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Research Article

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Studies on Human Plasma $C\bar{1}$ Inactivator-Enzyme Interactions

II. STRUCTURAL FEATURES OF AN ABNORMAL $C\bar{1}$ INACTIVATOR FROM A KINDRED WITH HEREDITARY ANGIONEUROTIC EDEMA

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ABSTRACT The function and several of the structural features of the $C\bar{1}$ inactivator protein isolated from the plasma of a mother and daughter with the variant form of hereditary angioneurotic edema have been examined. These abnormal inhibitors shared immunologic identity with the normal $C\bar{1}$ inactivator protein; however, they were inactive in inhibiting the functional activity of $C\bar{1}s$. Analysis of the abnormal inhibitors by sodium dodecyl sulfate (SDS) acrylamide gel electrophoresis suggested that each consisted of a single polypeptide chain, the mobility of which was slower than that of the normal $C\bar{1}$ inactivator. The apparent molecular weight of the patients' inhibitors was 109,000 daltons as contrasted to 105,000 daltons, that of the normal $C\bar{1}$ inactivator. The abnormal inhibitors failed to form a complex with $C\bar{1}s$ or plasmin as analyzed by SDS-acrylamide gels. The large proteolytic derivatives resulting from the plasmin- and trypsin-induced degradation of the abnormal inhibitors were approximately 3,000 daltons heavier than the corresponding products derived from normal $C\bar{1}$ inactivator. Thus, the structural abnormality identified appeared to be a property of the core molecule. Treatment of the inhibitors with neuraminidase failed to demonstate a difference between the normal and patient-derived $C\bar{1}$ inactivator molecule. Neither were major differences found between the amino acid composition of the defective and normal inhibitors; however, the acidic amino acids tended to be higher in the patients' inhibitors, and the phenylalanine content lower. Thus, these studies have identified both structural and functional abnormalities in the $C\bar{1}$ inactivator protein isolated from two related patients with hereditary angioneurotic edema. Examination of the interaction between endopeptidases and the inhibitors has further delineated the abnormal structural features.

INTRODUCTION

Patients with hereditary angioneurotic edema (HANE)[1] have been shown to lack functional $C\bar{1}$ inactivator,[2] the plasma inhibitor of the activated first component of complement (1). Immunochemical studies have defined two major forms of this inhibitor deficiency. Most affected individuals have low serum concentrations of the inhibitor antigen, whereas in the variant form,

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Part of this study was performed by Dr. Harpel while a Visiting Investigator at Scripps Clinic and Research Foundation. This is publication number 871. Drs. Harpel and Cooper are recipients of U. S. Public Health Service Research Career Development Awards, 1 KO4 HE50, 285 and 5 K4 A1 33,630. Dr. Hugli is an Established Investigator of the American Heart Association (72, 175).

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[1] *Abbreviations used in this paper:* ALMe, *N*-α-acetyl-L-lysine methyl ester; HANE, hereditary angioneurotic edema; SDS, sodium dodecyl sulfate.

[2] The complement terminology used conforms to the recommendations of the World Health Organization Committee on Complement Nomenclature (1968. *Bull. W. H. O.* **39**: 935–938).

patients have normal or elevated concentrations of a functionally inactive antigen (2, 3).

The structural features of the abnormal inhibitor protein from patients with hereditary angioneurotic edema responsible for the deficient function have not, as yet, been identified. In the present study, the $C\bar{1}$ inactivator protein has been isolated from the plasma of a mother and daughter with the variant form of the disease and some of its essential structural features compared to those of the $C\bar{1}$ inactivator prepared from normal plasma. The structural interactions of these abnormal inhibitors with $C\bar{1}s$, plasmin, and trypsin were examined and also compared to those reactions detailed in the accompanying paper (4) involving the normal inhibitor. Furthermore, comparisons were made of the amino acid composition of the normal and abnormal inhibitors.

METHODS

All chemicals used were reagent grade and were obtained from sources detailed in the accompanying paper (4).

Human plasma $C\bar{1}$ inactivator from normal individuals was purified as described in detail in the accompanying paper (4) and included the following sequential steps: fractional precipitation with polyethylene glycol, DEAE-cellulose followed by gel filtration (Bio-Gel A-5m) column chromatography, preparative Pevikon block electrophoresis, and concanavalin A-Sepharose (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.) affinity chromatography. To significantly increase the yield of the $C\bar{1}$ inactivator protein isolated from the patients studied with HANE, the purification procedure was terminated after the Bio-Gel filtration step. This was found to be feasible as preliminary studies using normal plasma established that the protein band corresponding to $C\bar{1}$ inactivator could be unambiguously separated and identified by electrophoretic analysis on sodium dodecyl sulfate (SDS) acrylamide gels at this stage in the purification procedure. Further, the electrophoretic mobility in SDS-acrylamide gels of the $C\bar{1}$ inactivator band after gel filtration chromatography was the same as that found for more highly purified preparations of the inhibitor. Also, the interactions between $C\bar{1}$ inactivator and $C\bar{1}s$, plasmin, and trypsin were the same as those reported for more highly purified preparations of the inhibitor (4).

The fractions containing the patients' $C\bar{1}$ inactivator at each stage in the isolation procedure were identified by double diffusion analysis using rabbit anti-$C\bar{1}$ inactivator antiserum obtained from Behring Diagnostics, Inc. (subsidiary of the American Hoechst Corp., Woodbury, N. Y.). The $C\bar{1}$ inactivator protein from the HANE plasma was purified in a manner identical to that of normal inhibitor. The major contaminants in both normal and functionally deficient $C\bar{1}$ inactivator preparations after the gel filtration procedure, as identified by immunoelectrophoretic analysis, were albumin, IgA, and IgG. The final material for analysis was concentrated by ultrafiltration to a protein concentration of 1.5 mg/ml and stored at −70°C.

Plasmin spontaneously activated in 50% glycerol, containing 77 Sgouris caseinolytic U/ml, was obtained from the Michigan Department of Public Health, Lansing, Mich. In some experiments, plasmin was obtained by activating affinity column-purified plasminogen as previously described (4).

$C\bar{1}s$ inhibitory activity of the plasma and isolated $C\bar{1}$ inactivator protein from the patients with HANE was tested in a functional hemolytic assay (4) as well as by an esterolytic assay using *N*-α-acetyl-L-lysine methyl ester HCl (ALMe) (Cyclo Chemical, Division Travenol Laboratories, Inc., Los Angeles, Calif) (5).

Alkaline acrylamide gel electrophoresis was performed as described by Davis (6), cellulose acetate electrophoresis according to the instructions supplied with the electrophoresis apparatus (Beckman Microzone System, Beckman Instruments, Inc., Fullerton, Calif.), and SDS-acrylamide gel electrophoresis (5% gels) was carried out by the methods of Weber and Osborn (7). The method for the preparation of the samples for analysis in SDS-gels has been detailed in the accompanying paper (4), as have the standards used to establish the apparent molecular weight of the various $C\bar{1}$ inactivator preparations and of their proteolytic derivatives.

Immunochemical quantitation of $C\bar{1}$ inactivator antigen concentration in normal and HANE plasma and in the partially purified preparations of patients' inhibitor was performed by single diffusion analysis (8).

Enzyme-treated $C\bar{1}$ inactivator preparations were prepared as follows. Purified normal $C\bar{1}$ inactivator or patients' inhibitor protein (7.5 μg inhibitor antigen per system as quantified by single diffusion analysis) was incubated with buffer, glycerol-activated plasmin (0.06 Sgouris caseinolytic U per system), or bovine trypsin (2.5 μg per system, obtained from Worthington Biochemical Corp., Freehold, N. J.) for 30 min at 37°C. The $C\bar{1}$ inactivator preparations (7.5 μg inhibitor antigen per system) were also incubated with neuraminidase (5–15 U per system; *Vibrio cholerae* neuraminidase from Calbiochem, San Diego, Calif.) for varying time periods (1–24 h) at 37°C, pH 6.0, in 0.1 M acetate buffer as described by Rosen, Alper, Pensky, Klemperer, and Donaldson (3). These incubation mixtures were then reduced with dithiothreitol and analyzed by acrylamide gel electrophoresis in the presence of SDS as detailed in the accompanying paper (4). The neuraminidase-treated $C\bar{1}$ inactivator preparations were also analyzed by cellulose acetate electrophoresis and by alkaline acrylamide gel electrophoresis (7.5% gels).

Amino acid composition of $C\bar{1}$ inactivator preparations was determined from analysis of sliced SDS-acrylamide gels. 200–300 mg of acrylamide gel, containing approximately 50 μg of $C\bar{1}$ inactivator, was excised from stained gels and the slices were lyophilized in 16 × 150-mm Pyrex tubes. Hydrolysis was performed for 22 h at 110°C according to the method described by Houston (9). Cysteic acid determinations were made on independent gel slices after oxidation according to the procedure of Moore (10). Proteins stained with Coomassie Blue were found to become clear as the oxidation proceeded; therefore, clarification of the stained gel slices served to indicate completion of the oxidative reaction. Amino acid analyses were performed according to the procedure of Spackman, Stein, and Moore (11) using an instrument (Beckman Amino Acid Analyzer, model 121C) modified for accelerated analysis (12).

RESULTS

Functional and immunologic activity of $C\bar{1}$ inactivator from two patients with HANE. Neither the plasma nor the partially purified inhibitor from the mother (M. A.) and daughter (M. S.) with HANE inhibited the functional activity of purified $C\bar{1}s$. Lack of inhibition was documented in a hemolytic assay which tested the ability of the patients' plasma and isolated inhibitor to prevent the inactivation of $C\bar{4}$ by $C\bar{1}s$ (4). In addition,

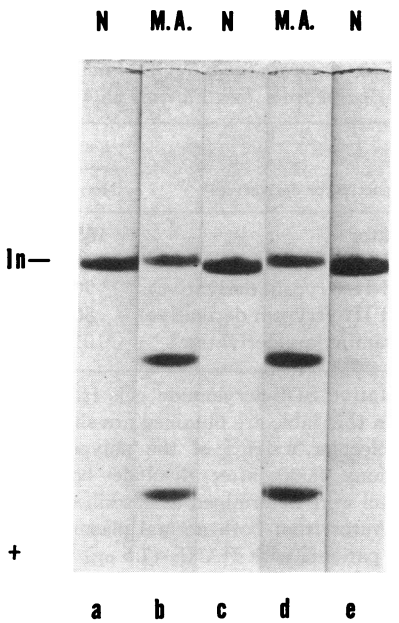

FIGURE 1 SDS gel (5% acrylamide) electrophoretic analysis of a preparation of C1 inactivator from a normal individual (N; gels a, c, and e) and from patient M. A. (gels b and d) with HANE. The samples (10 μg inhibitor antigen per gel) were applied to consecutive gels in one electrophoresis chamber to facilitate the comparison of electrophoretic mobilities. The top band in each gel is the C1 inactivator (In).

the abnormal C1 inactivator preparations did not inhibit the esterase activity of C1s with ALMe as a substrate (5). In contrast to the absence of functional activity, the patients' plasma contained material recognized by antibodies to normal C1 inactivator. The immunoelectrophoretic mobility of these antigens was similar to that of normal plasma both in agar and agarose, and a reaction of identity between the antigens in the patients' plasmas and normal plasma was observed by double diffusion analysis. The concentration of C1 inactivator antigen in the plasma of the patients with HANE was 13.1 (M. A.) and 12.0 mg per 100 ml (M. S.) as determined by radial immunodiffusion or approximately 70% of the normal plasma concentration (3). Thus, these patients represented the variant form of HANE, possessing a nonfunctional protein antigenically similar to normal C1 inactivator (2, 3).

Electrophoretic analysis in SDS-acrylamide gels of C1 inactivator preparations from HANE plasma. The C1 inactivator band in partially purified preparations of the inhibitor was identified by its position on electrophoresis in SDS-acrylamide gels, its high carbohydrate content as established by periodic acid-Schiff staining of the gels, the similarity of its plasmin and trypsin derivatives when compared to those formed from normal C1 inactivator, and its amino acid composition.

Both patient C1 inactivator preparations demonstrated an abnormal mobility as analyzed on acrylamide gels in the presence of SDS (Figs. 1 and 2). Comparison of the electrophoretic mobilities of the C1 inactivator band in the highly purified normal preparation and the less purified patient preparations was found to be feasible since preliminary studies demonstrated that the migration of the normal inhibitor band was unaffected by the degree of purification. The migration of the normal and abnormal inhibitors was compared between consecutive gels in an individual electrophoresis chamber (Fig. 1) since our studies have found that the mobility of replicate samples of these inhibitors varied less than 1% within a given chamber. Under these conditions, the abnormal C1 inactivator always demonstrated an electrophoretic migration measurably slower than that of the normal inhibitor. The electrophoretic mobility of the C1 inactivator band from patients M. A. and M. S. was found to be similar in both reduced and unreduced samples, supporting the concept that these inhibitor proteins consisted of a single polypeptide chain. The apparent molecular weight of the abnormal C1 inactivator was 109,000 daltons, as compared to 105,000 daltons, the molecular weight established for normal C1 inactivator (4). The preparation of C1 inactivator obtained from patient M. A. contained a low concentration of a material migrating slightly faster than the abnormal C1 inactivator band, but this mate-

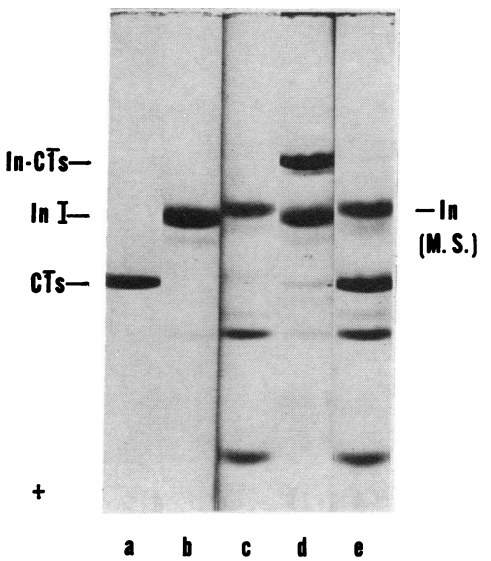

FIGURE 2 SDS gel (5% acrylamide) electrophoretic analysis of incubation mixtures of C1s with normal C1 inactivator and a C1 inactivator preparation from a patient (M. S.) with HANE. C1s (gel a) was incubated with C1 inactivator (gel d) or with the abnormal inhibitor (gel e) in equimolar concentrations. Both normal inhibitor (gel b) and patients' inhibitor (gel c) are included for comparison.

rial was only visualized with higher concentrations of the inhibitor preparation than shown in Figs. 1 and 3. Similar material was identified in the preparation from patient M. S. (Fig. 2, gel c) raising the possibility that this may represent a lower molecular weight form of the abnormal $C\bar{1}$ inactivator analogous to the band II material of the normal $C\bar{1}$ inactivator described in the accompanying paper (4).

As previously demonstrated, $C\bar{1}$ inactivator and $C\bar{1}s$ formed a 1:1 stoichiometric complex resistant to the denaturing agents SDS and urea (4) (Fig. 2, gel d). The abnormal inhibitors, which failed in functional assays to inhibit $C\bar{1}s$, did not form a complex with this enzyme, as illustrated for patient M. S. (Fig. 2, gel e). Similar studies documented that the abnormal inhibitors also failed to form a complex with plasmin.

Studies on the interaction between $C\bar{1}$ inactivator from patients with HANE and the enzymes plasmin, trypsin, and neuraminidase (Fig. 3, Table I). Glycerol-activated plasmin, in equimolar concentration, converted both patients' $C\bar{1}$ inactivator into a lower molecular weight derivative which displayed a slower migration in SDS gels than did the plasmin derivative from functionally normal inhibitor.[3] A representative experiment (patient M. A.) is shown in Fig. 3 (gels c and d). In an analogous manner, the two $C\bar{1}$ inactivator derivatives which resulted from interaction of the abnormal inhibitor protein with trypsin, had an apparent higher molecular weight than did the normal inhibitor's trypsin derivative chains (Fig. 3, gels e and f). The estimated mean molecular weights as outlined in Table I, showed a constant relationship between the abnormal and normal proteolytic derivative chains, the abnormal derivatives in all cases demonstrating a slower electrophoretic mobility on SDS-acrylamide gels and having an apparent molecular weight approximately 3,000 daltons greater than their normal counterpart.

To examine the possibility that an abnormality in neuraminic acid might be responsible for the apparent increase in molecular weight of the nonfunctional inhibitors, the inhibitor preparations were incubated with varying concentrations of neuraminidase for

TABLE I

Molecular Weight Estimates of $C\bar{1}$ Inactivator and its Plasmin, Trypsin, and Neuraminidase Derivative Chains from Two Patients with HANE

$C\bar{1}$ inactivator derivatives*	Estimated mol wt $\times 10^{-3}$‡		
	Normal	M. A.	M. S.
$C\bar{1}$ Inactivator	105.0	109.0	109.0
$C\bar{1}$ In band II (plasmin derivative)	96.0	99.0	98.5
$C\bar{1}$ In band II (trypsin derivative)	96.0	99.5	99.0
$C\bar{1}$ In band III (trypsin derivative)	86.0	89.0	88.5
$C\bar{1}$ In neuraminidase derivative§	101.0	104.0	104.0

* Representative SDS-acrylamide gels from which the data presented in this table are obtained are shown in Fig. 3.

‡ Mean molecular weights of the polypeptide chains (two determinations each) after disulfide bond cleavage with dithiothreitol were determined as described in Methods.

§ $C\bar{1}$ inactivator from both normal plasma and from plasma of the two patients with HANE (7.5 μg) was incubated with neuraminidase (*V. cholerae*, 5 U per system) at pH 6.0 2 h, 37°C.

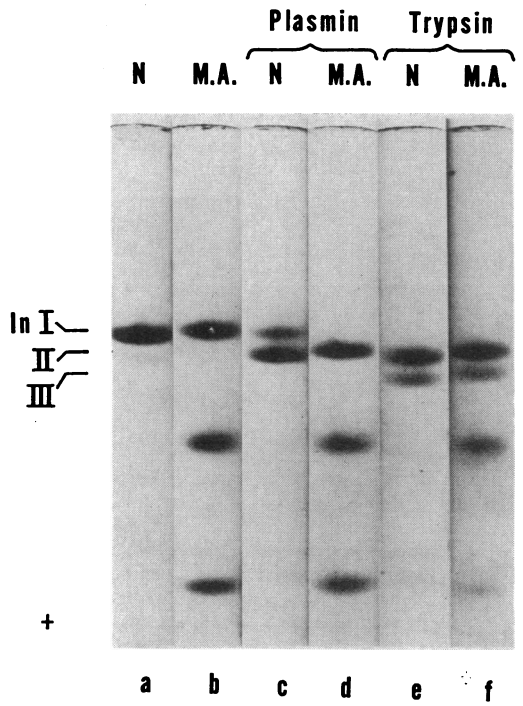

FIGURE 3 SDS gel (5% acrylamide) electrophoretic analysis of incubation mixtures of $C\bar{1}$ inactivator preparations from a normal individual and from patient M. A. with HANE after incubation with buffer, plasmin, or trypsin. The normal (N) or HANE (M. A.) inhibitors were incubated with buffer (gels a and b) or with equimolar concentrations of plasmin (gels c and d) or trypsin (gels e and f) for 30 min at 37°C. The samples were then applied to the gels after reduction with dithiothreitol as described in the Methods.

[3] It is not clear why the glycerol-activated plasmin-$C\bar{1}$ inactivator complex failed to remain intact in the presence of SDS and urea. One possibility is suggested by the study of Takeda and Nakabayashi, which demonstrated that glycerol-activated plasmin underwent autodigestion and therefore possessed a lower molecular weight than did freshly activated plasmin (13). These considerations have suggested that the degraded enzyme may have a deficiency in binding sites and hence may form a complex with $C\bar{1}$ inactivator which can be dissociated by denaturing agents.

1–24 h, similar to studies described previously (3). Before treatment with neuraminidase, both abnormal inhibitor preparations had an electrophoretic mobility identical to the normal C1 inactivator protein when analyzed on alkaline acrylamide gels (7.5%) or by cellulose acetate electrophoresis. After incubation with neuraminidase, the net electric charge of the inhibitor was decreased, as has been described by Rosen et al. (3), and the various inhibitor preparations demonstrated a slower, but still identical electrophoretic mobility. Analysis of the neuraminidase-treated inhibitors by SDS-acrylamide gel electrophoresis demonstrated an apparent 5% decrease in molecular weight (Table I) of both the normal and abnormal inhibitor preparations. It appeared as if a similar amount of sialic acid was available for enzymic digestion by neuraminidase. Thus, under the experimental conditions employed, the structural defect in the nonfunctional inhibitor could not be ascribed to the sialic acid moiety.

Amino acid composition of normal and functionally abnormal C1 inactivator obtained from SDS-acrylamide gels. A comparison was made between the amino acid compositions of normal and abnormal C1 inactivator after acid hydrolysis of acrylamide gel slices containing the respective protein. A summary of these results, as well as the composition for purified C1 inactivator reported by Haupt, Heimburger, Kranz, and Schwick (14) is presented in Table II. Although major differences were not detected between the composition of the normal and the abnormal C1 inactivator obtained from two patients, it cannot be concluded that the protein moiety of the inhibitors are identical since minor compositional variations would not have been detected. The values for certain amino acids varied as much as 5% between individual analyses of the same preparation. This experimental variation was similar to the apparent percent molecular weight difference between the normal and abnormal inhibitors (e.g., 105,000 vs. 109,000 daltons). In general, the values obtained for aspartic acid, threonine, serine, and glutamic acid were slightly higher in the abnormal C1 inactivator preparations. Values for phenylalanine in the normal protein (3.38) exceeded experimental variation for the abnormal proteins (2.97, 2.95) and may thus reflect a significant difference between the normal and abnormal protein moiety of C1 inactivator.

A comparison of the amino acid composition for C1 inactivator recovered from acrylamide gels after electrophoresis (column 2, Table II) and that reported by Haupt et al. (14) (column 1, Table II) indicated a number of significant variations in relative amino acid content. One possible explanation of these discrepancies is that the procedures employed in the present study have achieved a further purification of the C1 inactivator.

TABLE II

Amino Acid Composition of Normal and Abnormal C1-Inactivator Obtained from SDS-Acrylamide Gels (% Amino Acid)

Amino acid	C1 inactivator*	Normal C1 inactivator	Abnormal C1 inactivator	
			M. A.	M. S.
Lysine	4.43	4.08	3.93	3.81
Histidine	1.59	1.53	1.75	1.50
Arginine	2.72	1.55	1.65	1.60
Aspartic acid	5.92	6.19	6.34	6.42
Threonine‡	5.88	6.42	6.66	6.72
Serine‡	4.34	5.94	6.31	6.48
Glutamic acid	7.64	6.93	7.44	7.07
Proline	3.53	3.97	3.50	3.53
Glycine	0.93	2.26	2.01	2.47
Alanine	2.49	4.08	4.28	4.28
Half-cystine§	0.55	0.68	0.68	0.68
Valine	3.59	4.52	4.34	4.39
Methionine	1.79	1.21	1.20	1.30
Isoleucine	2.26	2.28	2.33	2.34
Leucine	7.09	7.46	7.51	6.95
Tyrosine	2.64	1.15	0.99	1.09
Phenylalanine	4.14	3.38	2.97	2.95
Tryptophan‖	1.31	1.31	1.31	1.31
Carbohydrates‖	34.70	34.70	34.70	34.70
Total percent‖	97.5	99.6	99.9	99.6

* Amino acid composition of C1 inactivator as reported by Haupt et al. (14).

‡ Not corrected for partial destruction during hydrolysis.

§ Half-cystine was determined as cysteic acid in normal C1 inactivator.

‖ Neither the values for tryptophan nor carbohydrates were determined in the present study; therefore those values reported by Haupt et al. (14) were utilized for comparison. The total percent has excluded values obtained for ammonia.

DISCUSSION

This report has examined several of the structural features of two nonfunctional C1 inactivator proteins isolated from the plasma of a mother and daughter with the variant form of HANE. These individuals possessed a C1 inactivator protein immunologically identical to normal C1 inactivator, but which failed to inhibit either the proteolytic or esterolytic activity of C1s, the activated subunit of the first component of complement.

Analysis of the abnormal inhibitor proteins in the presence of denaturing and reducing agents by SDS-acrylamide gel electrophoresis suggested that each consisted of a single polypeptide chain, the mobility of which was slower than that of the normal inhibitor. The apparent molecular weight of both abnormal inhibitors was 109,000 daltons as contrasted to 105,000 daltons found for the normal inhibitor protein. The findings of

the accompanying communication (4) that plasmin and trypsin degraded $C\bar{1}$ inactivator in a sequential and restricted manner suggested that the use of these endopeptidases might further define the structural aberration responsible for the apparent molecular weight difference between the normal and abnormal inhibitors. Studies of the interaction between the patients' inhibitors and these enzymes indicated that the structural difference responsible for the slow mobility in SDS-acrylamide gels appeared to be a property of the core molecule which remained after proteolytic degradation. Plasmin, which failed to form a complex with the nonfunctional inhibitors, produced an inhibitor derivative whose apparent molecular weight was 3,000 daltons greater than the derivative formed by the interaction of normal $C\bar{1}$ inactivator and plasmin (Fig. 3, Table I). Furthermore, as was the case for plasmin, the pattern of degradation of the abnormal inhibitor molecule by trypsin was similar to that established for the normal protein; however, each of the two trypsin derivatives from the patients' $C\bar{1}$ inactivator had an apparent molecular weight 3,000 daltons greater than did the analogous polypeptide chain from the normal inhibitor (Fig. 3, Table I). Thus, the nonfunctional as well as the intact inhibitor molecules possessed plasmin- and trypsin-susceptible regions which appeared to be similar.

It is probable that the slower electrophoretic mobility of the abnormal inhibitors as well as of their plasmin and trypsin derivatives in SDS gel electrophoresis was due to an increase in the molecular weight of the molecules; however, factors other than molecular weight may be responsible for our observation. Thus, charge and conformational changes may decrease SDS binding (15, 16). Although a conformational change has not been ruled out, it is unlikely that a difference in net charge is responsible for our results since the electrophoretic migration of the abnormal inhibitors was similar to the normal in both cellulose acetate electrophoresis and alkaline acrylamide gel electrophoresis. Furthermore, the inhibitors shared identical elution patterns after anion exchange chromatography.

Although treatment of the different inhibitor proteins with neuraminidase failed to demonstrate a difference in susceptibility with respect to apparent molecular weight loss, this finding does not rule out the possibility that an alteration in sialic acid content, or, alternatively, that other abnormal carbohydrate moieties might be responsible for the electrophoretic pattern of the nonfunctional inhibitors.

No major difference in the amino acid composition of the abnormal inhibitors as compared to the normal was documented. The acidic amino acids tended to be only slightly higher in the patients' inhibitor than in the normal, and the phenylalanine content appeared to be somewhat reduced in the abnormal inhibitor preparations. The association between these relatively minor deviations in the amino acid content of the abnormal as compared to the normal $C\bar{1}$ inactivator preparation and the loss of functional activity is unresolved.

The study of Rosen and co-workers (3) established an impressive genetic heterogeneity in eight kindred with nonfunctional inhibitor protein characterized by the differences in the ability to bind $C\bar{1}s$, in electrophoretic mobility in agarose, and in the ability to block the esterolytic activity of $C\bar{1}s$. Additional evidence for the functional heterogeneity of the $C\bar{1}$ inactivator was documented by Gigli, Mason, Colman, and Austen (17), who found that the plasma from two patients with the variant form of HANE inhibited kallikrein but not $C\bar{1}s$. A further abnormality was demonstrated by Rosen et al. (3) in the sera of affected individuals from a kindred that contained over four times normal concentration of $C\bar{1}$ inactivator protein. These patients, as well as an individual reported previously by Laurell, Lindegren, Malmros, and Martensson (18) possessed an inactivator protein with two different electrophoretic mobilities, apparently explained by the noncovalent binding of the inhibitor to albumin.

In the present investigation, the abnormality of the $C\bar{1}$ inactivator molecule from the kindred studied was found to be a property of its covalent structure. In view of the documented heterogeneity of the inhibitor in patients with HANE, it is possible that each additional kindred examined might be found to possess an inhibitor molecule with a unique structural abnormality. Thus, this study has identified some of the features of the covalent structure of a nonfunctional $C\bar{1}$ inactivator protein from a mother and daughter with the variant form of HANE and has, in addition, provided an approach to the study of these inhibitor proteins using endopeptidases to further define their abnormal structure.

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