Ondetti, M. A., Pluščec, J., Sabo, E. F., Williams, N. J., Weaver, E. R., and Kocy, O. (1972a), *Medicina (Buenos Aires) 32, Suppl. 1,* 9.
Ondetti, M. A., Pluščec, J., Weaver, E. R., Williams, N., Sabo, E. F., and Kocy, O. (1972b), *Chem. Biol. Pept., Proc. Am. Pept. Symp., 3rd, 1972,* 525.
Ondetti, M. A., Rubin, B., and Cushman, D. W. (1977), *Science 196,* 441.
Ondetti, M. A., Williams, N. J., Sabo, E. F., Pluščec, J., Weaver, E. R., and Kocy, O. (1971), *Biochemistry 10,* 4033.
Ondetti, M. A., Williams, N. J., Sabo, E. F., Pluščec, J., Weaver, E. R., and Kocy, O. (1972c), *Prog. Pept. Res., Proc. Am. Pept. Symp., 2nd, 1970,* 251.
Oparil, S., Koerner, T., Tregear, G. W., Barnes, B. A., and Haber, E. (1973), *Circ. Res. 32,* 415.
Piquilloud, Y., Reinharz, A., and Roth, M. (1970), *Biochim. Biophys. Acta 206,* 136.
Piszkiewicz, D., Landon, M., and Smith, E. L. (1970), *Biochem. Biophys. Res. Commun. 40,* 1173.
Pluščec, J., Weaver, E. R., Williams, N., Sabo, E. F., Kocy, O., and Ondetti, M. A. (1973), in Peptides 1972, Hanson, H., and Jakubke, H. D., Ed., Amsterdam, Holland, North Holland Publishing Co., p 403.
Quiocho, F. A., and Lipscombe, W. N. (1971), *Adv. Protein Chem. 25,* 1.
Skeggs, L. T., Kahn, J. P., and Shumway, N. P. (1956), *J. Exp. Med. 103,* 295.
Šorm, F., and Pravda, Z. (1951), *Collect. Czech. Chem. Commun. 16,* 423.
Ställberg, G. (1957) *Ark. Kemi 12,* 131.
van Gorkom, M. (1966), *Tetrahedron Lett. 44,* 5433.
Vasilevskii, V. L., Georgiadia, K., and Abdel-Maged, I. M. (1970), *Zh. Org. Khim.,* 244.
Webb, J. L. (1963), Enzyme and Metabolic Inhibitors, Vol. I, New York, N.Y., Academic Press.
Yang, H. Y. T., Erdös, E. G., and Levin, Y. (1971), *J. Pharmacol. Exp. Ther. 177,* 291.

Angiotensin I Converting Enzyme from Human Plasma†

Joseph J. Lanzillo* and Barry L. Fanburg

ABSTRACT: The angiotensin I converting enzyme was purified 101 000-fold to homogeneity from human plasma by a combination of chromatographic and electrophoretic techniques. The enzyme is similar to other angiotensin I converting enzymes. It is an acidic glycoprotein consisting of a single polypeptide chain of molecular weight 140 000 with an isoelectric point of 4.6. The enzyme requires chloride ion for activity and is inhibited by ethylenediaminetetraacetic acid, angiotensin II, bradykinin, bradykinin potentiating factor nonapeptide, and 3-mercapto-2-D-methylpropanoyl-L-proline (SQ-14,225). The purified preparation cleaves bradykinin as well as angiotensin I and hippuryl-L-histidyl-L-leucine. Its specific activity with angiotensin I is 2.4 units per mg and with hippuryl-L-histidyl-L-leucine is 31.4 units per mg.

The renin–angiotensin system has been implicated as a major system in the pathogenesis of hypertension (Skeegs et al., 1976) and hypertensive vascular damage (Giese, 1973) in man. A component of this system, the angiotensin I converting enzyme, converts the relatively inactive decapeptide angiotensin I to the potent pressor octapeptide angiotensin II. Detailed physical properties of this enzyme from human tissue have not been available because of the difficulty of purifying the enzyme. One problem has been the lack of a suitable supply of human tissue. Another has been that the most readily available human tissue, outdated human plasma, contains only a small quantity of enzyme. To use human plasma as the starting material, a high-resolution protocol had to be developed to enable purification of the enzyme in a reasonable number of steps.

In this paper, we describe a method for purifying the enzyme 101 000-fold to homogeneity from human plasma. This protocol should be readily adaptable to purifying the enzyme from any animal tissue. Some properties of the human enzyme are described. A purified enzyme from a human source will allow immunologic studies on human material not otherwise possible due to species specificity of angiotensin I converting enzyme (Conroy et al., 1976; Polsky-Cynkin and Fanburg, 1977).

Materials and Methods

Materials. Outdated human plasma was obtained from the Blood Bank of the New England Medical Center Hospital. Hip-His-Leu[1] was custom synthesized by Vega-Fox Biochemicals. Angiotensin I was from Beckman Instruments, Inc.; BPP_{9a} and 3-mercapto-2-D-methylpropanoyl-L-proline (SQ-14,225) were gifts from Dr. D. W. Cushman, Squibb Institute for Medical Research. Bradykinin was from Schwarz/Mann. Whatman DE 42 microgranular DEAE-cellulose was from Reeve-Angel. Bio-Gel HTP hydroxylapatite was from Bio-Rad Labs. Sephadex G-200 was obtained from Pharmacia Fine Chemicals, Inc. All other chemicals were reagent grade.

Angiotensin I Converting Enzyme Activity. The assay used for monitoring column and electrophoretic procedures was

† From the New England Medical Center Hospital and the Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts 02111. *Received June 28, 1977.* This investigation was supported by Grants HL 06824 and HL 14456 from the National Institutes of Health, U.S. Public Health Service.

* Address all correspondence to this author at the New England Medical Center Hospital.

[1] Abbreviations used are: Hip-His-Leu, hippuryl-L-histidyl-L-leucine; BPP_{9a}, bradykinin potentiating factor nonapeptide; $NaDodSO_4$, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetate; DEAE, diethylaminoethyl; TLC, thin-layer chromatography; Bis, *N,N'*-methylenebis(acrylamide); Tris, tris(hydroxymethyl)aminomethane.

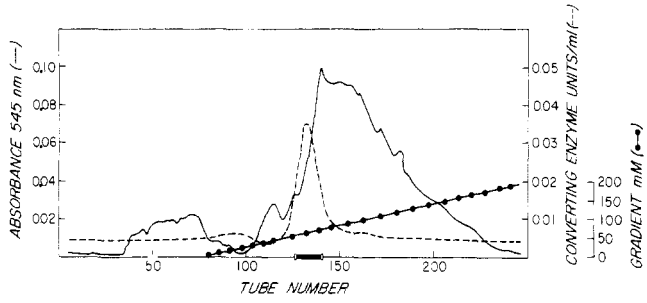

FIGURE 1: DEAE-cellulose chromatography of dialyzed human plasma. Tubes 126-140 were pooled for further purification. The 0-200 mM KCl gradient is indicated (●). Protein was determined by the biuret method (O'Brien et al., 1968).

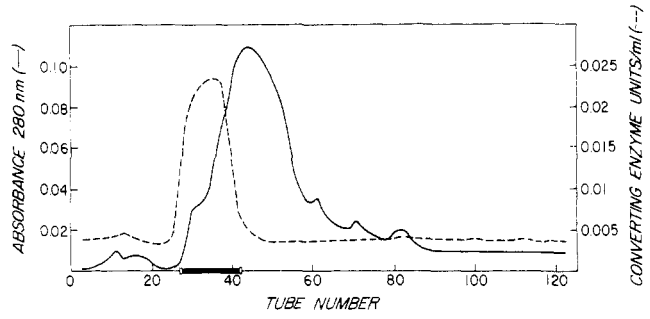

FIGURE 2: Hydroxylapatite chromatography of the pooled fractions from DEAE-cellulose with angiotensin I converting enzyme activity. Tubes 26-41 were pooled for further purification.

similar to the spectrophotometric method of Cushman and Cheung as previously described (Lanzillo and Fanburg, 1974). The final concentrations of the constituents of the assay medium in a 0.5-mL volume were as follows: 5 mM Hip-His-Leu, 300 mM NaCl, 100 mM potassium phosphate (pH 8.3), and angiotensin I converting enzyme. Incubation was carried out for 2 h at 37 °C.

A second radiometric assay used to determine the specific activity with 1.9 mM angiotensin I as substrate was performed as previously described (Lanzillo and Fanburg, 1976a).

Angiotensin I converting enzyme activity with bradykinin as substrate was determined qualitatively with and without chloride ion as follows. A 0.3-mL sample containing 3 mM bradykinin, 155 mM potassium phosphate, 500 mM NaCl (or no NaCl), and angiotensin I converting enzyme was incubated at 37 °C for 30 min. The reaction was stopped with 5 μL of concentrated HCl. Samples (10 μL) were applied to Eastman plastic-backed silica gel TLC plates. Control samples of bradykinin and the dipeptides Phe-Arg, Ser-Pro, and Gly-Phe at 1 mg/mL were also applied to the plate at 3-cm intervals. The plate was subjected to electrophoresis and peptides were visualized with Fluram as previously described (Lanzillo and Fanburg, 1976a).

Inhibition Studies. The Hip-His-Leu assay in the presence of inhibitors was performed as described above using either 10^{-3} M angiotensin II, 10^{-4} M bradykinin, 10^{-6} M BPP_{9a}, or 10^{-7} M SQ-14,225 as inhibitor. The assay mixture was prepared by adding both substrate and inhibitor prior to addition of enzyme. Other inhibition assays were performed either with 10^{-4} M EDTA or without chloride ion in the standard Hip-His-Leu incubation mixture.

Units. One unit of angiotensin I converting enzyme activity is defined as the amount that hydrolyzes 1 μmol of either angiotensin I or Hip-His-Leu per min under standard assay conditions. The specific activity is expressed as the number of units per mg of protein. Protein was determined by the Lowry method (Lowry et al., 1951).

Enzyme Purification. DEAE-cellulose. One liter of outdated human plasma was dialyzed against 12 L of 15 mM K_2HPO_4 (pH 8.3) for 5 days at 5 °C with a change of buffer daily. It was then pumped at 300 mL/h onto a 5 × 100 cm column of DEAE-cellulose equilibrated with the same buffer.[2] The column was developed at 200 mL/h with 4 L of a 0-200 mM linear gradient of KCl in phosphate buffer. Fractions (25 mL) were collected. Enzymatic activity was eluted with a gradient strength of 45-65 mM KCl.

Hydroxylapatite. The enzymatically active fractions from the DEAE-cellulose column were pooled and dialyzed overnight at 5 °C against 12 L of 12 mM K_2HPO_4 (pH 6.8). The pool was then pumped at 100 mL/h onto a 5 × 60 cm column of hydroxylapatite previously equilibrated with 12 mM K_2HPO_4 (pH 6.8). The column was washed with equilibrating buffer and 25-mL fractions were collected. The unbound enzymatically active fractions were pooled.

Sephadex G-200. Two batches of enzyme from the hydroxylapatite column were pooled and concentrated in an Amicon Model 202 cell with a PM-30 membrane to a final volume of 8 mL. This concentrate was pumped at 15 mL/h onto a 5 × 100 cm column of Sephadex G-200 equilibrated with 0.1 M Tris-HCl (pH 8.5). The column was developed with equilibrating buffer and 10-mL fractions were collected. The enzyme was eluted between 780 and 920 mL with an elution volume (V_e) of 850 mL. The column void volume (V_0), determined with blue dextran, was 730 mL; thus, V_e/V_0 was 1.16.

Preparative Polyacrylamide Gel Electrophoresis. The Sephadex G-200 pool was concentrated in an Amicon Model 52 cell with an XM-50 membrane to a final volume of 6 mL. This sample was subjected to electrophoresis in an ISCO Colura Ultraphor apparatus cooled to 9 °C by a refrigerated circulating system. A 0.7 × 14 × 7 cm slab gel was cast in 0.375 M Tris-HCl (pH 8.5[3]), and allowed to gel for 3 h at 9 °C. Total monomer (acrylamide + Bis) was 9% and cross-linker (Bis) was 2.3%. The electrode buffer was 0.0083 M Tris-0.38 M glycine (pH 8.5). The 6-mL sample, mixed with 1 mL of glycerin and 10 μL of bromophenol blue, was applied by syringe under the electrode buffer. Elution buffer was 0.3 M Tris-HCl (pH 8.5), flowing at 30 mL/h while the counter-elution buffer was 1.5 M Tris-HCl (pH 8.5), flowing at 60 mL/h. The power setting was 9 W constant power for 20 min, then 50 W constant power for 41 h. Fractions of 10 mL were collected at 25 °C. Enzymatically active tubes no. 61-68 eluted after 20-23 h were pooled, dialyzed overnight at 5 °C against 6 L of 0.02 M K_2HPO_4 (pH 7.5), and concentrated to a final volume of 10 mL.

Polyacrylamide Gel Electrophoresis and $NaDodSO_4$-Polyacrylamide Gel Electrophoresis. Polyacrylamide gel electrophoresis was performed in 9% polyacrylamide gels cast in 0.375 M Tris-sulfuric acid (pH 9.0), and $NaDodSO_4$ electrophoresis was performed in 4-8% polyacrylamide gels containing sodium dodecyl sulfate as previously described (Lanzillo and Fanburg, 1976b). Proteins were stained for sugar moieties using Schiff reagent. Noncarbohydrate proteins were detected with Coomassie brilliant blue R-250 (Maurer and Allen, 1972).

Isoelectric Focusing. Isoelectric focusing was performed in

[2] All column procedures were carried out at 5 °C in a cold room.

[3] The pH of Tris buffers was adjusted to 8.5 at 9 °C.

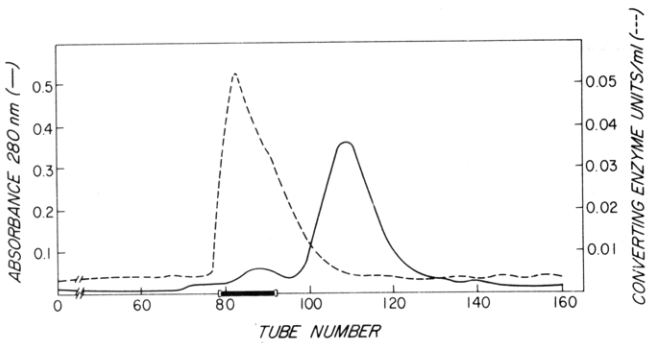

FIGURE 3: Sephadex G-200 chromatography of the pooled and concentrated fractions from hydroxylapatite with angiotensin I converting enzyme. Tubes 79–92 were pooled for further purification.

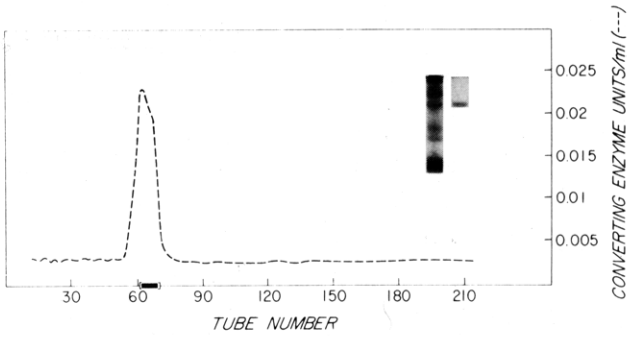

FIGURE 4: Angiotensin I converting enzyme activity eluted from the preparative gel slab. The distribution of protein and the elution order from the preparative gel slab are simulated by analytical polyacrylamide gel electrophoresis: (left gel) 40 μg of Sephadex G-200 pool before preparative gel electrophoresis; (right gel) 6 μg of pooled samples from preparative gel electrophoresis with angiotensin I converting enzyme activity. The 9% monomer gels were prepared as previously described (Lanzillo and Fanburg, 1976b). The power setting was 1 mA/tube for 15 min and then 3 mA/tube for 1.5 h.

polyacrylamide gel [7% T, 3.85% C (Bis), containing 2% LKB Ampholine, pH range 3.5–9.5] in a Canalco apparatus with 20 mM sodium hydroxide as catholyte (top), and 10 mM phosphoric acid as anolyte (bottom) at 25 °C. Cylindrical gels measuring 5 × 60 mm were photopolymerized with riboflavin for 2 h at 25 °C. The gels were stored at 5 °C overnight before use. Samples containing 25% glycerin and 2% Ampholine were applied to the tops of the gels and overlaid with a solution of 5% glycerin and 2% Ampholine. Sperm whale myoglobin, horse heart myoglobin, and carbonic anhydrase were used as marker proteins. Focusing was carried out at 0.5 mA/tube for 1 h, and then 100 V constant voltage for 18 h. Gels were removed from the running tubes, fixed, and stained in Coomassie brilliant blue G-250 with urea according to Vesterberg (Vesterberg et al., 1977). The pH profile was obtained by slicing a duplicate gel with a Canalco lateral gel slicer, suspending each slice in 1 mL of 20 mM potassium chloride, and recording the pH at 25 °C.

Results

Purification of Angiotensin I Converting Enzyme from Human Plasma. The angiotensin I converting enzyme was purified 101 000-fold from human plasma by the sequential use of DEAE-cellulose (Figure 1), hydroxylapaptite (Figure 2), Sephadex G-200 (Figure 3), and preparative polyacrylamide gel electrophoresis (Figure 4). Results of each step are shown by $NaDodSO_4$–polyacrylamide gel electrophoresis in Figure 5. A summary of the procedure is presented in Table I.

Physical and Catalytic Properties. The final specific activity with angiotensin I as substrate is 2.4 units per mg and with Hip-His-Leu as substrate is 31.4 units per mg. The enzyme has a molecular weight of 140 000 by $NaDodSO_4$–gel electrophoresis and consists of a single polypeptide chain containing carbohydrate moieties, as determined by a positive Schiff stained band on gel electrophoresis. It is an acidic protein with an isoelectric point of 4.6.

Criteria for Purity. The preparation from preparative gel electrophoresis appeared homogeneous in three systems. Only a single band was observed on gel electrophoresis (Figure 4), $NaDodSO_4$–gel electrophoresis (Figure 5), and by isoelectric focusing (Figure 6).

Effect of Inhibitors on Enzymatic Activity. Complete inhibition of angiotensin I converting enzyme was observed by the following known angiotensin I converting enzyme inhibitors at selected concentrations: 10^{-3} M angiotensin II, 10^{-4} M bradykinin, 10^{-6} M BPP_{9a}, and 10^{-4} M EDTA. The recently synthesized inhibitor SQ-14,225 was shown to be the most powerful inhibitor, requiring only 10^{-7} M to completely inhibit

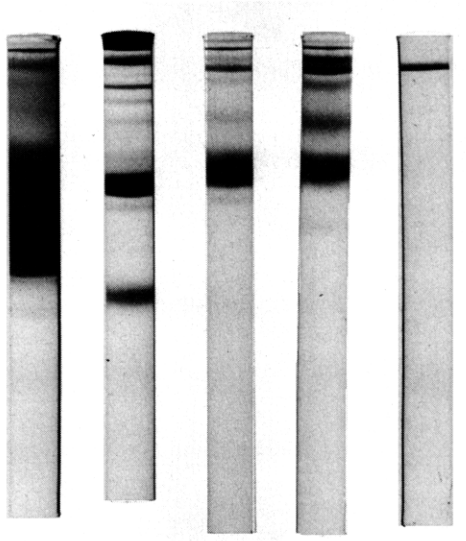

FIGURE 5: $NaDodSO_4$–polyacrylamide gel electrophoresis, from left to right: 300 μg of human plasma, 125 μg of DEAE-cellulose pool, 35 μg of hydroxylapatite pool, 40 μg of Sephadex G-200 pool, 4 μg of preparative gel electrophoresis pool. The 7% monomer gels were prepared as previously described (Lanzillo and Fanburg, 1976b). The power setting was 8 mA/tube for 3.5 h.

enzymatic activity. There was no detectable cleavage of either angiotensin I or Hip-His-Leu in the absence of chloride ion.

Bradykinase Activity. With bradykinin as substrate, carboxy-terminal dipeptides were cleaved both with and without chloride ion.

Discussion

The success of our purification scheme was dependent on careful control of several parameters. For example, the large increase in specific activity obtained on hydroxylapatite was observed only under nonbinding conditions at pH 6.8 and 12 mM K_2HPO_4. If a pH higher than 6.8 or a buffer concentration of 15 mM or greater was used, significant amounts of contaminating protein remained unbound along with the enzyme. When buffer concentrations below 10 mM were used, the enzyme was adsorbed by the hydroxylapatite and required a phosphate buffer gradient for elution. However, significant amounts of contaminating protein were collected with the enzyme during elution.

TABLE I: Purification of Angiotensin I Converting Enzyme from Human Plasma.[a]

Fraction	Total protein (mg)	Total act. (units)	Sp act. (Hip-His-Leu) (units/mg)	Purification (-fold)	Act. recovered (%)
A. Dialyzed human plasma	128000	40.2	0.00031	1	100
B. DEAE-cellulose	17900	28	0.0016	5.2	70
C. Hydroxylapatite	63	19	0.30	970	47
D. Sephadex G-200	3.2	7.8	2.4	7860	19
E. Prep-polyacrylamide gel electrophoresis	0.080	2.5	31.4	101000	6.2

[a] Results are based on 2000 mL of expired plasma. Enzyme activity in expired plasma approximates that of fresh serum from normal subjects (Fanburg et al., 1976).

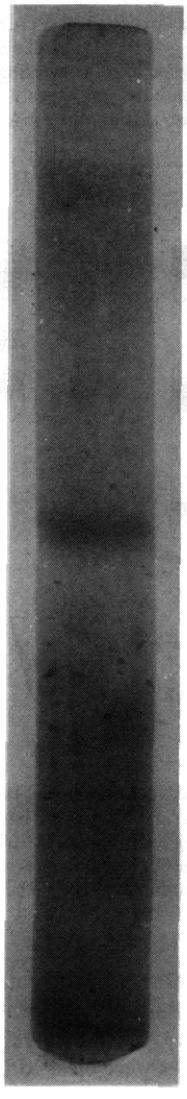

FIGURE 6: Isoelectric focusing of 4 μg of angiotensin I converting enzyme from the preparative gel electrophoresis pool.

The buffer used with Sephadex G-200 was determined by the requirements of the preparative polyacrylamide gel electrophoresis sample. It is important to adjust the Tris buffer pH to 8.5 at the running temperature for preparative gel electrophoresis (i.e. 9 °C). This is because Tris has a large temperature coefficient. As the temperature of a Tris buffer solution is lowered, the pH increases approximately 0.03 pH unit per °C. This Tris buffer pH dependence on temperature is most critical with the preparative gel electrophoresis system. When the original running conditions of Maurer and Allen were used, the Tris buffer pH was adjusted to 8.5 at 25 °C (Maurer and Allen, 1972). This resulted in an actual running pH of 9.4 at 9 °C with subsequent loss of enzymatic activity. The human plasma angiotensin I converting enzyme is most stable at a pH range of 8.0–8.8 and loses activity at pH values above 8.8. It later was found by trial and error that the best compromise between preservation of enzymatic activity and protein migration through the gel was obtained with pH 8.5 at 9 °C. This results in an actual running pH of 8.8 in the gel which is probably the result of factors other than the temperature coefficient of Tris. At this pH there is decreased mobility of high molecular weight contaminants, and the resolution is superior to that obtained at higher pH values.

Even though the enzyme is bound to the endothelial cell membrane in tissues (Caldwell et al., 1976; Ryan et al., 1976), it can be solubilized readily with detergent and remains in solution when detergent is removed (Lanzillo and Fanburg, 1974). Therefore, the purification technique reported here should be applicable to most, if not all, purifications from animal tissue. Unlike the purification scheme recently reported for rabbit serum angiotensin I converting enzyme (Das et al., 1977), which was based on the use of an antibody affinity column, this technique does not require the prior isolation of pure antigen or cross-reacting antigen to generate antibodies.

The only other attempt to purify angiotensin I converting enzyme from human tissue utilized human lung as starting material (Fitz and Overturf, 1972). Whether or not this technique yielded a homogeneous preparation is open to question. The molecular weight reported for the enzyme was 480 000 as determined by Sephadex G-200 gel filtration. We have shown previously that this technique gives anomalous molecular weight values with angiotensin I converting enzyme (Lanzillo and Fanburg, 1976b). However, if that molecular weight is accurate, it suggests that a membrane fragment and not the pure enzyme was isolated. No criteria for homogeneity or support of the molecular weight value, such as with Na-DodSO$_4$–gel electrophoresis or sedimentation data, were presented by Fitz and Overturf.

The specific activity of the human plasma angiotensin I converting enzyme with angiotensin I as substrate is 2.4 units per mg and with Hip-His-Leu as substrate is 31.4 units per mg. The physical properties of the human plasma angiotensin I converting enzyme such as quaternary structure, glycoprotein nature, acidic isoelectric point, and molecular weight are similar to those reported for the enzyme from other species (Lanzillo and Fanburg, 1976b; Nakajima et al., 1973; Oshima et al., 1974; Soffer et al., 1974). The behavior of selected inhibitors on human plasma enzymatic activity is similar to that on angiotensin I converting enzyme from other sources. The enzyme was inhibited by angiotensin II, bradykinin, BPP$_{9a}$, EDTA, and the absence of chloride ion. The recently synthesized inhibitor SQ-14,225 was the most powerful inhibitor

tested, requiring only 10^{-7} M to completely inhibit human plasma angiotensin I converting enzyme activity. This supports the observation of Ondetti et al. (1977) using SQ-14,225 in rats and with the rabbit lung angiotensin I converting enzyme. Our qualitative results show that human plasma angiotensin I converting enzyme also is capable of cleaving bradykinin either with or without chloride in the assay system.

Thus, the angiotensin I converting enzyme from human plasma appears quite similar in physical properties to those enzymes isolated from other species. The procedure for purification of the angiotensin I converting enzyme from human plasma offers a convenient route for isolation and subsequent study of this enzyme.

Acknowledgments

We thank Dr. E. Berkman for the supply of outdated human plasma, and Dr. D. W. Cushman for the generous amounts of SQ-14,225 and BPP_{9a}. The expert technical assistance of Dana MacNamee is greatly appreciated.

References

Caldwell, P. R. B., Seegal, B. C., Hsu, K. C., Das, M., and Soffer, R. L. (1976), *Science 191,* 1050.

Conroy, J. M., Hoffman, H., Kirk, E. S., Hirzel, H. O., Sonnenblick, E. H., and Soffer, R. L. (1976), *J. Biol. Chem. 251,* 4828.

Das, M., Hartley, J. L., and Soffer, R. L. (1977), *J. Biol. Chem. 252,* 1316.

Fanburg, B. L., Schoenberger, M. D., Bachus, B., and Snider, G. L. (1976), *Am. Rev. Respir. Dis. 114,* 525.

Fitz, A., and Overturf, M. (1972), *J. Biol. Chem. 247,* 581.

Giese, J. (1973), *Am. J. Med. 55,* 315.

Lanzillo, J. J., and Fanburg, B. L. (1974), *J. Biol. Chem. 249,* 2312.

Lanzillo, J. J., and Fanburg, B. L. (1976a), *Biochim. Biophys. Acta 445,* 161.

Lanzillo, J. J., and Fanburg, B. L. (1976b), *Biochim. Biophys. Acta 439,* 125.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193,* 265.

Maurer, H. R., and Allen, R. C. (1972), *Z. Klin. Chem. Klin. Biochem. 10,* 220.

Nakajima, T., Oshima, G., Yeh, H. S. J., Igic, R., and Erdös, E. G. (1973), *Biochim. Biophys. Acta 315,* 430.

O'Brien, D., Ibbot, F. A., and Rodgerson, D. O. (1968), in Laboratory Manual of Pediatric Microbiochemical Techniques, New York, N.Y., Harper and Row, pp 279-281.

Ondetti, M. A., Rubin, B., and Cushman, D. W. (1977), *Science 196,* 441.

Oshima, G., Gecse, A., and Erdös, E. G. (1974), *Biochim. Biophys. Acta 350,* 26.

Polsky-Cynkin, R., and Fanburg, B. L. (1977), *Am. Rev. Respir. Dis. 115,* 364.

Ryan, V. S., Ryan, J. W., Whitaker, C., and Chiu, A. (1976), *Tissue Cell 8,* 125.

Skeggs, L. T., Dorer, F. E., Kahn, J. R., Lentz, K. E., and Levine, M. (1976), *Am. J. Med. 60,* 737.

Soffer, R. L., Reza, R., and Caldwell, P. R. B. (1974), *Proc. Natl. Acad. Sci. U.S.A. 71,* 1720.

Vesterberg, O., Hansen, L., and Sjösten, A. (1977), *Biochim. Biophys. Acta 491,* 160.

Rabbit Liver Transglutaminase: Physical, Chemical, and Catalytic Properties†

Takashi Abe,‡ Soo Il Chung, Richard P. DiAugustine, and J. E. Folk*

ABSTRACT: Transglutaminase (R-glutaminyl-peptide:amine α-glutamyl-yltransferase [EC 2.3.2.13]) has been purified to apparent homogeneity from extracts of rabbit liver. The enzyme is a single polypeptide chain of approximately 80 000 molecular weight containing one catalytic site per molecule. That the isolated enzyme is the rabbit counterpart of the well-characterized guinea pig liver transglutaminase is evidenced by the similarities in their amino acid compositions and in their enzymic activities toward several substrates, together with the fact that the isolated rabbit enzyme is immunologically distinct from both rabbit plasma and rabbit platelet blood coagulation factor XIII. A striking difference between the catalytic activities of the rabbit and guinea pig enzymes is the low activity of rabbit transglutaminase for hydroxylamine incorporation into benzyloxycarbonyl-L-glutaminylglycine, a reaction for which the guinea pig enzyme shows a high reactivity. This finding reveals the cause of error in an earlier report (Tyler, H. M., and Laki, K. (1967) *Biochemistry 6,* 3259) that rabbit liver contains little, if any, of the enzyme. Preparation of, and analytical data on, several glutamine-containing peptide derivatives used in this study are reported here.

Early investigators reported enzymic activity responsible for incorporation of amines into proteins in the livers of a number of mammals (Sarkar et al., 1957) as well as in other tissues (Clarke et al., 1959; review: Waelsch, 1962). Species tested included guinea pig, rat, mouse, rabbit, and calf. Because guinea pig liver was particularly rich in this transglutaminase activity (Clarke et al., 1959; Wajda et al., 1963), subsequent studies centered on the enzyme from this species, and little attention was given to the transglutaminases from other mammals.

A combination of specific substrates, chromatographic techniques, and antiserum to the purified liver enzyme was

† From the Section on Enzyme Chemistry, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20014 (S.I.C. and J.E.F.), and The Laboratory of Pharmacology, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina 27709 (R.P.D.). *Received July 11, 1977.* A preliminary account of portions of this work has been given (Abe and DiAugustine, 1976).

‡ Present address: The Institute of Physical and Chemical Research, Wako-Shi, Saitama, 351, Japan.