

CHARACTERIZATION OF HUMAN
HIGH MOLECULAR WEIGHT KININOGEN
Procoagulant Activity Associated with the Light Chain of
Kinin-Free High Molecular Weight Kininogen

BY RUSSELL E. THOMPSON,* ROBERT MANDLE, JR., AND ALLEN P. KAPLAN

(From the Allergic Diseases Section, Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014)

High molecular weight (HMW)¹ kininogen has been shown to be a critical factor which functions at the initial step of the Hageman factor-dependent pathways. Thus, plasmas deficient in HMW kininogen have a markedly prolonged partial thromboplastin time and diminished kaolin-activatable fibrinolysis (1-4). The Hageman factor substrates, prekallikrein and factor XI, circulate bound to HMW kininogen (5, 6) and are adsorbed to negatively charged surfaces where they interact with surface-bound Hageman factor. HMW kininogen augments the ability of activated Hageman factor or Hageman factor fragments to activate prekallikrein (7-10) and factor XI (7-11), and it also augments the rate of Hageman factor activation (8) and cleavage (7, 12) by kallikrein. Kallikrein also cleaves the HMW kininogen to liberate the vasoactive peptide bradykinin.

We have previously reported that kinin-free HMW kininogen could still function as a coagulation factor (2) and this observation was confirmed by Schiffman et al. (13). However, Chan et al. (14) reported that kallikrein digestion of human HMW kininogen progressively diminished its coagulant activity, while Matheson et al. (15) assessed bovine HMW kininogen in human HMW kininogen-deficient plasma and found that the kinin-free protein possessed <1% of the coagulant activity of the native molecule. In this report we investigate the structural changes that occur in purified human HMW kininogen as a consequence of kallikrein digestion. The critical portion of the molecule that is responsible for its coagulant activity has been isolated, as reported in preliminary form (6), and is shown to possess an antigenic determinant that distinguishes HMW kininogen from low molecular weight (LMW) kininogen.

* Predoctoral candidate, Department of Biochemistry, Georgetown University, Washington, D. C.

¹ *Abbreviations used in this paper:* CM, carboxymethyl; DFP, diisopropylfluorophosphate; HMW, high molecular weight; LMW, low molecular weight; PAGE, polyacrylamide gel electrophoresis; PTT, partial thromboplastin time; QAE, quaternary aminoethyl; SDS, sodium dodecyl sulfate.

Materials and Methods

Materials. Materials were obtained as follows: bradykinin triacetate; Sandoz Ltd., Basel, Switzerland); hexadimethrine bromide and diisopropylfluorophosphate (DFP) (Aldrich Chemical Co., Inc., Milwaukee, Wis.); dithiothreitol and iodoacetamide (Calbiochem, San Diego, Calif.); benzamidine hydrochloride (Eastman Kodak Co., Rochester, N. Y.); quaternary aminoethyl (QAE) Sephadex A-50, carboxymethyl (CM) Sepharose, and Sephadex G-200 (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.); sodium dodecyl sulfate (SDS) (Fisher Scientific Co., Fairlawn, N. J.). Kininogen-deficient plasma (Williams Trait) was provided by Dr. Robert Colman (University of Pennsylvania, Philadelphia, Pa.).

Isolation of HMW Kininogen. HMW kininogen was isolated from pooled human plasma containing 0.38% sodium citrate by a modification of the method of Habal et al. (16). 4 liters of plasma was directly applied to an 18.5 × 30-cm column of QAE-Sephadex A-50 equilibrated in 10 mM Tris-Cl, pH 8.0, which contained 0.15 M NaCl, 0.1 mM DFP, and 20 mM benzamidine. The column was washed with 10 liters of this same Tris buffer containing 0.2 M NaCl. HMW kininogen was then eluted with 0.45 M NaCl and fractions were assayed for their ability to correct the partial thromboplastin time of HMW kininogen-deficient plasma. Solid ammonium sulfate was added to 50% saturation, and the suspension was stirred for 30 min, then centrifuged for 90 min at 1,200 *g*. The precipitate was collected and redissolved in ~1 liter of 30 mM sodium acetate buffer, pH 5.9, containing 0.1 mM DFP and 20 mM benzamidine, and the solution was adjusted to a conductivity of 30 mS with NaCl. The sample was then applied to a 5 × 20-cm column of CM-Sepharose equilibrated with the same buffer. HMW kininogen was eluted with a linear salt gradient which contained 0.6 M NaCl in the limiting buffer. The fractions containing coagulant activity were pooled, dialyzed against 0.1% ammonium bicarbonate containing 0.1 mM DFP, and lyophilized. Final purification utilized gel filtration through a 5 × 100-cm column of Sephadex G-200 equilibrated with 0.2 M ammonium bicarbonate containing 0.1 mM DFP. 10-ml samples were applied, 10-ml fractions were collected, and the flow was 40 ml/h. Fractions were concentrated by lyophilization and redissolved in 0.003 M phosphate-buffer containing 0.15 M NaCl, pH 7.4.

Purified HMW kininogen was assessed by reduced SDS polyacrylamide gel electrophoresis (SDS-PAGE) and revealed a single major band of 120,000 daltons (Fig. 3, first gel). In some preparations, trace amounts of cleavage products were observed which were subsequently shown to be analogous to kallikrein digestion products of HMW kininogen. The HMW kininogen contained no detectable Hageman factor, prekallikrein, factor XI, or the α -globulin inhibitors C₁INH, α_2 macroglobulin, α_1 antitrypsin, α_1 antichymotrypsin, antithrombin III, or inter- α -trypsin inhibitor.

Reduction and Alkylation of HMW-Kininogen. For reduction, samples were made 0.05 M in dithiothreitol and incubated for 1 h at 37°C. The samples were then alkylated with 0.12 M iodoacetamide and further incubated for 30 min at room temperature.

Preparation of Plasma Kallikrein. Prekallikrein was isolated by the procedure of Mandle and Kaplan (17). SDS gel electrophoresis of reduced prekallikrein yielded two bands of 88,000 and 85,000 daltons, respectively, as previously reported (17) and the preparation contained no detectable Hageman factor or factor XI. Active kallikrein was obtained by incubating 100 μ g of prekallikrein with 2 μ g of Hageman factor fragments (18) overnight at 4°C. Conversion to kallikrein was assessed after reduction and SDS-PAGE which yielded the characteristic kallikrein heavy chain (52,000 daltons) and light chains (36,000 and 33,000 daltons) (17). The resulting kallikrein (100 μ g/ml) was then serially diluted, and 200 μ l of each dilution was incubated with 200 μ l HMW kininogen (1 mg/ml) for 1 h at 37°C. Analysis of the cleaved HMW kininogen samples for bradykinin generation and by reduced SDS-PAGE indicated the optimal concentration of kallikrein necessary in order to obtain maximal cleavage of HMW kininogen in 1 h.

Preparation of Antibody Specific for HMW-Kininogen. Antibody to HMW kininogen was produced in a goat by intramuscular injection of 1.0 mg HMW kininogen emulsified in complete Freund's adjuvant. The goat was boosted intradermally 3 wk later with 0.5 mg HMW kininogen emulsified in incomplete Freund's adjuvant. The animal was bled after a further 3 wk. By immunoelectrophoresis (Fig. 1) this antiserum reacted with both the HMW and LMW kininogens found in normal plasma, and a reaction of partial identity was seen. No precipitin line was obtained when the antiserum was reacted with the kininogen-deficient plasma (Williams trait

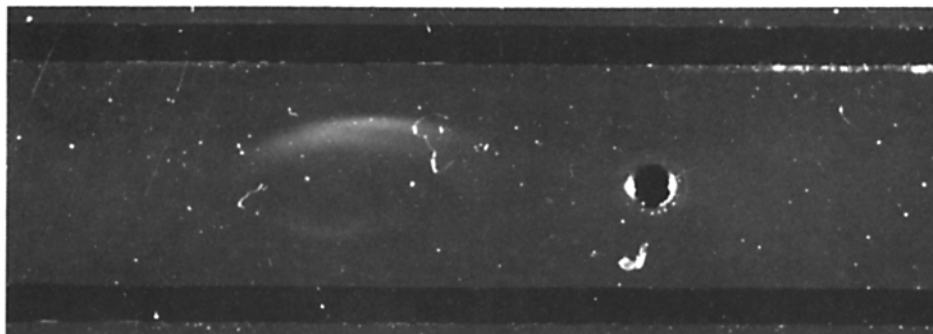


FIG. 1. Immunoelectrophoresis of normal plasma reacted against antibody to human kininogens (upper trough) and reacted against antibody rendered monospecific for HMW kininogen (lower trough). A line of partial identity between HMW and LMW kininogens is seen.

plasma). Adsorption of this antibody with Fitzgerald trait plasma, which contains only LMW kininogen (4) resulted in an antibody which was specific for the unique HMW kininogen antigenic determinants. The precipitin arc seen with this antiserum in the lower trough (Fig. 1) corresponds to the minor precipitin arc seen with the use of the anti-kininogen serum in the upper trough. This is consistent with previous estimates that HMW kininogen is $\cong 20\%$ of the total plasma kininogen (2, 19).

Coagulation Assay. The partial thromboplastin time (PTT) was measured by the method of Proctor and Rapaport (20) as modified by Colman et al. (2) using congenitally deficient plasmas.

Gel Analysis. SDS gel electrophoresis was performed either in cylindrical gels or in a slab apparatus (Bio-Rad Laboratories, Richmond, Calif.) by the procedure of King and Laemmli (21). For reduction, samples were made 10% in 2-mercaptoethanol (by volume) and were boiled for 2 min in 1% SDS before application to gels.

Urea disc gel electrophoresis was performed by the method of Reisfeld and Small (22). Samples were made 7 M in urea and incubated for 15 min at 37°C before the gels were loaded.

Immunoelectrophoresis. Immunoelectrophoresis was performed on microscope slides in 1% agarose containing Veronal buffer, pH 8.6, 0.06 μ , and 0.01 M EDTA (23).

Bradykinin Determination. Bradykinin was assayed by bioassay with the isolated guinea pig ileum (18).

Results

HMW kininogen was incubated with 1.0% plasma kallikrein by weight at 37°C for time intervals of 1, 2, and 9 h. Samples were removed and then subjected to SDS-PAGE. As seen in the nonreduced gels (Fig. 2), the starting material had a major band at 120,000 daltons and a second band that was diminished by $\cong 15,000$ daltons. Upon digestion with kallikrein for 1 h, virtually all of the material was found at 105,000 daltons; a minor band was seen at 100,000 daltons. The generation of bradykinin paralleled the formation of this 105,000-dalton kininogen. Prolonged digestion for up to 9 h revealed two major bands at 105,000 and 100,000 daltons, respectively, as well as evidence of further cleavage to yield a minor band at 90,000 daltons. Identical samples were also reduced and subjected to SDS gel electrophoresis. The starting HMW kininogen had a major band at 120,000 daltons and minor bands at 115,000 and 110,000, as well as a band at 85,000–90,000 daltons. Digestion with kallikrein resulted in loss of each of these bands and formation of heavy chains of 56,000–66,000 daltons, as shown in the gels in Fig. 3, and a light chain of 37,000

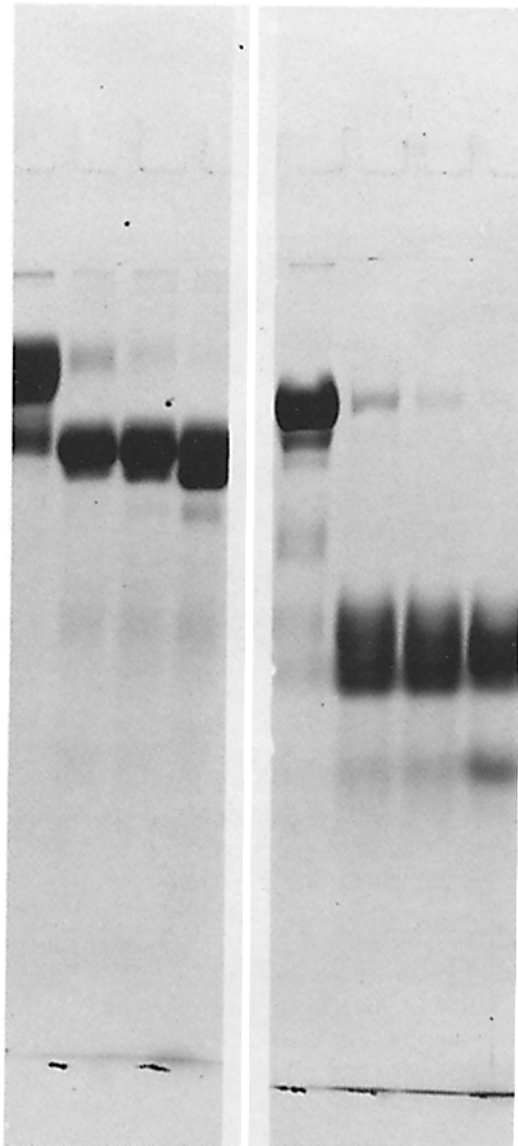


FIG. 2. Left, SDS-PAGE of 25 μ g HMW kininogen alone followed by HMW kininogen after digestion with 0.1% kallikrein for 1, 2, and 9 h. Right, SDS-PAGE of reduced samples containing 25 μ g HMW kininogen alone, followed by HMW kininogen after digestion with 0.1% kallikrein for 1, 2, and 9 h.

daltons. The light chain stained faintly and is best visualized in the final gel. Thus, in contrast to native HMW kininogen, the kinin-free protein appeared to consist of multiple chains that were linked by disulfide bonds. A time course of digestion of a second preparation of HMW kininogen using 1.0% kallikrein by weight was next performed and samples were taken at intervals of up to 1 h. The samples were reduced and assessed by SDS-PAGE. As shown in Fig. 3, there was progressive cleavage of the starting material (120,000 daltons) to

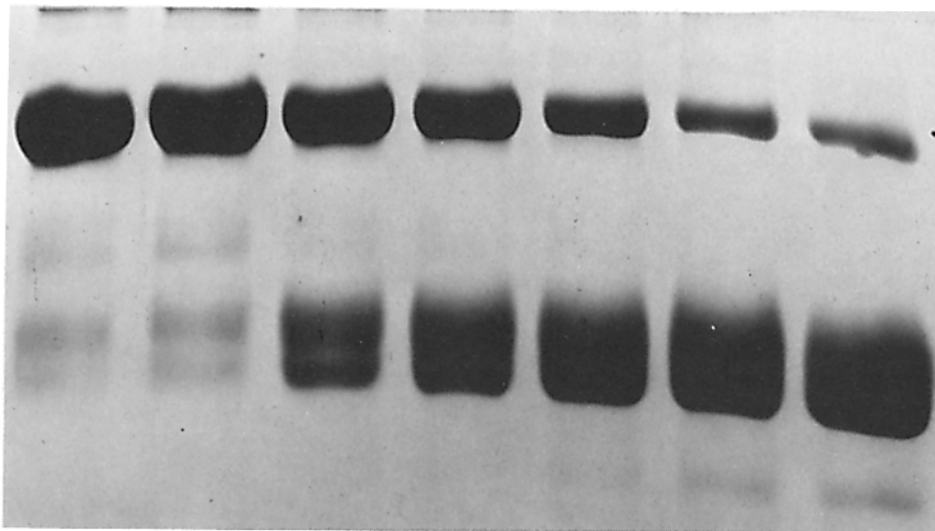


FIG. 3. SDS-PAGE of reduced samples after incubation of 500 μg HMW kininogen with 5 μg kallikrein at 37°C. 30 μg of protein was applied to the gels. The starting material is shown at the left followed by samples taken after digestion for 1, 5, 10, 20, 30, and 40 min. Two heavy-chain bands at 66,000 and 56,000 daltons are seen, as well as a light-chain band at 37,000 daltons.

yield heavy chains at 66,000 and 56,000 daltons, and a light chain at 37,000 daltons. A small fraction of the starting HMW kininogen preparation was already cleaved, as indicated by the double-banded heavy chain; however, the major protein present was clearly a single chain. The 66,000- plus 37,000-dalton bands total 103,000 daltons, thus approximating the molecular weight of the kinin-free protein; the 56,000-dalton band may represent further cleavage of the HMW kininogen heavy chain.

Experiments were next performed to determine the effect of kallikrein digestion and disulfide bond cleavage on the ability of HMW kininogen to correct the PTT of HMW kininogen-deficient plasma. Equivalent amounts of HMW kininogen or reduced and alkylated HMW kininogen were each incubated with 1% kallikrein by weight for 1 h at 37°C. The samples were then serially diluted and assayed for clotting activity. As seen in Fig. 4, neither digestion with kallikrein nor prior disulfide bond cleavage, nor a combination of the two procedures appeared to have any effect upon the clotting activity. Furthermore, reduction of disulfide bonds after kallikrein digestion did not alter the coagulant activity.

We therefore proceeded to purify the portion of the HMW kininogen molecule that was responsible for its coagulant activity. 1 mg of HMW kininogen was digested with 2% kallikrein for 1 h at 37°C. The cleavage products were then subjected to gel filtration through Sephadex G-200 equilibrated in 6 M guanidine-HCl in order to dissociate any noncovalently linked fragments. All of the clotting activity eluted with the major protein peak shortly behind the void volume of the column representing the kinin-free HMW kininogen. The only other peak of absorbance was located at approximately one gel bed volume of

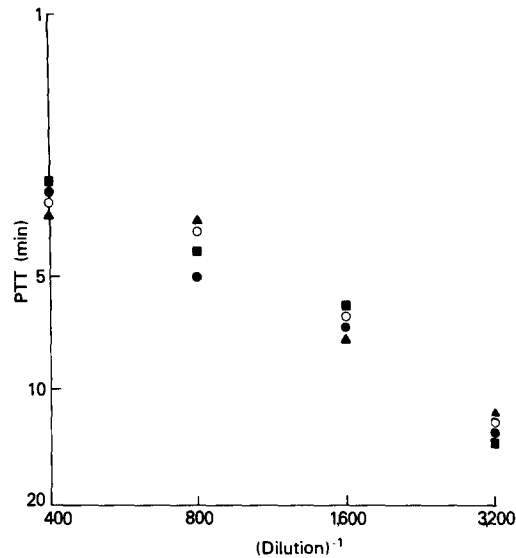


FIG. 4. Assessment of the partial thromboplastin time of HMW kininogen before and after cleavage with kallikrein. Identical samples of HMW kininogen were also reduced and alkylated (R/A) and assayed before and after digestion with kallikrein. (○), control HMW kininogen; (△), HMW kininogen + kallikrein; (●), R/A HMW kininogen; (■), R/A HMW kininogen + kallikrein.

the column. The initial protein peak was then reduced and alkylated with 0.05 M dithiothreitol and 0.12 M iodoacetamide, respectively, and reappplied to the same Sephadex column. As seen in Fig. 5, two absorption peaks at 220 nm were obtained and all the clotting activity was associated with the second peak. SDS-PAGE analysis of column fractions (shown above) revealed that the first peak contained the HMW kininogen heavy chain while the second peak contained the HMW kininogen light chain (Fig. 5). When the same fractions were subjected to disc gel electrophoresis in 7 M urea (Fig. 6), two light chain bands were resolved. When a replicate sample of gel 6 was sliced and eluted, and the eluates were assayed for their ability to correct the coagulation defect of HMW-kininogen deficient plasma, both light chain bands were shown to possess coagulant activity. The denser band had about three times the activity of the minor band. When the heavy- and light-chain peaks were dialyzed, concentrated, and assessed by immunoelectrophoresis with monospecific antibody to HMW kininogen (lower trough; Fig. 1), a precipitin arc was obtained with the light chain, while the antiserum did not react with the heavy chain.

Discussion

Purified human HMW kininogen is a single chain molecule of 120,000 daltons as assessed by SDS gel electrophoresis. LMW kininogen shares antigenic determinants with HMW kininogen (24), and antibody to purified LMW kininogen has been used to prepare an immunoabsorbant that will bind both HMW kininogen and LMW kininogen (2, 19). Yet LMW kininogen possesses no known coagulant activity. It is therefore of particular interest to determine

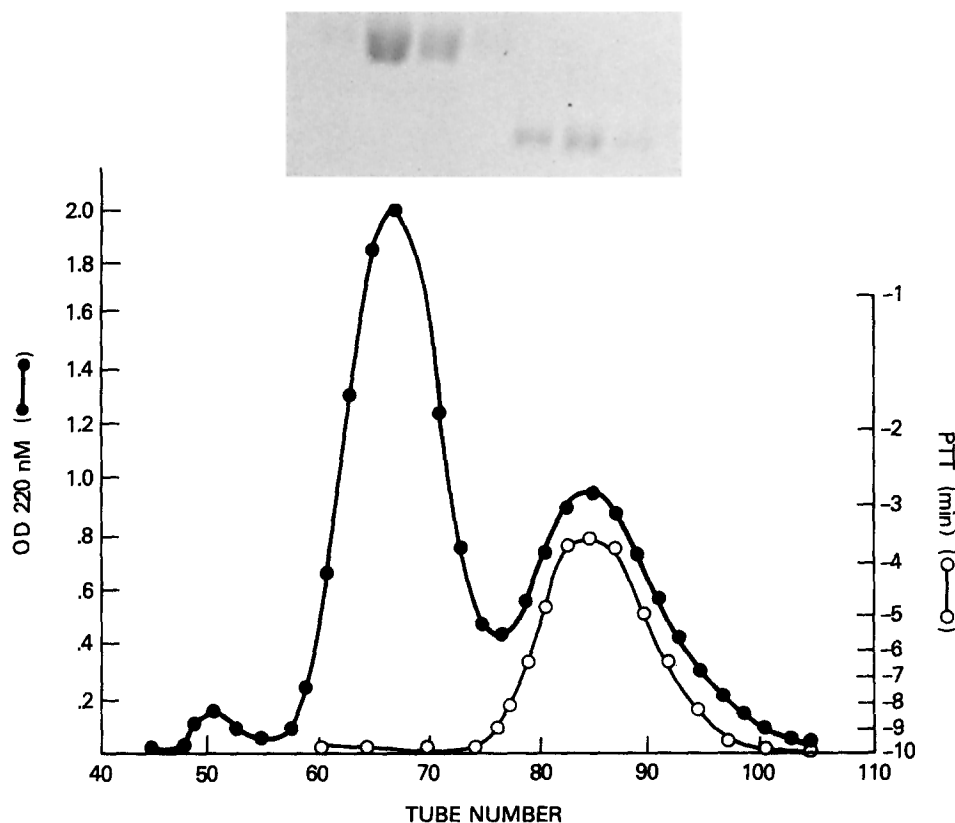


FIG. 5. Sephadex G-200 gel filtration of reduced kinin-free HMW kininogen. The absorbance at 220 nm is shown in addition to the ability of portions to correct the partial thromboplastin time of HMW kininogen-deficient plasma. Above (inset) is shown the SDS-PAGE pattern obtained after electrophoresis of 100 μ l of tubes 60, 65, 70, and 75 (heavy chain), followed by electrophoresis of 100 μ l of a 10-fold concentration of tubes 80, 85, and 90 (light chain).

the structural features of HMW kininogen that are required for its coagulant activity and to compare them to LMW kininogen.

Detailed analysis of bovine HMW kininogen has revealed two molecular forms: one a single chain molecule, whereas the other possessed two chains that were linked by disulfide bonds (25). Since the bradykinin moiety was not at either end of the single-chain HMW kininogen, two peptide bond cleavages were required to liberate it. However, both forms of HMW kininogen were found to possess full kinin-generating capacity. It was therefore proposed that the second form might represent a kininogen which has been cleaved at a single position within a disulfide bond. Digestion of the bovine HMW kininogen not only yields bradykinin but also liberates a fragment of 14,000 daltons which is designated fragment 1.2 (15, 25). Subsequent cleavage of fragment 1.2 by prolonged digestion with kallikrein yields fragment 1—a glycopeptide (26–28)—and fragment 2—a histidine-rich peptide (25). When human HMW kininogen was isolated rapidly, in the presence of proteolytic inhibitors, >90% of the isolated product was a single-chain molecule. It therefore appears likely

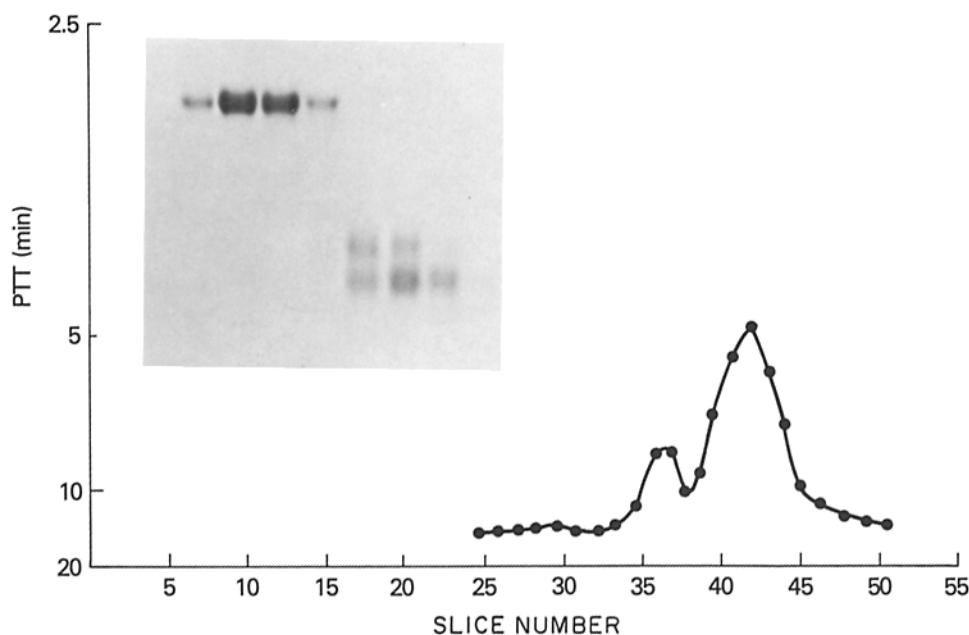


FIG. 6. Urea disc gel electrophoresis of samples taken from the Sephadex G-200 gel filtration of reduced kinin-free HMW kininogen (Fig. 5). 100 μ l of tubes 60, 65, 70, and 75 followed by 100 μ l of a 20-fold concentration of tubes 80, 85, and 90 were applied. A replicate gel of sample 6 (second from right) was sliced, each slice was eluted with 0.2 ml PBS, and the eluates were assayed for their ability to correct the partial thromboplastin time of HMW kininogen-deficient plasma. The peaks of coagulant activity seen at slices 36-38 and 40-44 correspond to the two bands seen in the gel.

that two-chain forms of human HMW kininogen reported by others (29), as well as one of the forms of bovine HMW kininogen, have been cleaved during the process of purification. Our findings are in general agreement with those of Nagasawa and Nakayasu (30) who purified human HMW kininogen in the presence of both benzamidine and DFP, and isolated a single-chain form. Although a reduction in molecular weight of kallikrein-digested human HMW kininogen is observed (Fig. 2), we have not isolated the peptides, if any, that are released.

Evidence that human HMW kininogen is heterogeneous, however, does exist. Reduced SDS gel electrophoresis of the starting material reveals two or three bands differing by 5,000-10,000 daltons, each of which can be cleaved by plasma kallikrein. In addition, the unreduced kinin-free kininogen is seen as two bands (Fig. 2) and, upon reduction, the heavy chain is seen as two or more bands (Figs. 2, 3). The sum of these two major forms of heavy chain (Fig. 3) totals 122,000, which exceeds the size of the kinin-free material. It therefore appears unlikely that each is a separate chain of kinin-free HMW kininogen. Furthermore, the isolated heavy chain appears homogeneous by antigenic analysis, and the sum of the molecular weight of the heavy chain and light chain approximates that of kinin-free HMW kininogen. Thus, kallikrein-digested, kinin-free HMW kininogen appears to consist of a single heavy-chain disulfide linked to a single light chain. The heterogeneity of the heavy chain

bands observed may reflect the size heterogeneity of the starting material and/or represent further proteolysis by plasma kallikrein. Clearly, in Fig. 2 we see gradual conversion to the 100,000 dalton molecule of a portion of the kinin-free HMW kininogen seen at 105,000 daltons. Although the light chain appears homogeneous by size it, too, demonstrates heterogeneity when assessed by urea disc gel electrophoresis (Fig. 6).

As shown in Fig. 4, digestion of HMW kininogen with plasma kallikrein does not affect its ability to function as a coagulation factor and neither reduction and alkylation of HMW kininogen before or after interaction with kallikrein, nor exposure to 6 M guanidine hydrochloride affects its clotting activity. An apparent loss of $\approx 15,000$ daltons accompanies kallikrein cleavage. Thus, neither bradykinin nor any other liberated peptide is required for the expression of this function. Our data therefore do not agree with reports that kallikrein digestion of human (14) or bovine (15) HMW kininogen destroys its coagulant activity and confirms the earlier observation that the kinin-free protein retains full activity (2). It is possible that species incompatibility accounts for the fact that digested bovine HMW kininogen will not clot human HMW kininogen-deficient plasma. Consistent with this suggestion is a recent report indicating that the clotting activity of the undigested bovine HMW kininogen may represent only a fraction of that obtained with equal quantities of the human protein (31). Nevertheless, the coagulant activity of the starting material was retained upon digestion with urinary kallikrein, and a chain containing bovine fragment 1.2 attached to light chain was shown to be responsible for the activity (31). Perhaps the human counterpart of fragment 1.2 is not cleaved from the light chain by plasma kallikrein.

It is also possible that one or more peptides could adhere to the human kinin-free protein or to its isolated light chain by noncovalent interaction and thereby contribute to the coagulant activity seen. However, when the human kinin-free protein is reduced, alkylated, and exposed to 6 M guanidine, the isolated light chain contains all of the coagulant activity. Furthermore, the isolated light chain (Fig. 5) does not differ in size from that observed when kallikrein-digested HMW kininogen is reduced and subjected to SDS gel electrophoresis (Fig. 2). The two forms of light chain observed upon urea disc gel electrophoresis may reflect charge heterogeneity (Fig. 6). However, we also considered the possibility that one band might represent a small peptide bound to light chain that resists dissociation in urea and guanidine and that might be important for the observed coagulant activity. Since both forms of light chain possessed coagulant activity, if binding of a small peptide were responsible for the heterogeneity, it would not be critical for functional activity. We therefore conclude that the light chain of human kinin-free HMW kininogen is the coagulant part of the molecule. The light chain also contains a unique antigenic determinant that distinguishes HMW kininogen from LMW kininogen. This finding is in agreement with observations that the amino acid compositions of the heavy chains of bovine HMW and LMW kininogens are identical (32). Thus, kininogens appear to share heavy chains. It is possible that Williams trait plasma (2), which is deficient in both forms of kininogen, has a defect involving the heavy chain, while Fitzgerald trait plasma (4), which has no

HMW kininogen but has about one-third to one-half normal LMW kininogen, has a defect in the HMW kininogen light chain. Further studies are now in progress to evaluate the structural features required for binding of HMW kininogen to surfaces and to bind prekallikrein and factor XI.

Summary

Human high molecular weight (HMW) kininogen has been isolated and was found to be a single chain protein of $\approx 120,000$ daltons. Upon digestion with plasma kallikrein bradykinin is generated, and SDS gel electrophoresis of the kinin-free protein reveals an apparent loss in size of 15,000 daltons. The kinin-free kininogen retains full activity as a coagulation factor and consists of two chains: a heavy chain of $\approx 66,000$ daltons disulfide-linked to a light chain of 37,000 daltons. The heavy chain of HMW kininogen shares antigenic determinants with LMW kininogen and possesses no detectable coagulant activity. The isolated light chain is shown to be responsible for the coagulant activity of HMW kininogen and contains a unique antigenic determinant that distinguishes HMW kininogen from low molecular weight kininogen.

Received for publication 13 September 1977.

References

1. Wuepper, K. D., D. R. Miller, and M. J. LaCombe. 1975. Flaujeac trait: deficiency of human plasma kininogen. *J. Clin. Invest.* 56:1663.
2. Colman, R. W., A. Bagdasarian, R. C. Talamo, C. F. Scott, M. Seavey, J. A. Guimaraes, J. V. Pierce, and A. P. Kaplan. 1975. Williams trait: human kininogen deficiency with diminished levels of plasminogen proactivator and prekallikrein associated with abnormalities of the Hageman factor dependent pathways. *J. Clin. Invest.* 56:1650.
3. Saito, H., O. D. Ratnoff, R. Waldmann, and J. P. Abraham. 1975. Fitzgerald trait. Deficiency of a hitherto unrecognized agent, Fitzgerald factor, participating in surface-mediated reactions of clotting, fibrinolysis, generation of kinins, and the property of diluted plasma enhancing vascular permeability (PF/Dil). *J. Clin. Invest.* 55:1082.
4. Donaldson, V. H., H. I. Glueck, M. A. Miller, H. Z. Movat, and F. Habal. 1976. Kininogen deficiency in Fitzgerald trait: Role of high molecular weight kininogen in clotting and fibrinolysis. *J. Lab. Clin. Med.* 87:327.
5. Mandle, R., Jr., R. W. Colman, and A. P. Kaplan. 1976. Identification of prekallikrein and HMW kininogen as a circulating complex in human plasma. *Proc. Natl. Acad. Sci. U. S. A.* 73:4179.
6. Thompson, R. E., R. Mandle, Jr., and A. P. Kaplan. 1977. Association of factor XI and high molecular weight kininogen in human plasma. *J. Clin. Invest.* 60:1376.
7. Griffin, J., and C. G. Cochrane. 1976. Mechanism for the involvement of high molecular weight kininogen in surface-dependent reactions of Hageman factor. *Proc. Natl. Acad. Sci. U. S. A.* 73:2559.
8. Meier, H. L., C. F. Scott, R. Mandle, Jr., M. E. Webster, J. V. Pierce, R. W. Colman, and A. P. Kaplan. 1977. Requirements for contact activation of human Hageman factor. *Ann. N. Y. Acad. Sci.* 283:93.
9. Meier, H. L., J. V. Pierce, R. W. Colman, and A. P. Kaplan. 1977. Activation and function of human Hageman factor. The role of high molecular weight kininogen

- and prekallikrein. *J. Clin. Invest.* 60:18.
10. Liu, C. Y., C. F. Scott, A. Bagdasarian, J. V. Pierce, A. P. Kaplan, and R. W. Colman. 1977. Potentiation of the function of Hageman factor fragments by high molecular weight kininogen. *J. Clin. Invest.* 60:7.
 11. Schiffman, S., and P. Lee. 1975. Partial purification and characterization of contact activation cofactor. *J. Clin. Invest.* 56:1082.
 12. Revak, S. D., C. G. Cochrane, and J. H. Griffin. 1977. The binding and cleavage characteristics of human Hageman factor during contact activation. A comparison of normal plasma with plasmas deficient in factor XI, prekallikrein, or high molecular weight kininogen. *J. Clin. Invest.* 59:1167.
 13. Schiffman, S., P. Lee, D. I. Feinstein, and R. Pecci. 1977. Relationship of contact activation cofactor (CAC) procoagulant activity to kininogen. *Blood.* 49:935.
 14. Chan, J. Y. C., F. M. Habal, C. E. Burrowes, and H. Z. Movat. 1976. Interaction between Factor XII (Hageman factor), high molecular weight kininogen and prekallikrein. *Thromb. Res.* 9:423.
 15. Matheson, R. T., D. R. Miller, M. J. LaCombe, Y. N. Han, S. Iwanaga, H. Kato, and K. D. Wuepper. 1976. Flaujeac factor deficiency. Reconstitution with highly purified bovine high molecular weight kininogen and delineation of a new permeability-enhancing peptide released by plasma kallikrein from bovine high molecular weight kininogen. *J. Clin. Invest.* 58:1395.
 16. Habal, F. M., H. Z. Movat, and C. E. Burrowes. 1974. Isolation of two functionally different kininogens from human plasma. Separation from proteinase inhibitors and interaction with plasma kallikrein. *Biochem. Pharmacol.* 23:2291.
 17. Mandle, R., Jr., and A. P. Kaplan. 1977. Hageman factor substrates. II. Human plasma prekallikrein. Mechanism of activation by Hageman factor and participation in Hageman factor-dependent fibrinolysis. *J. Biol. Chem.* 252:6097.
 18. Kaplan, A. P., and K. F. Austen. 1970. A prealbumin activator of prekallikrein. *J. Immunol.* 105:802.
 19. Pierce, J. V., and J. A. Guimaraes. 1976. Further characterization of highly purified human plasma kininogens. In *The Kallikrein System in Health and Disease*. J. J. Pisano and K. F. Austen, editors. Fogarty International Center Proceedings. U. S. Govt. Printing Office, Washington, D. C. 27:121.
 20. Proctor, R. R., and S. I. Rapaport. 1961. The partial thromboplastin time with kaolin. A simple screening test for first stage clotting deficiencies. *Am. J. Clin. Pathol.* 35:212.
 21. King, J., and U. K. Laemmli. 1971. Polypeptides of the tail fibres of bacteriophage T₄. *J. Mol. Biol.* 62:465.
 22. Reisfeld, R. A., and P. A. Small. 1966. Electrophoretic heterogeneity of polypeptide chains of specific antibodies. *Science (Wash. D. C.)*. 152:1253.
 23. Scheidegger, J. J. 1955. Une micro-méthode d'immunoélectrophorèse. *Int. Arch. Allergy Appl. Immunol.* 7:103.
 24. Pierce, J. V., and M. E. Webster. 1966. The purification and some properties of two different kallidinogens from human plasma. In *Hypotensive Peptides*. E. G. Erdos, N. Back, and F. Sicuteri, editors. Springer-Verlag, New York Inc., New York. 130.
 25. Kato, H., Y. N. Han, S. Iwanaga, T. Suzuki, and M. Komiya. 1975. Bovine plasma HMW and LMW-kininogens. Isolation and characterization of the polypeptide fragments produced by plasma and tissue kallikreins. In *Kinins—Pharmacodynamics and Biological Roles*. F. Sicuteri and N. Back, editors. Plenum Publishing Corp., New York. 135.
 26. Han, Y. N., H. Kato, S. Iwanaga, and T. Suzuki. 1974. Studies on the primary structure of bovine high molecular weight kininogen. *J. Biochem.* 77:55.
 27. Han, Y. N., H. Kato, S. Iwanaga, and T. Suzuki. 1976. Bovine high molecular

- weight kininogen: The amino acid sequence of fragment 1 (glycopeptide) released by the action of plasma kallikrein and its location in the precursor protein. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 63:197.
28. Han, Y. N., H. Kato, S. Iwanaga, and T. Suzuki. 1976. Primary structure of bovine plasma high-molecular-weight kininogen. The amino acid sequence of a glycopeptide portion (fragment 1) followed the C-terminus of the bradykinin moiety. *J. Biochem.* 79:1201.
 29. Habal, F. M., B. J. Underdown, and H. Z. Movat. 1975. Further characterization of human plasma kininogens. *Biochem. Pharmacol.* 24:1241.
 30. Nagasawa, S., and O. Nakayasu. 1974. Enzymatic and chemical cleavages of human kininogens. In *Chemistry and Biology of the Kallikrein-kinin System in Health and Disease*. J. J. Pisano and K. F. Austen, editors. Fogarty International Center Proceedings. U. S. Govt. Printing Office, Washington, D. C. 27:139.
 31. Waldmann, R., A. G. Scicli, G. M. Scicli, J. A. Guimaraes, O. A. Carretero, H. Kato, Y. N. Han, and S. Iwanaga. 1977. Significant role of fragment 2 plus light chain of bovine high molecular weight kininogen in contact mediated coagulation. *Thromb. Haemostasis.* 38:14. (Abstr.).
 32. Kato, H., Y. N. Han, S. Iwanaga, T. Suzuki, and M. Komiya. 1976. Bovine plasma HMW and LMW kininogens. Structural differences between heavy and light chains derived from the kinin-free proteins. *J. Biochem.* 80:1299.