Activation of Bovine Factor VII (Proconvertin) by Factor XII_a (Activated Hageman Factor)†

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ABSTRACT: Bovine factor VII (proconvertin) is a plasma glycoprotein that participates in the extrinsic pathway of blood coagulation. It has a molecular weight of 45 500 and is composed of a single polypeptide chain with an amino-terminal alanine residue. Factor VII is readily converted to factor VII_a by factor XII_a (activated Hageman factor) employing an enzyme to substrate weight ratio of 1:50. Factor VII_a is composed of a light and a heavy chain held together by a disulfide bond(s). The heavy chain, which is formed from the carboxyl-terminal region of the precursor, contains an amino-terminal sequence of Ile-Val-Gly-Gly-. The heavy chain also contains the active-site sequence of -Phe-Cys-Ala-Gly-Tyr-Thr-Asp-Gly-Thr-Lys-Asp-Ala-Cys-Lys-Gly-Asp-Ser-Gly-Gly-Pro-His-. This sequence is homologous with the active-site region of a number of plasma serine proteases. These data indicate that factor VII is a typical precursor of a serine protease which is converted to an enzyme by factor XII_a by the cleavage of a single, internal peptide bond.

Bovine factor VII (proconvertin, precursor of serum prothrombin conversion accelerator)[1] is a trace plasma protein which participates in the extrinsic pathway of blood coagulation. This plasma protein in the presence of tissue factor converts factor X to factor X_a (Flynn and Coon, 1953; Hjort, 1957; Hougie, 1959; Straub and Duckert, 1961; Deutsch et al., 1964; Williams and Norris, 1966; Nemerson and Pitlick, 1970; Osterud et al., 1972; Jesty and Nemerson, 1974; Fujikawa et al., 1974). In this reaction, a specific arginyl–isoleucine peptide bond in the heavy chain of factor X is cleaved giving rise to an activation peptide and factor $X_{a\alpha}$ (Fujikawa et al., 1975). Present evidence indicates that factor VII is activated prior to its participation in the activation of factor X. The enzyme(s) responsible for this activation has not been identified, but presumably involves some activated coagulation factor which is formed prior to the participation of factor VII in the coagulation process. Once factor X_a and thrombin are formed, they will in turn activate any remaining factor VII (Radcliffe and Nemerson, 1975, 1976). Accordingly, it has been suggested that the activation of factor VII by factor X_a and thrombin may constitute an important feedback control mechanism.

Other pathways for the activation of factor VII have also been implicated. For instance, the proteins that participate in the contact system involving glass, celite, or kaolin may play a role in the activation of factor VII (Rapaport et al., 1955; Soulier and Prou-Wartelle, 1960; Altman and Hemker, 1967; Shanberge and Matsuoka, 1966; Gjonnaess, 1972a,b). Indeed, factor XII_a, kallikrein, factor IX_a, and plasmin have been reported recently to be directly involved in the activation of factor VII (Laake and Ellingsen, 1974; Laake and Osterud, 1974; Saito and Ratnoff, 1975).

In the present communication, we report the activation of bovine factor VII by factor XII_a employing pure preparations of bovine factors VII (Kisiel and Davie, 1975) and XII_a (Fujikawa et al., 1977a). In this reaction, factor VII is converted to factor VII_a by the cleavage of an internal peptide bond giving rise to a two-chain molecule held together by a disulfide bond(s).

Experimental Section

Materials. Soybean trypsin inhibitor (type II-S), bovine serum albumin, ovalbumin, carbonic anhydrase, myoglobin, imidazole (grade I), *p*-aminobenzamidine hydrochloride, dithiothreitol, and 2-(*N*-morpholino)ethanesulfonic acid were obtained from Sigma Chemical Co., St. Louis, Mo. Benzamidine hydrochloride, cyclohexylmorpholinoethylcarbodiimide, diisopropyl phosphorofluoridate (DFP)[2] and 4-vinylpyridine were purchased from Aldrich Chemical Co., Milwaukee, Wis. DEAE-Sephadex A-50, Sephadex G-25, G-75, and G-150 were products of Pharmacia Fine Chemicals, Piscataway, N.J. 2-Mercaptoethanol and *N,N,N′,N′*-tetramethylethylenediamine were purchased from Eastman Kodak Co., Rochester, N.Y. Acrylamide, *N,N′*-methylenebisacrylamide and Bio-Gel A-15m (agarose) were obtained from Bio-Rad Laboratories, Richmond, Calif. Guanidine hydrochloride was purchased from Heico, Inc., Delaware Water Gap, Pa. Diisopropyl *N*-[1-^{3}H]fluorophosphate (0.9 Ci per mmol) was obtained from New England Nuclear, Boston, Mass. Sodium dodecyl sulfate was purchased from British Drug House, Poole, England. Urea (ultrapure) was obtained from Schwarz/Mann, Van Nuys, Calif. Cyanogen bromide and the sequenator reagents (Sequanol grade) were obtained from Pierce Chemical Co., Rockfold, Ill. Bovine brain thromboplastin and bovine factor VII deficient plasma were prepared as previously described (Kisiel and Davie, 1975). Bovine factor XII_a was purified to homogeneity (Fujikawa et al., 1977a). Phosphatidylcholine and phosphatidylserine purified from bovine brain were obtained from Applied Science Laboratories, State College, Pa. Dialysis tubing was treated according to McPhie (1971) prior to use. All other chemicals were commercial preparations of the highest quality available.

Methods. Amino acid analyses were performed according to Moore and Stein (1963) employing a Durrum Model D500 amino acid analyzer. Samples were hydrolyzed at 110 °C in 6 N HCl for 24, 48 and 72 h in evacuated tubes. Valine, isoleucine, and leucine values were those determined for the 72-h

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[1] The nomenclature for the various coagulation factors is that recommended by an international nomenclature committee (Wright, 1959).

[2] Abbreviations used are: DFP, diisopropyl phosphorofluoridate; DEAE, diethylaminoethyl; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol.

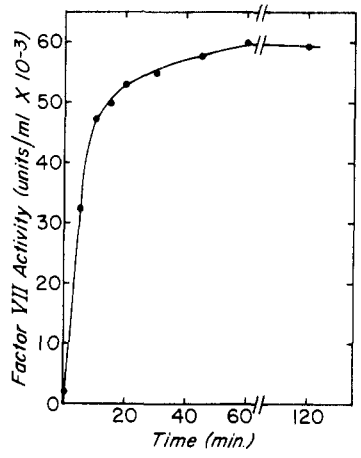

FIGURE 1: Time curve for the activation of factor VII by factor XII_a. Factor VII (1 mg) was incubated at 37 °C with factor XII_a (20 μg) in 0.05 M Tris-HCl, pH 7.5, containing 0.1 M NaCl. The final volume was 1.5 mL. At the desired times, 10 μL of the reaction mixture was withdrawn and assayed for factor VII activity as described under Methods.

hydrolysis. Threonine and serine content was determined by extrapolation to zero-time hydrolysis on semilogarithmic paper. Half-cystine was determined as pyridylethylcysteine according to Friedman et al. (1970).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed as previously described (Kisiel et al., 1976) employing 10% acrylamide gels.

Reduced and alkylated heavy and light chains of factor VII_a were prepared as follows: Factor VII (10 mg) was incubated at 37 °C with 200–300 μg of purified factor XII_a in the presence of 0.05 M Tris-HCl/0.1 M NaCl (pH 7.5) in a final volume of 10 mL. The reaction was terminated after 60 min by the addition of 0.5 mL of 1 M DFP. The reaction mixture was then desalted by gel filtration on a Sephadex G-25 column (2.6 × 30 cm) previously equilibrated with 0.1 M NH_4HCO_3. Salt-free protein, obtained by lyophilization, was reduced and *S*-pyridylethylated according to Friedman et al. (1970). Salt and excess reagents were removed by gel filtration on a Sephadex G-25 column (2.6 × 65 cm) equilibrated with 2.3 M formic acid. Void volume fractions from the column were combined and lyophilized. The heavy and light chains were separated by gel filtration on a Sephadex G-150 column (1.6 × 95 cm) previously equilibrated with 2.3 M formic acid containing 3 M urea. Salt-free protein was obtained by gel filtration on Sephadex G-25 equilibrated with 1.2 M formic acid followed by lyophilization.

Cyanogen bromide digestion was carried out by dissolving 15 mg of salt-free pyridylethylated factor VII and 30 mg of cyanogen bromide in 15 mL of 18.7 M formic acid and incubating the reaction mixture at 4 °C for 24 h. The reaction was terminated by a tenfold dilution with water followed by lyophilization. Cyanogen bromide fragments of pyridylethylated factor VII were separated by gel filtration on a Sephadex G-75 column (1.6 × 95 cm) previously equilibrated with 2.3 M formic acid in 3 M urea. Urea was separated from the peptides by gel filtration on a Sephadex G-25 column previously equilibrated with 1.2 M formic acid, and the salt-free peptides were subsequently lyophilized.

Amino-terminal sequence analyses were performed with a Beckman sequencer (Model 890A) utilizing the dimethylbenzylamine system developed by Hermodson et al. (1972). Phenylthiohydantoin amino acids were quantitated by gas chromatography after silylation or directly by high-pressure liquid chromatography (Bridgen et al., 1976). The protein mass subjected to sequence analysis was determined by amino acid analysis. The analysis of the cyanogen bromide fragment containing the active site of factor VII was carried out on two different preparations of the *S*-pyridylethyl peptide. In each case, approximately 100 nmol of the peptide (assuming a molecular weight of 8500) was subjected to analysis. Amino-terminal analyses of the S-pyridylethylated heavy and light chains of factor VII_a were performed on a single preparation of each peptide. In these experiments, approximately 50 nmol of each peptide was subjected to sequence analysis (assuming a molecular weight of 34 000 and 23 000 for the heavy and light chains, respectively).

Purification of Bovine Factor VII. Factor VII was partially purified from bovine plasma by a combination of barium citrate adsorption, ammonium sulfate fractionation, and DEAE-Sephadex A-50 column chromatography. The isolation procedure was carried out at 4 °C and was essentially that described by Stenflo (1976) for the isolation of vitamin K dependent proteins, except that Trasylol was replaced with crude soybean trypsin inhibitor. Factor VII was then purified to homogeneity by a modification of the procedure previously described (Kisiel and Davie, 1975). The fractions from DEAE-Sephadex column chromatography containing factor VII activity were pooled, concentrated by ultrafiltration to 100 mL, and applied to a benzamidine-agarose column (2.6 × 65 cm) previously equilibrated with 0.05 M imidazole hydrochloride/0.1 M NaCl/1 mM benzamidine (pH 6.5). After application of the sample, the column was washed with 3–4 column volumes of 0.05 M imidazole hydrochloride/1.0 M NaCl/1 mM benzamidine (pH 6.5). Factor VII was eluted from the column with 500 mL of the above washing buffer containing 1 M guanidine hydrochloride. The fractions containing factor VII activity were pooled and dialyzed overnight against 4 L of 0.05 M imidazole hydrochloride/0.1 M NaCl/1 mM benzamidine (pH 6.5). The dialyzed sample was rechromatographed on a benzamidine-agarose column (1.6 × 60 cm) under the same conditions as the previous chromatography except that factor VII was eluted from the column with a linear gradient of guanidine hydrochloride arising from 250 mL of 0.05 M imidazole hydrochloride/1 M NaCl/1 mM benzamidine (pH 6.5) and 250 mL 0.05 M imidazole hydrochloride/1 M NaCl/1 M guanidine hydrochloride/1 mM benzamidine (pH 6.5). Factor VII obtained from benzamidine-agarose rechromatography contained approximately 30% prothrombin. The latter was separated from factor VII by preparative discontinuous electrophoresis (Kisiel and Davie, 1975). Factor VII obtained from preparative electrophoresis was concentrated by ultrafiltration in the presence of 10 mM benzamidine and stored at −20 °C. Throughout the isolation procedure, factor VII was assayed as previously described (Kisiel and Davie, 1975).

Results

Activation of Bovine Factor VII. A time curve for the activation of bovine factor VII by factor XII_a is shown in Figure 1. In these experiments, an enzyme–substrate ratio of 1:50 (w/w) was employed. The increase in coagulant activity was approximately 30-fold. The activation reaction was essentially complete in 60 min and no loss of activity occurred during the next 60 min. The increase in coagulant activity varied in different experiments and ranged from about 10- to 30-fold. In control experiments employing factors VII and XII, no activation occurred after 60 min. In the presence of kaolin, approximately 5% activation was observed after 60 min employing a 1:50 ratio of factor XII to factor VII. Factor VII_a, like factor VII, still had an absolute requirement for tissue

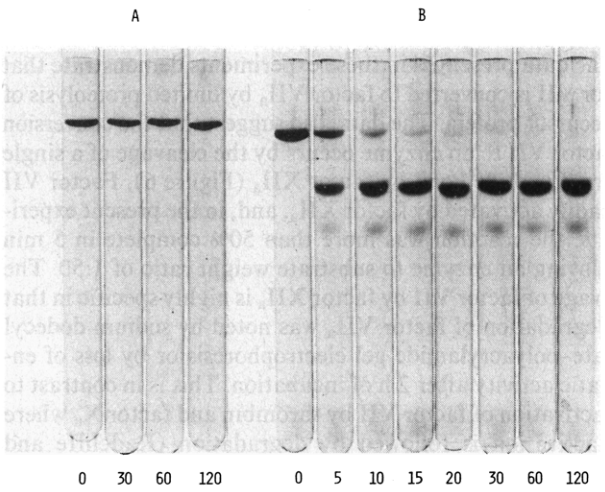

FIGURE 2: Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of factor VII following activation by factor XII_a. Each sample (20 μL containing 15 μg of protein) was removed from a reaction mixture corresponding to that shown in Figure 1 and incubated at 100 °C for 5 min with 5% sodium dodecyl sulfate in the absence (panel A) or presence (panel B) of 5% 2-mercaptoethanol. Electrophoresis was carried out as described under Methods. The numbers at the bottom of the gels represent incubation times. The anode is at the bottom of the gels.

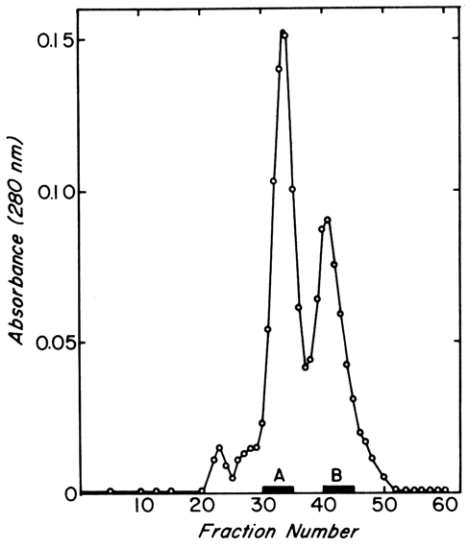

FIGURE 3: Separation of the heavy and light chains of factor VII_a on a Sephadex G-150 column. The S-pyridylethylated heavy and light chains of factor VII_a (5 mg) were applied to a Sephadex G-150 column (1.6 × 95 cm) previously equilibrated with 2.3 M formic acid containing 3 M urea. Elution was carried out with 2.3 M formic acid containing 3 M urea at a flow rate of 7.5 mL/h. Fractions containing 2 mL/tube were collected. Fractions (solid bars) containing the first peak (heavy chain) and the second peak (light chain) were pooled separately, desalted, and lyophilized as described under Methods.

factor in the coagulant assay. Substitution of bovine brain cephalin (Bell and Alton, 1954) or a sonicated aqueous dispersion of an equimolar mixture of phosphatidylserine and phosphatidylcholine for thromboplastin resulted in complete loss of factor VII activity. In these experiments, the final concentration of phospholipid was 0.1 mg/mL.

The molecular changes in factor VII that occurred during the activation reaction were then examined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. In these experiments, aliquots were removed at various times from an activation mixture corresponding to those shown in Figure 1 and analyzed by gel electrophoresis (panel A, Figure 2). At zero time, a major protein band corresponding to factor VII was observed. After 30, 60, and 120 min, a single protein band was still present, and this protein migrated at the same rate as factor VII. These experiments indicate that little, if any, change in the molecular weight of factor VII occurred during the activation reaction.

A change in the structure of factor VII during the activation reaction was noted, however, following reduction of the samples with 2-mercaptoethanol (panel B, Figure 2). At zero time, a single band was observed for factor VII corresponding to the single-chain precursor molecule. During the first few minutes of incubation, two new faster-moving bands appeared. After 60 min, all of the single-chain factor VII was converted to the two-chain molecule. These two bands indicate the presence of a light and a heavy chain in factor VII_a. The heavy chain has an apparent molecular weight of 34 000, and the light chain has an apparent molecular weight of 23 000 as determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The formation of the heavy and light chains occurred in parallel with the activation of factor VII. These data indicate that factor VII is activated by the cleavage of an internal peptide bond, and this leads to the formation of factor VII_a, an enzyme which is composed of a heavy and a light chain held together by a disulfide bond(s).

Characterization of Bovine Factor VII_a. In order to characterize further factor VII_a, the enzyme was incubated for 1 h at room temperature with 1×10^{-6} M radiolabeled DFP, reduced, pyridylethylated, and the heavy and light chains were separated by gel filtration on a Sephadex G-150 column (Figure 3). Peak A corresponded to the heavy chain of factor VII_a, and peak B corresponded to the light chain of factor VII_a as shown by sodium dodecyl sulfate–polyacrylamide gel electrophoresis experiments. All of the radioactivity was located in the heavy chain. Amino-terminal analyses of the heavy and light chains were then carried out in a Beckman sequenator. The amino-terminal sequence for the light chain was Ala-Asn-Gly-Phe-. This corresponds to the amino-terminal sequence of the intact protein (Kisiel and Davie, 1975). The yield of amino-terminal alanine was approximately 0.8 equiv/mol of protein assuming a molecular weight of 23 000. The yield for residues 2, 3, and 4 was not quantitated. The amino-terminal sequence for the heavy chain was Ile-Val-Gly-Gly-. The yield of amino-terminal isoleucine was approximately 0.8 equiv/mol of protein assuming a molecular weight of 34 000. The yield for residues 2, 3, and 4 was 0.6, 0.4 and 0.4 equiv, respectively. These data demonstrate that the heavy chain of factor VII_a originated from the carboxyl-terminal end of the precursor protein. Furthermore, the active-site region is located in the heavy chain of the enzyme.

Active-Site Region of Bovine Factor VII. It was also of interest to examine the amino acid sequence of factor VII in the active-site region. The possibility of readily isolating a peptide containing this region of factor VII seemed feasible, since an amino acid analysis of this protein indicated the presence of two methionine residues per mole of protein (Kisiel and Davie, 1975). Furthermore, it seemed probable that one of the two methionine residues would be located 15–20 residues prior to the active-site serine, since methionine in this position is highly conserved in various serine proteases. Accordingly, a cyanogen bromide digestion was made of *S*-pyridylethyl factor VII. The polypeptide fragments were fractionated by gel filtration on Sephadex G-75 (Figure 4). Three major polypeptide peaks were obtained. The second peak migrated as a single band on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (insert, Figure 4) and had a molecular weight of approximately 8500. The amino acid composition of this polypeptide in resi-

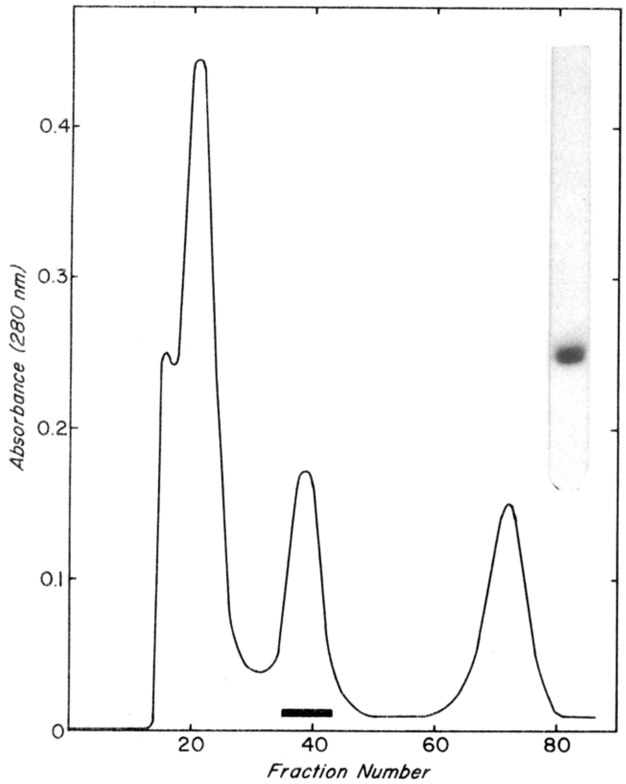

FIGURE 4: Gel filtration of a cyanogen bromide digest of S-pyridylethylated bovine factor VII. The lyophilized digest (15 mg) was dissolved in 2 mL of 2.3 M formic acid containing 3 M urea and applied to a Sephadex G-75 column (1.6 × 95 cm) previously equilibrated with the same solution. The cyanogen bromide peptides were eluted with 2.3 M formic acid in 3 M urea at a flow rate of 23 mL/h. Fractions were collected in 2 mL/tube. The fraction shown by the solid bar was pooled, lyophilized, and employed for sequence analysis. Insert: A sodium dodecyl sulfate–polyacrylamide gel electrophoresis pattern of peak 2. The anode is at the bottom of the gel.

dues per 8500 g of protein was as follows: Lys, 3.4; His, 2.8; Arg, 6.2; Asp, 5.3; Thr, 6.5; Ser, 8.1; Glu, 4.5; Pro, 1.7; Gly, 14.7; Ala, 10.4; Val, 4.2; Ile, 1.6; Leu, 4.6; Tyr, 3.2; and Phe, 5.2. Trp, Cys, and carbohydrate were not included in the analysis. Phenylalanine (approximately 0.8 equiv/8500 g of protein) was identified as the amino-terminal residue of this polypeptide, and no other amino acids were detected. The sequence of the first 21 residues of this polypeptide is shown in Figure 5. The repetitive yields for the degradations were about 95%. The active-site serine corresponding to serine-195 in chymotrypsin appeared in position 17 of the peptide. These data indicate that bovine factor VII contains an amino acid sequence which is characteristic of the active-site regions of a number of serine proteases that participate in blood coagulation.

Discussion

The data presented in these experiments demonstrate that factor VII is converted to factor VII_a by limited proteolysis of a precursor protein. The data also suggest that the conversion of factor VII to an enzyme occurs by the cleavage of a single internal peptide bond by factor XII_a (Figure 6). Factor VII is readily activated by factor XII_a, and, in the present experiments, the reaction was more than 50% complete in 5 min employing an enzyme to substrate weight ratio of 1:50. The cleavage of factor VII by factor XII_a is highly specific in that no degradation of factor VII_a was noted by sodium dodecyl sulfate–polyacrylamide gel electrophoresis or by loss of enzymatic activity after 2 h of incubation. This is in contrast to the activation of factor VII by thrombin and factor X_a where the activation is followed by degradation (Radcliffe and Nemerson, 1976). The mechanism of activation of factor VII by factor XII_a, however, appears to be essentially identical to that described by Radcliffe and Nemerson (1976) for the activation of factor VII by factor X_a or thrombin. These investigators showed that either factor X_a or thrombin cleaved a specific arginyl–isoleucine bond during the activation reaction.

Factor VII contains an active-site sequence which is homologous with other serine proteases (Figure 5). Accordingly, it seems likely that the basic mechanism for the proteolytic activation of factor VII is similar to that for the activation of the pancreatic proteases such as chymotrypsinogen and trypsinogen. During the activation of these proenzymes, a new amino-terminal isoleucine is generated and this residue forms an ion pair with the carboxyl group of the aspartic acid adjacent to the active serine (Sigler et al., 1968; Stroud et al., 1975). These reactions lead to the charge-relay network which is characteristic of the pancreatic serine proteases (Matthews et al., 1967; Sigler et al., 1968; Blow et al., 1969; Shotton and Watson, 1970). Factor VII also contains an aspartic acid six residues prior to the active-site serine. This residue is located in the bottom of the binding pocket in trypsin (Stroud et al., 1971) and forms an ion pair with a basic amino acid residue in the substrate (Mares-Guia and Shaw, 1965; Ruhlmann et al., 1973; Blow et al., 1974; Sweet et al., 1974; Krieger et al., 1974). Thus, it seems likely that the aspartic acid occupying a similar position in factor VII_a gives this enzyme a specificity toward basic amino acids. This suggestion is consistent with the role of factor VII_a in the activation of factor X. In this reaction, factor VII_a is apparently the enzyme that cleaves a specific arginyl–isoleucine bond between residues 51 and 52 in the heavy chain of factor X (Fujikawa et al., 1975). It should be pointed out, however, that factor IX_a also cleaves this bond, but differs somewhat in its active-site sequence.

It is also of interest that factor VII as well as factor XI contain lysine three residues prior to the active-site serine

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Factor VII	Phe	Cys	Ala	Gly	Tyr	Thr	-	Asp	Gly	Thr	Lys	-	-	Asp	Ala	Cys	Lys	Gly	Asp	SER	Gly	Gly	Pro	His
Thrombin	Phe	Cys	Ala	Gly	Tyr	Lys	Pro	Gly	Glu	Gly	Lys	Arg	Gly	Asp	Ala	Cys	Glu	Gly	Asp	SER	Gly	Gly	Pro	Phe
Factor IX_a	Phe	Cys	Ala	Gly	Tyr	His	-	Glu	Gly	Gly	Lys	-	-	Asp	Ser	Cys	Gln	Gly	Asp	SER	Gly	Gly	Pro	His
Factor X_a	Phe	Cys	Ala	Gly	Tyr	Asp	-	Thr	Gln	Pro	Glu	-	-	Asp	Ala	Cys	Gln	Gly	Asp	SER	Gly	Gly	Pro	His
Factor XI	Val	Cys	Ala	Gly	Tyr	Arg	-	Glu	Gly	Gly	Lys	-	-	Asp	Ala	Cys	Lys	Gly	Asp	SER	Gly	Gly	Pro	?
Factor XII	Leu	Cys	Ala	Gly	Phe	Leu	-	Glu	Gly	Gly	Thr	-	-	Asp	Ala	Cys	Gln	Gly	Asp	SER	Gly	Gly	Pro	Leu

FIGURE 5: Active-site sequence of bovine factor VII and several other coagulation proteins. Amino acid residues in factor VII that are identical with other coagulation factors are shown in blocks. Dashes refer to spaces that have been inserted to bring the six proteins into alignment for better homology. The question mark refers to an amino acid not known. The active-site serine analogous to serine-195 in chymotrypsin is shown in capital letters. The active-site sequences of thrombin, factors IX_a, X_a, XI, and XII are taken from Magnusson et al. (1975). Enfield et al. (1974), Titani et al. (1975), Koide et al. (1977), and Fujikawa et al. (1977b), respectively.

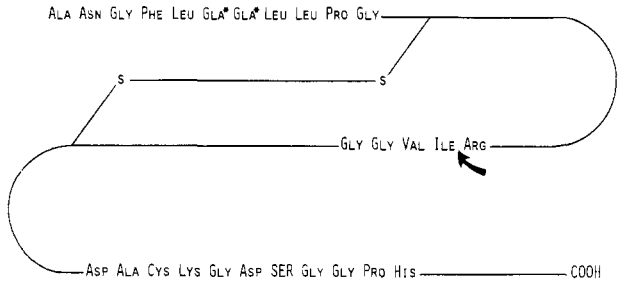

FIGURE 6: Partial structure of bovine factor VII. The arrow indicates the site of cleavage in the protein during its conversion to factor VII_a. The resulting light and heavy chains of factor VII_a are held together by a disulfide bond(s). The Gla residues (γ-carboxyglutamic acid) shown in positions 7 and 8 are tentative. (Taken in part from Kisiel and Davie (1975) and Radcliffe and Nemerson (1976).)

(Figure 5). This position corresponds to methionine-192 in chymotrypsin, glutamine in factor IX_a, factor X_a, and trypsin, and glutamic acid in thrombin. In trypsin and chymotrypsin, residue 192 is located in the substrate-binding pocket. Furthermore, alkylation of methionine-192 in chymotrypsin is accompanied by a tenfold increase in the Michaelis constant over that of the unmodified enzyme (Lawson and Schramm, 1965). Thus, it is possible that some of the differences in the specificity of the various coagulation enzymes may be due to the different amino acids present in this position.

The physiological significance for the activation of factor VII by factor XII_a is not clear. It is possible that this pathway provides an important mechanism for the activation of factor VII prior to its participation in the extrinsic pathway of blood coagulation. Accordingly, the contact activation system could be a major pathway for initiating both the intrinsic and extrinsic pathways of blood coagulation. It is also possible that the activation of factor VII by factor XII_a may be stimulated by high-molecular-weight kininogen, since this protein influences the activation of prekallikrein and factor XI by factor XII_a (Schiffman et al., 1975; Griffin and Cochrane, 1976; Chan et al., 1976).

The size of factors VII and VII_a appears to be identical as judged by sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The molecular weight of factor VII as determined by sedimentation equilibrium studies indicates that it has a minimal molecular weight of about 45 000. Thus, it seems probable that factor VII_a also has a molecular weight of 45 000. The molecular weights estimated for the heavy and light chains for factor VII_a by sodium dodecyl sulfate–polyacrylamide gel electrophoresis were 34 000 and 23 000, respectively. These values are probably high, however, since glycoproteins tend to bind less amounts of detergent resulting in high-molecular-weight estimates (Segrest and Jackson, 1972). Accordingly, the correct values for these chains may be closer to about 27 000 and 18 000, respectively. A summation of these two molecular weights would then yield a value of 45 000 for factor VII_a. More experiments are required, however, to clarify this question.

The mechanism by which factor VII_a is inactivated in serum is not known. Antithrombin III in the presence or absence of heparin has essentially no effect on the bovine enzyme (W. Kisiel, unpublished results). Similar results have been published previously by Osterud et al. (1976) employing human factor VII. Thus, other plasma serine protease inhibitors or inactivation by proteolysis may be of importance in the inactivation of factor VII.

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Carbon-13 Nuclear Magnetic Resonance Studies of the Binding of Selectively ^{13}C-Enriched Oxytocins to the Neurohypophyseal Protein, Bovine Neurophysin II†

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ABSTRACT: Complex formation between bovine neurophysin II and oxytocin molecules containing 85% ^{13}C enrichment in specific amino acid residues was studied using ^{13}C nuclear magnetic resonance spectroscopy. Chemical shift and relaxation time values of the analogue [^{13}C-Leu^3]oxytocin, [^{13}C-Gly^9]oxytocin, and the doubly labeled [^{13}C-Ile^3 Gly^9]oxytocin were obtained for the hormones in the absence and presence of neurophysin. The results showed that certain ^{13}C nuclear magnetic resonance parameters of residue 3 but not of residue 9 of oxytocin are altered upon binding to neurophysin. These observations suggest that residue 3 but not residue 9 is involved in the protein–hormone interaction, and they demonstrate the general applicability of selective ^{13}C enrichment for the study of peptide–protein interactions.

Nuclear magnetic resonance (NMR) spectroscopy can give detailed molecular information about biochemical systems in solution (Roberts and Jardetzky, 1970; Dwek, 1973; e.g., Lasker and Milvy, 1973). With the recent availability of signal enhancement by Fourier transform techniques, ^{13}C NMR studies of biomolecules became feasible and several observations of individual carbon resonances in natural abundance spectra of proteins have been reported (Allerhand et al., 1973; Oldfield et al., 1974; Shindo and Cohen, 1976). ^{13}C NMR studies have also provided new information about peptides and peptide hormones (Deslauriers and Smith, 1975). The studies of selectively ^{13}C-enriched oxytocins reported here were undertaken, first, to demonstrate the utility of ^{13}C enrichment in the study of peptide hormones themselves since the natural abundance of ^{13}C is only 1.1% and, second, to explore the value of selective ^{13}C enrichment in studying the molecular interactions between oxytocin and the neurohypophyseal hormone binding protein, bovine neurophysin (e.g., Walter, 1975). Complex formation between neurophysin and the neurohypophyseal hormones provides a model system for the study of

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