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Isolation and identification of hydroxyproline analogues of bradykinin in human urine

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Hydroxyproline (Hyp) analogues of bradykinin and lysyl-bradykinin, in which the third residue of bradykinin, proline, is replaced by hydroxyproline, were isolated from human urine. Their amino acid sequences were confirmed by both amino acid and sequence analyses, and also by comparison of their chromatographic behavior with that of synthetic peptides. The possibility that Lys-Ala^3-bradykinin, isolated by Mindroiu et al. [(1986) J. Biol. Chem. 261, 7407–7411] from human urine, was actually Lys-Hyp^3-bradykinin is discussed.

Kinin; Hydroxyproline; Bradykinin; Kallikrein; Lysyl-bradykinin; (Human urine)

1. INTRODUCTION

We have recently isolated and identified a hydroxyproline (Hyp) analogue of bradykinin (BK), Hyp^3-BK, from the ascitic fluid of a patient with gastric cancer [1]. Sasaguri et al. [2] have also described the literature of a Hyp analogue of Lys-BK from human plasma proteins (possibly kininogens) by digestion with hog pancreatic kallikrein. However, little is known concerning the functions of these new types of bradykinin.

One of the physiological and pathological functions of kallikrein is to regulate renal blood flow by the intrarenally generated kinins. To understand the function of renal kallikrein fully, it is necessary to identify all kinins occurring in urine. Three kinds of kinin, i.e. BK, Lys-BK and Met-Lys-BK, have been detected thus far in human urine [3,4]. Furthermore, Mindroiu et al. [5] have recently reported the occurrence of a new kinin, Lys-Ala^3-BK, in human urine. We report here the isolation and identification of two kinins, Hyp^3-BK and Lys-Hyp^3-BK, from human urine and discuss the possibility that the Lys-Ala^3-BK described by Mindroiu et al. was actually Lys-Hyp^3-BK.

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Abbreviations: BK, bradykinin; Hyp, hydroxyproline

2. MATERIALS AND METHODS

Synthetic BK, Lys-BK and Lys-Hyp^3-BK were purchased from the Peptide Institute (Osaka). Lys-Ala^3-BK was a product of Peninsula Labs (Belmont, CA). Hyp^3-BK was synthesized as in [1]. Sephadex G-25 superfine was purchased from Pharmacia (Japan). Urine, collected from healthy males who showed no abnormality in kidney function, was stored at −20°C until use. Kinin was assayed by contraction of the estrous rat uterus using authentic BK as standard, as reported [6]. The amino acid composition and sequence were determined with a Pico-Tag HPLC system (Waters Associates, Bedford, MA) and a gas-phase protein sequencer (model 470A) equipped with a 120A PTH analyzer by the on-line system (Applied Biosystems, Foster City, CA) according to [1]. PTH-hydroxyproline was identified from the two characteristic peaks of PTH-hydroxyproline stereoisomers on HPLC, as in [1]. Peptides were separated by reversed-phase HPLC with an ODS-120A column (4.6 mm × 25 cm) or a column (4.6 mm × 17 cm) of ODS-120T from Toyo Soda (Tokyo), using a linear gradient of acetonitrile in 0.1% trifluoroacetic acid or by isocratic elution with 17% acetonitrile as reported in [1], and by ion-exchange chromatography with

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an SP-5PW column (1 × 8 cm, Toyo Soda) with a linear NaCl gradient in 10 mM ammonium formate containing 10% acetonitrile and at a flow rate of 1 ml/min.

3. RESULTS

The supernatant fraction of a mixture of 2 l of the urine and 8 l ethanol was concentrated in vacuo and applied to a Sephadex G-25 colun (3 × 90 cm), equilibrated with 10% acetic acid. Kinin activity was thereby eluted as a single peak. The kinin-containing fractions were pooled, lyophilized and subjected to reversed-phase HPLC with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid. Kinin activity was eluted at 30–35% acetonitrile. The kinin fractions were combined, lyophilized and further subjected to reversed-phase HPLC with isocratic elution of 17% acetonitrile. As shown in fig.1A, kinin activity was detected in three fractions designated I–III. The retention times of fractions I–III were identical with those of synthetic Lys-Hyp^3-BK, Hyp^3-BK (Lys-BK) and BK, respectively (see lower part of fig.1A). Upon further reversed-phase HPLC, fraction I gave a single peak (not shown). The identity of this fraction with Lys-Hyp^3-BK was confirmed from the amino acid composition (table 1) and also by the sequence analysis (fig.2). Fraction II was not yet homogeneous and could be separated into two peaks (IIa,IIb) by ion-exchange chromatography on an SP-5W column (fig.1B). The chromatographic behavior of fractions IIa and IIb was identical with that of synthetic Hyp^3-BK and Lys-BK, respectively. The homogeneity of fraction IIa was confirmed by further reversed-phase HPLC (not shown). Determination of amino acid compositions (table 1) and sequence analysis (fig.2) confirmed that fractions IIa and IIb were actually Hyp^3-BK and Lys-BK, respectively. The yields of Lys-Hyp^3-BK, Hyp^3-BK and Lys-BK from 2 l of human urine (calculated from the recovery in amino acid sequence analysis) were 0.51, 0.18 and 0.11 nmol, respectively. Fraction III was not further characterized due to the low yield. However, its retention time on reversed-phase HPLC indicates that it is BK and not Lys-Ala^3-BK (fig.1).

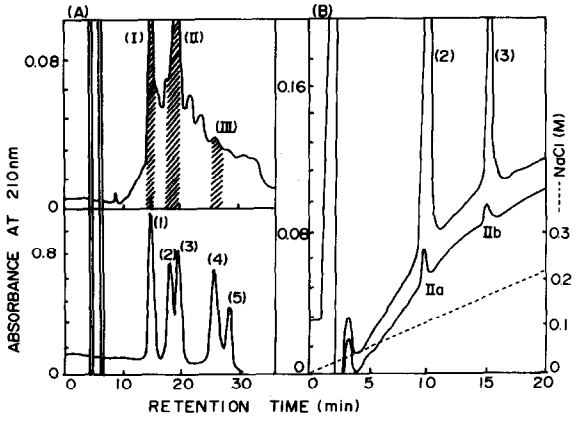

Fig.1. (A) Reversed-phase HPLC of kinins from human urine (upper) and synthetic kinins (lower). Kinins were applied to a column (4.6 mm × 25 cm) of ODS-120A and separated using an isocratic system. Shaded areas show the fractions with kinin activity. (1) Lys-Hyp^3-BK, (2) Hyp^3-BK, (3) Lys-BK, (4) BK and (5) Lys-Ala^3-BK. (B) Ion-exchange chromatography of fraction II on a column (1 × 8 cm) of SP-5PW. Fraction II and a mixture of Hyp$_3$-BK (2) and Lys-BK (3) were applied separately to the column and separated by a linear NaCl gradient.

Table 1

Amino acid compositions of kinins isolated from human urine

Amino acid	Fraction		
	I	IIa	IIb
Hydroxyproline	0.9 (1)	0.6 (1)	–
Serine	1.1 (1)	1.1 (1)	1.3 (1)
Glycine	1.4 (1)	1.4 (1)	1.4 (1)
Arginine	1.8 (2)	2.0 (2)	2.1 (2)
Proline	1.7 (2)	2.1 (2)	2.6 (3)
Phenylalanine	1.8 (2)	2.0 (2)	2.0 (2)
Lysine	1.4 (1)	–	0.8 (1)

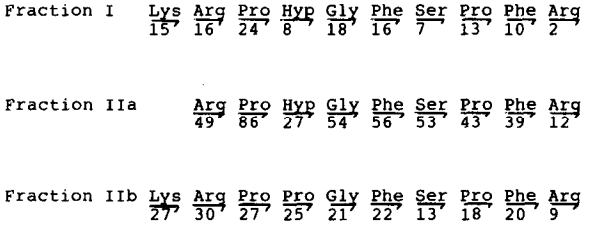

Fig.2. Amino acid sequences of fractions I, IIa and IIb. Arrows indicate the PTH-amino acids assigned by the gas-phase sequencer. Values beneath arrows represent pmol PTH amino acid.

4. DISCUSSION

The results reported above clearly indicate that human urine contains Hyp^3-BK and Lys-Hyp^3-BK in addition to Lys-BK and BK. Met-Lys-BK, which is known to be present in human urine, could not be detected in this study. Further work is still needed to elucidate the biological significance of the two Hyp analogues of BK. It should be noted in this connection that the yields of Lys-Hyp^3-BK and Hyp^3-BK from human urine were significantly higher than those of Lys-BK and BK.

Although Mindroiu et al. [5] have reported the occurrence of Lys-Ala^3-BK in human urine as a new kinin, we were unable to detect this type. To us it seems very likely that their kinin was not Lys-Ala^3-BK but Lys-Hyp^3-BK. The reasons for this argument are 4-fold. (i) Like our Lys-Hyp^3-BK, their kinin was eluted before Lys-BK on reversed-phase HPLC. (ii) Although they identified the fourth residue of their kinin as alanine, the presence of alanine in their preparation is not certain, because the amino acid composition is not given. (iii) In HPLC one of the two PTH-Hyp stereoisomer peaks shows a retention time very similar to that of PTH-alanine [1]. (iv) Finally, synthetic Lys-Ala^3-BK was eluted after Lys-BK, but not before Lys-BK as reported in their study.

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