Angiotensinase Activity of Dipeptidyl Aminopeptidase I (Cathepsin C) of Rat Liver

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SUMMARY

Dipeptidyl aminopeptidase I (cathepsin C) purified from rat liver was shown to have marked angiotensinase activity arising from its ability to catalyze the rapid removal of two dipeptide fragments, in succession, from the NH$_2$ terminus of a variety of angiotensin II analogues. The most rapid rates of degradation were observed on Asn$^1$-angiotensin II (Hypertensin, Ciba), angiotensin II (bovine), and Ile$^1$-angiotensin II (human). Kinetic studies with the first ($K_m = 0.44$ mm) and the last ($K_m = 0.34$ mm) showed essentially the same turnover number (5220 min$^{-1}$) for the removal of the first dipeptide at pH 5.0 and 37°. The $K_m$ for $\alpha$-Asp-$\beta$-naphthylamide (a model, fluorogenic substrate) was 0.31 mm. Unnatural, biologically active analogues such as $\beta$-Asp$^1$-angiotensin II and $\alpha$-$\delta$-Asp$^1$-angiotensin II were also degraded, although at lower rates. No action was detected on Asn$^1$-$\delta$-Arg$^2$-angiotensin II. The degradation of Asn$^1$-angiotensin II and Ile$^1$-angiotensin II by dipeptidyl aminopeptidase I described in this report was pronounced over a wide range of pH (3 to 7.5) with a maximum between pH 5 and 6. About 20% of the (pH 7.3) activity of the purified enzyme was manifested when added to rat blood plasma that did not contain added Cl$^{-}$ and $-SH$ activators. The predominant products and course of angiotensin II degradation at pH 5.5 by isolated rat liver lysosomes were identical with those produced by purified dipeptidyl aminopeptidase I, thereby demonstrating that this enzyme is probably the major contributor to the angiotensinase activity of liver lysosomes.

Numerous investigators (1-6) have attempted to show that the concentration of circulating angiotensin II, Asp$^1$-Arg$^2$-Val$^3$-Tyr$^4$-Val$^5$-His$^6$-Pro$^7$-Phe$^8$, the hypertensive component of the renin-angiotensin system, is governed by the relative activities of converting and inactivating enzymes in the blood. More recent studies have shown, however, that the blood level of angiotensin II is probably under the control of peptidases residues in tissue vascular beds. Ng and Vane (7, 8) have shown, for example, that angiotensin I is rapidly converted to angiotensin II in the pulmonary circulation, and not by an enzyme in the blood. The formed angiotensin II subsequently disappears in other vascular beds in which its rate of disappearance is much too rapid to be caused by angiotensinases in the blood (8-10). Numerous studies (11-15) have shown a central role for the liver in the metabolism of circulating angiotensin II. Metabolic studies (16-18) with radioactive analogues have ascribed its disappearance to the degradative activity of tissue peptidases.

Earlier reports (19-21) from this laboratory have described the ability of dipeptidyl aminopeptidase I to degrade numerous polypeptide hormones by catalyzing the successive removal of dipeptide moieties from their NH$_2$ termini. As was previously noted (19), Asn$^1$-angiotensin II was similarly degraded by dipeptidyl aminopeptidase I. It appears, however, that the angiotensinase activity of this enzyme has remained unrecognized by workers seeking to identify the enzyme (or enzymes), in liver and kidney responsible for the inactivation of circulating angiotensin II. Thus far, the acid angiotensinase activity of the liver (22, 23) and kidney (24) has been attributed primarily to a lysosomal carboxypeptidase.

In this report, a highly purified preparation of rat liver dipeptidyl aminopeptidase I was used to characterize the kinetics and substrate specificity of this lysosomal enzyme on a variety of angiotensin II analogues, and to show that the acid angiotensinase activity contained in rat liver lysosomes is primarily attributable to dipeptidyl aminopeptidase I.

MATERIALS AND METHODS

Preparation and Assay of Dipeptidyl Aminopeptidase I—The enzyme was prepared from the livers of fasted rats using a method based on that reported by Metrione et al. for the preparation of the beef spleen enzyme (23). The purification results, properties, and substrate specificity for the rat liver enzyme have already been described (19). The enzyme used in this study had a specific activity of at least 20 units per mg of protein. One unit of dipeptidyl aminopeptidase I is defined as the amount of enzyme hydrolyzing 1 µmole of Gly-Phe-$\beta$-naphthylamide per min at pH 6.0 under the conditions of the assay (19). 2-Mercaptoethanol hydrochloride was used to satisfy the -SH requirements of dipeptidyl aminopeptidase I. The dipeptidyl aminopeptidase I used in the peptide digests was first treated with DIPF to suppress contaminating traces of a lysosomal carboxypeptidase (28). This carboxypeptidase...
was inactivated by adding 50 μl of 0.1 M DIPF in isopropanol to 1 ml of dipetidyln amineopeptidase I (1 mg of protein per ml) in cold 0.1 M Na₂HPO₄-0.05 M citric acid buffer, pH 5.0.

Protein concentrations were established by the method of Lowry et al. (29), using crystalline bovine serum albumin obtained from Pentex, Inc. (Kankakee, Ill.) as the standard.

**Time Course Chromatography of Angiotensin Digests**—Chromatographic separations were conducted on thin layers (0.25 mm) of Avicel microcrystalline cellulose obtained as precoated glass plates (20 × 20 cm) from Brinkmann Instruments, Inc. (Westbury, N. Y.). Digest aliquots equivalent to 3 nmoles of angiotensin II were spotted at each origin, and the plates were developed with 2-butanol-3%-aqueous NH₃ (75:30, v/v) (concentrated NH₄OH was assumed to be 30% NH₃). The peptides were detected with a polychromatic ninhydrin reagent (30) containing 50 ml of 0.2% ninhydrin in absolute ethanol, 10 ml of glacial acetic acid, and 2 ml of 2,4,6-collidine. The plates were heated for 2 min at 110°C.

**Identification of Angiotensin Fragments**—A preparative amount of each split product of angiotensin II was isolated as a band on thin layers using the chromatographic system described above. A 100-μl quantity of angiotensin II digest (equivalent to about 0.3 μmole of angiotensin II) was combined with 200 μl of absolute methanol and streaked along a 10-cm origin with a Rodder automatic streaker (Rodder Instruments, Los Altos, Ca.). The plate was developed with the 2-butanol-NH₃ solvent. The edges of the plate were sprayed to locate the peptide bands. The peptides thus located were recovered for acid hydrolysis by scraping from the plate the appropriate bands of cellulose. The peptide was eluted from the cellulose, contained in a small glass filter tube, with about 1.5 ml of 10% HCl. The eluates were dried under vacuum and the peptide residues subjected to acid hydrolysis. The constituent amino acids were identified by comparing their mobilities with standards in two solvent systems: methanol-chloroform-9%/aqueous NH₃ (2:2:1, v/v), and methanol-water-pyridine (20:5:1, v/v). The characteristic color reactions obtained with the polychromic ninhydrin spray also served to confirm the identity of the amino acids.

**Measurement of Hydrolysis Rates on Angiotensin II**—Aliquots (0.1 ml) were taken at specific periods from reaction mixtures to establish the rate of removal of the NH₂-terminal dipeptide (Asn-Arg or Asp-Arg) as well as the penultimate dipeptide (Val-Tyr) from the angiotensin II substrates. Aliquots were inactivated with 0.4 ml of 0.2 M sodium citrate-HCl buffer, pH 2.2, and analyzed on a Beckman/Spinco model 120B amino acid analyzer equipped with a (570 mm) high sensitivity cuvette and expanded range recorder. This method was an adaptation of one previously reported (31). The dipeptides Asn-Arg and Val-Tyr were conveniently analyzed on the 10-cm basic column filled to 5.5 cm with Beckman/Spinco type AA-27 spherical resin maintained at 57°C. To separate Asn-Arg and Val-Tyr from contaminants the following development was necessary: Step 1, to follow the sample onto the column with 3.0 ml of 0.2 M sodium citrate-HCl buffer, pH 3.25; Step 2, to elute with 8.65 ml of this same buffer for about 5 min; and then Step 3, to complete the development with 0.35 M sodium citrate-HCl buffer, pH 5.25, at a flow rate of 68 ml per hour. Val-Tyr eluted at 24 min with a color value of 11.5 A per μmole (compared with 60.16 for a leucine reference), and Asn-Arg eluted at 65 min with a color value of 16.01 A per μmole. To analyze for Asp-Arg, simply omit Step 2 above. Asp-Arg eluted at 20 min with a color value of 6.65 A per μmole and was adequately separated from NH₂ (64 min) and other contaminants.

**Preparation of Rat Liver Lysosomes on Sucrose Density Gradients**—Sprague-Dawley rats were decapitated and their livers quickly transferred to cold 0.25 M sucrose. A 5% (w/v) suspension of minced liver in sucrose was homogenized according to de Duve et al. (32) in a Potter-Elvehjem homogenizer of the B type having a chamber clearance of 0.005 to 0.007 inch; it was expanded range recorder. This method was an adaptation of one previously reported (31). The dipeptides Asn-Arg and Val-Tyr were conveniently analyzed on a Beckman/Spinco model 120B amino acid analyzer equipped with a (570 mm) high sensitivity cuvette and expanded range recorder. This method was an adaptation of one previously reported (31). The dipeptides Asn-Arg and Val-Tyr were conveniently analyzed on the 10-cm basic column filled to 5.5 cm with Beckman/Spinco type AA-27 spherical resin maintained at 57°C. To separate Asn-Arg and Val-Tyr from contaminants the following development was necessary: Step 1, to follow the sample onto the column with 3.0 ml of 0.2 M sodium citrate-HCl buffer, pH 3.25; Step 2, to elute with 8.65 ml of this same buffer for about 5 min; and then Step 3, to complete the development with 0.35 M sodium citrate-HCl buffer, pH 5.25, at a flow rate of 68 ml per hour. Val-Tyr eluted at 24 min with a color value of 11.5 A per μmole (compared with 60.16 for a leucine reference), and Asn-Arg eluted at 65 min with a color value of 16.01 A per μmole. To analyze for Asp-Arg, simply omit Step 2 above. Asp-Arg eluted at 20 min with a color value of 6.65 A per μmole and was adequately separated from NH₂ (64 min) and other contaminants.
RESULTS

Products and Relative Rates of Hydrolysis of Angiotensin II Analogues by Dipeptidyl Aminopeptidase I—As illustrated in Fig. 1a, dipeptidyl aminopeptidase I catalyzed the consecutive removal of the first two dipeptides, Asn-Arg and Val-Tyr, from the NH$_2$ terminus of Asn$_1$-angiotensin II (Ciba hypertensin). The initial appearance of free Asn-Arg was accompanied by the concomitant accumulation of a transient hexapeptide intermediate. After 30 min of reaction, this intermediate was further degraded, giving rise to the simultaneous appearance of free Asn-Arg and the formed hexapeptide intermediate. The cleavage of the Tyr$_4$-Val$_5$ bond in human angiotensin II occurred as readily as the Tyr$_4$-Val$_5$ bond in the bovine analogue. The tetrapeptide product, Ile$_4$-His$_5$-Pro$_6$-Phe$_7$, was similarly resistant to further breakdown by dipeptidyl aminopeptidase I.

The hydrolysis of two unnatural, but biologically-active analogues of angiotensin II are shown in Fig. 1c. Both the β-L-Asp$_1$- and α-d-Asp$_1$ analogues of angiotensin II were hydrolyzed, and at comparable rates, by dipeptidyl aminopeptidase I. Although at least 25 times as much enzyme was required to achieve these rates, this level of activity may still be physiologically significant, since the molar ratio of enzyme to substrate (3.3 x 10$^{-5}$) is still relatively low, and the liver is especially rich in dipeptidyl aminopeptidase I. By comparison, absolutely no activity was detected when the same amount of enzyme was incubated with Asn$_1$-Arg$_2$-angiotensin II. This analogue resembles that used in Fig. 1a, except that the penultimate arginyl residue has the α configuration. The reaction mixtures contained the enzyme, substrate, buffer system containing sulfhydryl and chloride activators as described above, except that 0.8 μg of DIPF-treated dipeptidyl aminopeptidase I was used, giving a molar ratio of 1.3 x 10$^{-5}$. c, hydrolysis of β-L-Asp$_1$-angiotensin II. The results shown for this analogue are similar to those obtained on α-d-Asp$_1$-angiotensin II. These reactions were conducted with 20 μg of DIPF-treated dipeptidyl aminopeptidase I, giving a molar ratio of 3.3 x 10$^{-4}$.

Effect of pH—Dipeptidyl aminopeptidase I exhibited angiotensinase activity over a wide range of pH in a citrate-phosphate buffer system containing sulfhydryl and chloride activators as employed in Fig. 1. A substantial rate of removal of the first dipeptide occurred between pH 3 and 7 on both human (Asp$_1$-Ile$_5$) and Ciba (Asn$_1$-Val$_5$) analogues. Although the pH...
curves were broad and lacked a well defined maximum, this was not attributed to a possible inhibitory effect of the competitive (hexapeptide) intermediate. A similarly broad pH curve was previously observed (19) for the removal of Gly-Arg from Gly-Arg β-naphthylamide. The latter determination was based on initial rates of hydrolysis established by direct fluorometric analysis, and the rates were not complicated by the production of an inhibitory product. The hexapeptide intermediates of both the Ile₈ and Asn₁ analogues accumulated to a detectable extent only on the acid side of the pH curve, indicating that the more acidic conditions favored the removal of the first dipeptide (Asp-Arg or Asn-Arg) as compared with the second dipeptide (Val-Tyr).

Kinetic Studies—Initial velocities, v, were measured over a critical range of substrate concentration to determine the rate of removal of the NH₂-terminal dipeptide moieties from Asp-Arg-angiotensin II, Ile₈-angiotensin II, and Asp-Arg-β-naphthylamide by dipeptidyl aminopeptidase I in the same reaction medium as described in Fig. 1. The V_max values obtained for the two angiotensin analogues were similar; based on these values, the turnover number was estimated to be about 87 μmoles of angiotensin II hydrolyzed (NH₂-terminal dipeptide released) per s per μmole of dipeptidyl aminopeptidase I, using a molecular weight of 200,000 for the rat liver enzyme (19). The Kₘ values were estimated to be 0.44 mM for Asn-angiotensin II and 0.34 mM for Ile₈-angiotensin II at pH 5.9 and 37°.

A somewhat greater rate response to substrate concentration was obtained on Asp-Arg-β-naphthylamide. However, as a result of the substrate inhibition that occurred at concentrations in excess of 0.5 mM, its rate of hydrolysis at 1 mM was essentially the same as the rates observed on the angiotensin analogues at 1 mM. The Kₘ value for Asp-Arg-β-naphthylamide was estimated to be 0.31 mM. Since the values for Kₘ and V_max resembled those obtained for the analogues of angiotensin II, it became apparent that Asp-Arg-β-naphthylamide might serve as a useful fluorogenic model substrate for estimating the angiotensinase activity of dipeptidyl aminopeptidase I in relatively crude preparations.

Effect of Plasma on Activity of Purified Dipeptidyl Aminopeptidase I—When it was found that rat liver dipeptidyl aminopeptidase I possessed significant angiotensinase activity at pH 7.0 (>85% of the pH 5.5 rate), an attempt was made to determine whether the purified enzyme retained activity when combined with blood, in which competitive substrates or inhibitory components might obscure its activity. For this purpose, extremely small amounts of purified dipeptidyl aminopeptidase I were added to aliquots of rat plasma, and the proportion of detectable activity was assessed fluorometrically. To achieve maximum sensitivity, Gly-Arg-β-naphthylamide was used as the assay substrate of choice since, as previously reported, it possesses the lowest Kₘ (0.11 mM) and the highest turnover number (1,300 s⁻¹) of all the fluorogenic substrates thus far reported for dipeptidyl aminopeptidase I (19). Consequently, it was possible to subject aliquots of the plasma-containing digests to large dilutions before making fluorescence measurements to quantitate the amount of β-naphthylamide arising from the hydrolysis of Gly-Arg-β-naphthylamide. It was thereby possible to dilute out the fluorescence and quenching contributions made by the normal components of plasma. These dilutions also rendered undetectable the contributions of endogenous plasma aminopeptidases also capable of degrading Gly-Arg-β-naphthylamide, but at a relatively low rate compared with the added dipeptidyl aminopeptidase I. As seen in Fig. 2, the greatest rate of Gly-Arg-β-naphthylamide hydrolysis was exhibited by the system containing purified rat liver dipeptidyl aminopeptidase I at about 2 μg per ml in phosphate-buffered saline, pH 7.3, containing the necessary sulfhydryl and chloride activators. This rate was taken to represent 100% of the added activity. When undiluted rat plasma was substituted for 0.9% NaCl solution, comprising 80% of the reaction volume, about 20% of the added activity was still manifest. This amount was considered to represent a significant proportion of the added activity, since only a very small amount of enzyme had been added to preclude the masking of plasma inhibitors, exogenous sulfhydryl and chloride activators had not been added to the plasma, and the plasma had not been protected from the effects of aeration. Under the conditions of the assay, buffered plasma alone (without added enzyme) exhibited an undetectable level of activity on Gly-Arg-β-naphthylamide.

Hydrolysis of Angiotensin II by Extract of Rat Liver Lysosomes—Equilibrium density centrifugation was used to obtain a lysosome-rich fraction from rat liver. The details of the procedure are described under "Materials and Methods." The distribution of activities in the sucrose gradient are shown in Fig. 3. Dipeptidyl aminopeptidase I, an established lysosomal enzyme (21), was located using Asp-Arg-β-naphthylamide as a model of the NH₂ terminus of angiotensin II.

The lysosomal carboxypeptidase of rat liver that inactivates angiotensin II by removing the COOH-terminal phenylalanine was assayed on Z-Pro-Phe at pH 5.5. This activity has been observed in liver and kidney preparations by various workers, and has been reported under such names as acid angiotensinase (22), angiotensinase C (24), prolylcarboxypeptidase (35), and cathpeptic carboxypeptidase C (28). Since these activities are, in all probability, attributable to a common enzyme, the term "prolylcarboxypeptidase," as introduced by Yang and Erdős...
is used herein to refer to the DIPF-sensitive, lysosomal carboxypeptidase activity measured on Z-Pro-Phe at pH 5.5.

As illustrated in Fig. 3, dipeptidyl aminopeptidase I and prolylcarboxypeptidase had a coincident location in the sucrose gradient, with maximum activities occurring in tube 16 (d = 1.21). Assays conducted with Gly-Phe-β-naphthylamide, a more conventional substrate for dipeptidyl aminopeptidase I, also showed maximal activity in tube 16. Characteristically, the activity of dipeptidyl aminopeptidase I in this tube was strongly inhibited by p-chloromercuriphenyl sulfonate, and the activity of prolylcarboxypeptidase by DIPF. As shown in Fig. 3, the activity of dipeptidyl aminopeptidase I contained in tube 16, as measured on Asp-Arg-β-naphthylamide, was about 26 times greater than the activity of prolylcarboxypeptidase measured on Z-Pro-Phe.

An aliquot of the rat liver lysosome fraction contained in tube 16 was incubated with Asn'-angiotensin II in an attempt to elucidate the primary route of angiotensin II degradation at pH 5.5 by the concerted action of all of the lysosomal peptidases. As illustrated in Fig. 4, the time course chromatographic analysis of the products of angiotensin II degradation revealed products, the RF values and order of appearance of which could only be attributed to the action of dipeptidyl aminopeptidase I. Typical of the time course results obtained with purified dipeptidyl aminopeptidase I (Fig. 1), the substrate disappeared with the concomitant appearance of products with RF values corresponding to split products such as Asn-Arg and the hexapeptide intermediate. The Asn-Arg spots near the origin were somewhat distorted by the relatively high level of solute contained in this crude lysosomal digest. The fragments typically produced by the action of purified dipeptidyl aminopeptidase I, together with some free amino acids, were included as standards on the chromatogram. It can be seen that the product corresponding to the hexapeptide intermediate subsequently disappeared with the concomitant appearance of products corresponding to Val-Tyr and Val-His-Pro-Phe. The amount of the tetrapeptide was seen to become more faint with time and in longer time courses (not shown) actually disappeared. This was attributed to the terminal action of prolylcarboxypeptidase, inasmuch as free...
phenylalanine was seen to appear in conjunction with a spot with an Rf value corresponding to a product previously identified as Val-His-Pro (28). The results of this study make it apparent that, at pH 5.5, the angiotensinase activity contributed by dipeptidyl aminopeptidase I in rat liver lysosomes predominates over that of prolylcarboxypeptidase.

**DISCUSSION**

It is evident from this study that purified dipeptidyl aminopeptidase I from rat liver has potent angiotensinase activity on both the bovine (Asp1,Val5) and human (Asp1,Ile6) forms of angiotensin II, as well as on Ciba hypertensin (Asn4,Val7). Such results were anticipated in the light of earlier findings (19) that showed rat liver dipeptidyl aminopeptidase I to have its greatest rates of hydrolysis on dipeptide derivatives with penultimate basic residues, for example Gly Arg β-naphthylamide and Gly-Lys-OMe. Unlike the traditional aminocarboxy aminopeptidases, dipeptidyl aminopeptidase I also hydrolyzed, although at lower rates, unnatural analogues of angiotensin II such as the β-L-Asp1 and α-Asp1 analogues. Such a lack of specificity could have physiological significance since it has been reported (10) that the vasopressor activity of β-L-Asp1-angiotensin II, which is resistant to the angiotensinase activity of plasma (36) and purified aminopeptidase A (37), disappears from the circulation almost as readily as that of its α analogue.

Based on the known minimal structural requirements for the biological activity of angiotensin II (38), a complete loss of pressor activity would be expected to result from the removal of the first dipeptide by dipeptidyl aminopeptidase I. This effect can be compared with a resultant 50% loss of activity for the removal of the NH2-terminal residue only.

Although the L-configuration was not required at the NH2 terminus of angiotensin II to permit hydrolysis by dipeptidyl aminopeptidase I, it was essential that the penultimate residue have the natural configuration. Such a requirement was evident from the inability of the enzyme to hydrolyze Asn4, d-Arg4-angiotensin II, a result that agrees with a similar2 limitation found for β-Tyr1, Lys8,18,β18-corticotropinamide.

Dipeptidyl aminopeptidase I was found to have angiotensinase activity over a wide range of pH. The activity at pH 7.5, measured in terms of the rate of Asp-Arg removal, was about half that at the pH 5.5 optimum. Although dipeptidyl aminopeptidase I is known to have polymerase activity between pH 7.5 and 8.0 on dipeptide amides (39) and β-naphthylamides (19), its action on the various analogues of angiotensin II revealed only split products. In addition, the enzyme was shown to manifest considerable activity in a plasma environment not containing exogenous activators. These results suggest that enzyme released into the blood as a consequence of injury or disease would be free to exert its angiotensinase activity in the general circulation.

Since other studies (8–10) have shown that the rapid inactivation of circulating angiotensin II cannot be attributed to plasma peptidases in nonpathological states, a number of investigators have therefore attempted to identify angiotensinases residing in the tissues. Since the rapid inactivation of unnatural analogues such as β-L-Asp1-angiotensin II precluded a significant contribution by (aminocarboxy) aminopeptidases, several workers have emphasized the importance of a carboxypeptidase attack. For example, Johnson and Ryan (40) reported that rabbit liver extracts showed a carboxypeptidase attack at pH 7.4. Phenyl-

1 J. K. McDonald and S. Ellis, unpublished data.

2 J. K. McDonald and S. Ellis, unpublished data.
both natural and unnatural biologically active analogues of angiotensin II. By means of exocytosis, the digested contents of these heterolysosomes could subsequently be extruded. Such a process would be compatible with the results of studies conducted with radioisotopically-labelled angiotensin II showing that the initial uptake of angiotensin II is soon followed by the appearance of inactive fragments in the circulation.

A possible contribution for the angiotensinase activity of dipeptidyl aminopeptidase I seems particularly probable in various pathological states. de Duve and Beaufay (47) have shown that the rapid release of lysosomal enzymes is one of the earliest detectable changes in the ischemic liver. Since tissue anoxia and acidosis are central components in the pathophysiology of shock (48), and since these conditions, as reported by Janoff et al. (49), favor the labilization and even rupture of tissue lysosomes, it is not surprising that lysosomal hydrolases have been reported in the blood of shocked animals (49, 50). Since our studies show that dipeptidyl aminopeptidase I can exert activity in plasma, it seems probable that it could contribute to the elevated plasma angiotensinase activity and lowered blood pressure seen in dogs rendered hypotensive by hemorrhage or injected endotoxin (51), and in patients suffering various forms of liver disease (52).

The foregoing observations do not prove that dipeptidyl aminopeptidase I makes an important contribution to the inactivation of circulating angiotensin II. However, such a role is favored in the light of observations reported here. On the other hand, the assumed contributions of certain other plasma and tissue peptidases now seem less likely in the light of more recent studies concerned with the biological fate of administered natural and unnatural analogues of angiotensin II.

In summary, dipeptidyl aminopeptidase I exhibits a strong affinity and high rate of hydrolysis on angiotensin II; it catalyzes the hydrolysis and complete inactivation of various natural and unnatural analogues over a wide range of pH; it is, significantly, most abundant in the liver, but is also present in large amounts in other tissues noted for their angiotensinase activity; and it appears to be responsible for the major part of the angiotensinase activity of liver lysosomes. It therefore seems most probable that the angiotensinase activity of dipeptidyl aminopeptidase I plays an important role in the metabolism of angiotensin II under both normal and certain pathological conditions.

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