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PREPARATION OF HUMAN KININOGEN—III

ENZYMATIC DIGESTION AND MODIFICATION*

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Abstract—Purified human plasma kininogen of mol. wt 70,000 has been shown to be resistant to carboxypeptidase B treatment and to be a substrate for both plasma and urinary kallikrein. Two kininogen-containing peaks isolated from fresh plasma by stepwise column chromatography on Sephadex DEAE-A50 were shown to have identical mobility upon disc gel electrophoresis in 8 M urea or after neuraminidase treatment and to have sedimentation coefficients slightly smaller and slightly larger than that of albumin. These data, together with a difference in partial specific volume, suggest a role for carbohydrate content in accounting for the apparent heterogeneity of kininogens.

HUMAN PLASMA kininogen of mol. wt 70,000, purified as previously described,[1,2] has been further examined with regard to its inactivation by carboxypeptidase B and its ability to serve as a substrate for purified preparations of human plasma or urinary kallikrein.

Because of the still unresolved question of the number and characteristics of human kininogen(s),[3–5] a single, rapid chromatographic step‡ was employed to separate two kininogen-containing fractions from fresh human plasma. These kininogens have been examined with regard to certain of their physicochemical characteristics and their relationship to the purified kininogen previously described.[1,2]

EXPERIMENTAL

Reagents. Hexadimethrine bromide (Polybrene) was purchased from Aldrich Chemical Co., Milwaukee, Wis.; 1,10-phenanthroline, from Eastman Kodak Co., Rochester, N.Y.; 8-hydroxyquinoline, from K & K Laboratories, Plainview, N.Y.; bradykinin, from New England Nuclear Corp., Boston, Mass.; Clostridial neuraminidase, from Worthington Biochemical Corp., Freehold, N.J.; and recrystallized, lyophilized bovine pancreatic trypsin (195–200 U/mg), from Worthington Biochemical Corp., Freehold, N.J.

Monospecific antiserum against human kininogen was prepared as described.[2] Antisera against albumin, α_2-macroglobulin, inter-α-trypsin inhibitor, α_1-antitrypsin, and inter-α-chymotrypsin inhibitor were purchased from Behring Diagnostics

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‡ M. Webster, personal communication.

(Woodbury, N.Y.); antiserum against the inhibitor of the first component of complement ($C\bar{1}INH$) was obtained from Miles-Yeda, Kankakee, Ill. These antisera were employed in counter-immunoelectrophoresis assays[6] to detect the presence of the relevant antigen.

Kininogen preparation. Fresh human plasma was collected in the presence of Na_2EDTA and hexadimethrine for the purification of kininogen by ion exchange chromatography, gel filtration and disc gel electrophoresis.[1,2] A rapid, single-step procedure for separating kininogens from plasma at room temperature employed a column of Sephadex DEAE-A50 in Tris chloride buffer at pH 8·0. Kininogen-containing fractions were eluted with stepwise cuts of the same buffer containing increasing concentrations of sodium chloride.* Characterization of the kininogens by disc gel electrophoresis in the presence of 8 M urea or after neuraminidase treatment, and by sucrose density gradient and isopycnic centrifugation, was performed as described.[2]

Treatment of kininogen with carboxypeptidase B. The kininogen-containing fractions were assessed during their purification for susceptibility to carboxypeptidase B. Twenty-five microlitres of a solution of 40 μg/ml carboxypeptidase B, commercially available after diisopropylfluorophosphate (DFP) treatment to inactivate trypsin and chymotrypsin (70 U/mg, Worthington Biochemical Corp., Freehold, N.J.), was added to 0·1–0·5 ml of kininogen preparations in 0·05 M phosphate at pH 7·5. To assess the carboxypeptidase activity in the presence of the kininogen-containing fractions, 100 ng of bradykinin was added to duplicate tubes of kininogen. After incubation for 10 min at 37°, the carboxypeptidase was inactivated by boiling for 15 min. To release kinin from the heat-inactivated kininogen, 200 μg of trypsin was introduced and the mixture incubated for 30 min at 37°.[7] The trypsin was inactivated by boiling for 15 min before assaying the kinin on the guinea-pig terminal ileum.

Preparation of kallikreins and enzymatic release of kinin from kininogen. Hageman factor fragments and prekallikrein were isolated from human plasma as described[8,9] and incubated together to generate plasma kallikrein.

Human urinary kallikrein was prepared from pooled human urine which had been concentrated 16-fold by ultrafiltration (Amicon UM 10, Amicon Corp., Lexington, Mass.). At each step, urinary kallikrein was detected by its ability to release kinin from heat-inactivated plasma, prepared from blood collected without glass contact in Na_2EDTA (9 mg/10 ml) and hexadimethrine (0·1 mg/10 ml of a 36 mg/ml solution). The plasma was heated for 2 hr at 61° and dialyzed overnight at 4° against saline containing Na_2EDTA (800 mg/l.) and hexadimethrine (200 mg/l.). After overnight dialysis of the concentrated urine against 0·05 M potassium phosphate, pH 6·5, 110 ml was applied to a 5 × 100 cm DE-52 cellulose (Whatman, Reeve Angel, Clifton, N.J.) column equilibrated with the dialysis buffer. After collection of the effluent, a linear gradient was applied consisting of 2 l. of the dialysis buffer and 2 l. of the same buffer containing 1·0 M KCl. Fractions generating kinin from heat-inactivated plasma were located as a broad peak in the area of 12 mS salt concentration, and were pooled and concentrated by ultrafiltration to 9 ml. The pH was adjusted to 5·0 with acetic acid and the concentrate applied to a 5 × 100 cm Sephadex G-100 column equilibrated with 0·05 M sodium acetate at pH 5·0, and eluted with the same buffer.

* M. Webster, personal communication.

Enzymatically active urinary kallikrein was located in the region of apparent mol. wt 25,000–30,000 using bovine serum albumin and chymotrypsinogen A as reference proteins.[10] The active fractions were pooled, concentrated by ultrafiltration to 10 ml, dialyzed against distilled water and lyophilized. This material had neither kinin nor kininase activity, and showed two prealbumin bands, an albumin band and a faint beta contaminant upon alkaline disc gel electrophoresis. A somewhat similar method of preparing human urinary kallikrein has been described by Pierce and Nustad.[11]

Kininogen obtained from plasma after DE-52 cellulose chromatography and gel filtration[1,2] was incubated at 37° with either plasma or urinary kallikrein, mixtures of the two enzymes, or first with one enzyme and then with the other. The kinin released was assayed on the guinea-pig ileum and compared to a synthetic bradykinin standard; the biologic activity generated was not further identified as either bradykinin or kallidin. The kininogen preparations[1,2] used were shown to be free of kinin-generating and kininase activity.

RESULTS

Purification of a pretransferrin kininogen

Preparation. Kininogen, purified as previously described,[1,2] was assayed for its apparent molecular weight by gel filtration, its sedimentation coefficient by sucrose density gradient ultracentrifugation, and its partial specific volume by isopycnic centrifugation; these values agree with those previously published,[2] resulting in a calculation of mol. wt = 70,000. Two modifications of this purification procedure were assessed: the effect of collecting plasma in citrate, and the use of ammonium acetate during the gel filtration step.

In order to assess the efficacy of employing hexadimethrine bromide and Na_2EDTA in the collection and dialysis of plasma for kininogen preparation and the use of hexadimethrine in the column chromatography, blood was collected without glass contact in a standard acid–citrate dextrose solution (0·15 ml/ml blood, U.S.P. Formula A) or in hexadimethrine bromide and Na_2EDTA as for the preparation of kininogen. After harvesting the plasma, paired aliquots were stored for 20 hr at 37 or 4° with or without kaolin (1 mg/0·1 ml plasma). Each sample was then divided in two, brought to 1·0 ml with 0·2% acetic acid and boiled for 30 min. After reneutralization with 1·0 N NaOH, one aliquot of each pair received 200 μg trypsin. All aliquots were incubated at 37° for 30 min[7] and then boiled for 15 min prior to assay on the guinea pig ileum. None of the trypsin-free controls contained residual kinin. The citrated plasma sample incubated for 20 hr at 37° contained 8 μg bradykinin equivalents of kininogen/ml plasma after incubation with kaolin, and 9 μg without; the hexadimethrine–Na_2EDTA sample contained 10 and 12 μg respectively. At 4°, the citrated plasma showed about 90 per cent loss of kininogen with or without kaolin, while the hexadimethrine and Na_2EDTA-prepared sample contained 8 μg with kaolin and 10 μg without. That the loss of kininogen seen with citrate is due to activation of plasma prekallikrein was supported by the finding that Fletcher factor plasma, genetically deficient in prekallikrein,[12] did not lose kininogen when stored in citrate at 4°. After incubation for 20 hr, the sample without kaolin contained 11·7 μg of bradykinin equivalents/ml plasma, and the sample with kaolin, 10·5 μg/ml.

Substitution of 0·02 M ammonium acetate (pH 7·0) for phosphate buffer during the gel filtration step yielded kininogen eluting as a single peak with the void volume, suggesting an apparently larger molecule than was routinely recovered in phosphate buffer. Further examination of this kininogen by sucrose density gradient centrifugation indicated a sedimentation coefficient slightly smaller than that of albumin, while its electrophoretic mobility was like that of the aggregated kininogen previously described.[2] After dialysis against phosphate buffer, this kininogen migrated into the pretransferrin position upon alkaline disc gel electrophoresis. (Kininogen prepared in ammonium acetate was not employed in studies described elsewhere in this paper.)

Carboxypeptidase B treatment. To determine whether kininogen exists in a native form susceptible to inactivation of the kinin moiety, or whether such susceptibility[13] is created due to partial denaturation or proteolytic digestion during purification, the resistance of kininogen was examined after purification. To subsequently determine the capacity of the kininogen to serve as a source of kinin, the carboxypeptidase B required inactivation prior to the addition of a kinin-releasing enzyme. Preincubation of carboxypeptidase B for 30 min with the chemical inhibitors, 1,10-phenanthroline or 8-hydroxyquinoline, at concentrations of 4×10^{-3} M, failed to protect 100 ng of bradykinin from complete inactivation in 2 min. Na_2EDTA, 3×10^{-3} M, gave 60 per cent inhibition after 15 min and 90 per cent inhibition after 30-min preincubation with carboxypeptidase B. Immersion in boiling water for 15 min yielded complete inactivation and was used to inactivate carboxypeptidase B; because heating decreases the susceptibility of kininogen to kallikrein,[14] trypsin was then used as the kinin-releasing enzyme. As shown in Table 1, kininogen after gel filtration and disc gel electrophoresis was resistant to carboxypeptidase B activity. That the carboxypeptidase was active in these incubation mixtures was shown by the observation that 100 ng of exogenous bradykinin was not detectable when these samples were assayed.

Enzymatic release of kinin from kininogen. Kininogen isolated by ion exchange chromatography and gel filtration was free of the kallikrein inhibitors α_2-macroglobulin[15] and the inhibitor of the first component of complement ($C\bar{1}INH$)[16,17]

TABLE 1. TREATMENT OF KININOGEN WITH CARBOXYPEPTIDASE B

Kininogen source	Carboxypeptidase B (μg)	Bradykinin added (ng)	Kinin released (ng)
Sephadex G-100 gel filtration			
Expt. 1	0	0	100
	7·2	0	100
	7·2	100	100
Expt. 2	0	0	110
	0·9	0	110
	0·9	100	110
Polyacrylamide gel eluates			
Expt. 1	0	0	25
	7·2	0	25
Expt. 2	0	0	100
	0·9	0	100
	0·9	100	100

TABLE 2. RELEASE OF KININ FROM KININOGEN WITH PLASMA OR URINARY KALLIKREIN OR COMBINATION OF BOTH ENZYMES

Kininogen (ml)	Enzyme*	Incubation time (min)	Kinin release (ng)
Expt. 1			
0·1	1 μg urinary kallikrein	2	40
0·1	1 μg urinary kallikrein	5	50
0·1	20 μg urinary kallikrein	5	45
0·1	20 μg urinary kallikrein	10	50
0·1	20 μg urinary kallikrein	30	45
0·1	30 μl plasma kallikrein	2	40
0·1	30 μl plasma kallikrein	10	50
0·1	50 μl plasma kallikrein	10	40
0·1	30 μl plasma kallikrein followed by 1 μl urinary kallikrein	4 2	50
Expt. 2			
0·1	none	5	0
0·1	10 μg urinary kallikrein	5	25
0·1	20 μg urinary kallikrein	5	20
0·1	30 μl plasma kallikrein	5	15
0·1	60 μl plasma kallikrein	5	20
0·1	30 μl plasma kallikrein mixed with 10 μg urinary kallikrein	5	20

* Because the prekallikrein has only been functionally purified, the plasma kallikrein derived from it is expressed in terms of volume.

as assessed by counter-immunoelectrophoresis. Of the other plasma protease inhibitors examined, inter-α-trypsin inhibitor had been removed, the α_1-chymotrypsin inhibitor was present in trace quantity and α_1-antitrypsin was prominent. Removal of α_1-antitrypsin by disc gel electrophoresis yielded a kininogen with reduced reactivity with plasma kallikrein. Since a highly purified preparation of this inhibitor has not been found to inhibit plasma kallikrein[18] or urinary kallikrein (J. Spragg and A. D. Schreiber, unpublished observations), the material isolated at the gel filtration step was used to examine the reactivity of kininogen with these enzymes. Either plasma or urinary kallikrein released kinin from kininogen as shown in Table 2. Incubation of 1 μg of purified human urinary kallikrein with kininogen for 2 min yielded 40 ng of kinin. This yield was only slightly greater when the enzyme concentration or the time of interaction with the substrate was increased. Thirty μl of Hageman factor activated plasma kallikrein, exhibiting a similar *p*-tosyl-L-arginine methyl ester (TAMe) esterase activity,[19] as the lower urinary kallikrein dose used (0·01 μM/min) also yielded 40 ng of kinin during 2 min of interaction with kininogen. As seen with urinary kallikrein, increasing the time of incubation or the concentration of plasma kallikrein did not increase the kinin released. Incubation of kininogen with plasma kallikrein for 4 min and then with urinary kallikrein for 2 min yielded 50 ng of kinin, a value not significantly greater than the amount released with either enzyme alone. Table 2 also shows the results obtained using a similar preparation of kininogen and a mixture of enzymes compared to doubling each enzyme alone. Both enzymes appear to act on the same substrate as indicated by the fact that the quantity of kinin formed with the lower concentration of each alone is similar to that achieved with a mixture of the two enzymes.

Isolation of pretransferrin and posttransferrin kininogens

Column fractionation. The single-step separation of kininogens resulted in two regions of activity as depicted in Fig. 1. The 0·2 M salt eluate yielded two protein peaks, the second of which was associated with kininogen activity identified by the ability to serve as a heat-inactivated substrate for the trypsin-induced release of kinin. The 0·4 M salt eluate yielded a third protein peak which also contained kininogen. The inhibitor of the first component of complement was identified in both the 0·2 and 0·4 M salt fractions, and α_2-macroglobulin was found in the former. Because plasma kallikrein inhibitors were present antigenically, the ability of the unheated fractions to inhibit kallikrein functionally was determined using plasma kallikrein and heat-inactivated plasma as a substrate. Under such circumstances the column fractions

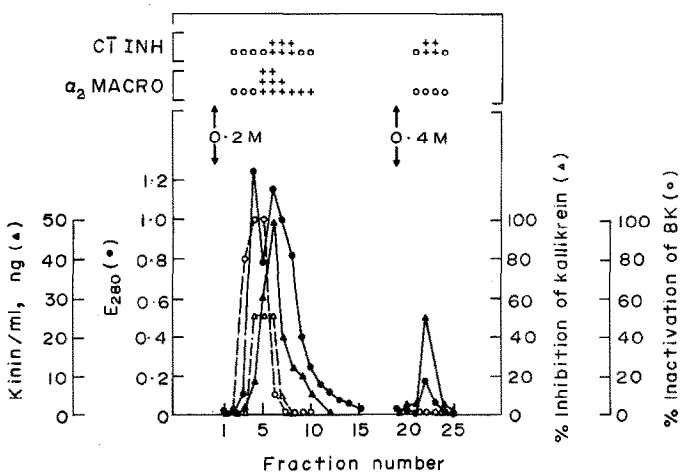

FIG. 1. Separation of two kininogen-containing regions on Sephadex DEAE-A50. Kininogen activity is associated with the second protein peak eluting with pH 8 Tris buffer containing 0·2 M NaCl, and with the protein peak eluting with the same buffer containing 0·4 M NaCl. Kinin-destroying and kallikrein inhibitory activities are located overlapping the first kininogen peak. Counter-immunoelectrophoresis identified the plasma kallikrein inhibitors C1INH. in the kininogen-containing region of the 0·2 and 0·4 M salt eluates. and α_2-macroglobulin. in the 0·2 M salt eluate.

contribute both inhibitors and substrate, although the heat-inactivated plasma is the principle substrate and lacks inhibitor and kininase activity. Fractions overlapping the 0·2 M kininogen eluate gave partial inhibition, and a five-fold concentrate of the 0·4 M eluate completely inhibited plasma kallikrein. Kininase activity, identified by the ability of unheated fractions to inactivate 100 ng of bradykinin upon incubation at 37° for 5 min, was located earlier than and overlapping the 0·2 M kininogen. Because of contamination with plasma kallikrein inhibitors and kininase activity, these kininogen preparations were not examined with regard to their activity with plasma or urinary kallikrein and further studies were limited to their physicochemical characteristics.

Mobility in acrylamide disc gels. After electrophoresis in alkaline disc gels, kininogens were located at two different regions, both functionally (Fig. 2) and antigenically. The kininogen eluted with 0·2 M salt migrated to the pretransferrin region and the

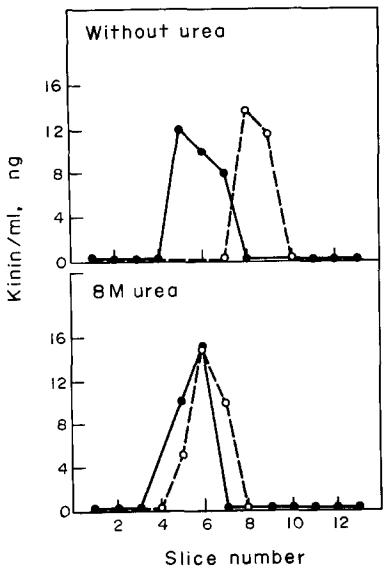

FIG. 2. Alkaline polyacrylamide disc gel electrophoresis of the kininogens eluted from Sephadex DEAE-A50 with buffer containing 0·2 M NaCl (●——●) or 0·4 M NaCl (○——○). The anode is to the left. The upper panel shows the results obtained in the absence of urea, and the bottom panel, the results obtained when the electrophoresis is performed in 8 M urea. Kininogen was identified by trypsin treatment and bioassay of the kinin released.

kininogen obtained in the 0·4 M eluate was identified in the β region of the gel. When each kininogen was electrophoresed in disc gels containing 8 M urea, no difference in mobility was observed (Fig. 2), and both kininogens moved in the pretransferrin region. The loss of mobility differences upon electrophoresis in 8 M urea could be attributed to disaggregation, bonding disruption by urea, or to minor charge differences negated in the presence of 8 M urea. The role of minor charge differences contributed by sialic acid residues on the carbohydrate sidechains was investigated by treatment of both kininogens with Clostridial neuraminidase. The mobility of the kininogens eluted with 0·2 and 0·4 M salt, when detected by antigenic activity, were $R_f = 0{\cdot}52$ and 0·43, respectively, while after neuraminidase treatment, the mobility of both kininogens was $R_f = 0{\cdot}33$.

Sucrose density gradient centrifugation. Centrifugation of both kininogens in 5–25 per cent linear gradients of sucrose followed by trypsin treatment and bioassay of the eluted fractions revealed both kininogens to have sedimentation coefficients similar to that of albumin (Fig. 3). The kininogen eluted with 0·2 M salt was slightly lighter than albumin and the kininogen recovered in the 0·4 M salt eluate was slightly heavier. Because of the large amount of contaminating protein in these preparations, more accurate calculation of the sedimentation coefficient was not attempted. Incubation of these kininogens with neuraminidase under conditions which alter mobility on disc gel electrophoresis did not appear to alter the sedimentation coefficient, nor did freezing and thawing.

Isopycnic centrifugation. Cesium chloride density gradient centrifugation of kininogens eluted at 0·2 and 0·4 M salt indicated a difference in density when detected antigenically with monospecific antikininogen (Fig. 4). The former had a density of

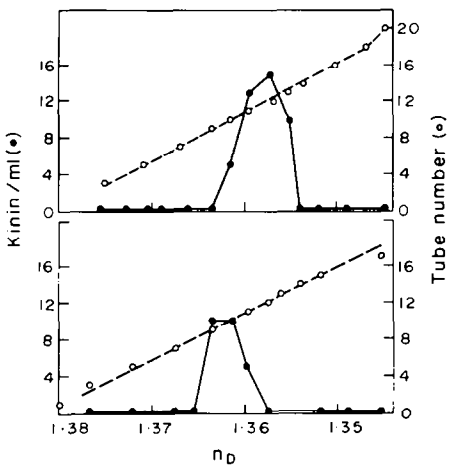

FIG. 3. Sucrose density gradient ultracentrifugation of the kininogens eluted as shown in Fig. 1 with buffer containing 0·2 M NaCl (top) or 0·4 M NaCl (bottom). Kininogen was located by trypsin treatment and bioassay of the kinin released, expressed here in terms of ng bradykinin/ml bath volume. The peak of albumin activity, located at n_D = 1·3600, was determined by counter-immunoelectrophoresis.

1·3114 ($\bar{v}$ = 0·76) and the latter, a density of 1·3917 ($\bar{v}$ = 0·72). The partial specific volumes were 0·76 and 0·73, respectively, in a separate experiment employing another preparation of these kininogens, in which the fractions collected after centrifugation were assessed functionally following removal of the cesium chloride by dialysis.

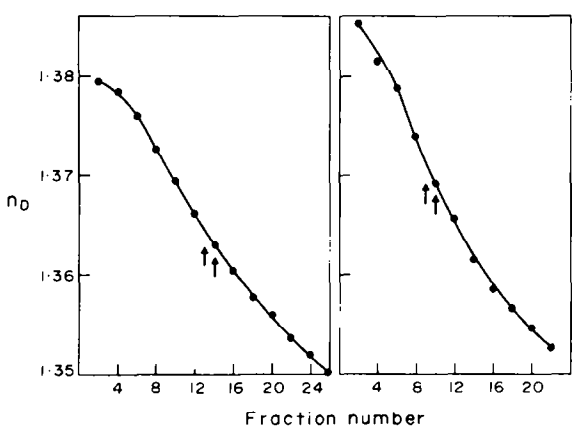

FIG. 4. Isopycnic centrifugation in 30% (w/w) cesium chloride of the kininogens eluted from Sephadex DEAE-A50 with buffer containing 0·2 M NaCl (left) or 0·4 M NaCl (right). After centrifugation, fractions were collected from the bottom of the tubes. The kininogen (arrows) was located by counter-immunoelectrophoresis employing monospecific antikininogen, and the densities were calculated as described.[2]

DISCUSSION

The present study was undertaken to further characterize the kininogen of mol. wt 70,000[1,2] and to examine certain properties of kininogen-containing material obtained without extensive purification. The 70,000 mol. wt material appeared to contain a

kininogen which served as a substrate for both purified plasma kallikrein and purified urinary kallikrein (Table 2). It was also resistant to inactivation by carboxypeptidase B as assessed by its capacity to yield kinin after heat denaturation and trypsin treatment (Table 1), suggesting protection of the carboxy terminal arginine of the kinin moiety by either internal localization or steric effects. The above result is in accord with the finding of Suzuki *et al.*[20] that carboxypeptidase B releases a limited amount of arginine from purified bovine kininogen of mol. wt 70,000 and that upon cyanogen bromide treatment the same material yields a peptide containing the kallidin sequence at the amino terminus and very little of the free decapeptide. These authors suggest that kininogens in plasma have the kinin moiety located internally, and that the carboxypeptidase B-sensitive material increases as a result of purification.[20] The purification and characterization of a circulating, enzymatically active, carboxypeptidase B-like enzyme, the anaphylatoxin inactivator, together with a survey of plasma proteins of known structure, have led to the suggestion that lysine or arginine residues should not be found at the carboxy terminus of human plasma proteins.[21] Thus, it would appear that the kinin moiety is located internally in human and bovine kininogen and that the appearance of kininogen susceptible to inactivation by carboxypeptidase B represents limited proteolysis of the native molecule.

Two kininogen-containing fractions, releasing kinin after heat denaturation and trypsin treatment and reacting with antibody prepared against the 70,000 mol. wt. kininogen,[2] have been separated by rapid chromatography at 25° on DEAE Sephadex (Fig. 1). The material eluting with 0·2 M salt appears similar to that previously described[2] with regard to mobility on alkaline disc gel electrophoresis, retardation of electrophoretic mobility after neuraminidase treatment, sedimentation rate and partial specific volume. Although the kininogens eluting from DEAE Sephadex were not examined functionally because of the contaminants described above, certain of their physicochemical characteristics were compared (Figs. 2–4). It was apparent that the introduction of 8 M urea (Fig. 2) or neuraminidase treatment eliminated the differential mobility on disc gel electrophoresis. The small difference in sedimentation coefficients (Fig. 3) suggests that these mobilities are mainly a function of charge rather than size. This observation is further strengthened by the finding that the kininogen eluting at 0·4 M salt has a lower partial specific volume than does the kininogen eluting with 0·2 M salt (Fig. 4), an indication of less carbohydrate content.[22] The results described above are consistent with the view that variation in carbohydrate sidechains participates in determining the differentiating physicochemical properties of the kininogens isolated here. Such differences in carbohydrate content have been described for other glycoproteins[23] and have recently been reported to account for the separation on Sephadex DEAE-A50 of two forms of bovine clotting factor X.[24]

The partial purification of human plasma kininogen(s) and examination with regard to physicochemical characterization, susceptibility to various kininogenases, and localization of the kinin moiety have been described by several authors (and reviewed[3–5]). Pierce and Webster[13] identified two kininogens eluting from hydroxylapatite, and exhibiting different susceptibility to inactivation by carboxypeptidase B and different electrophoretic mobilities. A mixture of the two kininogens had a molecular weight of approximately 50,000 by equilibrium ultracentrifugation.[3] Jacobsen and Kriz[25] have isolated two kininogens having molecular weights and sedimentation coefficients of 197,000, $S_{20,w} = 7{\cdot}68$; and 57,000 and $S_{20,w} = 3{\cdot}65$.

The high molecular weight kininogen had a mobility on disc gel electrophoresis of a beta globulin and the low molecular weight kininogen, that of an alpha globulin.[25] The high molecular weight kininogen reacted rapidly with a plasma pseudoglobulin preparation, used as a source of plasma kallikrein, while both the high and low molecular weight kininogens reacted rapidly with human saliva, used as a source of glandular kallikrein,[26] leading to the suggestion that kininogens of mol. wt 200,000 represent the only substrate for plasma kallikrein.[4] More recently, Habal and Movat[27] have described the preparation of two kininogens at room temperature. Although their higher molecular weight material eluted just after gamma globulin on Sephadex G-200 and had the mobility of a beta globulin on disc gel electrophoresis, it had a sedimentation coefficient of only 4·2. The low molecular weight kininogen eluted from Sephadex G-200 just prior to human serum albumin, had a sedimentation coefficient of 3·8, and an α_1-mobility on disc gel electrophoresis. Under conditions where both substrates reacted about equally with trypsin, an enzyme which prefers denatured substrate,[14] the high molecular weight kininogen reacted better with plasma kallikrein than did the low molecular weight kininogen.

Cochrane and Wuepper[28,29] have reported the isolation of a single kininogen from human plasma using a inhibitor of Hageman factor activation.[1] This material, with mol. wt 79,000 as determined by sodium dodecyl sulfate gel electrophoresis, and $S_{20,\omega} = 3{\cdot}8$, was demonstrated to be a substrate for purified human plasma kallikrein, human urinary kallikrein and trypsin. Recent examination of bovine kininogens indicates a high degree of peptide homology between 70,000 and 50,000 mol. wt material.[30] The authors have proposed a model of a single kininogen with the kinin moiety located internally which may undergo limited proteolysis to yield both altered high molecular weight kininogens and low molecular weight kininogen.[30] The present study demonstrates the protected localization of the kinin moiety within human kininogen of mol. wt 70,000, confirms the reactivity of this kininogen with both purified plasma and urinary kallikreins, and indicates a role for the carbohydrate sidechains in determining certain of the physicochemical differences between kininogens. Thus, variations in carbohydrate content and the degree of limited proteolysis may account for the apparent heterogeneity of the plasma protein containing the nonapeptide bradykinin sequence.

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