*Biochemical Pharmacology*, Vol. 35, No. 12, pp. 1957–1963, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00
Pergamon Journals Ltd.

# STUDIES OF THE DIGESTION OF BRADYKININ, LYSYL BRADYKININ, AND KININ-DEGRADATION PRODUCTS BY CARBOXYPEPTIDASES A, B, AND N*

IFTIKHAR A. SHEIKH and ALLEN P. KAPLAN

Division of Allergy, Rheumatology, and Clinical Immunology, Department of Medicine, SUNY-Stony Brook, Health Sciences Center, Stony Book, NY 11794–8161, U.S.A.

(*Received* 17 *July* 1985; *accepted* 12 *November* 1985)

**Abstract**—We have compared the digestion of bradykinin, lysyl bradykinin, and kinin degradation products by carboxypeptidases N, B and A (CPN, CPB and CPA). Carboxypeptidase N removed the C-terminal arginine from bradykinin or lysyl bradykinin to leave the des-Arg derivative of each, and no further degradation occurred regardless of enzyme concentration or time of incubation. However, both CPB and CPA degraded the des-Arg derivatives to remove the C-terminal phenylalanine. The inhibitory effect of phosphate ions upon this activity of CPB (but not CPA) suggests that CPA may be responsible for the formation of free phenylalanine seen upon degradation of kinins in plasma or serum. However, angiotensin converting enzyme degraded des-Arg$^9$-bradykinin in plasma or serum prior to such Phe removal to yield the pentapeptide Arg-Pro-Pro-Gly-Phe and the tripeptide Ser-Pro-Phe. We demonstrated that CPB degraded Arg-Pro-Pro-Gly-Phe but not Ser-Pro-Phe; this reaction was also inhibited by phosphate ions. Carboxypeptidase A, on the other hand, liberated Phe from both peptides in phosphate-buffered saline and accounted, at least in part, for the free phenylalanine detected. Carboxypeptidase N did not digest the aforementioned pentapeptide or tripeptide. It is clear that carboxypeptidase B and carboxypeptidase A had overlapping activities, depending upon the substrate tested, and were distinguished by the effects of different ionic environments. We further suggest a role for carboxypeptidases other than CPN in the degradation of kinins in human plasma or serum.

The inactivation of bradykinin in plasma or serum has been shown to be caused by the action of two kininases. One of these, termed carboxypeptidase N (CPN) [1] or Kininase I, removes the C-terminal arginine from bradykinin to form the octapeptide des-Arg$^9$-bradykinin. The angiotensin converting enzyme (ACE) is the second kininase [2]. We have studied the ability of ACE to digest bradykinin, (BK), lysyl bradykinin (LBK) and des-Arg$^9$-bradykinin (DBK) in detail [3]. However, preliminary studies on the degradation of bradykinin in plasma and serum [4, 5] revealed the presence of free phenylalanine, a product which is not known to be formed by either kininase, and presented evidence to suggest that serum may have small quantities of enzymes having the specificities of both carboxypeptidase A (CPA) and carboxypeptidase B (CPB). In this paper we focus upon kininase I and compare its activities to the other carboxypeptidase to determine which, if any, can lead to the release of free phenylalanine from BK, or directly degrade either DBK or Arg-Pro-Pro-Gly-Phe and Ser-Pro-Phe, the products of digestion of DBK by angiotensin converting enzyme [6]. Our results, reported herein, indicate that carboxypeptidase B or carboxypeptidase A removed the C-terminal Phe from kinin degradation products, but carboxypeptidase N did not.

* Supported by Grant 5P50 AI 1633706.

## MATERIALS AND METHODS

### *Materials*

Bradykinin, des-Arg$^9$-bradykinin, and lys-bradykinin were obtained from Sigma (St. Louis, MO). Purified rabbit pulmonary angiotensin converting enzyme (EC 3.4.15.1), having a specific activity of 0.1 units/$\mu$g of protein (one unit releases 1 $\mu$mole of hippuric acid from Hip-His-Leu per min at 37°), was provided by Dr. Richard L. Soffer, Cornell Medical Center, New York, NY. Purified human plasma carboxypeptidase N (EC 3.4.12.7), having a specific activity of 0.14 units/$\mu$g of protein (one unit removes 1 $\mu$mole of Lys from Benz-Ala-Lys per min at 37°) was a gift from Dr. Ervin G. Erdos, University of Texas, Dallas, TX. Bovine pancreatic carboxypeptidase A (EC 3.4.12.2) and diisopropyl fluorophosphate (DFP)-treated bovine pancreatic carboxypeptidase B (EC 3.4.12.3) were obtained from Sigma as well as from the Worthington Biochemical Corp. (Malvern, PA). Phe-Arg, Ser-Pro and the pentapeptide Arg-Pro-Pro-Gly-Phe were purchased from Serva Biochemicals (Garden City, NY); the tripeptide Ser-Pro-Phe was prepared in our laboratory as described below. HPLC-grade acetonitrile, EDTA, and all inorganic salts used were purchased from Fisher (Springfield, NJ); trifluoroacetic acid was from Pierce (Rockford, IL).

### *Preparation of Ser-Pro-Phe*

Bradykinin (500 $\mu$l, 10 $\mu$g/$\mu$l) was incubated with

3 μl of carboxypeptidase-B DFP (2.2 μg/μl) at 37° for 30 min in the presence of 60 μl of Tris–HCl buffer (0.5 M; pH 7.5). High performance liquid chromatographic analysis of 1 μl of the reaction mixture demonstrated the complete conversion of the BK into des-Arg$^9$-bradykinin. After addition of 20 μl of 3 M NaCl, the resulting des-Arg$^9$-bradykinin was next digested with 35 μl of the angiotensin converting enzyme (350 ng/μl) at 37° for 1 hr. The analysis of 1 μl of the reaction mixture showed complete degradation of the DBK into the tripeptide Ser-Pro-Phe plus the tetrapeptide Arg-Pro-Pro-Gly and free Phe (the latter two products are the result of degradation of the pentapeptide Arg-Pro-Pro-Gly-Phe by the CPB [4, 7] already present in the reaction mixture). Ser-Pro-Phe was separated from the other products by HPLC and lyophilized to complete dryness to give 1.37 mg (88% yield) of the pure peptide. The identity of the material was confirmed by amino acid analysis.

*Enzyme digestions*

*Arg removal.* Incubations of BK and LBK to remove the C-terminal arginine were performed with CPN and CPB at 37° in a total volume of 100 μl containing 100 mM NaCl and buffered at pH 7.4 with 3.0 mM sodium phosphate to approximate the concentration of these salts in human plasma [8]. The final concentrations of the substrates were 20 ng/μl (20 μM), while CPN and CPB were 40 ng/μl (0.14 μM) and 0.3 ng/μl (0.009 μM) respectively. The enzyme/substrate ratios using CPN and CPB were 0.7 and 0.05%. The concentration of CPN was selected to equal that in human plasma [9]; the substrate concentrations were chosen so as to yield clear peaks by HPLC.

*Phe removal.* Incubation of DBK, DLBK (LBK plus CPB), pentapeptide (Arg-Pro-Pro-Gly-Phe), tripeptide (Ser-Pro-Phe), and Phe-Arg were performed with CPN, CPB and CPA at 37° in a total volume of 100 μl using various buffer systems at pH 7.4. The final concentration of CPN was 3.5 μM (1.0 μg/μl), whereas that of the substrates was ~20 μM (DBK and DLBK = 20 ng/ml; pentapeptide = 10 ng/μl; tripeptide and Phe-Arg = 7 ng/μl). The concentrations of CPB and CPA varied according to the nature of the substrate: i.e. for DBK, DLBK, tripeptide, and Phe-Arg, CPB = 73 ng/μl (2.1 μM) and CPA = 150 ng/μl (4.4 μM); for pentapeptide, CPB = 3.5 ng/μl (0.1 μM) and CPA = 7 ng/μl (0.2 μM).

*Analysis of the reaction mixtures.* At timed intervals, 30-μl aliquots were quenched with 250 μl of trifluoroacetic acid solution [0.1% (v/v) in water], pH 2.1, and directly loaded onto the HPLC column for analysis.

*High performance liquid chromatography*

HPLC analyses were performed with a Walters Associate system containing a set of 6000 A pumps, Data Module 730, System Controller 720, Automated Injector (Wisp) 710-B, and Extended Wavelength Module 440 absorbance detector. Peptide products were applied to a μ-Bondapack C-18 reverse phase column (3.9 mm i.d. × 30 cm) of 10 micron particle size (Waters). The separations were carried out with different linear gradients and isocratic systems of 0.065% (v/v) acetonitrile: trifluoroacetic acid (Solvent B) and 0.1% (v/v) trifluoroacetic acid in deionized water (Solvent A). For the analysis of the BK, LBK, DBK and DLBK digested-products, a linear gradient of B with increasing concentrations from 10 to 50% in a span of 10 min was applied; for pentapeptide products, the gradient was 10 to 30% in 5 min. Isocratic systems for tripeptide and Phe-Arg reaction mixtures were 87% A: 13% B and 90% A: 10% B respectively. A flow rate of 1.5 ml/min was maintained at ambient temperature in all the gradients and isocratic systems. The products detected at 214 nm were identified by co-elution with peptide standards and were confirmed by amino acid analyses. Substrate degradation was calculated by comparing the integrated peak area to the peak area of a known amount of authentic standard.

*Amino acid analyses*

Amino acid analyses were performed by Dr. Marshal Elzinga, Brookhaven National Laboratories, Upton, NY, as follows: the peak obtained by HPLC was lyophilized and exposed to 6 N KCl overnight at 110°. The sample was dried under nitrogen, dissolved in 0.2 M sodium citrate buffer, pH 2.0, and loaded into the column. Separation and identification were performed as described by Hewick *et al.* [10].

## RESULTS

We first tested the ability of purified human carboxypeptidase N to digest bradykinin. As shown in Fig. 1, there was progressive conversion of bradykinin to des-Arg$^9$-bradykinin, and this product was stable to futher digestion for over 24 hr. Increasing the enzyme concentration 10-fold also had no effect upon des-Arg$^9$-bradykinin. A similar result was obtained with lysyl-bradykinin (LBK); arginine was released and the residual peptide was des-Arg$^9$ Lys-bradykinin (DLBK). Because of the additional lysine residue, the retention times of LBK and DLBK were slightly less than that of BK or DBK respectively. This is corroborated by the data in Fig. 2 in which a mixture of BK and LBK was digested, showing their progressive conversion to DBK and DLBK and their separation from each other. The rates of digestion of BK and LBK in the mixture were no different from the rates of digestion when each was tested separately.

Carboxypeptidase B, when tested at 0.3 ng/μl, rapidly removed the C-terminal arginine whereas carboxypeptidase N, at 2.4 ng/μl (equimolar concentration), was far less active. This is shown in Fig. 3, in which rapid degradation of LBK or BK was seen within a 6-min time interval, while at this concentration CPN had no discernable effect. For Figs. 1 and 2, CPN was used at a concentration 17 times greater than in Fig. 3, and the assay time was extended to 60 min. In contrast to our prior study of ACE [3], we found no significant effect of the concentration of NaCl, KCl, $NH_4Cl$, phosphate ion, or calcium, magnesium, copper, or zinc ions on the rate of arginine removal from BK or LBK by either CPB or CPN. The rate of digestion was, however,

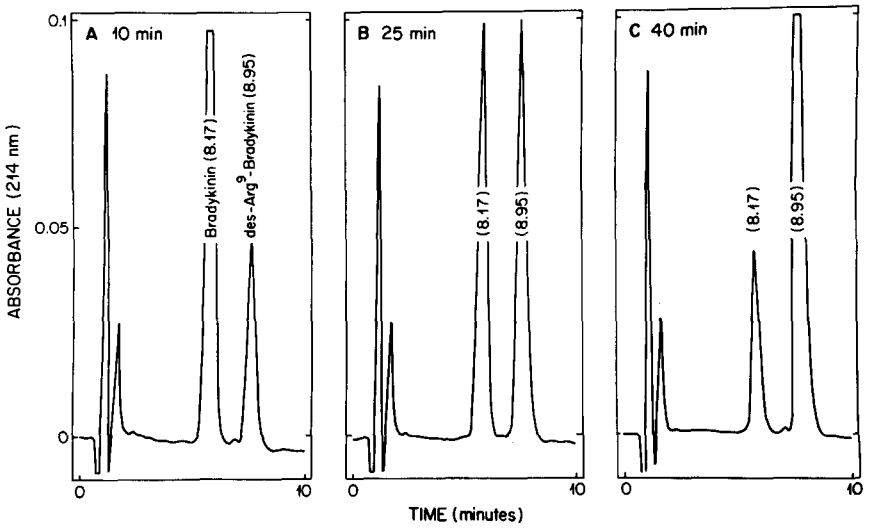


Fig. 1. Elution pattern of bradykinin degradation by CPN as assessed by reverse phase high pressure liquid chromatography as described in Materials and Methods. Assessment of time points at 10, 25, and 40 min are shown for parts A, B, and C respectively. In part A, the identity of each peak indicated, and its retention time, in minutes, is given in parentheses; only the latter is given in B and C.

pH dependent. The rate of digestion of BK and LBK was increased between pH 5 and 8. A further increase in pH slightly diminished the rate of LBK digestion but had no effect upon the rate of BK digestion (Fig. 4).

Our earlier results suggested that carboxypeptidase B is capable of removing the C-terminal Phe from des-Arg$^9$-bradykinin [4, 5]. DFP-treated material behaved similarly, making it unlikely that it was due to contaminating trypsin or chymotrypsin. An example is shown in Fig. 5 in which progressive digestion of des-Arg$^9$-bradykinin by CPB (73 ng/$\mu$l) converted it to the heptapeptide Arg-Pro-Pro-Gly-Phe-Ser-Pro plus free phenylalanine. This effect was seen at a concentration as low as 10 ng/$\mu$l but required over 3 hr of incubation; at 73 ng/$\mu$l, complete conversion required only 1 hr. When LBK was similarly digested, free Phe was also formed, leaving the octapeptide Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro which eluted 30 sec prior to the retention time of the heptapeptide. Carboxypeptidase A is known to rapidly remove C-terminal Phe from peptides [11]; thus, it was reasonable to question whether the carboxypeptidase B might have been contaminated with carboxypeptidase A. The manufacturer claimed that the potential contamination of CPB by CPA would not exceed 4%. Assuming such contamination, we found that CPB at 73 ng/$\mu$l depleted 55% of DBK in 15 min (Table 1). Testing CPA at 2 and 4% concentrations by weight upon DBK gave only 5 and

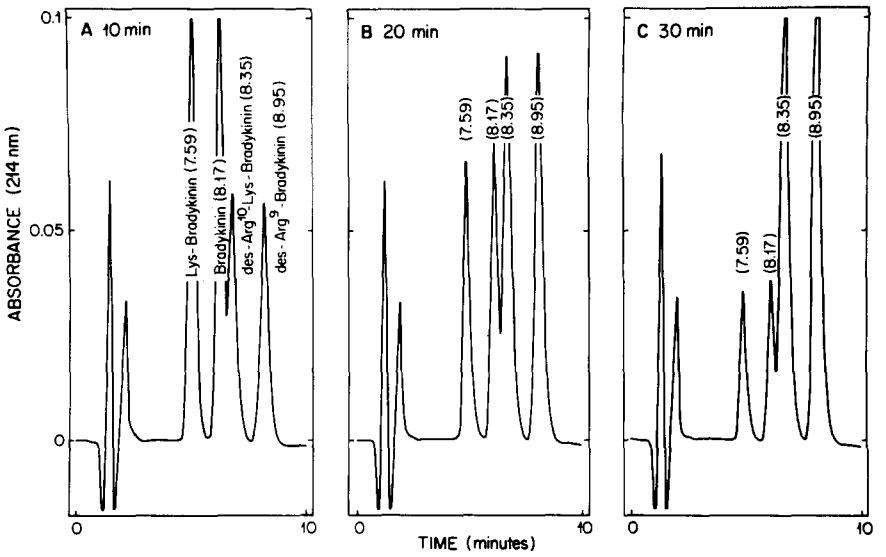


Fig. 2. Elution pattern of degradation of a mixture of LBK and BK (10 ng/$\mu$l each) by CPN (40 ng/$\mu$l) as assessed by HPLC. Time points obtained at 10, 20, and 30 min are shown in parts A, B, and C.

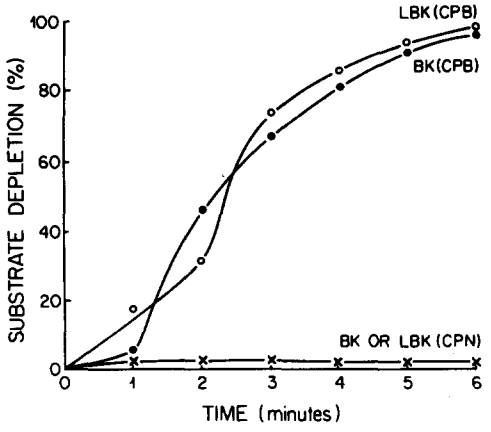


Fig. 3. Time curves of degradation of BK and LBK by either CPB (0.3 ng/μl) or CPN (2.4 ng/μl). The percent depletion at each time point was monitored by HPLC and the result plotted against time in minutes.

11% depletion, respectively, in the same amount of time. Increasing CPA to 150 ng/μl was required to approximate the rate of digestion of DBK seen with CPB. Thus, CPB removed Phe from DBK more readily than CPA did. To test for a possible synergistic effect, we added the equivalent of 2 and 4% CPA to the CPB and demonstrated 61 and 65% DBK depletion (Table 1), an additive but not synergistic result.

We next compared the effects of various ions on digestion of DBK by CPB versus CPA (Fig. 6, A and B). When CPB was tested (Fig. 6A), zinc acetate had no effect, sodium chloride had a slightly inhibitory effect, cupric acetate and EDTA were strongly inhibitory, and 3 mM sodium phosphate buffer, pH 7.4, completely eliminated the reaction. In contrast, when CPA was examined (Fig. 6B), 100 mM sodium chloride dramatically augmented the reaction; EDTA (4 nM), sodium phosphate buffer,

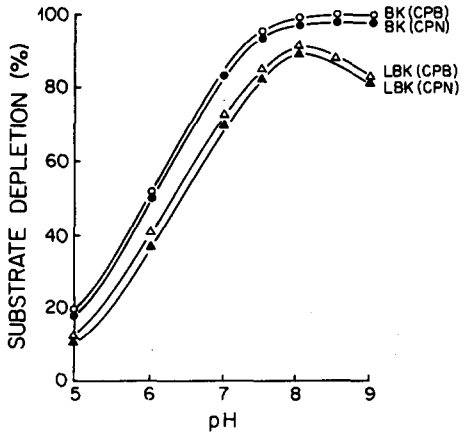


Fig. 4. Percent depletion of BK and LBK by CPB or CPN at differing pH (range 5–9) prepared in sodium phosphate buffer. The effect of CPB on BK or LBK was determined at 5 and 4 min of digestion respectively. The effect of CPN on BK or LBK was determined at 50 and 40 min of digestion respectively.

Table 1. Comparison of Phe removal from DBK by CPB and increasing concentrations of CPA

| Final conc. of enzyme/μl | % DBK depletion |
|---|---|
| (1) 73 ng CPB | 55 |
| (2) 2.4 ng CPA | 5 |
| (3) 4.8 ng CPA | 11 |
| (4) 9.6 ng CPA | 17 |
| (5) 19.2 ng CPA | 27 |
| (6) 38.4 ng CPA | 35 |
| (7) 150 ng CPA | 57 |
| (8) 73 ng CPB + 2.4 ng CPA | 61 |
| (9) 73 ng CPB + 4.8 ng CPA | 65 |

DBK and the enzymes were incubated in deionized water for 15 min at 37°. The reaction mixtures were quenched and analyzed by HPLC as described in Materials and Methods.

or cupric acetate buffer also augmented the rate of the reaction, but to a lesser degree. Zinc acetate buffer, however, was mildly inhibitory. As is evident, the effects of different buffer systems upon the ability of each carboxypeptidase to remove Phe from DBK were completely different. These results strongly suggest that CPA cannot account for the digestion of DBK seen with CPB.

Another possibility to account for the above result would be contamination of CPB by prolyl carboxypeptidases [12–14] which can remove a C-terminal Phe if the penultimate residue is proline. To test this hypothesis we determined the ability of CPB to remove the C-terminal Phe from the pentapeptide Arg-Pro-Pro-Gly-Phe and the tripeptide Ser-Pro-Phe, the degradation products of DBK by ACE [3].

The effect of CPB on the pentapeptide is shown in Fig. 7. There was progressive digestion of this peptide in deionized water to yield the tetrapeptide Arg-Pro-Pro-Gly plus free phenylalanine. Thus, a penultimate Pro, which is not present in this peptide, is not required for the effect of CPB and makes contamination with a prolyl carboxypeptidase unlikely. In fact, the rate of cleavage of this pentapeptide was about 600 times faster than with DBK (6 sec vs 1 hr) at the same molar ratio of enzyme/substrate. CPN, again, had no effect upon the pentapeptide at any concentration tested up to 1 μg/μl for 24 hr. When digestion of this pentapeptide by CPB was compared to CPA using the same buffer systems as in Fig. 6, we again demonstrated a striking difference in requirements between the two enzymes. As shown in Fig. 8A, the magnitudes of digestion of pentapeptide in water, 100 mM sodium chloride or 0.1 mM zinc acetate were essentially the same. However, increasing inhibition was seen with cupric acetate buffer, EDTA or 3 mM sodium phosphate buffer. When CPA digestion of pentapeptide was similarly tested (Fig. 8B), none of the buffer systems was inhibitory. Zinc acetate has no effect. EDTA, cupric acetate buffer, or sodium phosphate buffer augmented the reaction rate, while 100 mM sodium chloride gave a prominent increase in rate. In general, the effects of these buffers upon the ability of each carboxypeptidase to digest either DBK or pentapeptide were similar in each instance. A different result was obtained when the tripeptide Ser-Pro-Phe was tested as a substrate. Neither CPN

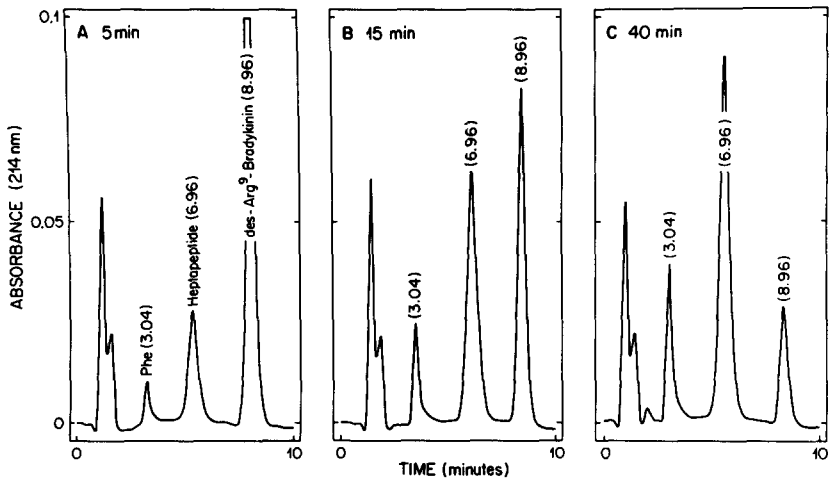


Fig. 5. Elution pattern of digestion of des-Arg$^9$-BK by CPB as assessed by reverse phase HPLC showing time points obtained at 5, 15, and 40 min in parts A, B, and C. The reaction was performed in deionized water as described in Materials and Methods.

nor CPB had any effect in any buffer system mentioned above. CPA at 150 ng/$\mu$l removed 50% of the Phe in 4 hr in saline phosphate buffer (Fig. 9). Thus, this peptide is not very susceptible to digestion by any of the carboxypeptidases. The resistance to digestion of the Pro-Phe bond of the tripeptide also makes contamination of CPB with prolyl carboxypeptidase unlikely.

We also tested the dipeptidase Ser-Pro and Phe-Arg (degradation products of BK or LBK by ACE) for susceptibility to each of the carboxypeptidases studied. Ser-Pro was not digested at all, whereas Phe-Arg was readily cleaved by CPB and was slowly cleaved by CPN but not by CPA.

## DISCUSSION

Bradykinin and lysyl bradykinin are known to be degraded by carboxypeptidase N to form the des-Arg$^9$-derivatives of each [1]. Carboxypeptidase B behaves similarly [15], but it is known to be much more active on a molar basis [16, 17]. Utilizing a variety of synthetic substrates with C-terminal Arg, the $K_{cat}/K_m$ for human or bovine carboxypeptidase B was 10- to 150-fold greater than for CPN [17]. When we added bradykinin to plasma, our experiments suggested [4, 5] the formation of free phenylalanine, a product that was not anticipated. One possible explanation is that the Phe-Arg that is liberated by angiotensin converting enzyme [2] is digested by CPN to release free Phe and Arg. When we added des-Arg$^9$-bradykinin to plasma, however, free Phe was also seen, suggesting its cleavage either directly from des-Arg$^9$-BK or from some other later degradation product. When we examined the digestion of DBK by CPN and CPB, we found that CPB, when used in high concentration, removed the C-terminal Phe, whereas CPN was inactive in this regard. Cleavage of a Pro-Phe bond at the C-terminus of a peptide is not a commonly recognized

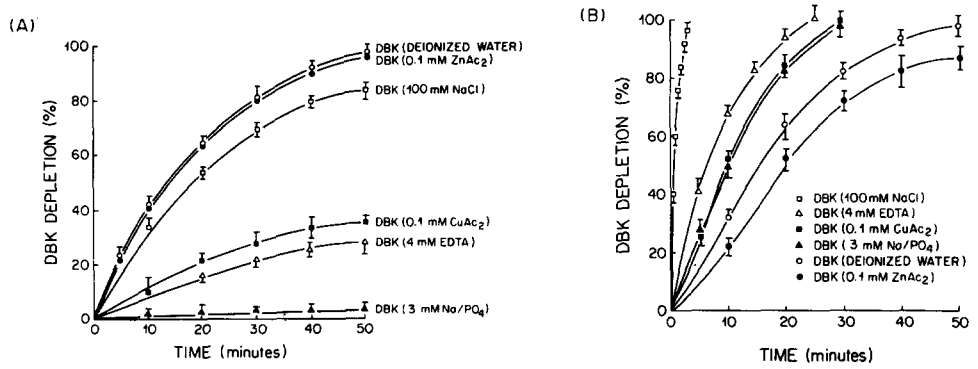


Fig. 6. (A) Time course of digestion of DBK by CPB in various buffer systems. The percent depletion at each time count was monitored by HPLC and the result plotted against time in minutes. (B) The same experiment substituting CPA for CPB, utilizing concentrations of each as described in Materials and Methods. The mean and standard error for three experiments is shown for each time point in panels A and B.

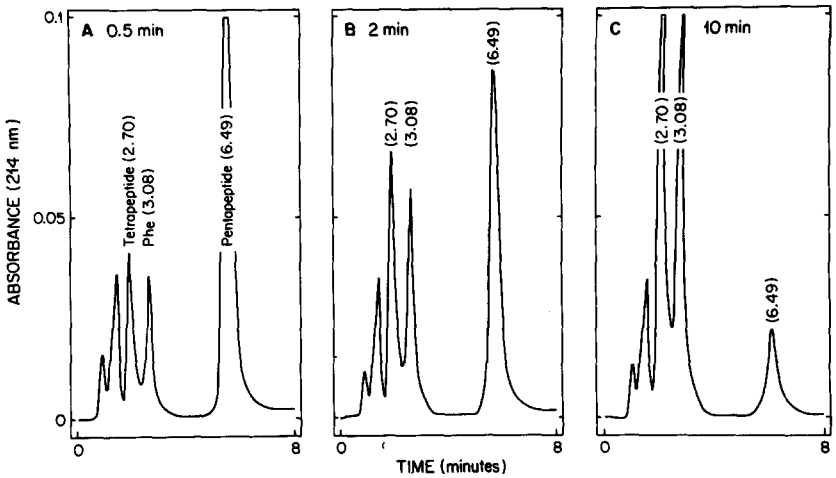


Fig. 7. Elution pattern of digestion of Arg-Pro-Pro-Gly-Phe by CPB in deionized water showing time points obtained at 0.5, 2, and 10 min, as shown in parts A, B, and C.

function of CPB. However, a report by Wintersberger *et al.* [7] demonstrated that CPB is capable of digesting Carbenzoxy-Gly-Phe, a substrate of CPA. This has not been confirmed using either synthetic or physiologic substrates. Therefore, we considered the possibility that our preparation was contaminated by either tryptic or chymotryptic enzymes, carboxypeptidase A, or prolyl carboxypeptidases. DFP-treated material was no less active than untreated CPB, indicating absence of significant tryptic or chymotryptic-like activity. Also, most prolyl carboxypeptidases [11–14] are DFP-sensitive, thus making this possibility less likely. In addition we demonstrated that the C-terminal Phe from the peptide Arg-Pro-Pro-Gly-Phe was readily liberated by CPB (about 600 times faster than the rate seen with des-Arg$^9$-BK). In this case, there is no penultimate Pro; hence, the activity seen is not consistent with a prolyl carboxypeptidase. Conversely, Ser-Pho-Phe was not susceptible to CPB but would be digested by a prolyl carboxypeptidase.

Of the possible contaminants, attention was focused upon CPA, particularly since some contamination of CPB preparations by CPA is possible. However, our data do not support this explanation. First, if we had 4% CPA contamination (Table 1), it is insufficient to account for the activity seen. In fact, CPB was more effective than CPA in removing the C-terminal Phe from DBK, DLBK, and the pentapeptide. Further, the effects of various ions upon the abilities of the two enzymes to remove Phe were completely different (Fig. 6, A and B). Finally, CPA slowly degraded Ser-Pro-Phe, whereas CPB did not.

Other enzymes have been described that are capable of removing the C-terminal Phe from DBK; these were derived from rat brain [18], rabbit brain [19], rat dental pulp [20], or beef spleen [21]. The rabbit brain kininase is DFP inhibitable and is a form of prolyl endopeptidase, while the catheptic enzyme derived from beef spleen is an acid protease. These activities differ from our observations using CPB. The rat brain and rat dental pulp kininases are of interest in that they are neutral proteases which sequentially remove C-terminal Arg and then Phe from bradykinin. One author suggested that this represents a new type of carboxypeptidase [20]. Our results with CPB are similar, but such activity has not been reported previously in man. Further, the ability of the rat enzymes to release phenylalanine

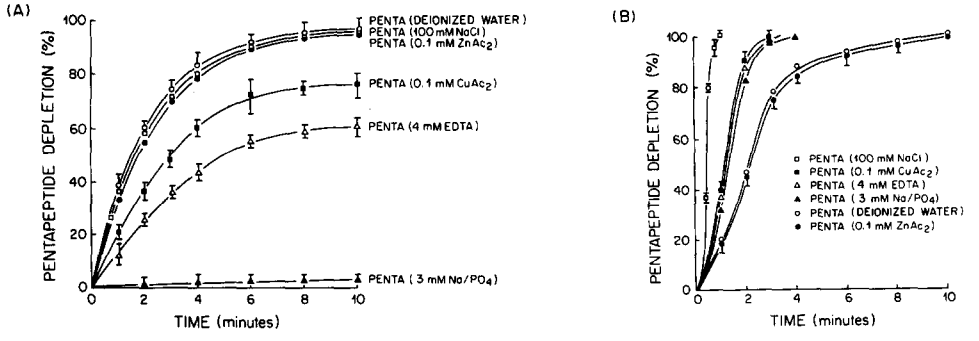


Fig. 8. (A) Time course of digestion of Arg-Pro-Pro-Gly-Phe by CPB on various buffers systems as indicated. The percent depletion at each time point was monitored by HPLC and the result plotted against time in minutes. (B) The same experiment substituting CPA for CPB, utilizing concentrations of each as described in Materials and Methods. The mean and standard error for three determinations at each time point is shown in panels A and B.

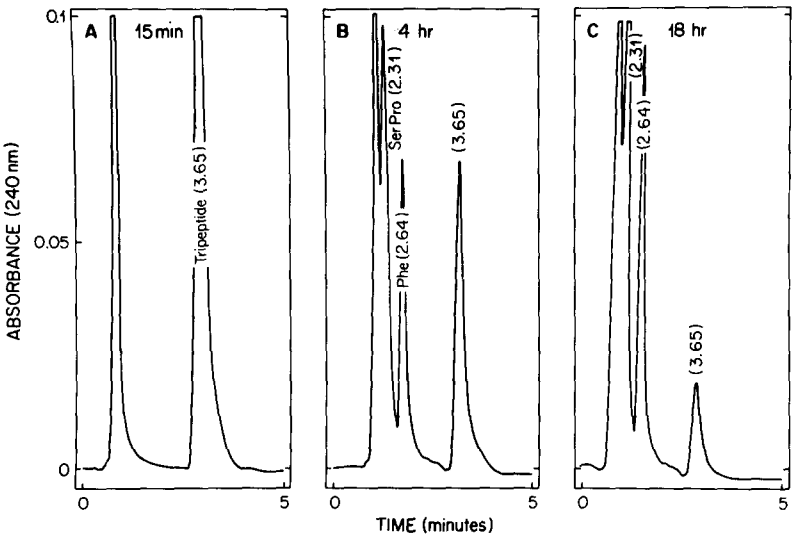


Fig. 9. Elution pattern of digestion of Ser-Pro-Phe by CPA showing time points obtained at 15 min, 4 hr, and 18 hr as shown in parts A, B, and C. The experiment was performed in saline (100 mM), phosphate (3 mM) buffer as described in Materials and Methods.

was not EDTA inhibitable, whereas CPB was inhibited (Fig. 6A). We conclude that CPB is indeed capable of removing Phe from DBK, DLBK, and Arg-Pro-Pro-Gly-Phe, lending support to the original observation that the spectrum of activity of CPB can overlap that of CPA [7].

CPN or CPB removed the C-terminal Arg equally well in water, phosphate buffer, sodium chloride, or buffers containing calcium, magnesium, copper, or zinc but the activity of CPB was ⩾CPN as indicated earlier [17]. However, Phe removal by CPB was exceedingly sensitive to the ionic environment, as shown in Figs. 6A and 8A.

Our earlier studies [4, 5], which prompted this investigation, suggest that human serum also possesses an activity that degrades bradykinin to lead to free phenylalanine formation. Although carboxypeptidase B and/or A like activities might explain this observation, the complete inhibition of this activity of CPB by 3 mM phosphate favors the presence of a serum CPA like enzyme. However, we have demonstrated that Arg removal from bradykinin in serum occurs at a rate exceeding that of heparinized plasma and is more rapid than can be attributed to CPN.* We conclude, therefore, that both CPB and CPA were present and contributed to Arg and Phe removal respectively.

Since these studies were all performed *in vitro*, one cannot extrapolate the results to the *in vivo* situation. Clearly the rate of kinin degradation and the nature of the final products seen may be different. Of particular interest, however, are studies of bradykinin degradation in rabbit tissues, e.g. lung, kidney and liver [22], which report free phenylalanine and arginine, Phe-Arg, Ser-Pro, pentapeptide, and heptapeptide, among the degradation products, although differences in relative quantity were found when one tissue was compared with another.

* I. Sheikh and A. P. Kaplan, manuscript submitted for publication.

## REFERENCES

1. E. G. Erdos and E. M. Sloane, *Biochem Pharmac.* **11**, 585 (1962).
2. H. Y. T. Young and E. G. Erdos, *Nature, Lond.* **215**, 1402 (1967).
3. I. Sheikh and A. P. Kaplan, *Biochem. Pharmac.* **35**, 1951 (1986).
4. I. Sheikh and A. P. Kaplan, *Fed. Proc.* **44**, 1242 (1985).
5. A. P. Kaplan, I. Sheikh and M. H. Frendscho, *Int. Archs Allergy appl. Immun.* **77**, 64 (1985).
6. J. I. Inokuchi and A. Nagamutsu, *Biochim. biophys. Acta* **662**, 300 (1981).
7. E. Wintersberger, D. J. Cox and H. Neurath, *Biochemistry* **1**, 1069 (1962).
8. P. L. Lehninger, *Biochemistry*, 2nd Ed, p. 843. Worth, New York (1876).
9. V. Bokisch and H. J. Muller-Eberhard, *J. clin. Invest.* **49**, 2427 (1970).
10. R. M. Hewick, M. W. Hunkapiller, L. E. Hood and W. J. Dreyer, *J. biol. Chem.* **256**, 7990 (1981).
11. K. Hofmann and M. Bergmann, *J. biol. Chem.* **134**, 225 (1940).
12. M. Koida and W. Roderich, *J. biol. Chem.* **251**, 7593 (1976).
13. R. Walter, W. H. Simmons and T. Yoshimoto, *Molec. cell. Biochem.* **30**, 111 (1980).
14. T. Yoshimoto, M. Fishl, R. C. Orlowski and R. Walter, *J. biol. Chem.* **10**, 3708 (1978).
15. E. G. Erdos, A. G. Renfrew, E. M. Sloane and J. R. Wohler, *Ann. N.Y. Acad. Sci.* **104**, 222 (1963).
16. E. G. Erdos and H. Y. T. Yang, *Handbk. exp. Pharmac.* **25**, 289 (1970).
17. T. J. McKay, A. W. Phelan and T. H. Plummer, Jr., *Archs Biochem. Biophys.* **197**, 487 (1979).
18. T. Shikima and H. Iwata, *Biochem. Pharmac.* **19**, 1399 (1970).
19. M. Orlowski, E. Wilk, S. Pearce and S. Wilk, *J. Neurochem.* **33**, 461 (1979).
20. E. Maita, Y. Endo and Y. Ogura, *J. dent. Res.* **63**, 1067 (1984).
21. L. M. Greenbaum and K. Yamafuji, *Life Sci.* **4**, 657 (1965).
22. M. A. Cicilini, H. Caldo, J. D. Berti and A. C. M. Camargo, *Biochem. J.* **163**, 433 (1977).