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Ile-Ser-bradykinin (T-kinin) and Met-Ile-Ser-bradykinin (Met-T-kinin) are released from T-kininogen by an acid proteinase of granulomatous tissues in rats

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An acid proteinase of granulomatous tissues in rats with carrageenin-induced inflammation released kinin from T-kininogen. The kinin isolated by *n*-butanol extraction was separated by reverse-phase high-performance liquid chromatography into T-kinin and a T-kinin derivative. From determination of its amino acid composition and its immunoreactivity toward anti-bradykinin antiserum, the T-kinin derivative was identified as Met-Ile-Ser-bradykinin (Met-T-kinin).

T-Kinin; Met-T-kinin; Acid proteinase; (Rat granulomatous tissue)

1. INTRODUCTION

Previously, we have reported that the release of T-kinin from T-kininogen occurred by consecutive cleavage by a cathepsin E-like proteinase and a 72 kDa proteinase in rat spleen [1]. However, it has not yet been clarified whether T-kinin could be released from T-kininogen by granulomatous tissues in rats with carrageenin-induced inflammation, although T-kininogen and free T-kinin have been reported to increase in the plasma and pouch fluid of rats with carrageenin-induced inflammation [2,3]. This paper reports that both Ile-Ser-bradykinin (T-kinin) and Met-Ile-Ser-bradykinin (Met-T-kinin) are released from T-kininogen by an acid proteinase of granulomatous tissues in rats with carrageenin-induced inflammation.

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Abbreviation: HPLC, high-performance liquid chromatography

2. MATERIALS AND METHODS

T-kininogen (spec. act. 9.2 μg bradykinin equiv./mg protein) was prepared from rat plasma as in [4]. Synthetic bradykinin, Lys-bradykinin, Met-Lys-bradykinin and T-kinin were purchased from the Protein Research Foundation (Osaka). Granulomatous tissues were collected on days 5–20 after carrageenin injection into rats, as described [2]. The acid proteinase from granulomatous tissues was partially purified by chromatography on DEAE-Sephadex A-50 and Sephadex G-100, according to [1]. The specific activity of the acid proteinase was 0.67 U/A_{280} with hemoglobin as substrate. Acid proteinase and kinin-releasing activities were measured as in [1]. The amount of kinin was determined by a bioassay using rat uterus [5] and by an enzyme immunoassay [6].

3. RESULTS

Purified T-kininogen (2 mg protein) was digested with the acid proteinase (0.4 U) at 37°C

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for 60 min in 12.0 ml of 0.2 M glycine-HCl buffer (pH 3.6) containing 2 mM EDTA. The reaction was terminated by acidifying the mixture to pH 2.5 with 1 N HCl. The liberated kinin (18 μg bradykinin equiv.) was extracted with *n*-butanol [7] and subjected to reverse-phase HPLC

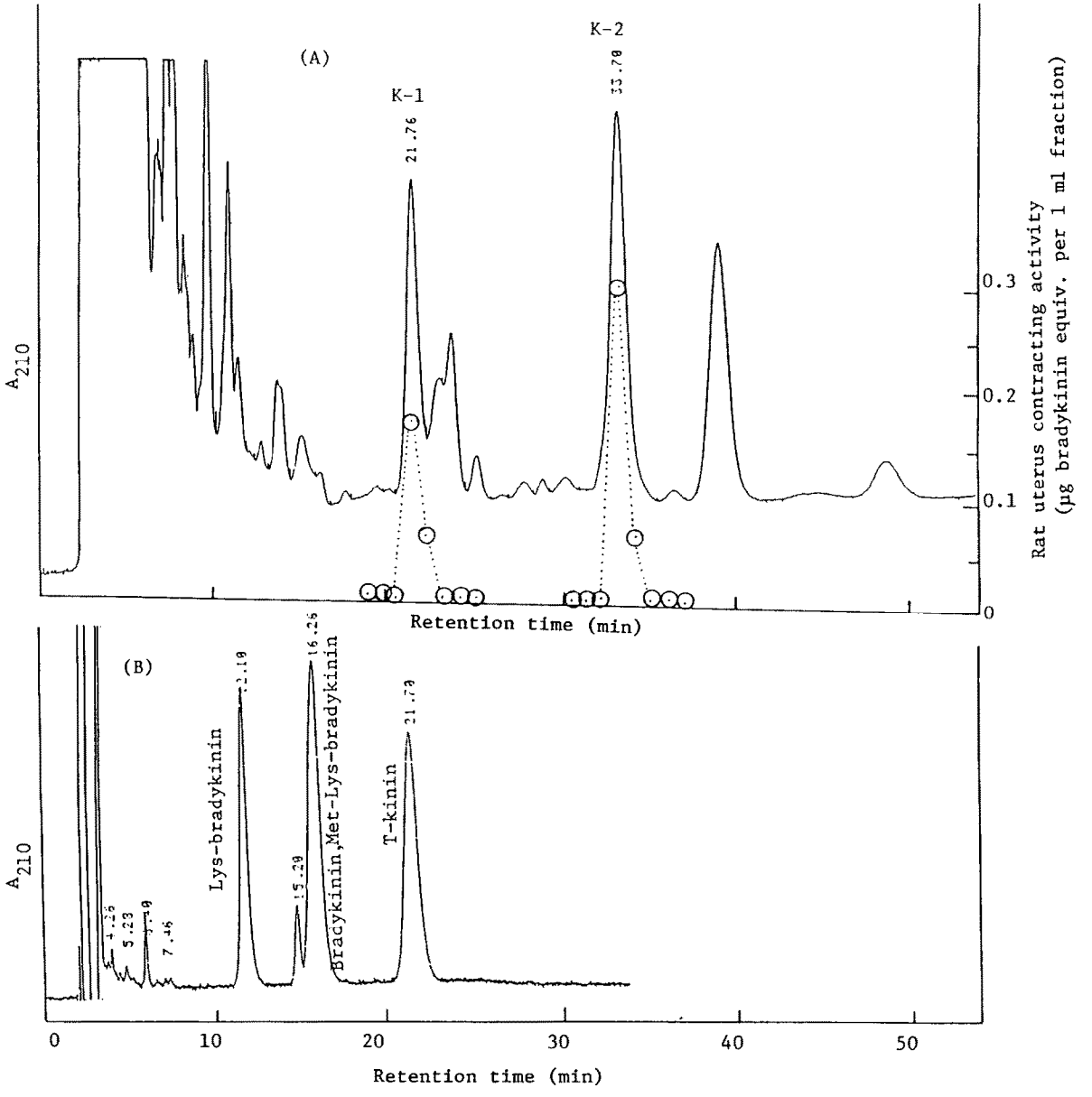

Fig.1. Reverse-phase HPLC of kinin released from T-kininogen by acid proteinase (A) and of synthetic kinins (B). (A) The released kinin (0.7 μg bradykinin equiv.), which was extracted with *n*-butanol and lyophilized according to Okamoto and Greenbaum [7], was injected into the reverse-phase column (ODS-120T, 0.46 × 25 cm). The column was eluted isocratically with 20% acetonitrile in 0.05% trifluoroacetic acid at a flow rate of 1.0 ml/min. Fractions of 1.0 ml were collected. (⊙···⊙) Rat uterus contracting activity. (B) 200 ng synthetic bradykinin and its derivatives. The kinin activity of each fraction was determined by a bioassay using rat uterus and enzyme immunoassay using bradykinin as a standard.

Table 1

Amino acid compositions of kinins (K-1 and K-2) released from T-kininogen by acid proteinase of granulomatous tissues in rats

Amino acid	K-1			K-2		
	nmol	mol/mol Ile	Residue/ molecule	nmol	mol/mol Ile	Residue/ molecule
Ser	5.3	1.9	2	6.7	1.8	2
Pro	9.1	3.2	3	11.6	3.1	3
Gly	3.8	1.4	1	4.6	1.2	1
Ile	2.8	1.0	1	3.7	1.0	1
Phe	5.3	1.9	2	7.6	2.1	2
Arg	5.0	1.8	2	7.3	2.0	2
Met	–	–	–	2.8	0.8	1

Purified K-1 (3.3 μg) and K-2 (5.5 μg) were hydrolyzed with 6 N HCl at 110°C for 24 h in evacuated and sealed tubes. The hydrolysates were evaporated and analyzed in a Hitachi-835 amino acid analyzer. The amounts of kinins were calculated from the area of HPLC

(ODS-120 T, Toyo Soda, Japan). As shown in fig.1A, the kinin, determined by measuring rat uterus contracting activity, was thereby separated into two fractions (K-1, K-2). The retention time (21.76 min) of K-1 was identical with that of synthetic T-kinin (fig.1B). Therefore, K-1 was identified as T-kinin. However, the retention time (33.70 min) of K-2 differed from those of Met-Lys-bradykinin, Lys-bradykinin and bradykinin. The weight ratio of K-1 and K-2 liberated from T-kininogen was estimated to be 0.48 from peak areas on HPLC.

In order to characterize K-2, its amino acid composition, immunoreactivity in the enzyme immunoassay, and uterus contracting activity were compared with those of T-kinin (K-1). The amino acid composition of K-2 showed one additional Met residue in comparison with that of T-kinin, as shown in table 1. In the enzyme immunoassay, K-2 had virtually equal immunoreactivity to T-kinin, but the contracting activity on rat uterus was 63% in comparison with that of T-kinin. From these results the amino acid sequence of K-2 is assumed to be: Met-Ile-Ser-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg (Met-T-kinin, M_r 1315).

4. DISCUSSION

In mammals, four types of kinin, i.e. bradykinin, Lys-bradykinin, Met-Lys-bradykinin and Ile-Ser-bradykinin (T-kinin), can be generated from three types of kininogen [8]. Here, it has been shown that a fifth kinin can be released from T-kininogen by an acid proteinase of granulomatous tissues and this kinin has been identified as Met-Ile-Ser-bradykinin (Met-T-kinin). From nucleotide sequencing of rat T-prekininogen I mRNA, Nakanishi et al. [9] have reported that T-kininogen harbors an Ile-Ser-bradykinin sequence preceded by the dipeptide Met-Met. Therefore, the acid proteinase of granulomatous tissues seems to release both T-kinin and Met-T-kinin by cleavage of the Met–Met and Met–Ile bonds. However, it is not yet clear whether T-kinin was produced from Met-T-kinin or directly from T-kininogen by the acid proteinase, because the acid proteinase preparation used in this study still showed several bands on SDS-polyacrylamide gel electrophoresis. In a preliminary experiment, where the acid proteinase was further purified by column chromatography on DEAE-Sephadex A-50 and pepstatin-Sepharose 4B, two types of acid proteinase seemed to be responsible for the kinin release, as was the case for the release of T-kinin by consecutive cleavage by the cathepsin E-like proteinase and the 72 kDa proteinase of rat spleen [1]. The characterization of acid proteinase (kinin-releasing enzyme) of granulomatous tissues is now underway.

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