Biochimica et Biophysica Acta, 662 (1981) 300–307
Elsevier/North-Holland Biomedical Press

BBA 69418

TRIPEPTIDYL CARBOXYPEPTIDASE ACTIVITY OF KININASE II (ANGIOTENSIN-CONVERTING ENZYME)

JIN-ICHI INOKUCHI and ATSUO NAGAMATSU

Faculty of Pharmaceutical Sciences, Fukuoka University, Nanakuma, Nishi-ku, Fukuoka 814-01 (Japan)

(Received May 18th, 1981)

Key words: Tripeptidyl carboxypeptidase; Kininase II; Angiotensin-converting enzyme

The degradation of des-Arg^9-bradykinin and its analogues by highly purified preparations of hog lung and kidney kininase II (angiotensin-converting enzyme; peptidyldipeptide hydrolase, EC 3.4.15.1) was studied. The degradative peptide fragments were separated and isolated by high performance liquid chromatography and identified by amino acid analysis. Both enzymes released C-terminal tripeptides from des-Arg^9-bradykinin, des-Arg^9-(Leu^8)-bradykinin, Pro-Pro-Gly-Phe-Ser-Pro-Phe, Pro-Gly-Phe-Ser-Pro-Phe, Gly-Phe-Ser-Pro-Phe, Bz-Gly-Ser-Pro-Phe and Bz-Gly-Ala-Pro-Phe. Hydrolysis of Phe-Ser-Pro-Phe, Bz-Gly-His-Pro-Phe, Bz-Gly-Phe-Pro-Phe and Bz-Gly-Gly-Pro-Phe by both enzymes was negligible. These data indicate that kininase II can release C-terminal tripeptides of substrates having a proline residue in the penultimate position such as des-Arg^9-bradykinin and its analogues, and that this enzyme is able not only to act as a dipeptidyl carboxypeptidase but also acts as a tripeptidyl carboxypeptidase. The tripeptidyl carboxypeptidase activity of this enzyme was sensitive to inhibition by kininase II inhibitors.

Introduction

A number of enzymes have been isolated from mammalian tissue which have dipeptidyl carboxypeptidase activity in that they can convert angiotensin I to angiotensin II by releasing the C-terminal dipeptide His-Leu. In 1956, Skeggs et al. [1] first described an angiotensin-converting enzyme isolated from horse plasma. Further work by other investigators showed that this enzyme was not specific for angiotensin I, but could release the C-terminal dipeptide from bradykinin and several other small oligopeptides having a free carboxyl group at the C-terminal residue [2,3]. This enzyme could not, however, hydrolyse the imide bond of a proline residue or release a dipeptide from a substrate having a C-terminal glutamic acid residue [2]. Enzymes with similar catalytic activities have been isolated from human plasma, urine and lung as well as from various animal tissues. Generally, these enzymes are chloride-activated and are inhibited by dipeptides, bradykinin, bradykinin-potentiating peptides and EDTA. Studies designed to elucidate the in vivo action of this class of enzyme have indicated that angiotensin-converting enzyme plays a part in the pathogenesis of hypertension [4,5]. Although the name 'angiotensin-converting enzyme' appears to be commonly used, other names such as dipeptidyl carboxypeptidase [6], kininase II [6], peptidase P [7], carboxycathepsin [2] and angiotensin I (Phe^8-His^9) hydrolase [8] have also been used to reflect differing physical and chemical properties and/or sources of various preparations.

We have studied the enzymes from hog kidney and lung and have used the name 'kininase II'. Our interest in the enzyme was based on its potential for having general proteolytic activity in releasing dipeptides

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; SDS, sodium dodecyl sulfate.

from substrates and, therefore, its use as a tool to study the primary structure of polypeptides and proteins.

Kininase II has very broad specificity in sequentially releasing dipeptides from the C terminus of a wide variety of polypeptides and hyrolysis terminates when a proline residue appears in the penultimate position, such as angiotensin II and angiotensin III, however, the results of the present work demonstrate that kininase II can release C-terminal tripeptides of substrates having a proline residue in the penultimate position such as des-Arg^9-bradykinin and its analogues.

Regoli et al. [9] have disclosed that des-Arg^9-bradykinin which normally possesses about 1% potency of bradykinin, is 10-times as potent as bradykinin on isolated rabbit aorta strips. Also potent release of adrenal catecholamines by des-Arg^9-bradykinin has been observed by Damas and Cession-Fossion [10].

The aim of the present paper is to describe the actions of kininase II on des-Arg^9-bradykinin and its analogues.

Materials and Methods

Materials. Bradykinin, des-Arg^9-bradykinin, des-Arg^9-(Leu^8)-bradykinin, angiotensin I, angiotensin II, angiotensin III, Bz-Gly-His-Leu, His-Leu and bradykinin-potentiator C (Pyr-Gly-Leu-Pro-Pro-Gly-Pro-Pro-Ile-Pro-Pro) were obtained from the Protein Research Foundation, Minoh, Japan. Other peptides used were synthesized in this laboratory. All synthesized peptides were homogeneous on TLC on silica gel (*n*-BuOH/AcOH/H_2O, 4 : 1 : 2 and *n*-BuOH/AcOH/pyridine/H_2O, 4 : 1 : 1 : 2) and on HPLC. SQ 14,225 (2-D-methyl-3-mercaptopropanoyl-L-proline) was obtained from Squibb Pharmaceutical Co.

Hog kidney and lung kininase II were prepared as described previously [11]. Both purified enzyme preparations gave a single protein band on SDS-polyacrylamide gel electrophoresis [12] and have the same molecular weight (300 000) based on gel filtration by Sephadex G-200. A unit of dipeptidyl carboxypeptidase activity was defined as the amount of enzyme cleaving 1 μmol Bz-Gly-His-Leu at 37°C (pH 7.4). The specific activities of the purified hog lung and kidney enzymes used in the present studies were 4.3 units/mg protein and 12.5 units/mg protein, respectively.

Methods. HPLC was carried out using a Waters Associated Model 204 liquid chromatograph, including Model U6K injector, two Model 6000A pumps and Model 660 programmer, and coupled to a Soma variable ultraviolet detector S-310A. Samples were injected onto a 4 mm × 30 cm reverse phase column (μ Bondapak C_{18}, Waters Associates). A linear gradient was established between 15% methanol in 0.05 M KH_2PO_4 adjusted to pH 2.5 with phosphoric acid and pure methanol [13]. The gradient was programmed for an increase of 2%/min of the methanol concentration. The total flow was 1.5 ml/min and the effluent was monitored continuously at 210 nm. Fractions corresponding to discrete peaks were collected in large enough amounts for amino acid analysis, evaporated under a stream of nitrogen, and the residue was hydrolyzed in vacuo in 6 N HCl for 18 h at 105°C. The hydrolyzate was evaporated under nitrogen and the residue was subjected to amino acid analysis using a Model JLC-5AH, JEOL Co.

Assay of kininase II activity with different substrates. In all experiments, incubations were carried out at 37°C in 13 × 100 mm siliconized glass tubes. The following assay components, in a final volume of 1 ml, were incubated for 5–30 min: 0.05 M Hepes buffer (pH 7.5)/5 mM each substrate/100 mM NaCl/0.5–6.0 munits hog lung or kidney kininase II. At specified times following incubation, the reaction was stopped by the boiling water bath for 5 min and 100 μl of the incubation mixtures were subjected to HPLC analysis. The rates of hydrolysis were calculated directly from the peak heights of the recordings and were expressed as nmol peptide released/min in the 1 ml incubation mixture. The analytical system was calibrated with the peptide products released from various substrates as shown in Table I.

Enzyme kinetic studies. All values of K_m and V are averages of duplicate values. K_m and V values of kininase II for various substrates were determined from Lineweaver-Burk plots at optimal conditions for enzymic hydrolysis of the individual substrate given in Table I. Initial velocities of substrate hydrolysis were determined from the amount of product obtained after 2, 5 and 10 min incubation with hog lung or kidney kininase II, using the HPLC assay method described above.

TABLE I

OPTIMAL CONDITIONS FOR ENZYMIC HYDROLYSIS AND PRODUCTS MEASURED OF THE INDIVIDUAL SUBSTRATE

The incubation mixture of 1 ml contain substrate and Cl^- in 0.05 M Hepes buffer. Hog lung and kidney kininase II have the same optimal conditions for enzymic hydrolysis of the individual substrate.

Substrate	Substrate conc. (mM)	Optimum pH	NaCl (mM)	Product(s) measured
Bz-Gly-His-Leu	3	8.3	300	Bz-Gly or His-Leu
Angiotensin I	0.1	7.5	100	His-Leu
Bradykinin	0.01	7.5	10	Phe-Arg
Des-Arg^9-bradykinin	1	7.5	100	Ser-Pro-Phe
Des-Arg^9-(Leu^8)-bradykinin	1	7.5	100	Ser-Pro-Leu
Pro-Pro-Gly-Phe-Ser-Pro-Phe	1	7.5	500	Ser-Pro-Phe
Pro-Gly-Phe-Ser-Pro-Phe	1	7.5	500	Ser-Pro-Phe
Gly-Phe-Ser-Pro-Phe	1	7.5	500	Ser-Pro-Phe
Bz-Gly-Ser-Pro-Phe	5	7.5	500	Bz-Gly or Ser-Pro-Phe
Bz-Gly-Ala-Pro-Phe	5	7.5	500	Bz-Gly or Ala-Pro-Phe

Results

HPLC on a reverse phase column was capable of resolving all peptide products from each other and from unreacted substrate. Under the conditions of HPLC described in the Materials and Methods, the retention times of the various peptides were estimated (Table II). HPLC analysis of the incubation mixture of des-Arg^9-bradykinin with hog kidney kininase II revealed two components (Fig. 1). Amino acid analysis of the resolved components was consistent with the structure Arg-Pro-Pro-Gly-Phe and Ser-Pro-Phe establishing a point of cleavage at the Phe^5-Ser^6 bond. The chromatogram of HPLC analysis of the incubation mixture of bradykinin with hog kidney kininase II is shown in Fig. 2. Two dipeptides, Phe-Arg and Ser-Pro, were released sequentially from bradykinin. Fig. 3 shows the conversion of angiotensin I to angiotensin II by this enzyme. The same results were obtained with hog lung kininase II. No hydrolysis of angiotensin II and angiotensin III by both enzymes occurred.

The relationship between hydrolysis of des-Arg^9-bradykinin or bradykinin and chloride concentration is shown in Fig. 4. The rate of cleavage of des-Arg^9-bradykinin by kininase II was greatly enhanced in the presence of Cl^-, however, the rate of cleavage of bradykinin was enhanced to a lesser degree.

In order to confirm the substrate specificities of the tripeptidyl carboxypeptidase activity of hog lung and kidney kininase II, we have done extensive kinetic studies using lower C-terminal homologues of des-Arg^9-bradykinin and those analogues with systematic structural modifications of the amino acid residue in locations close to the scissible bond (Table III). As shown in Table III, the K_m and V values of hog lung and kidney kininase II for des-Arg^9-bradykinin, des-Arg^9-(Leu^8)-bradykinin, Pro-Pro-Gly-Phe-Ser-Pro-Phe, Pro-Gly-Phe-Ser-Pro-Phe, Gly-Phe-Ser-Pro-Phe, Bz-Gly-Ser-Pro-Phe and Bz-Gly-Ala-Pro-Phe were determined from Lineweaver-Burk plots at their optimal pH and chloride concentrations. Hydrolysis of Phe-Ser-Pro-Phe, Bz-Gly-His-Pro-Phe, Bz-Gly-Phe-Pro-Phe and Bz-Gly-Gly-Pro-Phe by both enzymes was negligible. Among various peptide substrates shown in Table III, des-Arg^9-bradykinin was most rapidly hydrolyzed by both enzymes. Des-Arg^9-(Leu^8)-bradykinin, which is a competitive inhibitor of the contractive action of bradykinin and des-Arg^9-bradykinin on isolated rabbit aorta strips [9], was also hydrolyzed by both enzymes at the Phe^5-Ser^6 bond and released C-terminal tripeptide Ser-Pro-Leu. The V values of both enzymes for des-Arg^9-(Leu^8)-bradykinin were slightly lower than those for des-Arg^9-bradykinin, and the V/K_m values for des-Arg^9-(Leu^8)-bradykinin were about one-half of those for

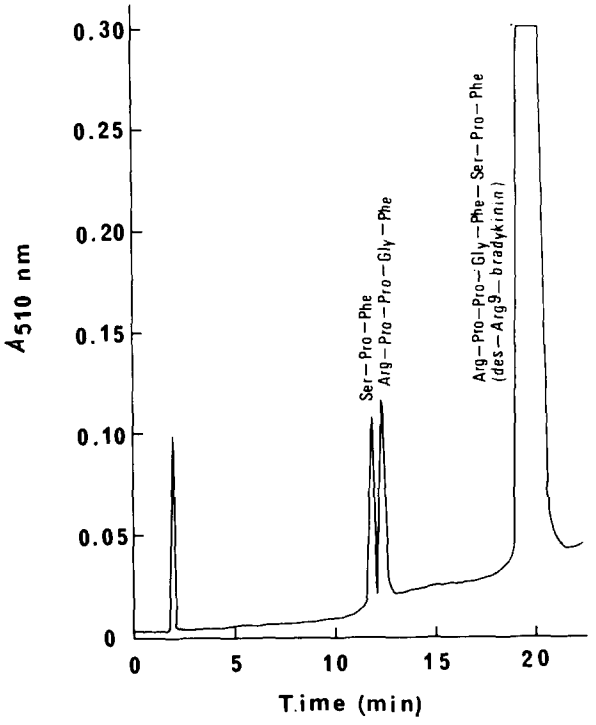

Fig. 1. Reverse phase HPLC separation of peptide products formed by the action of kininase II on des-Arg^9-bradykinin. Incubation time: 5 min. Assays were performed using 0.47 μg hog kidney enzyme and 1 μmol des-Arg^9-bradykinin.

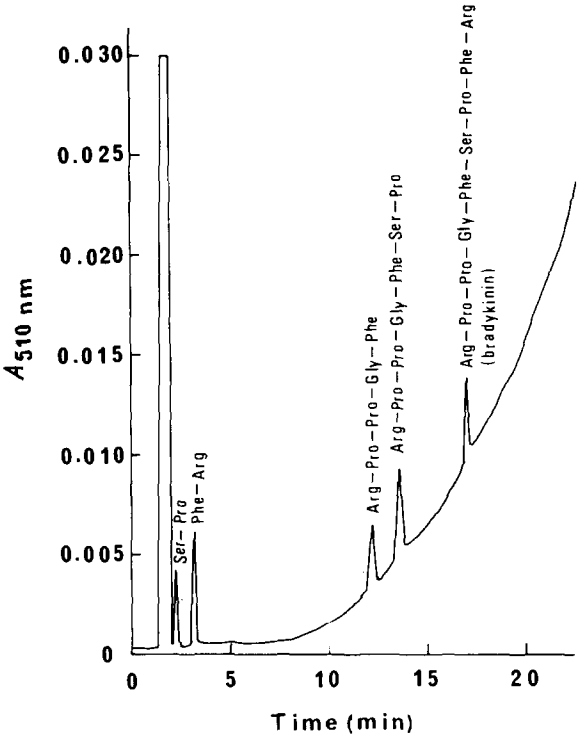

Fig. 2. Reverse phase HPLC separation of peptide products formed by the action of kininase II on bradykinin. Incubation time: 5 min. Assays were performed using 0.47 μg hog kidney enzyme and 10 nmol bradykinin.

TABLE III

KINETIC PARAMETERS FOR TRIPEPTIDYL CARBOXYPEPTIDASE ACTIVITY OF HOG LUNG AND KIDNEY KININASE II ON VARIOUS SUBSTRATES

The kinetic parameters K_m, V *and* V/K_m were determined at optimal pH and Cl concentrations for enzymic hydrolysis of the individual substrates as given in Table I. V values are expressed as μmol/min per mg enzyme.

Substrate	Lung enzyme			Kidney enzyme		
	K_m (μM)	V	V/K_m	K_m (μM)	V	V/K_m
Phe-Ser-Pro-Phe	negligible rate of hydrolysis					
Gly-Phe-Ser-Pro-Phe	130	3.6	0.028	160	10.7	0.067
Pro-Gly-Phe-Ser-Pro-Phe	120	18.1	0.151	130	20.2	0.155
Pro-Pro-Gly-Phe-Ser-Pro-Phe	130	38.1	0.293	165	61.7	0.374
Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe (des-Arg^9-bradykinin)	130	40.3	0.310	170	70.3	0.414
Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu (des-Arg^9-(Leu^8)-bradykinin)	250	34.0	0.136	380	48.8	0.128
Bz-Gly-Ser-Pro-Phe	360	9.1	0.025	410	30.2	0.074
Bz-Gly-Ala-Pro-Phe	1 080	3.0	0.003	1 200	10.3	0.009
Bz-Gly-His-Pro-Phe	negligible rate of hydrolysis					
Bz-Gly-Phe-Pro-Phe	negligible rate of hydrolysis					
Bz-Gly-Gly-Pro-Phe	negligible rate of hydrolysis					

TABLE II

HPLC SEPARATION OF VARIOUS PEPTIDES

Amino acid composition was determined by amino acid analysis.

Peptide	Retention time (min)
Ser-Pro	2.4
Phe-Arg	3.4
His-Leu	3.5
Gly-Phe	8.4
Ser-Pro-Leu	8.5
Bz-Gly	9.1
Pro-Phe	9.9
Pro-Gly-Phe	10.9
Ser-Pro-Phe	11.8
Arg-Pro-Pro-Gly-Phe	12.3
Ala-Pro-Phe	13.3
Arg-Pro-Pro-Gly-Phe-Ser-Pro	13.5
Pro-Pro-Gly-Phe	15.3
Phe-Ser-Pro-Phe	16.1
Bz-Gly-His-Leu	16.3
Bz-Gly-His-Pro-Phe	16.6
Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg (bradykinin)	16.8
Arg-Val-Tyr-Ile-His-Pro-Phe (angiotensin III)	17.1
Asp-Arg-Val-Tyr-Ile-His-Pro-Phe (angiotensin II)	17.3
Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu (des-Arg^9-(Leu^8)-bradykinin)	18.1
Bz-Gly-Ser-Pro-Phe	18.5
Pro-Gly-Phe-Ser-Pro-Phe	19.5
Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe (des-Arg^9-bradykinin)	19.6
Bz-Gly-Ala-Pro-Phe	20.0
Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu (angiotensin I)	20.4
Bz-Gly-Gly-Pro-Phe	20.7
Bz-Gly-Phe-Pro-Phe	23.5
Bz-Gly-Ser-Pro-Phe-OEt	25.1
Bz-Gly-Ala-Pro-Phe-OEt	27.1

des-Arg^9-bradykinin (Table III). The V and V/K_m values of both enzymes for lower C-terminal homologues of des-Arg^9-bradykinin decreased with decreasing peptide chain length (Table III). Bz-Gly-Ser-Pro-Phe and Bz-Gly-Ala-Pro-Phe were released Ser-Pro-Phe and Ala-Pro-Phe, respectively. The V/K_m values of both enzymes for Bz-Gly-Ser-Pro-Phe were 8-fold greater than those for Bz-Gly-Ala-Pro-Phe (Table III). The ethyl esters of N-protected tetrapeptides described above could not be hydrolyzed.

Kinetic parameters for dipeptidyl carboxypeptidase activity of hog lung and kidney kininase II on angiotensin I, bradykinin and Bz-Gly-His-Leu are shown in Table IV. The V values of the dipeptidyl carboxypeptidase activity of hog lung and kidney kininase II for bradykinin are 1.22 and 2.13 μmol/min per mg protein, respectively, and the K_m values are 0.9 and 1 μM (Table IV). On the other hand, the V values of the tripeptidyl carboxypeptidase activity of hog lung and kidney kininase II for des-Arg^9-bradykinin are 40.3 and 70.3 μmol/min per mg protein, respectively, and the K_m values are 130 and 170 μM (Table III). Therefore, there is a several-fold difference between the V/K_m values of bradykinin and des-Arg^9-bradykinin (Tables III and IV).

The tripeptidyl carboxypeptidase activity of hog kidney kininase II was inhibited by agents well known to be effective antagonists for kininase II. The data in Table V show the inhibitory effects of SQ 14,225 and bradykinin-potentiator C in the assay sys-

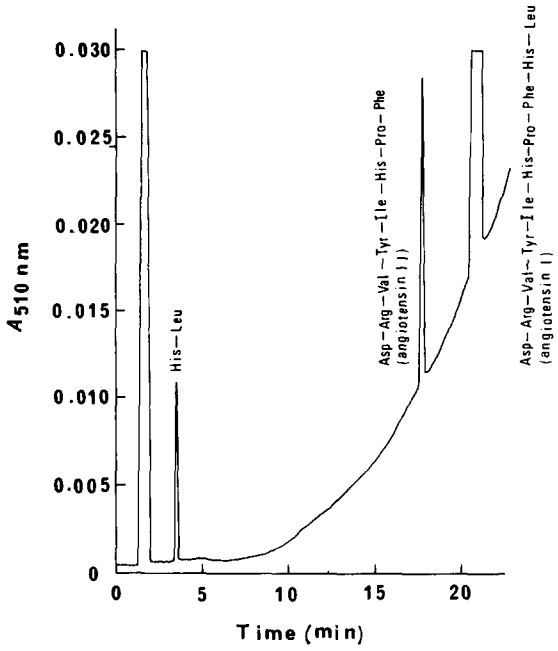

Fig. 3. Reverse phase HPLC separation of peptide products formed by the action of kininase II on angiotensin I. Incubation time: 5 min. Assays were performed using 0.47 μg hog kidney enzyme and 100 nmol angiotensin I.

tem. Both compounds inhibited the hydrolysis of des-Arg^9-bradykinin in a dose-dependent manner as well as the hydrolysis of angiotensin I and bradykinin by this enzyme. However, SQ 14,225 was about 50-times as effective as an inhibitor, producing a 50% inhibition at a concentration of $9.0 \cdot 10^{-8}$ M, whereas bradykinin-potentiator C produced 50% inhibition at a concentration of $4.8 \cdot 10^{-6}$ M. The I_{50} values obtained using hog lung kininase II with angiotensin I, bradykinin and des-Arg^9-bradykinin as substrates agreed quite closely with I_{50} values obtained using hog kidney kininase II.

Discussion

The results of our experiments show that highly purified kininase II obtained from hog lung and kidney is able not only to act as a dipeptidyl carboxypeptidase but also as a tripeptidyl carboxypeptidase. The tripeptidyl carboxypeptidase activity of kininase II was dependent on the presence of Cl^- and was sensitive to inhibition by SQ 14,225 and bradykinin-potentiator C.

(19)

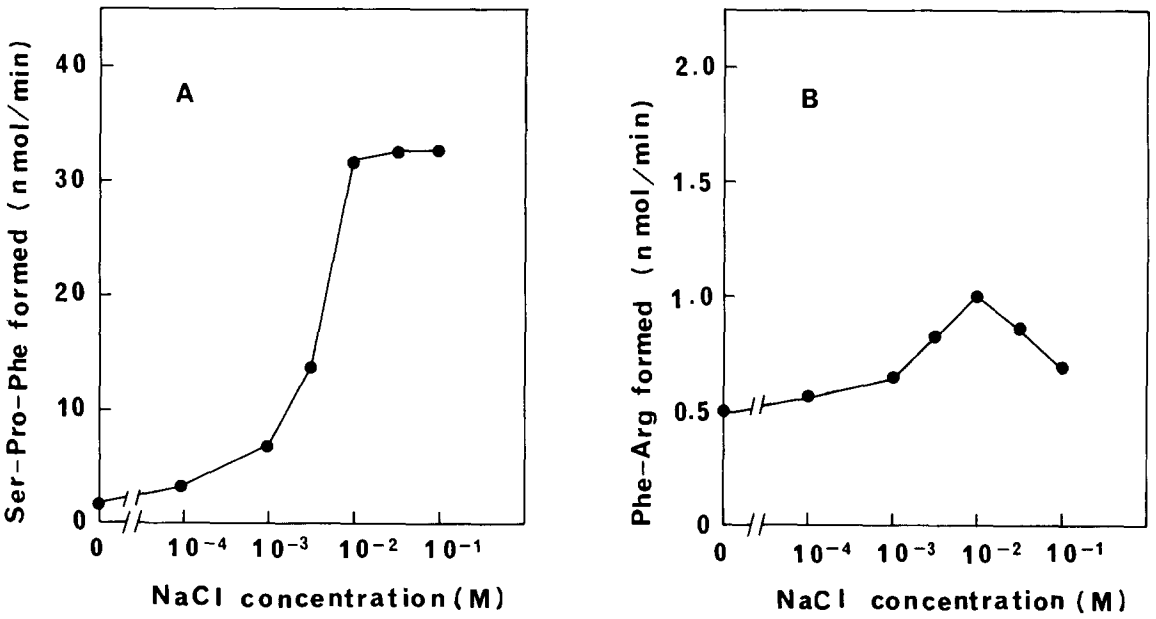

Fig. 4. Effect of Cl^- on the rates of hydrolysis of des-Arg^9-bradykinin and vradykinin by kininase II. Assays were performed using 0.47 μg hog kidney and 1 μmol des-Arg^9-bradykinin (A) or 10 nmol bradykinin (B).

TABLE IV

KINETIC PARAMETERS FOR DIPEPTIDYL CARBOXYPEPTIDASE ACTIVITY OF HOG LUNG AND KIDNEY KININASE II ON ANGIOTENSIN I, BRADYKININ AND Bz-Gly-His-Leu

The kinetic parameters K_m, V and V/K_m were determined at optimal pH and Cl concentrations of the individual substrates as given in Table I. V values are expressed as μmol/min per mg enzyme.

Substrate	Lung enzyme			Kidney enzyme		
	K_m (μM)	V	V/K_m	K_m (μM)	V	V/K_m
Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu (angiotensin I)	25	3.60	0.144	30	6.57	0.219
Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg (bradykinin)	0.9	1.22	1.356	1	2.13	2.130
Bz-Gly-His-Leu	270	10.80	0.040	320	26.60	0.083

TABLE V

I_{50} VALUES FOR INHIBITORS OF KININASE II ACTIVITY ON ANGIOTENSIN I, BRADYKININ AND DES-Arg^9-BRADYKININ

The inhibitor was preincubated with hog kidney kininase II for 5 min prior to the addition of each substrate. Incubations were carried out at optimal pH and Cl concentrations for enzymic hydrolysis of the individual substrates as given in Table I.

Substrate	I_{50} (M)	
	SQ 14, 225	Bradykinin-Potentiator C
Angiotensin I	$5.0 \cdot 10^{-8}$	$2.3 \cdot 10^{-5}$
Bradykinin	$4.3 \cdot 10^{-8}$	$2.5 \cdot 10^{-5}$
Des-Arg^9-bradykinin	$9.0 \cdot 10^{-8}$	$4.8 \cdot 10^{-6}$

The cleavage of des-Arg^9-bradykinin by kininase II provides an example of the tripeptidyl carboxypeptidase nature of this enzyme (Fig. 1). The data in Table III suggest that kininase II can release C-terminal tripeptides from peptides consisting of five or more residues and N-protected tetrapeptides, where a penultimate proline residue and an antepenultimate serine or alanine residue are present. The ethyl esters of N-protected tetrapeptides (Bz-Gly-Ser-Pro-Phe and Bz-Gly-Ala-Pro-Phe) could not be hydrolyzed. This is consistent with reports that the dipeptidyl carboxypeptidase activity of this enzyme requires a free carboxyl group to effect hydrolysis [2,3].

The effect of Cl^- on kininase II depends on the structure of the substrate used [14]. The hydrolysis of angiotensin I, Bz-Gly-His-Leu and other substrates stops almost entirely in Cl-free medium, but that of bradykinin proceeds at about half the optimal rate. In the present experiment the addition of Cl^- strongly accelerated the cleavage of des-Arg^9-bradykinin, which is dependent on the tripeptidyl carboxypeptidase activity of kininase II, as well as the conversion of angiotensin I to angiotensin II.

The resolution of the observed initial velocity into the two components V/K_m (proportional to velocity at low substrate concentration) and V (velocity at saturating concentration) allows us to look at the 'binding' and 'hydrolytic' reactions individually. The V and V/K_m values of both enzymes for lower C-terminal homologues of des-Arg^9-bradykinin decreased with decreasing peptide chain length (Table III). Thus, the decrease in V/K_m which takes place between des-Arg^9-bradykinin and its lower C-terminal homologues on the tripeptidyl carboxypeptidase activity of kininase II leads to speculation that eight residue peptides must be needed for optimal binding of substrate to occur.

Based on our observations, the significant differences between bradykinin and des-Arg^9-bradykinin on substrate specificity of kininase II are considered to be caused only by the C-terminal arginine residue in bradykinin molecule. Thus, it seems very probable that kininase II has a physiological role as a tripeptidyl carboxypeptidase in the regulation of bradykinin, when the cleavage of C-terminal arginine from bradykinin by kininase I (arginine carboxypeptidase) occurs in vivo with subsequent cleavage of des-Arg^9-bradykinin.

References

1 Skeggs, L.T., Kahn, J.R. and Shumway, N.P. (1956) J. Exp. Med. 103, 295–299
2 Elisseeva, Y.E., Orekohovich, V.N., Pavlikhina, L.V. and Alexeenko, L.P. (1971) Clin. Chim. Acta 31, 413–419
3 Yang, H.Y.T., Erdös, E.G. and Levin, Y. (1971) J. Pharmacol. Exp. Ther. 177, 291–300
4 Erdös, E.G. (1979) in Handbook of Experimental Pharmacology (Erdös, E.G., ed.), Vol. 25 Suppl., pp. 427–487, Springer-Verlag, Berlin
5 Cushman, D.W. and Ondetti, M.A. (1980) Biochem. Pharmacol. 29, 1871–1877
6 Yang, H.Y.T., Erdös, E.G. and Levin, Y. (1970) Biochim. Biophys. Acta 214, 374–376
7 Erdös, E.G. and Yang, H.Y.T. (1967) Life Sci. 6, 569–574
8 Overturf, M., Wyatt, S., Boaz, D. and Fitz, A. (1975) Life Sci. 16, 1669–1682
9 Regoli, D., Barabe, J. and Park, W.K. (1977) Can. J. Physiol. Pharmacol. 55, 855–867
10 Damas, J. and Cession-Fossion, A. (1973) C.R. Soc. Biol. (Paris) 167, 1478–1482
11 Nagamatsu, A., Inokuchi, J. and Soeda, S. (1980) Chem. Pharm. Bull. 28, 459–464
12 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412
13 Mönch, W. and Dehnen, W. (1977) J. Chromatogr. 140, 260–262
14 Igic, R., Nakajima, T., Yeh, H.S.J., Sorrels, K. and Erdös, E.G. (1973) in Symposium on Kinin Peptides, Proc. 5th Int. Congr. Pharmacol. (Acheson, G.H., ed.), San Francisco, Vol. 5, pp. 307–319, Karger, Basel