PRIMARY STRUCTURE OF BOVINE HIGH MOLECULAR WEIGHT KININOGEN: CHEMICAL COMPOSITIONS OF KININ-FREE KININOGEN AND PEPTIDE FRAGMENTS RELEASED BY PLASMA KALLIKREIN

Yong Nam HAN, Masanobu KOMIYA*, Hisao KATO, Sadaaki IWANAGA and Tomoji SUZUKI
Division of Plasma Proteins, Institute for Protein Research, Osaka University, Suita, Osaka-565, Japan

Received 2 July 1975

1. Introduction

High molecular weight (HMW) kininogen found in mammalian blood plasma is one of the functional glycoproteins, which participates in the kallikrein-kinin system [1]. Quite recently, a new function of this protein has been suggested by findings of Flaujeac and Williams traits with a deficiency of the kininogen [2,3]. The deficient plasma does not release kinin upon incubation of kallikrein and also has a prolonged activated partial thromboplastin time and inability to form plasmin. These suggest a participation of the kininogen on the intrinsic blood coagulation and fibrinolysis, in addition to the kinin-forming system.

In order to realize these physiological functions, it seems important to study the chemical structure of kininogen. Previously, we reported that a few of peptide fragments, in addition to kinin, were liberated from HMW kininogen on the digestion with plasma kallikrein [4]. One of the fragments was found to contain an abnormally high level of histidine (named tentatively histidine-rich peptide), and its amino acid sequence was established [5]. This communication describes further studies on the chemical compositions of kinin-free kininogen and peptide fragments produced from HMW kininogen by plasma kallikrein.

* Present address: Research and Development Division, Dainippon Pharmaceutical Co., Ltd., Enoki-33-94, Suita, Osaka-564.

Abbreviations: HMW kininogen, high molecular weight kininogen; SDS, sodium dodecylsulfate; TAME, *N*-α-tosyl-L-arginine methylester.

2. Materials and methods

Highly purified HMW kininogen was prepared from fresh bovine plasma by the previous method [6]. Bovine plasma kallikrein was prepared from highly purified prekallikrein [7], by activating it with purified bovine prekallikrein activator (Factor XIIa) [8]. The specific activity of the kallikrein was 23.5 TAME units per mg protein [7]. Sephadex G-25 (super fine), G-50 (fine) and G-100 were products of Pharmacia, Uppsala, Sweden. Kinin activity was assayed in terms of its ability to cause smooth muscle contraction of isolated rat uterus [9]. For the analysis of amino acids, peptides were hydrolyzed in 0.5 ml of constant-boiling HCl in evacuated, sealed tubes at 110°C for 24, 48 and 72 h. The sample was analyzed with an amino acid analyzer, Model JLC-5AH, Japan Electron Optics, Ltd., by the method of Spackman et al. [10]. The N-terminal residues of the isolated peptides and whole proteins were determined by Edman's phenylisothiocyanate procedure [11] and Sanger's method [12]. For the analysis of carbohydrates, total hexoses were measured by the phenol-sulfuric acid method of Dubois et al. [13], using a standard solution containing equimolar amounts of D-galactose and D-mannose. The separation of glucosamine and galactosamine were made as described by Spiro [14], and hexosamines and sialic acid were estimated by the methods of Blix [15] and the periodate-thiobarbituric acid method of Warren [16], respectively.

3. Results

HMW kininogen (80 mg) was incubated with 78

North-Holland Publishing Company – Amsterdam

μg of plasma kallikrein (1.8 TAME units) in 5.0 ml of 0.2 M ammonium bicarbonate buffer, pH 8.0, at 37°C. After incubation for 2 h, 0.1 M DFP was added to give a final concentration of 10^{-3} M, and the mixture was stood for 4 h. The mixture was then gel-filtrated through a column of Sephadex G-50 equilibrated with 0.2 M ammonium bicarbonate buffer, pH 8.0, and the effluent was analyzed by u.v. absorption at 280 nm, ninhydrin reaction and smooth muscle contraction of isolated rat uterus. Through this procedure, three fragments showing u.v. absorption and one fragment with kinin activity were separated. The large fragment eluted in the void volume fraction was kinin-free kininogen, which showed no more release of the kinin upon incubation with kallikrein. The other two fragments, which were eluted in a low molecular weight fraction and separated from the kinin, were a glycopeptide (fragment 1) and histidine-rich peptide (fragment 2) [5].

The kinin-free kininogen was subsequently reduced and carboxymethylated [17] and the resulting S-alkylated material was separated on a Sephadex G-100 column equilibrated with the same buffer used before. The elution pattern is shown in Fig.1. Two large fragments, named heavy (H) and light (L) chains, were resolved clearly, and these were gel-electrophoretically pure, as shown in the inlet of the figure.

Table 1 shows amino acid and carbohydrate compositions of all the fragments thus isolated. The fragment 1 and fragment 2 contained extremely high levels of histidine and glycine, and their N-terminal residues were both serine. Moreover, the fragment 1 was found to be glycopeptide containing a total of 7.4% of carbohydrates.

On the other hand, the amino acid compositions of H- and L-chains derived from kinin-free kininogen were different for each other. Especially, L-chain showed a characteristic composition, in which a sum of five amino acid residues, Asp, Thr, Ser, Glu and Pro, comprises 57% of the total residues. Another difference between the two was the presence of galactosamine in L-chain but not in H-chain. Moreover, no clear N-terminal residue reactive to phenylisothiocyanate and 2,4-dinitrofluorobenzene was detected in H-chain as well as whole HMW kininogen [18], whereas the N-terminal threonine was identified evidently on L-chain. The C-terminal residue of L-chain was identified to be leucine, which was the same residue as that of whole HMW kininogen [18].

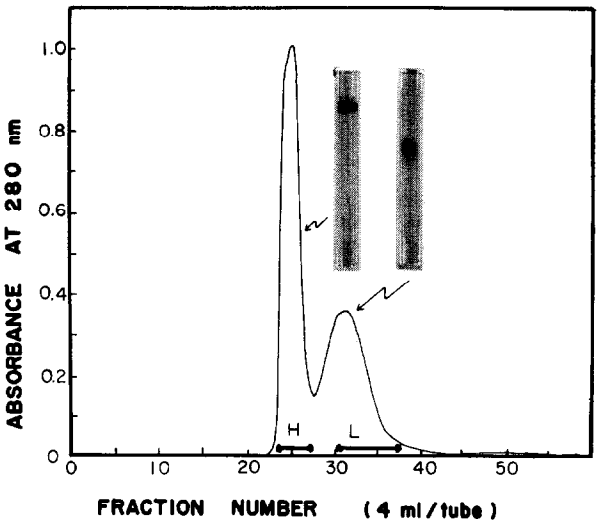

Fig.1. Separation of the heavy (H) and light (L) chains constituting kinin-free HMW kininogen on a Sephadex G-100 column. The S-alkylated sample (30 mg) dissolved in 1 ml of 0.2 M $(NH_4)HCO_3$ buffer, pH 8.0, was applied to the column (2 cm × 94 cm) and eluted with the same buffer at a flow rate of 20 ml/h. SDS polyacrylamide-gel (8%) electrophoresis was done at room temperature at a current of 8 mA per tube for 4 h, according to the method of Weber and Osborn [21].

4. Discussion

Bovine HMW kininogen having a molecular weight of 76 000 consists of a single chain polypeptide with a masked N-terminal residue and C-terminal leucine, and it carries the vasoactive peptide, bradykinin, in the interior of the chain bridged by a disulfide bond [18]. This kininogen liberates two peptide fragments, in addition to kinin, on the digestion with plasma kallikrein. One of the fragments, histidine-rich peptide, contains a total of 41 amino acid residues with the N-terminal serine and C-terminal arginine [5]. The other fragment is a glycopeptide containing also high level of histidine (Table 1), and the partial N-terminal sequence of Ser-Val-Gln-Val-Met-Lys-Thr-Glu-Gly- is the same as the C-terminal sequence of the CNBr-fragment containing kallidin [18,19]. Thus, this glycopeptide fragment must be connected with the C-terminus of the kinin along the polypeptide chain of kininogen.

The residual protein, named kinin-free kininogen, which consists of two chain polypeptides of H- and

Table 1

Chemical composition of kinin-free kininogen and fragments derived from HMW kininogen with plasma kallikrein.
Amino acid compositions of each fragment were corrected values from the hydrolysates for 24, 48 and 72 h at 110°C except fragment 1. The values of fragment 1 were from the hydrolysate for 24 h at 110°C and those of fragment 2 were from the complete amino acid sequence determined previously [5]

Amino acid	HMW kininogen[a]	Kinin-free kininogen	H-chain	L-chain	H+L	fragment 1	fragment 2	BK	Kinin-free kininogen + fragment 1 + fragment 2 + BK
			residues per mole						
Asp	63	60	45	15	60	4	4		68
Thr	41	40	26	14	40	4	—		44
Ser	49	44	32	13	45	6	1	1	52
Glu	64	57	44	14	58	9	2		68
Pro	39	35	21	13	34	3	—	3	41
Gly	39	20	17	3	20	11	11	1	43
Ala	31	31	23	7	30	1	—		32
1/2Cys	20	18	17	1	18	—	—		18
Val	38	35	28	6	34	4	—		39
Met	8	6	4	2	6	2	—		8
Ile	22	22	17	5	22	1	—		23
Leu	36	33	24	9	33	4	2		39
Tyr	19	17	14	3	17	—	1		18
Phe	20	20	15	5	20	—	—	2	22
Trp	8	6	4	2	6	1	1		8
His	28	12	9	2	11	10	11		33
Lys	43	35	28	5	33	6	7		48
Arg	13	11	9	1	10	2	1	2	16
Total	581	502	377	120	497	68	41	9	620
N-terminal amino acid	(Ser)[b]	Thr	—	Thr		Ser	Ser		
Hexose (%)	4.57	4.97	4.61	6.03		3.73			
Galactosamine (%)	1.53	1.43	0	5.15		1.55			
Glucosamine (%)	2.12	2.97	4.18	0		0			
Sialic acid (%)	4.35	5.45	3.82	10.09		2.09			
Total (%)	12.57	14.82	12.61	21.27		7.37			
Molecular weight	76 000[c]	66 000[c,d]	48 500[d]	16 000[d]		8 000[d]	4 600[d]		

a) From ref. 6.
b) Small amounts of serine (0.2 mole/mole) can be detected as N-terminal amino acid because HMW kininogen contains small amounts of kininogen which suffered limited proteolysis as discussed previously [18].
c) Determined by sedimentation equilibrium method.
d) Calculated from amino acid composition and carbohydrate content.

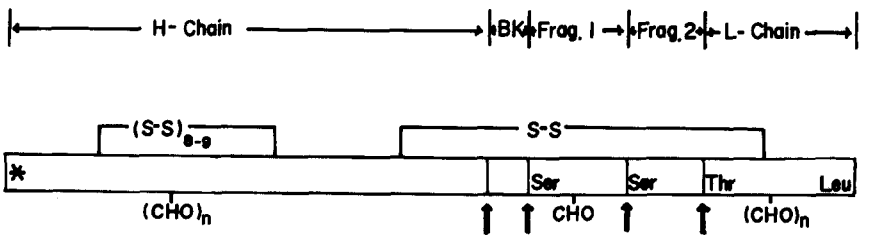

Fig.2. An alignment of the peptide segments and gross structural feature of bovine HMW kininogen. The arrows indicated show the sites cleaved by plasma kallikrein. CHO represents a carbohydrate chain. *Unreactive N-terminal residue with Edman's and Sanger's reagents. BK means bradykinin.

L-chains, has to constitute the N- and C-terminal portions of whole HMW kininogen, because their end groups are identical to those of the mother molecule. Since L-chain contains only one half-cystine residue, H- and L-chains constituting kinin-free kininogen are thought to be linked by a single disulfide bridge. Moreover, a sum of the total amino acid residues of H- and L-chains coincides with that of kinin-free kininogen and also the sum of those of kinin-free kininogen, fragment 1, fragment 2 and bradykinin with those of whole HMW kininogen produced by plasma kallikrein had been isolated.

Based on these results, it seems now possible to build up a linear peptide sequence of HMW kininogen molecule and its gross structural feature, as shown in Fig.2. Thus, there should be at least four peptide bond cleavages associated with the kallikreinic digestion of HMW kininogen, yielding the kinin-free kininogen, fragment 1 (glycopeptide), fragment 2 (histidine-rich peptide) and vasoactive peptide, bradykinin. The results also indicate that the C-terminal polypeptide portion comprising L-chain, fragment 1 and fragment 2 links oligosaccharide chains containing galactosamine, whereas the N-terminal portion comprising H-chain links those containing glucosamine. Moreover, it should be noted that HMW kininogen locates an abnormally histidine-rich peptide region near to the position of the kinin moiety along the polypeptide chain. Although the biological activities of fragments 1 and 2 are scarcely known, a preliminary experiment indicates that these retard the generation of plasma kallikrein activity induced by a glass contact activation of Hageman factor (Factor XII) [20]. The amino acid sequence studies of fragment 1 and L-chain described above are now in progress.

Acknowledgement

The assistance of Miss Masayo Kitaguchi in amino acid analysis is appreciated. This work was supported in part by grants from the Scientific Research Fund of the Ministry of Education of Japan.

References

[1] Habermann, E. (1970) in: Handbook of Experimental Pharmacology (Erdös, E. G., ed.) pp. 250–288, Springer-Verlag, Berlin-Heidelberg-New York.
[2] Wuepper, K. D., Miller, D. R. and Lacomb, M. J. (1975) Federation Proc. 34, 859.
[3] Colman, R. W., Bagdasarian, A., Talamo, R. C., Seavey, M., Scott, C. F. and Kaplan, A. P. (1975) Federation Proc. 34, 859.
[4] Komiya, M., Suzuki, T. and Han, Y. N. (1973) Seikagaku (in Japanese) 45, 682.
[5] Han, Y. N., Komiya, M., Iwanaga, S. and Suzuki, T. (1975) J. Biochem. 77, 55–68.
[6] Komiya, M., Kato, H. and Suzuki, T. (1974) J. Biochem. 76, 811–822.
[7] Takahashi, H., Nagasawa, S. and Suzuki, T. (1972) J. Biochem. 71, 471–483.
[8] Komiya, M., Nagasawa, S. and Suzuki, T. (1972) J. Biochem. 72, 1205–1218.
[9] Yano, M., Nagasawa, S. and Suzuki, T. (1971) J. Biochem. 69, 471–481.
[10] Spackman, D. H., Stein, W. H. and Moore, S. (1958) Anal. Biochem. 30, 1190–1206.
[11] Edman, P. (1970) in: Protein Sequence Determination (Needleman, S. B., ed.) pp. 211–255, Springer-Verlag, Berlin.
[12] Bailey, J. L. (1967) in: Techniques in Protein Chemistry, 2nd ed., pp. 163–182, Elsevier, New York.
[13] Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smity, F. (1956) Anal. Chem. 28, 350–356.

[14] Spiro, R. G. (1966) in: Methods in Enzymology (Neufeld, E. F. and Ginsburg, V., eds.) Vol. VIII, pp. 3–26.
[15] Blix, G. (1963) Acta Chem. Scand. 2, 467–473.
[16] Warren, L. (1959) J. Biol. Chem. 234, 1971–1975.
[17] Crestfield, A. M., Moore, S. and Stein, W. H. (1963) J. Biol. Chem. 238, 622–627.
[18] Komiya, M., Kato, H. and Suzuki, T. (1974) J. Biochem. 76, 823–832.
[19] Komiya, M., Han, Y. N., Iwanaga, S. and Suzuki, T. (1974) Seikagaku (in Japanese), 46, 704.
[20] Iwanaga, S., Han, Y. N., Komiya, M., Suzuki, T., Katori, N. and Ohishi, S. (1975) Life Sci. 16, 792.
[21] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.