Henry Ford Hospital Medical Journal

Volume 27
Number 3 *John W. Rebuck Testimonial Issue*

Article 8

9-1979

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Henry Ford Hosp Med Journal
Vol 27, No 3, 1979

The Role of High Molecular Weight Kininogen (Fitzgerald Factor) in the Activation of Various Plasma Proteolytic Enzyme Systems†

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Bovine high molecular weight kininogen (bHMWK) partially corrects the aPTT of Fitzgerald-trait plasma, which is congenitally deficient in HMWK. The relationship between the structure and activity of HMWK was clarified by studying the effects of different fragments of bHMWK on the aPTT of Fitzgerald-trait plasma. The peptides studied, all in equimolar concentrations, were lys-bradykinin-free HMWK, bradykinin-fragment 1-2-free HMWK, heavy chain, fragment 1-2-light chain, and light chain. Bradykinin-fragment 1-2-free HMWK, heavy chain, and light chain have little or no correcting activity upon Fitzgerald-trait plasma aPTT. Fragment 1-2 light chain has the same correcting activity as intact bHMWK, while that of lys-bradykinin-free HMWK appears to be higher. Both fragment 1-2 and fragment 2 inhibit the clotting time of normal human plasma. On a molar basis, fragment 2 is a more active inhibitor than fragment 1-2. Bovine plasma kallikrein released kinins from both bHMWK and hHMWK; however, while the correcting activity of bHMWK was completely destroyed after sixty minutes of incubation, that of hHMWK was fully retained. These data suggest that: (1) the active part of bHMWK is comprised of the fragment 1-2 light chain portion; (2) fragment 1-2 or fragment 2 is the binding site to negatively charged surfaces, while the light chain interacts with other components of the surface-mediated reactions; and (3) bovine plasma kallikrein releases kinins but probably does not cause the release of fragment 1-2 from hHMWK.

When normal plasma comes in contact with negatively charged surfaces such as glass, kaolin, and connective tissue, a group of plasma proteolytic enzymes is activated. The proteins that have been identified as being involved in this contact reaction are: Hageman factor (factor XII), Fletcher factor (prekallikrein), and Fitzgerald factor (high molecular weight kininogen, HMWK). The interaction of these plasma proteins on a negatively charged surface results in the activation of factor XI, prekallikrein, and plasminogen (1-11). Congenital deficiencies in any of these proteins result in abnormalities of the intrinsic clotting and fibrinolytic pathways and in the formation of kinins (5-11).

Until the Fitzgerald-trait (HMWK deficiency) was discovered (8), the only known role of HMWK was as a substrate for plasma kallikrein. Now, it is also recognized as a necessary component for the activation by contact of the above mentioned proteolytic pathways. Data from our laboratory (12) and from others (13) have shown that purified bovine high molecular weight kininogen (bHMWK) partially corrects the prolonged activated plasma thromboplastin time (aPTT) and the kaolin-activated euglobulin-lysis time of Fitzgerald-trait plasma. Several investigators have found that, while hydrolysis of human HMWK (hHMWK) by human plasma kallikrein results in kinin release, the ability of kinin-free HMWK to correct the abnormalities of Fitzgerald-trait plasma is not affected (11, 14,15). Hydrolysis of bHMWK by bovine plasma kallikrein results in the release of a large peptide (fragment 1-2) as well as bradykinin. The bradykinin-fragment 1-2 free bHMWK had almost no correcting activity when tested on Fitzgerald-trait plasma (12,13). These differences in the correcting activity of both human and bovine kininogens after hydrolysis may be due to a lack of fragment 1-2 cleavage from the hHMWK by plasma kallikrein.

To determine whether fragment 1-2 is necessary for bHMWK correcting activity, our laboratory tested kinin-free bHMWK with intact fragment 1-2 for its correcting activity upon the aPTT of Fitzgerald-trait plasma. Also, to examine whether bovine plasma kallikrein decreases the correcting activity of hHMWK, we incubated these two proteins and determined the correcting activity of the mixture.

† Supported in part by a grant from the Michigan Heart Association. These data were presented at the VI International Congress on Thrombosis and Haemostasis in Philadelphia, 1977 (Thrombos Haemost 1977; 38:14).

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To clarify the relationship between the structure and the function of HMWK, the correcting activity of various isolated fragments of bHMWK was studied. Since we previously reported that fragment 2 (histidin rich fragment) has an inhibitory effect upon the clotting time of normal human plasma, we also studied the inhibitory activity of fragment 1-2.

Materials and Methods

Reagents and chemicals

Sodium barbital, NaCl, acid-washed kaolin (Fisher Scientific Company, Pittsburgh, PA), and $CaCl_2$ anhydrous (J.T. Baker Chemical Products, Clifton, NJ) were commercially obtained. Mr. Fitzgerald himself provided us with HMWK deficient plasma. Nine parts of blood were mixed with one part of 3.8% sodium citrate. Plasma was separated by centrifugation, distributed in 3 ml aliquots in polystyrene tubes, and kept frozen at −70°C.

Purified bovine proteins

Intact bHMWK, Lys-bradykinin-free HMWK, bradykinin-fragment 1-2 free HMWK, fragment 1-2, fragment 2, and plasma kallikrein were obtained as previously described (16-18). Heavy chain and light chain were obtained by reduction and alkylation of the bradykinin-fragment 1-2 free bHMWK. Fragment 1-2-light chain was similarly obtained from the lys-bradykinin-free bHMWK (18). Purified hHMWK was obtained by modifying the method of Pierce and Guimaraes (19).

Kaolin aPTT

The correcting activity of HMWK and its fragment on the aPTT of Fitzgerald plasma were tested by adding the following in a polystyrene tube: 0.1 ml of Fitzgerald plasma, 0.1 ml of the test substance in Owren's buffer, and 0.1 ml of cephalin-kaolin suspension (10 mg of acid-washed kaolin suspended in 1.0 ml of cephalin). After this mixture was pre-incubated at 37°C for eight minutes, 0.1 ml of 0.02 M $CaCl_2$ was then added and the clotting time determined.

Inhibition of the aPTT of normal plasma by fragment 1-2 and fragment 2

Cephalin-kaolin suspension (0.1 ml) was incubated for one minute with an Owren's buffer solution of the fragment to be tested (0.1 ml). Normal plasma (0.1 ml) was added and the mixture incubated for three minutes. After 0.02 M $CaCl_2$ (0.1 ml) had been added, the clotting time was determined.

Comparison of the correcting activity of bHMWK and its fragments on the aPTT of Fitzgerald-trait plasma on a molar and on a weight basis

Intact bHMWK (M.W. 76,000), lys-bradykinin-free bHMWK (M.W. 75,000), bradykinin-fragment 1-2-free bHMWK (M.W. 66,000), and fragment 1-2-light chain (M.W. 48,500) were dissolved in Owren's buffer at concentrations of 1.32, 0.26, and 0.13 nMol/ml. The correcting activity of 0.1 ml of each solution was tested upon Fitzgerald-trait plasma aPTT. Simultaneously, the correcting activity of serial dilutions of a pool of normal human plasma and its fragments was determined. We then used this curve to calculate the correcting activity of bHMWK and its fragments in U/nMol. One unit of correction is defined as the correcting activity of 200 μl of normal human plasma.

To compare the correcting activity of bHMWK and its fragments on a weight basis, 100 μg of each of the prelyophilized test materials were dissolved in 1.0 ml of Owren's buffer, and 0.1 ml was used to determine their correcting activity upon Fitzgerald plasma aPTT.

Effects of bovine plasma kallikrein on the correcting activity of human and bovine HMWK

Purified bovine plasma kallikrein (0.28 μg) and hHMWK (95 μg) were dissolved in 1.0 ml of Owren's buffer (pH 7.5) and incubated at 37°C for thirty and sixty minutes. At both times two aliquots of 20μl each of the incubation mixture were taken. One was diluted by mixing it with 80 μl of Owren's buffer, and the correction upon the aPTT of Fitzgerald-trait plasma was tested immediately. The second aliquot was mixed with 1.980 μl of a solution of 0.1 M SBTI in 0.1 M Tris-HCl buffer (pH 7.4). This mixture was immersed in a boiling water bath for ten minutes, centrifuged, and the kinins measured. The same procedure was followed with bHMWK (50 μg) except that 100 μl of undiluted incubation mixture was used for testing its Fitzgerald correcting activity. When human and bovine HMWK were incubated with Trypsin (20), they released 4.7 and 80 μg of kinins/mg of protein, respectively. Kinins were tested by an RIA (21).

Results

The correcting activity of the different purified bovine proteins, when expressed in U/nMol, was not affected by the three different concentrations tested (Tables I-IV). The exception to this result was lys-bradykinin-free bHMWK, in which the specific activity doubled with a ten-fold dilution.

TABLE I

Molar Comparison of Correcting Activity of Bovine HMWK and Its Fragments

Protein	% of normal plasma	U/nMol
HMWK	2.5	0.10
Lys-bradykinin-free HMWK	5.6	0.22
Bradykinin and fragment 1-2-free HMWK	0.8	0.03
Fragment 1-2-light chain	2.5	0.10
Light chain	0.1	0.004
Heavy chain	No activity	0

All proteins were tested at 0.26 nMol/ml

TABLE II

Weight Comparison of Correcting Activity of Bovine HMWK and Fragments

Protein	U/mg
HMWK	1.2
Lys-bradykinin-free HMWK	1.7
Bradykinin and fragment 1-2 free HMWK	0.3
Fragment 1-2-light chain	2.6
Light chain	0.1
Heavy chain	0
Fragment 1-2	Inhibits
Fragment 2	Inhibits

All proteins were tested at 100 μg/ml

Discussion

High molecular weight kininogen seems to be essential for the contact activation of the intrinsic coagulation pathway (22). To determine which region of the HMWK molecule is responsible for the correction of Fitzgerald-trait plasma aPTT, we have used bHMWK, which is known to partially correct the abnormalities in the aPTT of HMWK-deficient human plasma (12,13).

Intact bHMWK has a molecular weight of about 76,000 daltons. Of these, 48,500 are contributed by the heavy chain, 1,000 by bradykinin, 12,600 by fragment 1-2, and 16,000 by the light chain (17). Since the fragments of bHMWK are present in the intact molecule in equimolar concentrations, their activity must be expressed on a molar basis in order to compare their relative correcting activity.

Our findings indicate that a peptide formed by fragment 1-2-light chain is responsible for the Fitzgerald correcting activity of bHMWK. Fragment 1-2-light chain is as active as intact bHMWK on a molar basis and virtually more than twice as active on a weight basis.

As expected, we found that the heavy chain of bHMWK has little or no correcting activity, since it is chemically and immunologically common to both low and high molecular weight kininogen; low molecular weight kininogen lacks correcting activity (12). Although the heavy chain is not essential to express the coagulant activity of bHMWK, it may play a permissive role, since lys-bradykinin-free bHMWK (which includes both the heavy chain and the fragment 1-2-light chain) is more active on a molar basis than fragment 1-2-light chain (Table I). It is also more active than intact bHMWK. Lys-brady-kinin-free bHMWK may have a better steric relationship with Hageman factor and/or prekallikrein and factor XI than fragment 1-2-light chain or bHMWK (Table II), thus favoring a more effective interaction on the surface.

We have observed an increase in the specific activity of lys-bradykinin-free bHMWK with higher dilutions. This increase may be due to the presence of trace amounts of a contaminant with inhibiting capacity, such as fragment 1-2 or fragment 2, in the preparation.

Fragment 1-2-light chain was obtained by reduction of the disulfide bonds of the lys-bradykinin-free bHMWK, followed by alkylation of the thiol groups. It retains full activity in the coagulation assay, which suggests that intact disulfide bonds are not necessary for the Fitzgerald correcting activity of bHMWK.

TABLE III

Inhibitory Effects of Fragment 1-2 and Fragment 2 on Normal Human Plasma aPTT

	nM/ml	aPTT seconds
Fragment 2 (M.W. 4,600)	21.7	320
Fragment 1-2 (M.W. 12,600)	19.8	139

Normal plasma aPTT was 45 seconds.

TABLE IV

Effect of Bovine Plasma Kallikrein on Correcting Activity of Human and Bovine HMWK

Time of incubation (min)	Human HMWK —	Human HMWK and bovine kallikrein	Bovine HMWK —	Bovine HMWK and bovine kallikrein
0	73	71	91	103
30	72	74 (240)	—	149 (265)
60	76	72	98	200

Number indicates aPTT in seconds. Numbers in parentheses indicate ng of kinins released by bovine plasma kallikrein.

The light chain without fragment 1-2 lacks functional activity, indicating that the fragment 1-2 peptide, or part of it (fragment 2), plays a crucial role in initiating the intrinsic coagulation system. It was also observed that fragment 1-2 and, to a greater degree, fragment 2 have a marked capacity to inhibit the surface activation of the coagulation pathways of normal human plasma, but only if the fragment 1-2 or fragment 2 were added before or during the incubation of normal plasma with kaolin. This observation suggests that when fragment 1-2 and fragment 2 bind to the negatively charged surface, they competitively inhibit the binding and activation of the proteins involved in the intrinsic coagulation pathway (Table III).

Considering these results, we propose that the fragment 1-2 portion serves to bind HMWK to the negatively charged surfaces, and that the light chain, or part of it, interacts with Hageman factor and/or prekallikrein and factor XI. Furthermore, since Wiggins, et al (23) have clearly shown that the activation of factor XI occurs only on the surface, we propose that the light chain without fragment 1-2 (binding site) lacks activity because it will remain in the fluid phase. It may be speculated that HMWK complexed with prekallikrein and factor XI in the fluid phase (24,25) can "carry" these proteins to negatively charged surfaces. On the surface, the interaction between Hageman factor, HMWK, prekallikrein, and factor XI will activate all the Hageman dependent pathways, such as intrinsic coagulation, fibrinolytic, kallikrein-kinin, and complement systems.

There are some differences as well as similarities between bovine and human HMWK. Bovine plasma kallikrein destroys the correcting activity of bHMWK, while human plasma kallikrein does not affect the activity of hHMWK. This difference may be due to an enzymatic contamination in the bovine plasma kallikrein preparation (Table IV). When hHMWK was incubated with bovine kallikrein, kinins were released, although there was no loss of functional activity. It is therefore unlikely that a contaminating enzyme is present in the bovine plasma kallikrein preparation, unless the hypothetical contaminating enzyme has species specificity. It is more probable that the different behavior of bHMWK and hHMWK after hydrolysis with homologous plasma kallikrein can be explained by structural variations between both substrates. The fact that the bovine plasma has only 7-12% (12,26) of the correcting activity of human plasma also points to partial structural differences between bHMWK and hHMWK. Furthermore, we have found that if fragment 1-2 remains attached to the light chain of bHMWK, the Fitzgerald correcting activity is also retained. Since hHMWK retains full correcting activity after hydrolysis with bovine or human plasma kallikrein, we conclude that neither enzyme is able to separate the basic peptide, equivalent to fragment 1-2, from hHMWK.

Recent evidence indicates that human kininogen shares the same basic structures with its bovine counterpart. Nagasawa, et al (27) have reported that hHMWK is a linear glycoprotein in which the bradykinin moiety is included in the inner portions. Human kallikrein acts upon hHMWK, releasing bradykinin and leaving a kinin-free HMWK which has a heavy and a light chain linked by a disulfide loop, as in bHMWK. It has been shown that antibodies against human low molecular weight kininogen (hLMWK) cross-react with hHMWK (19,28). Similarly, antibodies against bLMWK cross-react with bHMWK (17).

It is known that both bLMWK and hLMWK lack correcting activity upon Fitzgerald-trait plasma aPTT (10-13). Antibodies against hHMWK, after adsorption with Fitzgerald-trait plasma (which has LMWK), no longer cross-react with hLMWK. When tested against hHMWK, a precipitation line is observed (14,29), which suggests the presence of antigenic determinants in the HMWK molecule not present in the LMWK. It is logical to assume that these antigenic determinants are responsible for the HMWK coagulating activity.

Komiya, et al (16) and Kato, et al (17) have reported that the bLMWK and bHMWK differ in their light chains, and we have shown that the correcting activity of the bHMWK resides precisely in the fragment 1-2 light chain. To complete the resemblance between the bovine and human kininogen, it has been reported (29) that the light chain enables the hHMWK to correct the aPTT of Fitzgerald-trait plasma. However,a positively charged peptide similar to the bovine fragment 1-2 has yet to be identified in the light chain of the hHMWK.

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