of AP from factor XIII remain to be established.

Promotion of the initial step in the activation of factor XIII by polymeric fibrin may serve to ensure that significant amounts of factor XIII are not activated until its physiological substrate polymeric fibrin is present. This activation scheme would be expected to minimize both wasteful premature activation of factor XIII and deleterious cross-linking of other serum proteins. Additionally, the system appears to be regulated so as to avoid overproduction of catalytically active factor XIIIa, since enhancement of AP release dramatically falls, as the product of activation, factor XIIIa, begins to cross-link its fibrin substrates.

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Structural Evidence for Leucine at the Reactive Site of Heparin Cofactor II†

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Received April 23, 1985

ABSTRACT: The reaction products formed during the enzymatic inactivation of heparin cofactor II (HCII) by a proteinase isolated from *Echis carinatus* were analyzed by sodium dodecyl sulfate ($NaDodSO_4$)–polyacrylamide gel electrophoresis and by reverse-phase high-performance liquid chromatography. By $NaDodSO_4$–polyacrylamide gel electrophoresis, limited proteolysis of HCII was observed, which resulted in a decrease in the apparent molecular weight of the protein from ~68 000 to ~53 000. By reverse-phase high-performance liquid chromatography, at least 20 peptides were observed. Primary structure analysis of these peptides indicated that significant proteolysis had occurred in the NH_2-terminal region of the protein. HCII inactivation, however, coincided with the appearance of a peptide from the COOH-terminal region of the protein. The peptide differed from the previously identified reactive site peptide [Griffith, M. J., Noyes, C. M., & Church, F. C. (1985) *J. Biol. Chem. 260*, 2218–2225] by only one residue: a leucyl residue at the NH_2-terminal of the peptide. We conclude that leucine, as opposed to the expected arginine, is at the reactive site of HCII.

Several studies have been reported during the past few years in which the structural and functional properties of heparin cofactor II[1] (HCII)[2] have been investigated (Tollefsen et al., 1982; Wunderwald et al., 1982; Witt et al., 1983; Griffith et al., 1983, 1985; Griffith, 1983; Hurst et al., 1983; Griffith &

† Supported by National Institutes of Health Grants HL-32656, HL-07255, and HL-06350.

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[1] The term heparin cofactor II (Tollefsen et al., 1982), used in this study, corresponds to the second antithrombin heparin cofactor identified in human plasma. The activity corresponding to heparin cofactor II has been referred to in other work as heparin cofactor A (Briginshaw & Shanberge, 1974a,b; Griffith et al., 1983) and as antithrombin BM (Wunderwald et al., 1982; Witt et al., 1983).

0006-2960/85/0424-6777$01.50/0

Marbet, 1983; Tollefsen et al., 1983; Church & Griffith, 1984, 1985; Parker & Tollefsen, 1985; Church et al., 1985). HCII, like antithrombin III (ATIII), inhibits thrombin at an accelerated rate in the presence of heparin (Abildgaard, 1968; Briginshaw & Shanberge, 1974a,b). Both HCII and ATIII inhibit thrombin by forming stable 1:1 molar complexes in which the active site of thrombin is blocked (Harpel & Rosenberg, 1976; Tollefsen et al., 1982). Dissociation of thrombin complexes with both HCII and ATIII under alkaline conditions results in the cleavage and inactivation of the inhibitor (Fish et al., 1979; Griffith & Lundblad, 1981; Griffith et al., 1985). Recently, we reported that there is a significant degree of primary structure similarity between HCII and ATIII in the reactive site peptides, i.e., the peptides that are COOH-terminal to the thrombin cleavage sites in both proteins (Griffith et al., 1985). ATIII, however, inhibits a wide variety of trypsin-like enzymes (Travis & Salvesen, 1983) whereas HCII appears to inhibit only thrombin in this proteinase family (Parker & Tollefsen, 1985). This observation suggested that there are significant differences between the two proteins with respect to the amino acid residues either at the respective reactive sites or in the extended proteinase binding domains (reactive centers), which would account for the differences in proteinase specificity.

The present investigation was undertaken to further characterize the primary structure of HCII in the reactive site region of the protein. Earlier studies had shown that several snake venoms contain proteinases that inactivate ATIII by cleavage of the protein in the vicinity of the reactive site Arg-385[3] residue (Kress & Catanese, 1980, 1981). In recent work we have found that HCII is enzymatically inactivated by incubation with *Echis carinatus* venom (ECV) (unpublished) and have isolated a proteinase, termed proteinase 1, for use in this study. Our results indicate that proteinase 1 inactivates HCII by cleavage of the protein one residue from the NH_2-terminal of the reactive site peptide and that the limited proteinase specificity of HCII observed to date can be attributed in part to the presence of leucine as opposed to the expected arginine at the reactive site of HCII.

Experimental Procedures

Materials. Triethanolamine (TEA) and poly(ethylene glycol) (PEG, M_r 6000–8000) were purchased from Fisher. N^{α}-*p*-Tosylglycyl-L-prolyl-L-arginine-*p*-nitroanilide (TosGlyProArgNA) was purchased from Boehringer-Mannheim. *Echis carinatus* venom (ECV, lot 103C-1370) was purchased from Sigma. Protein molecular weight standards for polyacrylamide gel electrophoresis were purchased from Bio-Rad.

Heparin cofactor II (HCII) was isolated from human plasma as described previously (Griffith et al., 1985). Heparin cofactor II concentration was determined by absorbance at 280 nm with an extinction coefficient value of 0.915 mL mg^{-1} cm^{-1} (Griffith et al., 1985) and an M_r value of 65 600 (Tollefsen et al., 1982). Human α-thrombin was prepared by incubation of purified prothrombin with ECV (Franza et al., 1975) and sulfopropyl-Sephadex (Pharmacia Fine Chemicals) column chromatography as described previously (Lundblad et al., 1976). Thrombin concentration was determined by absorbance at 280 nm with an extinction coefficient value of 1.75 mL mg^{-1} cm^{-1} and an M_r value of 36 600 (Fenton et al., 1977). Thrombin solutions contained 0.1% PEG to prevent adsorption to surfaces (Wasiewski et al., 1976).

Proteinase Isolation from ECV. A proteinase, termed proteinase 1, was isolated from *Echis carinatus* venom for use in the present study.[4] All steps used in the isolation procedure were performed at room temperature (22 °C). Proteinase activity associated with HCII inactivation (anti-HCII activity) was measured by incubating samples obtained during the isolation procedure with 5.0 μM HCII for 2–4 h at room temperature, followed by the measurement of residual HCII activity as described below (Measurement of HCII activity). ECV (0.5 g) was added to 6.0 mL of a solution containing 0.05 M Tris-HCl (pH 7.4), 0.25 M NaCl, and 5.0 mM $CaCl_2$. After the solution was gently rocked for 2 h, a small amount of insoluble material was removed by centrifugation. The supernatant, containing 540 absorbance units at 280 nm, was applied to a 2.5 × 97 cm Sephacryl S-300 column equilibrated with 0.05 M Tris-HCl (pH 7.4), 0.25 M NaCl, and 5.0 mM $CaCl_2$. Fractions containing 6.9 mL were collected at a flow rate of 45 mL/h. Fractions 44–47, containing anti-HCII activity, were pooled and dialyzed for 16 h against 0.05 M Tris-HCl (pH 7.4)–2.0 mM $CaCl_2$. The dialyzed material was applied to a 1.1 × 10 cm heparin–agarose column equilibrated with 0.05 M Tris-HCl (pH 7.4)–2.0 mM $CaCl_2$. The flow-through material, containing anti-HCII activity, was pooled and applied to a 1.1 × 15 cm QAE-Sephadex A-50 column equilibrated with 0.05 M Tris-HCl (pH 7.4)–2.0 mM $CaCl_2$. The column was washed with approximately 100 mL of equilibration buffer and the anti-HCII activity eluted from the column with a 0–0.5 M NaCl gradient (0.2 L × 0.2 L). Anti-HCII activity was detected in fractions (6.9 mL) corresponding to 0.175–0.225 M NaCl in the gradient. Only one protein band (proteinase 1; $M_r \simeq 55\,000$) was observed by $NaDodSO_4$–PAGE in fractions corresponding to the peak of anti-HCII activity. Fractions containing purified proteinase 1 were pooled, and the pool was stored at 4 °C until use. An extinction coefficient value of 1.0 mL mg^{-1} cm^{-1} was used to estimate proteinase 1 concentration.

HCII Proteolysis and Analysis of Reaction Products. Purified proteinase was added to a solution containing 0.1 M TEA (pH 8.0), 0.1 M NaCl, 0.1% PEG, 5 mM $CaCl_2$, and 65 μM (4.25 mg/mL) HCII and the solution incubated at 37 °C. The final proteinase 1 concentration was 50 μg/mL in a total reaction volume of 1.7 mL. At timed intervals, samples (0.1 mL) were removed and added to 0.9 mL of a solution containing 0.1 M TEA (pH 8.0), 0.1 M NaCl, 0.1% PEG, and 10 mM EDTA. There was no further inactivation/degradation of HCII after dilution of the samples into buffer containing EDTA (unpublished). The last sample was taken after incubation for 150 min, and 0.1 mL of 0.1 M EDTA was added to the remainder (0.7 mL) of the reaction solution (final reaction solution).

The reaction products formed during the incubation time studies were analyzed by $NaDodSO_4$–PAGE. Aliquots (40 μL, 17 μg) of the diluted samples were subjected to $NaDodSO_4$–PAGE, under nonreducing conditions, on 10% gels according to the method of Weber & Osborn (1969). Prior to electrophoresis, the samples were denatured by heating for 3

[2] Abbreviations: HCII, heparin cofactor II; ATIII, antithrombin III; ECV, *Echis carinatus* venom; TEA, triethanolamine; PEG, poly(ethylene glycol); $NaDodSO_4$–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TosGlyProArgNA, N^{α}-*p*-tosylglycyl-L-prolyl-L-arginine-*p*-nitroanilide; EDTA, ethylenediaminetetraacetic acid; HPLC, high-performance liquid chromatography; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

[3] The reactive site arginine in antithrombin III corresponds to residue 385 in the primary structure of the protein as reported by Peterson et al. (1979) and to residue 393 in the primary structure as reported by Chandra et al. (1983a).

[4] Additional details regarding the purification and characterization of proteinase 1 will be published elsewhere.

min at 100 °C in the presence of 2% $NaDodSO_4$. After electrophoresis, the gels were stained with 0.2% Coomassie Blue and destained with 10% acetic acid. To determine the reactivity of the reaction products with thrombin, thrombin (20 μL, 13 μM) was added to 40 μL of the diluted samples, and the solutions were incubated for 20 min at 22 °C. Under these conditions, essentially 100% inactivation of native HCII was attained. The samples were then denatured and subjected to electrophoresis on 7.5% gels as described above.

The reaction products formed during the incubation time studied were also analyzed by reverse-phase high-performance liquid chromatography (HPLC). For analytical runs, aliquots (0.3 mL, 127 μg) of the diluted samples were applied to a 4.6 mm × 25 cm Vydac C-18 column (Catalog no. 218TP546). The flow rate was 1.0 mL/min, and the column temperature was 45 °C. A 0.1% (v/v) trifluoroacetic acid (solvent A)–2-propanol (solvent B) gradient was developed with a Beckman Model 324 gradient chromatograph. The 2-propanol concentration was increased linearly from 0 to 22% in 57 min, followed by an increase to 60% in 38 min. Peptide elution was detected by monitoring the effluent at 210 and 280 nm with an ISCO Model 1840 variable-wavelength detector and a Beckman Model 153 detector. To obtain peptides for primary structure analysis, a preparative HPLC run was made by applying 0.3 mL (1.12 mg, 17 nmol of native protein) of the final reaction solution to the column. Isolated peptides were subjected to primary structure analysis by amino-terminal amino acid sequence determination in a Beckman Model 890C protein sequencer as described previously (Noyes, 1983; Griffith et al., 1985). Automated Edman degradation was performed with 0.1 M Quadrol as the coupling buffer (Edman & Begg, 1967; Brauer et al., 1975). All sequencing runs were made with 3 mg of Polybrene as a carrier (Tarr et al., 1978; Klapper et al., 1978). The resulting phenylthiohydantoin amino acids were identified by HPLC on a 4.6 mm × 25 cm Beckman Ultrasphere-ODS column as described previously (Noyes, 1983). The repetitive yield obtained per sequencer cycle was 93%.

Measurement of HCII Activity. HCII inactivation by incubation with proteinase 1 as described above was quantitated by measuring the rate of loss of the antithrombin activity of HCII. The buffer solution used throughout the assay procedure consisted of 0.1 M TEA (pH 8.0), 0.1 M NaCl, and 0.1% PEG, and all incubations were performed at room temperature. The assay procedure was performed in two steps as follows: (1) Aliquots (20 μL) of the diluted samples obtained above were added to 0.2 mL of buffer containing 10 mM EDTA and 5 nM thrombin, and the solution was incubated for 30 min. (2) A total of 0.8 mL of buffer containing 1.5 mM Tos-GlyProArgNA was then added and the solution incubated for 3 min before the addition of 0.1 mL of acetic acid. The amount of TosGlyProArgNA hydrolyzed was determined by measuring the absorbance of the solution at 400 nm (A_{400}). Under these conditions, the A_{400} value was directly proportional to the residual thrombin concentration (Griffith, 1983). Since the HCII concentration was always at least 20-fold higher than the thrombin concentration, thrombin inhibition followed apparent pseudo-first-order kinetics (Griffith, 1983) and the A_{400} values were converted to the residual HCII concentration according to

$$[\text{HCII}] = -\ln [(A^{s}_{400}/A^{c}_{400})]/(k''_{app}t) \quad (1)$$

In eq 1, A^{s}_{400} corresponds to the absorbance of the sample solution (step 2) after incubation with thrombin for t = 30 min (step 1), and A^{c}_{400} corresponds to the absorbance of a control solution containing only thrombin in step 1. The apparent second-order rate constant value, k''_{app}, for thrombin inhibition by HCII was determined experimentally to be $k''_{app} = 5.0 \times 10^4\ M^{-1}\ min^{-1}$.

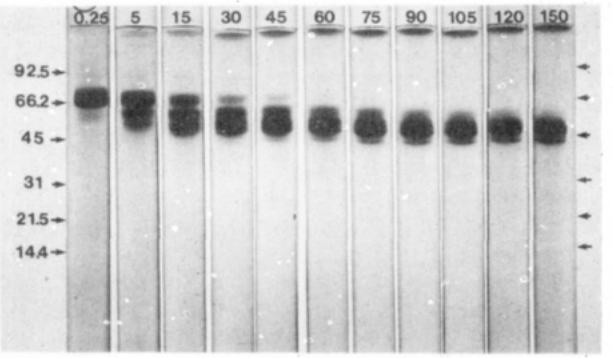

FIGURE 1: Electrophoretic analysis of the products formed by incubation of HCII with proteinase 1. HCII was incubated with proteinase 1 as described under Experimental Procedures. Samples obtained at the reaction times indicated were subjected to $NaDodSO_4$–PAGE under nonreducing conditions on 10% gels as described under Experimental Procedures. Arrows indicate the migration position of phosphorylase B (92 500), bovine serum albumin (66 200), ovalbumin (45 000), carbonic anhydrase (31 000), soybean trypsin inhibitor (21 500), and lysozyme (14 400). The tracking dye (bromophenol blue) comigrated with lysozyme.

Results

Incubation of HCII with purified proteinase 1 resulted in the rapid disappearance of the native protein and the appearance of several species with lower molecular weight values as observed by $NaDodSO_4$–PAGE. These results are shown in Figure 1. Proteolysis appeared to occur in two steps in which the accumulation of intermediate species (M_r 60 000) was followed by a gradual increase in species between M_r 53 000 and M_r 55 000. Samples obtained at the time intervals indicated in Figure 1 were incubated with thrombin and subjected to $NaDodSO_4$–PAGE as described under Experimental Procedures. Under the experimental conditions used, all of the native HCII and essentially all of the intermediate species (M_r 60 000) observed in the gels shown in Figure 1 appeared to be complexed with thrombin (not shown).

Low molecular weight peptides released during proteolysis were not detectable by $NaDodSO_4$–PAGE under the experimental conditions used. By reverse-phase HPLC, however, at least 20 peptides were found to be formed during the incubation time studied. The chromatogram obtained from a sample taken after 75 min of incubation is shown in Figure 2. Comparison of the chromatogram obtained from the samples taken over the incubation time studied indicated that several peptides (e.g., 7, 8, and 17) accumulated relatively rapidly, but then appeared to undergo further proteolysis. Other peptides (e.g., 2, 3, and 10) accumulated quickly and did not appear to increase or decrease after approximately 60 min. The appearance and disappearance of the major peptides identified during the incubation time studied is shown in Figure 3. The loss of HCII activity, shown also in Figure 3, appeared to coincide with the accumulation of peptide 20, which eluted at a similar point in the HPLC solvent gradient as the previously identified reactive site peptide of HCII (Griffith et al., 1985). Primary structure analysis of peptide 20 indicated that it differed from the reactive site peptide by only one residue, a leucyl residue at the NH_2-terminal of the peptide. While peptide 20 is from the COOH-terminal region of native HCII, all of the other peptides analyzed appeared to result from cleavage at the NH_2-terminal of native HCII. These results are summarized in Table I. Peptide 17 was found to overlap

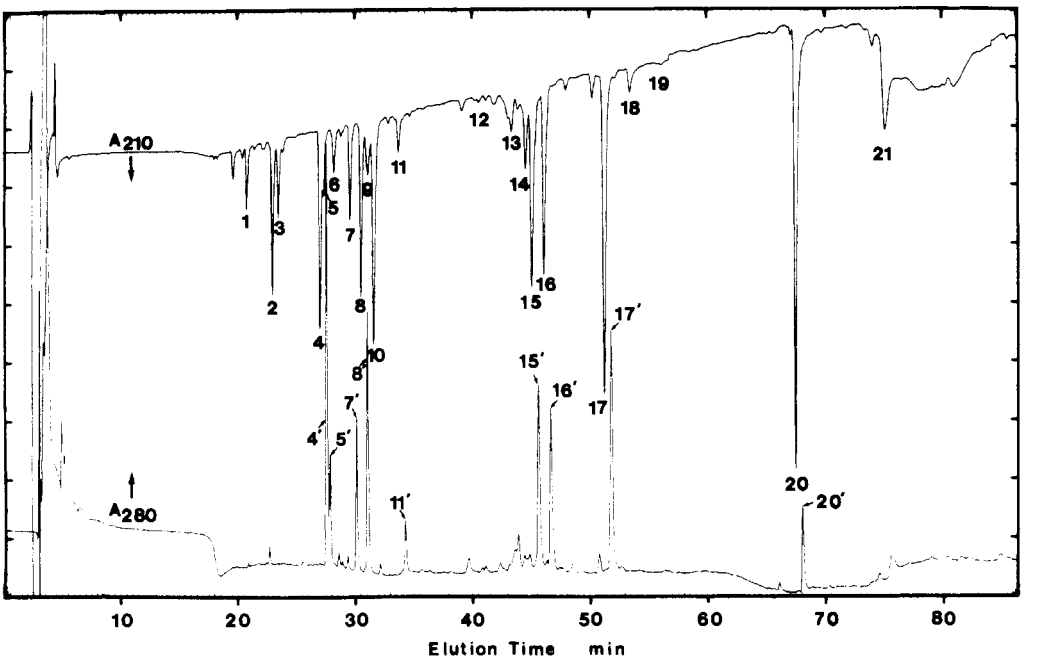

FIGURE 2: HPLC analysis of the products formed by incubation of HCII with proteinase 1. HCII was incubated with proteinase 1 as described under Experimental Procedures. At the times indicated in Figure 1, the reaction products were analyzed by reverse-phase HPLC as described under Experimental Procedures. Peptide elution was monitored by absorbance at 210 nm (top to bottom; 0.2 absorbance unit full scale) and at 280 nm (bottom to top; 0.02 absorbance unit full scale). Peaks identified by absorbance at 210 nm are numbered consecutively, and the corresponding peaks identified by absorbance at 280 nm are designated by prime numbers. The chromatogram of a sample (127 μg; 2 nmol of HCII) obtained after incubation for 75 min is shown.

Table I: Primary Structure of Peptides Obtained by Proteinase 1 Cleavage of HCII[a]

	5	10	15	20	25	30
Native NH_2-terminal (N)	G S K G P L D Q L E K G G E T A Q S A D P Q W E Q L N N K - L S M					
Peptide						
2	G S K G P L D Q					
3	G S K G P L D Q					
7		L E K G G E T A Q S A D P Q W E Q				
8		L E K G G E T A Q S A D P Q W E Q				
13		L E K G G E T A Q S A D P Q W E Q L				
5			A Q S A D P Q W E Q			
4			S A D P Q W E Q			
9					L N N K - L	
10					L N N K - L S M	

	5	10	15	20	25
NH_2-terminal dHCII			N D W I P E G E E D ...		
Peptide					
17	L L P A D F H K E N T V T N D W I P E G E E D D D Y L A T				
6	L L P A D				
14	L L P A D F				
16	F H K E N T V T N D W I P E G E E D D D Y L A T				
15	H K E N T V T N D W I P E G E E D D D Y L A T				

	1	1'	5'	10'	15'	20'	25'	30'	35'
Reactive Site Peptide		S T Q V R F T V D R P F L F L I Y E H P T S T L L F M G R V A N P S R Q							
Peptide									
20		L S T Q V R F T V ...							
Alpha$_1$-aChT Reactive Site Peptide		**L S** A L **V R - T** N - **R P F L** M I I V - - **P T** D **T** I F **F M** S K **V T N P S** K P...							
		E-T I F V-R					Q-N		

[a] Peptides (numbered as indicated in Figure 2) isolated by HPLC after incubation of HCII with proteinase 1 for 150 min were subjected to primary structure analysis as described under Experimental Procedures. Minor peptides (1, 11, 12, 18, 19) were not sequenced. The native NH_2-terminal sequence (residues 1–24), the NH_2-terminal sequence of degraded-HCII (dHCII), and the reactive site peptide sequence were determined previously (Griffith et al., 1985). The reactive site peptide sequence of α_1-antichymotrypsin (α_1-aChT) was reported by Chandra et al. (1983b).

the NH_2-terminal sequence of a degraded form of HCII previously shown to retain antithrombin activity (Griffith et al., 1985). While the major sequence identified for peptide 15 is that indicated in Table I, a second sequence of 11 residues was partially identified. The position of this peptide (Leu-X-Val-Ser-Pro-X-Glu-X-X-Val-Ala) in the primary structure of HCII could not be established. Material identified as peptide 21 in Figure 2, was found to contain NH_2-terminal sequences corresponding to peptide 20 and protein cleaved at the Gln-8–Leu-9 bond. This may represent the aggregated material

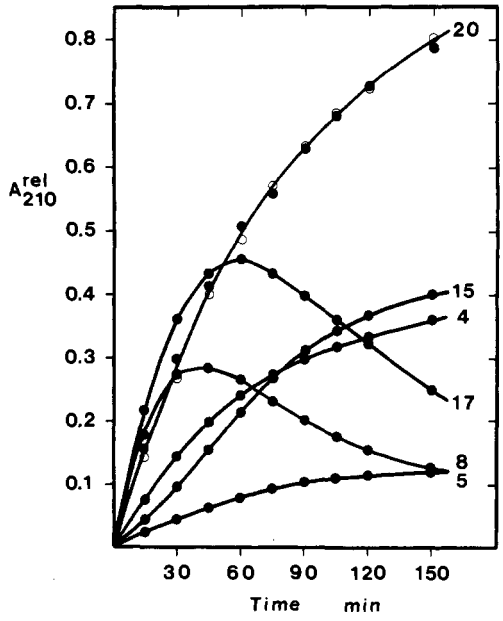

FIGURE 3: Correlation between HCII inactivation and peptide formation. HCII was incubated with proteinase 1 as described under Experimental Procedures. HCII inactivation was measured as described under Experimental Procedures. The relative amount of HCII inactivated (○) is shown as a function of incubation time. The relative absorbance at 210 nm [A^{rel}_{210} (●)] of selected peptides, determined by integration of the corresponding peaks shown in Figure 2, is also shown as a function of incubation time.

observed at the top of the gels shown in Figure 1. Finally, it is interesting to note that proteinase 1 appears to have a significant degree of specificity for Gln–X bonds (Table I). As such, proteinase 1 might prove to be a useful reagent in a variety of protein structure/function studies.

Discussion

Preliminary work in our laboratory indicated that incubation of HCII with proteinase 1 from ECV results in a progressive loss of both the antithrombin and heparin cofactor activities of HCII. In the present study, we have analyzed the reaction products formed during the inactivation process by NaDodSO$_4$–PAGE and by reverse-phase HPLC. The reaction products detectable by NaDodSO$_4$–PAGE corresponded to degraded species of HCII with molecular weight values between 53 000 and 60 000, while the products detectable by reverse-phase HPLC corresponded to the peptides (molecular weight values <5000) released from the NH$_2$-terminal and COOH-terminal regions of the native protein.

HCII proteolysis appeared to occur in two steps in which the transient appearance of an intermediate species (M_r 60 000) was followed by the appearance of species with molecular weight values between 53 000 and 55 000. The intermediate species of HCII was found to retain antithrombin activity. In earlier work, we isolated a degraded species of HCII (M_r 60 000), which also retained antithrombin (and heparin cofactor) activity (Griffith et al., 1985). Primary structure analysis of the degraded HCII (dHCII) indicated that it was missing a significant portion of the amino-terminal region of the native protein. The intermediate species of HCII identified in the present study was therefore suspected to correspond primarily to HCII in which proteolysis in the NH$_2$-terminal region had occurred.

Primary structure analysis of the peptides formed during HCII inactivation confirmed that significant proteolysis had occurred in the NH$_2$-terminal region of the protein. HCII inactivation, however, coincided with the appearance of a peptide from the COOH-terminal region of the protein. Thrombin cleavage of HCII, which occurs when the HCII–thrombin complex is incubated under alkaline conditions, also results in HCII inactivation and the appearance of a peptide, referred to as the reactive site peptide, from the COOH-terminal region of the protein (Griffith et al., 1985). On the basis of the primary structure similarity between the reactive site peptides of HCII and ATIII, the high degree of substrate specificity of thrombin for Arg–X peptide bonds (Blomback et al., 1977), and the presence of arginine at the reactive site of ATIII (Danielsson & Bjork, 1980), it was expected that arginine would also be present at the reactive site of HCII. This, however, does not appear to be the case. The peptide identified from the COOH-terminal of HCII in the present study differed from the reactive site peptide by only one residue, a leucyl residue at the NH$_2$-terminal. As such, the results obtained in the present study provide reasonably strong structural evidence for a leucyl residue at the reactive site of HCII.

Of the trypsin-like proteinases studied to date, only thrombin appears to react at a significant rate with HCII (Parker & Tollefsen, 1985). In view of the Arg–X, Lys–X substrate specificity of trypsin-like proteinases, the inability of these proteinases to interact with the reactive site leucine of HCII is not too surprising. While the reactivity of thrombin with HCII does not fit with the expected substrate specificity of thrombin, both thrombin and trypsin are inhibited by α_1-proteinase inhibitor in which a methionyl residue appears to be at the reactive site (Travis & Salvesen, 1983). It therefore seems reasonable to conclude that structural properties of HCII and α_1-proteinase inhibitor beyond the specific reactive site residues play an important role in proteinase binding and stable complex formation. With respect to HCII, recent studies in our laboratory have shown that incubation of the protein with 2,3-butanedione results in a loss of antithrombin activity, suggesting that arginyl residues in the vicinity of the reactive site are important for thrombin binding to HCII (unpublished).

The identification of leucine at the reactive site of HCII suggests that HCII might be an effective inhibitor of chymotrypsin-like proteinases (Church et al., 1985). There is a significant degree of structural similarity between the reactive site peptides of HCII and α_1-antichymotrypsin (Table I). α_1-Antichymotrypsin, however, reacts rapidly (k''_{app} = 3.1 × 10^9 M^{-1} min^{-1}) with cathepsin G, a chymotrypsin-like proteinase (Beatty et al., 1980), whereas the HCII/cathepsin G reaction is relatively slow (k''_{app} = 2.0 × 10^4 M^{-1} min^{-1}) (Parker & Tollefsen, 1985). While the HCII/cathepsin G reaction is accelerated approximately 6-fold by dermatan sulfate, the physiological significance of this reaction is uncertain at the present time (Parker & Tollefsen, 1985). Recent studies in our laboratory have shown that the HCII/chymotrypsin reaction is approximately 2 orders of magnitude faster than the HCII/thrombin reaction (Church et al., 1985). Unlike the HCII/thrombin reaction, which is greatly accelerated by both heparin (Briginshaw & Shanberge, 1974) and dermatan sulfate (Griffith & Marbet, 1983; Tollefsen et al., 1983), there does not appear to be a significant effect of either mucopolysaccharide on the HCII/chymotrypsin reaction rate (Church et al., 1985).

Finally, while it is reasonable to question whether or not the primary role of HCII is in the regulation of hemostasis, as has been established for ATIII (Egeberg, 1965), it is well established that HCII rapidly inhibits thrombin in the presence of mucopolysaccharides that could very well be prevalent at

the site of vasculature injury. The difference between leucine vs. arginine at the reactive site of the HCII, however, suggests that the proteinase specificity might be much greater with enzymes of the chymotrypsin family and that the primary physiological role of HCII might be considered in this regard.

Registry No. L-Leu, 61-90-5; heparin cofactor II, 81604-65-1; proteinase, 9001-92-7.

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