Hydrolysis of Bradykinin by Angiotensin-Converting Enzyme

By Frederic E. Dorer, Joseph R. Kahn, Kenneth E. Lentz, Melvin Levine, and Leonard T. Skeggs

ABSTRACT

Two dipeptides, phenylalanylarginine (Phe-Arg) and serylproline (Ser-Pro), are released sequentially from bradykinin by angiotensin-converting enzyme purified from hog lungs; chloride increases the rate of release of both dipeptides. Using an automated ninhydrin-reagent method, we studied the kinetics of bradykinin hydrolysis. The reaction proceeded in the absence of chloride; however, the addition of chloride increased the rate of hydrolysis by decreasing $K_m$ and increasing $V_m$. The $K_m$ values for bradykinin were $3.9 \times 10^{-6}$M in the absence of chloride and $0.85 \times 10^{-6}$M in the presence of $0.01$M NaCl (optimal concentration). Both of these $K_m$ values were well below the value of $30 \times 10^{-6}$M determined for angiotensin I at its optimal chloride concentration of $0.1$M. Hydrolysis of bradykinin had a pH optimum of 7 and was inhibited by low concentrations ($10^{-6}$M) of ethylenediaminetetraacetic acid or the nonapeptide pyroglutamyl (Pyr)-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro. It is concluded that one enzyme, acting as a dipeptidyl carboxypeptidase, catalyzes both the conversion of angiotensin I to angiotensin II and the hydrolysis of bradykinin.

KEY WORDS

phenylalanylarginine
dipeptidyl carboxypeptidase
enzyme kinetics

serylproline
chloride activation

hog lung

Bothrops jararaca nonapeptide

Extracts of mammalian lung contain an enzyme or enzymes capable of converting angiotensin I to angiotensin II and of destroying the biological activity of bradykinin (1). (Hydrolysis of any peptide bond in bradykinin [Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg] abolishes biological activity [2].) Recently, an enzyme purified from lung has been shown to possess both of these activities (3). Bradykinin is a competitive inhibitor of angiotensin conversion (4), implying a common enzyme. However, the hydrolysis of bradykinin by angiotensin-converting enzyme preparations is reported to be independent of the presence of chloride; this finding leads to speculation that the enzyme activities must differ in some way (4, 5). The availability of a purified enzyme preparation (6) and the development of a quantitative chemical assay for measuring the hydrolysis of bradykinin enabled us to investigate whether bradykinin and angiotensin are hydrolyzed by the same enzyme and whether chloride participates in the bradykininase reaction.

Methods

Angiotensin I, hippuryl (Hip)-Gly-Gly, and His-Leu were synthesized as described previously (6, 7). Bradykinin was purchased from Schwarz/Mann, and N-2-hydroxyethylpiperazine - N'-2-ethanesulfonic acid (HEPES) was purchased from Calbiochem. The dipeptides, serylproline (Ser-Pro), phenylalanylarginine (Phe-Arg), and Gly-Phe, were purchased from Fox Chemical Company. The concentrations of standard solutions of the peptides were determined by amino acid analysis following acid hydrolysis. The synthetic peptide pyroglutamyl (Pyr)-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro was a gift. The angiotensin-converting enzyme purified from hog lung was obtained from fraction G or fraction H as described previously (6). Fraction H has been shown to be homogeneous by disk gel electrophoresis at pH 9.5 (6). These two fractions are identical with regard to relative rates of bradykinin, angiotensin I, and Hip-Gly-Gly hydrolysis, chloride activation, and similarity of dipeptide formed from bradykinin.

Enzyme assays were performed by a modification of the automated method of Skeggs et al. (8) in which the rate of peptide bond hydrolysis is measured continuously by the ninhydrin reaction. Increased sensitivity was obtained by using the Technicon AutoAnalyzer II colorimeter, which permitted a twenty-fold electrical range expansion so that $6 \times 10^{-6}$M leucine gave a full-scale reading. The incubation mixtures (final volume 5 ml) contained enzyme in 0.025M HEPES buffer (pH 7.5) with 0.04% Brij-35 (a synthetic detergent) and NaCl at the indicated concentration. After a 10-minute incubation at 37°C, the substrate was added, and the mixture was sampled continuously for 8 minutes at 37°C. Enzyme velocities were calculated directly from the slopes of the recordings and were expressed as nanomoles of dipeptide formed per minute in the 5-ml incubation mixture. The analytical system was calibrated with His-Leu when angiotensin I was the substrate, with Gly-Gly when...
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Hip-Gly-Gly was the substrate, and with Ser-Pro or Phe-Arg (identical ninhydrin color values, 83% of leucine) when bradykinin was the substrate.

Results

The automated enzyme assay method used in this study for bradykinin hydrolysis yielded velocities that were linear with time during the 8-minute sampling period and enzyme concentrations that were in the range of 0.1 to 1.0 μg/ml. At pH 7.5, the K_m and V_m values for bradykinin hydrolysis (Fig. 1) were 3.9 x 10^{-6} M and 0.81 μmoles/min mg^{-1} protein, respectively, in the absence of chloride, and 0.85 x 10^{-6} M and 1.4 μmoles/min mg^{-1} protein in the presence of 0.01M NaCl. Similarly, the K_m and V_m values for angiotensin I hydrolysis were 3.0 x 10^{-6} M and 2.1 μmoles/min mg^{-1} protein, respectively, in the presence of 0.1M NaCl.

The relationship between bradykinin hydrolysis and chloride concentration is shown in Figure 2. The optimal chloride concentration is about one-tenth of that previously found for the angiotensin-conversion reaction (phosphate buffer, pH 8) (6). The observed velocity in the absence of added chloride did not appear to result from the presence of trace amounts of chloride in the reagents, because there was no hydrolysis of Hip-Gly-Gly, a reaction which absolutely requires chloride (6) under these conditions. Also, bradykinin that had been treated by chromatography on BioGel P-2 or with an ion-exchange resin (AG 2-X8 acetate) to remove any traces of chloride was still hydrolyzed at the same rate in the absence of added NaCl.

The pH-activity relationships for bradykinin and angiotensin I hydrolysis are shown in Figure 3. The shapes of the curves for bradykinin hydrolysis in the presence and the absence of chloride are similar. Figure 4 shows the time course of bradykinin hydrolysis with and without chloride. Chloride increased the rate of hydrolysis without changing the final value attained.

The hydrolysis of bradykinin (4 x 10^{-6} M) both in the presence and the absence of NaCl was inhibited more than 50% by 10^{-6} M ethylenediaminetetraacetic acid (EDTA) or 10^{-3} M Pyr-Trp-Pro-Arg.

Double reciprocal plot of bradykinin hydrolysis showing the effect of chloride on K_m and V_m. Velocities are expressed as nmols dipeptide formed/min. and substrate concentrations are expressed as nmols/ml. Assays were carried out as described in the text in the absence of NaCl with 2.5 μg of enzyme (solid circles) and 5.0 μg of enzyme (triangles) and in the presence of 0.01M NaCl with 1.0 μg of enzyme (crosses) and 2.5 μg of enzyme (open circles).

FIGURE 1

Bradykinin hydrolysis as a function of NaCl concentration. Assays were carried out as described in the text using 2.5 μg of enzyme and 20 nmols of bradykinin.

FIGURE 2
Pro-Gln-Ile-Pro-Pro, a bradykinin-potentiating peptide found in Bothrops jararaca venom (9).

The demonstration by chemical methods of the hydrolysis of bradykinin by angiotensin-converting enzyme was confirmed by the loss of biological activity of bradykinin. Incubation for 1 hour with an excess of enzyme (with or without NaCl) completely abolished the depressor effect of bradykinin (1 nmoles, iv) injected into an anesthetized rat (10).

To determine which peptide bonds were hydrolyzed, bradykinin (260 nmoles) was incubated for 1 hour with an excess of converting enzyme in a volume of 0.7 ml at pH 7.5; this procedure was followed by identification of products on the amino-acid analyzer. The results were the same with and without chloride; two peaks were observed that coincided with Ser-Pro (240 nmoles) and Phe-Arg (280 nmoles). No free arginine or Gly-Phe was found.

The effect of NaCl on the initial rate of release of Phe-Arg and Ser-Pro is shown in Figure 5. The bradykinin concentration used in this experiment...
was $4 \times 10^{-6}$M, the same as that used in the experiments considered in Figures 2 and 3 and twice that in the experiments considered in Figure 4. The rate of release of both dipeptides depended on chloride (Fig. 5); in the case of Phe-Arg, the stimulation by chloride was about 2.5-fold.

Discussion

Most of the data presented in this paper represent a mixed reaction, i.e., the actual quantity measured was the sum of Phe-Arg and Ser-Pro. Nevertheless, the initial mixed rate, at least under the experimental conditions of Figure 5, appears to represent the initial reaction, which was the release of Phe-Arg.

Evidence that chloride participates in the hydrolysis of bradykinin was found. Although it is not an absolute requirement, chloride does affect both $K_m$ and $V_m$. The data presented in Figure 5 show that chloride is required for maximal rate of release of both dipeptides. Since the bradykinin molecule becomes inactive when the Phe-Arg moiety is removed, the rate of Phe-Arg release should parallel the loss of biological activity (bradykininase activity). Thus, it is difficult to understand why the chloride effect has not been observed by other investigators (4, 5) using biological assays.

It is not known whether the pulmonary converting enzyme–bradykininase described in this paper is the enzyme responsible for bradykinin inactivation under physiological conditions in the intact animal. Bradykinin is hydrolyzed at several bonds during perfusion through the blood-free rat lung (2). However, the inactivation of bradykinin in the pulmonary circulation is inhibited by Bothrops jararaca peptides (5, 12).

Evidence that one enzyme, which requires chloride for maximal activity, catalyzes both the conversion of angiotensin I to angiotensin II and the hydrolysis of bradykinin has been presented. This conclusion is compatible with the dipeptidyl carboxypeptidase type of specificity previously described for this enzyme (3).

References


Correction

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Table 3, line five, should read

TABLE 3

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