IDENTIFICATION OF Ser–Leu–Met–Lys–BRADYKININ ISOLATED FROM CHEMICALLY MODIFIED HIGH-MOLECULAR-WEIGHT BOVINE KININOGEN

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1. Introduction

Bovine blood plasma contains at least two kininogens, viz., high-molecular weight (HMW) and low-molecular weight (LMW) kininogen; each consists of a single polypeptide chain with a molecular weight of 76 000 or 50 000, respectively [1]. Both of these include the bradykinin moiety in their inner portions surrounded by a disulfide loop [2]. Upon incubation with plasma kallikrein or snake venom kininogenase, they yield the corresponding kinin-free proteins which consist of a heavy and a light chain [2] derived, respectively, from the NH_2- and COOH-terminal portions of the parent molecule [3]. The heavy chains have the COOH-terminal sequence –Leu–Met–LysOH [4], giving rise to the speculation that the heavy chain is contiguous with the kinin moiety in both kininogens, inasmuch as Met–Lys–bradykinin has been isolated from bovine plasma [5]. However, this sequence does not agree with those of the two kinin-containing peptides, Ser–Arg–Met–Lys–bradykinin and Gly–Arg–Met–Lys–bradykinin, which were isolated by Hochstrasser and Werle [6] from the peptic digest of bovine plasma Cohn fraction IV-6. To resolve this contradiction, we have re-examined bovine kininogen and established the sequence of residues preceding the kinin moiety. The strategy used was to prepare a chemically modified HMW kininogen, digest it with pancreatic kallikrein, isolate the kinin-containing peptide from the digest, and determine its amino acid sequence. The results indicate that the sequence is Ser–Leu–Met–Lys–bradykinin, showing that the COOH-terminus of the heavy chain is indeed juxtaposed to the kinin moiety.

2. Materials and methods

Highly purified HMW kininogen was prepared from fresh bovine plasma by a method described previously [1]. Porcine pancreatic kallikrein was a generous gift from Dr T. Takami, Ajinomoto Co., Tokyo. Chloramine T and citraconic anhydride were obtained from Nakarai Co., Kyoto. Synthetic bradykinin and its derivatives were products of the Protein Research Foundation, Osaka. Kinin activity was assayed by its ability to cause smooth muscle contraction of isolated rat uterus, and the results were expressed in terms of the mass of purified bradykinin exhibiting equivalent activity [7]. Amino acid sequence was determined by the dansyl-Edman degradation procedure which was described in detail previously [8,9]. Reduced and carboxymethylated (RCM) HMW kininogen (360 mg), which was prepared according to the method of Crestfield et al. [10], was citraconylated at pH 8.4 in 15 ml of 8 M urea with 400 μl of citraconic anhydride [11]. Selective oxidation of the methionine residues of citraconyl-RCM HMW kininogen (212 mg) was performed at room temperature for 20 min in 30 ml of 0.2 M Tris–HCl buffer, pH 8.7, with 25 mg of chloramine T [12].

3. Experimental

The chemically modified HMW kininogen (210 mg) was incubated at 37°C with 1.3 mg of pancreatic kallikrein in 30 ml of 0.2 M NH_4HCO_3 buffer, pH 8.8. After incubation for 8 h, 0.1 M diisopropylphosphorofluoridate was added to give a final concentration of

6.6×10^{-3} M, whereupon the mixture was allowed to stand for 1 h at 37°C and then lyophilized. The kallikrein digest thus obtained was treated with 20 ml of 10% acetic acid for 6 h to remove the citraconyl groups, and the mixture was lyophilized. For reduction of methionine sulfoxide, the dried material was further treated with 6 ml of 20% aqueous 2-mercaptoethanol at room temperature for 2 days. The resulting deblocked and reduced product was then gel-filtered through a column of Sephadex G-50 in 10% acetic acid, and the fractions were assayed for kinin activity (fig.1). Those which exhibited activity were re-assayed after trypsin digestion. Most of the kinin activity was found in a symmetrical peak spanning fractions 101–110 (fig.1). These fractions were pooled and further purified on a column of Sephadex G-25 (superfine, 2 × 145 cm) in 10% acetic acid; a single peak of kinin activity was found at an elution volume of 240–262 ml. This portion of the eluate was lyophilized, and one fourth of the material obtained was applied to a column of CM-cellulose (CM-32) equilibrated with 0.05 M pyridine-acetate buffer, pH 5.0. Linear gradient elution was carried out as described in the legend of fig.2. Two peaks with kinin activity, designated K_1 and K_2, were detected (fig.2). Each exhibited a single spot after cellulose plate (E. Merck) thin layer chromatography in *n*-butanol/pyridine/acetic acid/water (15 : 10 : 3 : 12, by vol.,). The R_f values were as follows; K_1, 0.58; K_2, 0.68; bradykinin, 0.63; Lys–bradykinin, 0.53; Met–Lys–bradykinin. 0.57. The kinin specific activity of isolated K_1 and K_2 was about one third that of synthetic bradykinin. However, both showed almost the same activity as bradykinin after treatment with trypsin, indicating that both materials must contain bradykinin or an equivalent moiety.

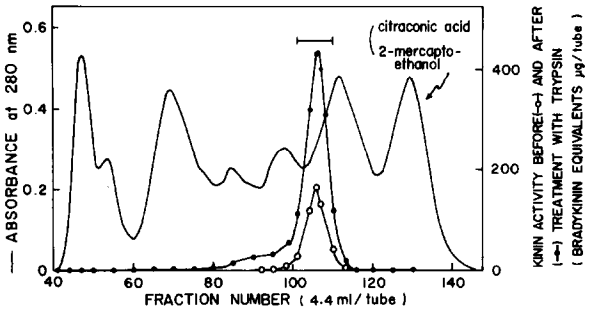

Fig.1. Fractionation of the pancreatic kallikrein digest of oxidized-citraconyl-RCM derivative of bovine plasma HMW kininogen on a column of Sephadex G-50. A sample (210 mg) of this derivative was digested with kallikrein, treated to remove the citraconyl groups and to reduce the methionine sulfoxide residues, applied to the column (2.3 × 139 cm) and subsequently eluted with 10% acetic acid at a flow rate of 25 ml/h.

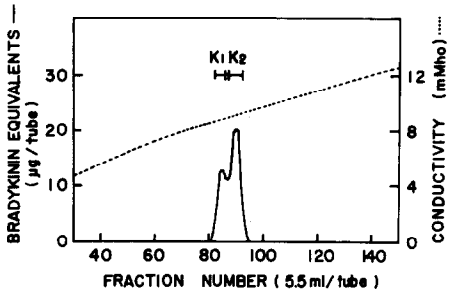

Fig.2. Separation of kinin-containing peptides by CM-cellulose (CM-32) column chromatography. A pooled peptide fraction with kinin activity was chromatographed on a column (1 × 37 cm) of CM-cellulose with a linear gradient (total volume 1200 ml) from 0.05–0.5 M pyridine–acetate buffer, pH 5.0. The kinin activity was assayed after trypsin digestion.

Table 1 shows the amino acid compositions of K_1 and K_2. Each contains one mole of Leu, Ser, Met, and Lys, in addition to the residues representing the amino acid composition of bradykinin. The NH_2-terminal sequence of both was found to be Ser–Leu–Met–Lys–Arg– . After the fourth step of the Edman degradation of K_1 and K_2 had been completed, the peptides were dansylated, and the resulting materials were subjected to cellulose plate thin layer chromatography (*n*-butanol/acetic acid/water = 4 : 1 : 5, v/v/v), along with the dansyl derivatives of bradykinin, Lys–bradykinin and Met–Lys–bradykinin. The DNS-derivatives of both peptides showed an R_f value identical with that of DNS-bradykinin. These results indicate that the total amino acid sequence of the two kinin-containing peptides, K_1 and K_2, must be as follows:

Ser–Leu–Met–Lys–Arg–Pro–Pro–Gly–Phe–Ser–Pro–Phe–Arg

After K_1 and K_2 were treated with 30% aqueous 2-mercaptoethanol at 37°C for 24 h, K_1 gave the same R_f value as K_2 on thin layer chromatography in the

Table 1
Amino acid compositions of the isolated kinin-containing peptides, K_1 and K_2

Amino acid	K_1	K_2	Bradykinin
	Residues per molecule		
Ser	1.8(2)	1.8(2)	1
Pro	3.0(3)	3.0(3)	3
Gly	1.1(1)	1.1(1)	1
Met	1.0(1)	1.0(1)	–
Leu	1.0(1)	1.0(1)	–
Phe	2.0(2)	2.0(2)	2
Lys	1.1(1)	1.1(1)	–
Arg	2.0(2)	1.9(2)	2
Total	13	13	9

Approximately 50 nmol of each sample was hydrolyzed in 0.5 ml of 5.7 N HCl at 110°C for 24 h and analyzed with a Model JLC-5AH amino acid analyzer [13].

system used above. On the other hand, the R_f value of K_2, 0.68, did not change with this treatment. It seems, therefore, that the chromatographic difference between K_1 and K_2 arose from the fact that the methionine residue in K_1 was still in the oxidized form.

4. Discussion

In the past it has proved very difficult to isolate active peptide containing portions of the kininogen molecule abutting the NH_2-terminus of the kinin moiety because the –Met–Lys–Arg– sequence is very susceptible to proteinases such as trypsin, plasmin and kallikrein. Therefore, in the present study we attempted chemical modification of the amino acid residues in this region prior to enzymic treatment of the kininogen. The success of this approach is indicated by the isolation of an active peptide with the sequence Ser–Leu–Met–Lys–bradykinin. The Leu–Met–Lys– sequence is consistent with the COOH-terminal sequence of the HMW heavy chain previously determined [4] and, moreover, proves the contiguity of the HMW heavy chain and kinin moiety in the kininogen.

In 1966, Habermann and Helbig [14] suggested that the residue preceding Met–Lys–bradykinin might be leucine, although the experimental data have not been reported. Our results confirm their suggestion, but do not support the sequence Ser (and Gly)–Arg–Met–Lys–bradykinin reported by Hochstrasser and Werle [6]. However, there remains the possibility that the peptic fragment isolated by the latter investigators could have been derived from an unknown kininogen other than that used in the present study. We have not yet identified a kinin-containing peptide, Ser–Leu–Met–Lys–bradykinin, from bovine LMW kininogen. It seems, however, that this sequence must occur in the corresponding region of the LMW kininogen, inasmuch as both HMW- and LMW heavy chains have the same COOH-terminal sequence, viz., Leu–Met–LysOH [4].

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