetylation of the alanine residue adjacent to the methionine. Our results did not indicate carboxyl-terminal processing of guinea pig liver transglutaminase.

Transglutaminases (protein-glutamine:amine γ-glutamyltransferase, EC 2.3.2.13) are calcium-dependent acyltransferases that catalyze the formation of an amide bond between the γ-carboxamide groups of peptide-bound glutamine residues and the primary amino groups in a variety of compounds, including the ϵ-amino group of lysine in certain proteins. These enzymes are widely distributed in most tissues and body fluids, and several are involved in diverse biological

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functions in which they catalyze the formation of ε-(γ-glutamyl) lysine cross-links with protein substrates [for reviews see Folk and Finlayson (1977), Folk (1980), and Lorand and Conrad (1984)]. These functions include stabilization of the fibrin structure in hemostasis (Lorand, 1972) and in endogenously occurring fibrinolysis (Sakata & Aoki, 1980, 1982), formation of the cornifying envelope in epidermal keratinocytes (Rice & Green, 1978), stiffening of the erythrocyte membrane (Siefring et al., 1978), wound healing (Mosher & Schad, 1979), formation of a postejaculation vaginal plug in rodents (Williams-Ashman, 1984), and cellular growth regulation and differentiation (Birckbichler & Patterson, 1978; Birckbichler et al., 1981; Kannagi et al., 1982; Murtaugh et al., 1984).

Liver transglutaminase is one of the most extensively studied transglutaminases, but its biological role is unknown. Slife et al. (1986) have reported that some of the transglutaminase activity in rat liver is associated with the plasma membrane and may be responsible for forming covalently cross-linked matrices of protein at sites of cell-to-cell contact. We have reported possible applications of transglutaminase reactions in various areas using guinea pig liver enzyme (Ikura et al., 1980, 1981, 1984, 1985a; Yoshikawa et al., 1982; Okumura et al., 1984). To produce the animal transglutaminase in bacteria and understand the structure–function relationship of this enzyme, we have cloned the cDNA of guinea pig liver transglutaminase and identified 2073 bases of the complete coding sequence for a polypeptide with 691 amino acid residues (Ikura et al., 1987, 1988). Amino acid sequences in both the amino- and carboxyl-terminal ends predicted from the cDNA were not consistent with those reported previously by Connellan et al. (1971). In this study, we isolated the amino- and carboxyl-terminal peptides of guinea pig liver transglutaminase and determined their structures to know whether or not the liver transglutaminase is processed posttranslationally at its terminal regions.

Materials and Methods

Materials. Guinea pig liver transglutaminase was purified on an immunoadsorbent column using monoclonal antibody as described previously (Ikura et al., 1985b). Other materials were obtained commercially from the sources indicated: Pronase (Calbiochem), TPCK-treated trypsin (Worthington), SP-Sephadex C-25 (Pharmacia), acylamino-acid-releasing enzyme and anhydrotrypsin agarose gel (Takara Shuzo Co., Ltd., Kyoto 600-91, Japan), reverse-phase Sep-Pak C_{18} cartridge (Waters).

Protease Digestions of Transglutaminase. We digested the enzyme with Pronase to obtain an amino-terminal peptide. To 8 mg (0.1 μmol) of transglutaminase in 1.5 mL of 10 mM Tris-HCl buffer (pH 8.2) containing 1 mM EDTA was added 0.1 mg of Pronase dissolved in 0.1 mL of the same buffer. Digestion was done at 37 °C. After 2 h, an additional 50 μg of Pronase was added, and the digestion was allowed to proceed for 15 h. The digest was cooled on ice and its pH adjusted to between 2 and 3 by the addition of 2 M HCl before we used the digest for ion-exchange chromatography with SP-Sephadex C-25. We digested the enzyme with trypsin to obtain a carboxyl-terminal peptide. To 0.9 mg (11 nmol) of transglutaminase in 400 μL of 0.2 M Tris-HCl buffer (pH 8.2) containing 20 mM $CaCl_2$ was added 8 μg of TPCK-treated trypsin dissolved in 8 μL of 1 mM HCl containing 20 mM $CaCl_2$. Digestion was done at 25 °C. After 3 h, an additional 4 μg of TPCK-treated trypsin was added, and the digestion was allowed to proceed for 13 h. The reaction was stopped by the addition of 4 μL of 0.1 M diisopropyl fluorophosphate. The pH of the digest had been adjusted to between 5.0 and 5.5 by the addition of 1 M acetic acid before we applied the digest to affinity chromatography with anhydrotrypsin agarose.

Purification of a Candidate for the Carboxyl-Terminal Peptide. The unadsorbed fraction (120 mL) of the anhydrotrypsin agarose gel chromatography (see Results) was pretreated with a reverse-phase Sep-Pak C_{18} cartridge, put into the cartridge, washed with 10 mL of water, and eluted with 5 mL of 65% acetonitrile/0.1% TFA.[1] The eluted fraction was lyophilized, dissolved in 400 μL of 0.1% TFA, and put on a Cosmosil $5C_{18}$ column (4.6 × 150 mm) equilibrated with 0.1% TFA, and a linear gradient of 0–64% acetonitrile/0.1% TFA was applied over 32 min at a flow rate of 1 mL/min. Fractions of the main peak were pooled as a fraction containing the candidate for the carboxyl-terminal peptide.

Amino Acid Analysis and Sequencing of Peptides. The amino acid composition of the peptide was analyzed with a PICO-TAG amino acid analyzing system (Waters) that includes hydrolysis with HCl gas under evacuated conditions and separation of phenylthiocarbamylated amino acids by reverse-phase HPLC. Amino acid sequences of the protein and isolated peptides were analyzed with a gas-phase protein sequencer (Applied Biosystems Model 477A). PTH amino acids were quantitated by comparison with known standards by use of an on-line PTH amino acid analyzer (Applied Biosystems Model 120A).

Treatment with Acylamino-Acid-Releasing Enzyme. To a peptide sample (2 nmol) in 80 μL of 10 mM sodium phosphate buffer (pH 7.2) was added 0.1 unit of acylamino-acid-releasing enzyme dissolved in 40 μL of 5 mM sodium phosphate buffer (pH 7.2) containing 1 mM 2-mercaptoethanol. The reaction mixture was incubated for 1 h at 37 °C and then put into reverse-phase HPLC to purify a deacylaminoacylated peptide. The HPLC was done under the conditions described in the legend to Figure 1.

Fast Atom Bombardment Mass Spectrometry (FABMS). Mass spectra were obtained by using a JMS-HX110 high-resolution mass spectrometer (JEOL). Samples (2–10 nmol) to be analyzed were dissolved in a small amount of glycerol and introduced into the ion source on a stainless probe tip via a conventional vacuum lock. The sample matrix was bombarded by xenon ion/atoms.

Results

Analyses of Amino-Terminal Peptides. Amino-terminal sequence analysis of guinea pig liver transglutaminase using a gas-phase protein sequencer failed to detect any amino-terminal residue. This result was compatible with the finding of Connellan et al. (1971) that the α-amino group of the amino-terminal residue of the liver enzyme is blocked. Therefore, we isolated an amino-terminal peptide by protease digestion and cation-exchange column chromatography. A Pronase digest prepared from 8 mg of transglutaminase was applied to a SP-Sephadex C-25 column (1.0 × 4.5 cm) equilibrated with 0.01 M formic acid at 4 °C. After the application, this column was washed with 10 mL of cold 0.01 M formic acid. The effluent that should contain acidic peptides corresponding to the blocked amino-terminal peptides was lyophilized, dissolved in 150 μL of 0.1% TFA, and separated on a reverse-phase HPLC (Figure 1). Two main peaks (NP-1 and NP-2) were found. As we expected, no amino-

[1] Abbreviations: HPLC, high-performance liquid chromatography; PTH, phenylthiohydantoin; TFA, trifluoroacetic acid; FABMS, fast atom bombardment mass spectrometry.

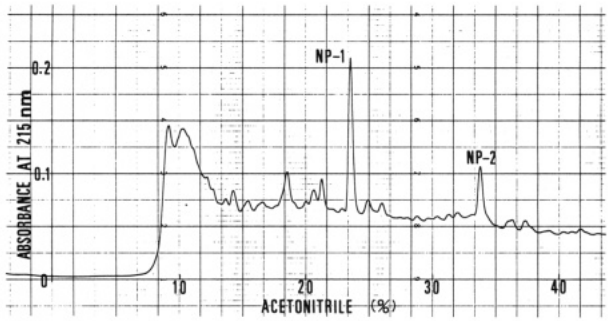

FIGURE 1: Separation of N^{α}-blocked peptides derived from the Pronase digest of guinea pig liver transglutaminase on reverse-phase HPLC. The unadsorbed fraction of the SP-Sephadex C-25 column chromatography (see text) was put on a Cosmosil $5C_{18}$ column (4.6 × 150 mm, Nakarai Chemicals) equilibrated with 0.1% TFA, and a linear gradient of 0–50% acetonitrile/0.1% TFA was applied over 30 min at a flow rate of 1 mL/min. Two main peaks were designated NP-1 and NP-2, respectively.

Table I: Amino Acid Composition of Acidic Peptides Isolated from Pronase Digest of Transglutaminase

peptide	amino acid	molar ratio	peptide	amino acid	molar ratio
NP-1	Ala	0.9	NP-2	Ala	1.2
	Glx[a]	1.2		Glx[a]	2.3
	Asp	0.8		Asp	1.1
	Leu	1.0		Leu	2.0
				Ile	1.0

amino-terminal sequence predicted from cDNA sequences:[b]

$$\overset{1}{\text{Ala}}\text{-Glu-Asp-Leu-}\overset{5}{\text{Ile}}\text{-Leu-Glu-Arg-Cys-}\overset{10}{\text{Asp}}$$

[a] Glx; Glu + Gln. [b] Ikura et al. (1988).

Table II: Amino Acid Composition of NP-1 and NP-2 Peptides Treated with Acylamino-Acid-Releasing Enzyme

peptide treated with enzyme	amino acid	molar ratio	peptide treated with enzyme	amino acid	molar ratio
NP-1	Glx[a]	0.8	NP-2	Glx[a]	2.0
	Asp	1.0		Asp	1.1
	Leu	1.0		Leu	2.1
				Ile	1.0

[a] Glx; Glu + Gln.

terminal residues were detected with both NP-1 and NP-2 peptides on the amino-terminal sequence analysis using a protein sequencer. The amino acid compositions of NP-1 and NP-2 peptides agreed well with those of the tetrapeptide and heptapeptide portions of the amino-terminal sequence predicted from the cDNA sequence (Ikura et al., 1988), respectively (Table I).

Evidence that the amino-terminal residue of these NP-1 and NP-2 peptides is acylalanine was obtained by using an acylamino-acid-releasing enzyme (*N*-acylaminoacyl-peptide hydrolase, EC 3.4.19.1). After incubation of NP-1 and NP-2 peptides with this enzyme, the peptides expected to be in a truncated form were isolated by reverse-phase HPLC. The results of amino acid analysis of the isolated peptides are shown in Table II. The peptides derived from NP-1 and NP-2 were sequenced to be Glu-Asp-Leu and Glu-Asp-Leu-Ile-Leu-Glu, respectively. These results, together with those shown in Table I, indicated that the amino-terminal residue of NP-1 and NP-2 peptides is acylalanine and that NP-1 and NP-2 should be the tetrapeptide and heptapeptide, respectively. Amino acid sequences of these peptides (NP-1, acylAla-Glu-Asp-Leu; NP-2, acylAla-Glu-Asp-Leu-Ile-Leu-Glu) were completely consistent with the predicted amino-terminal sequences corresponding to residues 1–4 and 1–7 (see Table I).

Table III: Amino Acid Composition of Candidate for Carboxyl-Terminal Peptide

amino acid	molar ratio	predicted molar ratio[a]	amino acid	molar ratio	predicted molar ratio[a]
Asx[b]	0.9	1.0	Gly	1.2	1.0
Val	0.8	1.0	Pro	0.9	1.0
Ile	2.1	2.0	Ala	1.0	1.0

[a] Calculated from the amino acid composition of the carboxyl-terminal heptapeptide of the predicted sequence (Ikura et al., 1988). [b] Asx; Asp + Asn.

FABMS was done to measure the protonated weight of NP-1 and NP-2 peptides directly and to find what kind of acylation had occurred at the amino-terminal alanine residue. With NP-1 peptide, the signal corresponding to the $(M + H)^+$ ion of the acetylAla-Glu-Asp-Leu was detected at m/z 489. The spectrum obtained with NP-2 peptide demonstrated that the $(M + H)^+$ ion at m/z 844 was identical with the value predicted for acetylAla-Glu-Asp-Leu-Ile-Leu-Glu. The spectrum also showed the signals corresponding to the values predicted for acetylAla-Glu-Asp-Leu-Ile-Leu-CO (m/z 697), acetylAla-Glu-Asp-Leu-Ile-CO (m/z 584), acetylAla-Glu-Asp-Leu-CO (m/z 471), and acetylAla-Glu-Asp-CO (m/z 358). These results indicated that the amino-terminal alanine residue of NP-1 and NP-2 peptides is acetylated. The partial sequence of NP-2 peptide deduced from the data of FABMS (–Leu-Ile-Leu-Glu) was compatible with the sequence data described above.

From these findings we concluded that the amino-terminal sequence of guinea pig liver transglutaminase is acetylAla-Glu-Asp-Leu-Ile-Leu-Glu, and this enzyme is processed posttranslationally at its amino-terminal portion as we presumed previously (Ikura et al., 1988).

Analyses of Carboxyl-Terminal Peptide. Since none of the experiments using carboxypeptidases P, Y, and A provided interpretable data on the carboxyl-terminal sequence of guinea pig liver transglutaminase, we decided to analyze the carboxyl-terminal peptide that should be obtained from the protease digest of the enzyme directly. We used trypsin digestion and affinity chromatography with anhydrotrypsin agarose gel (Arisaka et al., 1987) for the following reasons. The anhydrotrypsin agarose gel specifically adsorbs peptides having lysine or arginine residues at their carboxyl termini. None of the carboxypeptidase experiments released lysine and arginine from the liver transglutaminase during the early period of the peptidase reaction, suggesting that the transglutaminase has neither carboxyl-terminal lysine nor arginine residues. The carboxyl-terminal residue of the predicted sequence of this enzyme was alanine. Therefore, the carboxyl-terminal peptide in the trypsin digest of transglutaminase should be selectively recovered in the unadsorbed fraction on the affinity chromatography with anhydrotrypsin agarose gel. One-tenth of the trypsin digest prepared from 0.9 mg of transglutaminase was put on an anhydrotrypsin agarose gel column (0.8 × 2.0 cm) equilibrated with 50 mM sodium acetate buffer (pH 5.0) containing 20 mM $CaCl_2$. After this, the column was washed with 20 mL of the same buffer, and the effluent, which should contain the carboxyl-terminal peptide, was pooled. The unadsorbed fractions obtained from six chromatographies were collected and subjected to reverse-phase HPLC to purify the candidate for the carboxyl-terminal peptide (see Materials and Methods). The main peak fraction believed to contain a single peptide, the candidate peptide, was pooled. Analyses of amino acid composition and sequence were done on the peptide in the pooled fraction

Table IV: Sequence Analysis of Candidate for Carboxyl-Terminal Peptide[a]

cycle	PTH derivatives	quantity (pmol)	cycle	PTH derivatives	quantity (pmol)
1	Asn	64	5	Gly	9
2	Val	26	6	Pro	5
3	Ile	20	7	Ala	4
4	Ile	11	8	*b*	*b*

[a] The candidate peptide (100 pmol) was sequenced with a protein sequencer. [b] No PTH derivatives were identified in cycle 8.

(Tables III and IV). The amino acid composition of this peptide and its sequence agreed well with those of the predicted carboxyl-terminal heptapeptide sequence of transglutaminase. Therefore, we concluded that the carboxyl-terminal sequence of guinea pig liver transglutaminase is Asn-Val-Ile-Ile-Gly-Pro-Ala, and this enzyme is not processed posttranslationally in the carboxyl-terminal region.

Discussion

With guinea pig liver transglutaminase, amino acid sequences in both amino- and carboxyl-terminal ends predicted from the cDNA (NH_2-Ala-Glu- and -Pro-Ala-COOH; Ikura et al., 1988) were not consistent with those reported previously (pyroGlu-Ala- and -Ser-Gly-COOH; Connellan et al., 1971). Here we tried to confirm the terminal sequences and examine the possibility of terminal processing of this enzyme.

We took the following strategy to identify both the amino- and carboxyl-terminal sequences of guinea pig liver transglutaminase. First, the candidates for terminal peptides were isolated from a protease digest of the enzyme, and then the isolated peptides were sequenced. Finally their sequences were compared with the terminal ones predicted from the cDNA sequence. The complete agreement between the sequences of the candidate peptides obtained here and the predicted terminal sequences substantiated that these peptides are true terminal peptides of guinea pig liver transglutaminase. The reasons for the discrepancy between our data and those of Connellan et al. (1971) are not known. As we discussed in the previous paper (Ikura et al., 1988), a genetic polymorphism of guinea pig liver transglutaminase remains to be studied.

Our results verified the assumption that guinea pig liver transglutaminase is processed after or in the course of translation at its amino-terminal site, namely, the removal of the initiator methionine and a subsequent N^{α}-acetylation of the alanine residue adjacent to the methionine, probably by methionine aminopeptidase (Yoshida & Lin, 1972) and amino-terminal acetyltransferase (Tsunasawa & Sakiyama, 1984). This finding supported the paper of Boissel et al. (1985) that described the specificity of amino-terminal processing on eukaryotic proteins; the presence of alanine, serine, or glycine next to the initiator methionine favors removal of the initiator methionine and N^{α}-acetylation of the newly exposed amino-terminal residue. The N^{α}-acetylation of protein is presumed to confer protection against unwanted events such as proteolytic attack and nonenzymic modification of the amino group by reactive metabolites (Boissel et al., 1985). The functional role of the N^{α}-acetyl group in transglutaminase is an open question. Recently we have succeeded in expressing the transglutaminase cDNA covering the entire coding region in *Escherichia coli* and obtaining the active enzyme protein (unpublished experiments). This expression system may be useful in understanding the significance of the amino-terminal processing of this enzyme, because *E. coli* may produce an unprocessed transglutaminase, and it will also be possible to produce transglutaminases that have different amino-terminal structures by site-directed mutagenesis. A catalytic subunit of human factor XIII, a zymogenic transglutaminase, is also N^{α}-acetylated at the amino-terminal serine residue positioned next to the initiator methionine (Takahashi et al., 1986).

Our results did not indicate any carboxyl-terminal processing of guinea pig liver transglutaminase. In the case of the catalytic subunit of factor XIII, the methionine deduced as the carboxyl-terminal residue from its cDNA (Grundmann et al., 1986; Ichinose et al., 1986) is not present at the carboxyl-terminal end, and the carboxyl terminus is heterogeneous (Takahashi et al., 1986). This heterogeneity is considered to be attributable to posttranscriptional processing.

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Interaction of Clotting Factor V Heavy Chain with Prothrombin and Prethrombin 1 and Role of Activated Protein C in Regulating This Interaction: Analysis by Analytical Ultracentrifugation†

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ABSTRACT: Changes in the affinity of the heavy subunit of blood coagulation factor Va (Vh) for prothrombin are thought to be important in regulating the rate of thrombin production. Using analytical ultracentrifugation, we have measured the affinity of bovine Vh for prothrombin and for the prethrombin 1 fragment of prothrombin at 23.3 °C, pH 7.65, in 50 mM tris(hydroxymethyl)aminomethane, 0.1 M NaCl, 0.1 mM benzamidine, and either 2 mM Ca^{2+} or 2 mM ethylenediaminetetraacetate (EDTA). Under these conditions a 1:1 complex of Vh with prothrombin is formed that is governed by a dissociation constant (K_d) of 10 μM, regardless of whether the buffer contains Ca^{2+} or EDTA. An identical K_d is observed when prethrombin 1 is substituted for prothrombin. This indicates that the fragment 1 portion of prothrombin, containing the γ-carboxyglutamic acid residues, does not influence the association. Substitution of human prethrombin 1 for the bovine molecule also results in a 1:1 Vh–prethrombin 1 complex governed by a slightly weaker K_d (27 μM). Discrete proteolysis of bovine Vh by the anticoagulant activated protein C converts the Vh to a form with little or no affinity for prethrombin 1 ($K_d > 1$ mM), without detectable change in the mass of the Vh.

Previous studies have shown that factor Va is a critical regulatory protein in the factor Xa catalyzed activation of prothrombin (Mann, 1984). At least two separate roles have been postulated for factor Va function: (1) binding to factor Xa resulting in subsequent alteration of factor Xa conformation and improved catalytic efficiency (Mann, 1984; Nesheim et al., 1979; Tracy et al., 1981; Husten et al., 1987; Krishnaswamy et al., 1987; Rosing et al., 1980) and (2) binding to the prothrombin, thereby enhancing affinity for membrane surfaces and/or altering the conformation of the substrate (Hemker et al., 1967; Esmon et al., 1973; Van de Waart et al., 1984b; Guinto & Esmon, 1984). Support for the concept that prothrombin binding plays a role in the activation complex comes from the kinetics studies cited above, as well as the observation that factor V activation leads to the formation of a substrate binding site and that factor Va inactivation leads to the loss of this site (Esmon et al., 1973; Guinto & Esmon, 1984). Available information suggests that the major binding site for prothrombin is in the factor Va heavy chain (Vh)[1] and that calcium is not obligatory for the binding. However, no quantitative studies have been performed to evaluate the affinity of the interaction or to determine the role of the metal in this process (Guinto & Esmon, 1984). Further, while it is known that activated protein C proteolysis of Vh prevents binding of this chain to immobilized prothrombin, the extent the association is perturbed by proteolysis is not known.

We have used sedimentation equilibrium analysis to quantitate the structure–function relationships in the interaction between Vh and prothrombin. In these studies we have examined both prothrombin, which contains the γ-carboxyglutamic acid residues required for Ca^{2+} binding, and prethrombin 1, which lacks these residues. Further, we have examined the influence of activated protein C proteolysis of Vh

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[1] Abbreviations: EDTA, ethylenediaminetetraacetate; SDS, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; rms, root mean square; rpm, revolutions per minute; K_d, molar dissociation constant; Vh, heavy subunit of thrombin-activated blood coagulation factor V.

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