

AMINO ACID COMPOSITION AND ELECTROPHORETIC PROPERTIES OF HYPERTENSIN I

LEONARD T. SKEGGS, JR., PH.D., WALTON H. MARSH, PH.D.,
JOSEPH R. KAHN, M.D., AND NORMAN P. SHUMWAY, M.D.

*(From the Department of Medicine and Surgery, Veterans Administration
Hospital, and the Department of Pathology, Western Reserve
University, Cleveland)*

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The pressor substance hypertensin, which has been demonstrated in the blood of many patients with hypertensive cardiovascular disease (1), can be divided into two active fractions (2). One of these compounds, hypertensin I, is the initial product of the action of the renal enzyme renin upon its plasma substrate; the other, hypertensin II, is produced from hypertensin I by the action of a chloride-activated enzyme contained in the plasma. Hypertensin I has recently been obtained in a highly purified form. The method of preparation has been described in detail (3). The final product was obtained by countercurrent distribution and satisfied the criteria outlined by Craig for a single component (4). It has a specific pressor activity of 7050 Goldblatt units (5) per mg. of N or 1125 units per mg. of solid and a nitrogen content of 16 per cent. Preliminary two dimensional chromatograms of the acid hydrolysates indicated the presence of nine different amino acids.

The present paper gives the results of an amino acid analysis using the ion exchange column methods of Moore and Stein (6), an electrophoretic determination of its isoelectric point, and an example of its electrophoretic behavior in a starch medium.

EXPERIMENTAL

Amino Acid Analysis.—

A sample containing 4.85 mg. of solids or 0.776 mg. of N with 5460 Goldblatt units of activity was hydrolyzed in 2 ml. of 6 N HCl in a sealed tube at 105°C. for 20.5 hours. The hydrolyzed product which had little or no color was applied to a 100 cm. dowex 50 × 8 ion exchange column. The procedure of Moore and Stein for the development of the column was followed in exact detail (6). The effluent pattern of amino acids obtained is shown in Fig. 1. Since basic amino acids appeared it was necessary to prepare a second hydrolysate for application to the 15 cm. column. For this purpose a 2.45 mg. sample of hypertensin I, containing 0.392 mg. N₂ and 2760 units was hydrolyzed in similar fashion. The effluent pattern obtained from this chromatogram is illustrated in Fig. 2.

The quantitative results obtained by integration of the areas under the bands are given in Table I. The recovery of known residues amounted to

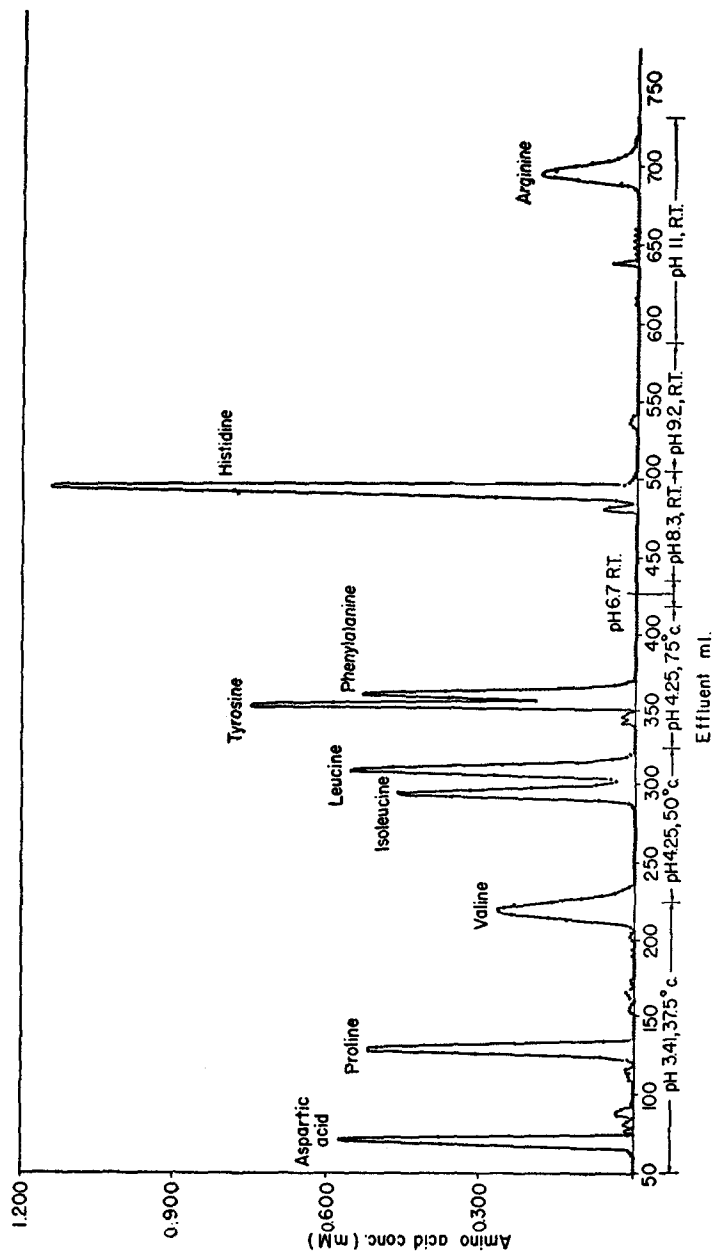


Fig. 1. Effluent patterns from a 100 cm. ion exchange column.

TABLE I
The Results of Amino Acid Analysis

	Nitrogen as per cent of total nitrogen	Amino acid residue per 100 gm. of sample	Molar proportions
Aspartic.....	6.2	9.4	1.03
Proline.....	6.2	8.1	1.03
Valine.....	6.2	8.2	1.03
Isoleucine.....	5.7	8.5	0.95
Leucine.....	6.5	9.7	1.09
Tyrosine.....	5.7	11.8	0.96
Phenylalanine.....	6.2	11.8	1.04
Histidine.....	33.4	19.7	1.87
Arginine.....	23.6	11.7	0.99
Total.....	99.7	98.9	

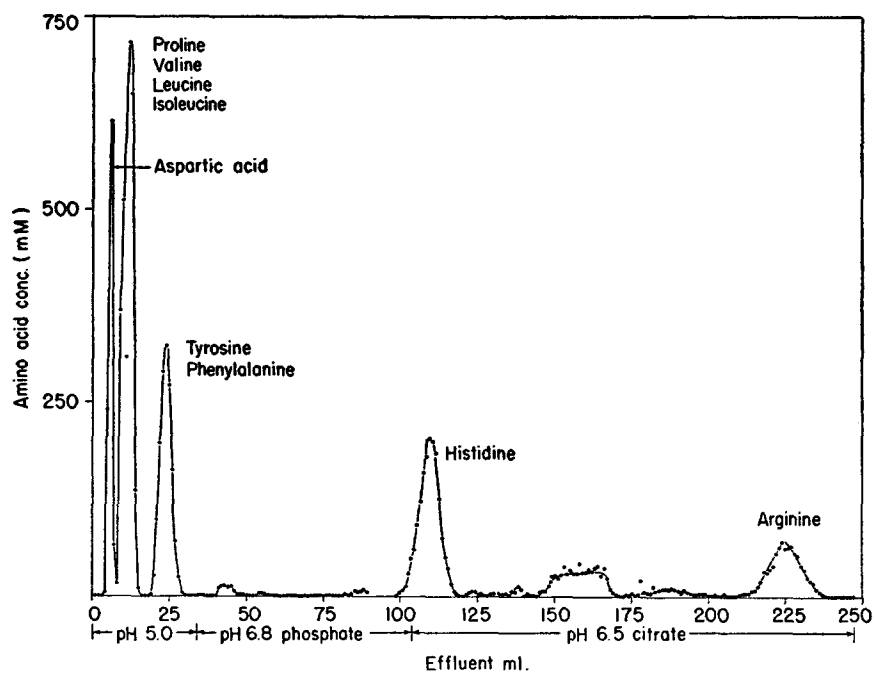


FIG. 2. Effluent patterns from a 15 cm. ion exchange column.

98.9 per cent of the weight of sample taken while the recovery of nitrogen was 99.7 per cent. The small non-symmetrical band appearing between histidine and arginine in Fig. 2 was thought to be ammonia which had its origin in the buffers and was therefore disregarded.

Isoelectric Point.—The paper electrophoretic methods of Kunkel, Taylor, and du Vigneaud (7) were used for the determination of the isoelectric point.

The apparatus available utilized paper lying horizontally on a siliconized plate. The temperature was controlled at 2°C. and the paper was enclosed to minimize evaporation. Samples

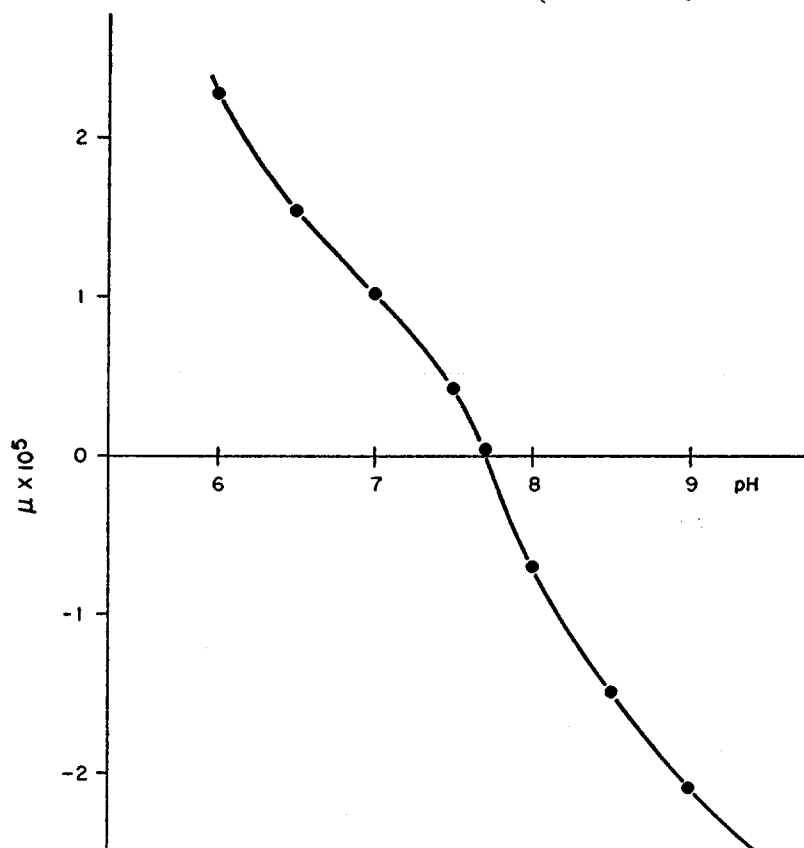


FIG. 3. Mobility determinations of hypertensin I at different pH values.

of 22 μg . were applied to the center of 50 cm. strips of Whatman 4 filter paper dampened with the appropriate buffer. The monovalent buffers, cacodylate, and barbiturate, with an ionic strength of 0.1 were prepared by titration of the sodium salts to the desired pH with hydrochloric acid. Electrophoresis was conducted for 3.5 hours under the influence of 300 volts. At the conclusion of the run the location of the material was revealed by means of Durrum's stain (8). Dextran was used as an indicator of the true point of origin. Round homogeneous spots were obtained in all cases. The mobilities obtained at eight different pH values between 5 and 9 are plotted in Fig. 3. Each point represents at least two separate determinations. The results reveal that the isoelectric point of hypertensin I is approximately 7.7.

The location of the physiologically active material was determined in several experiments at differing pH values by assay (9) of eluates of segments of

a second paper strip run in parallel to the one treated with Durrum's stain. The active material exhibited the same mobility as the stained spot in all cases. In four experiments the amount recovered averaged 55 per cent.

Electrophoresis.—Because of the small amount of sample which was available a starch-supporting pad was used for the electrophoretic medium (7) instead of the conventional Tiselius apparatus.

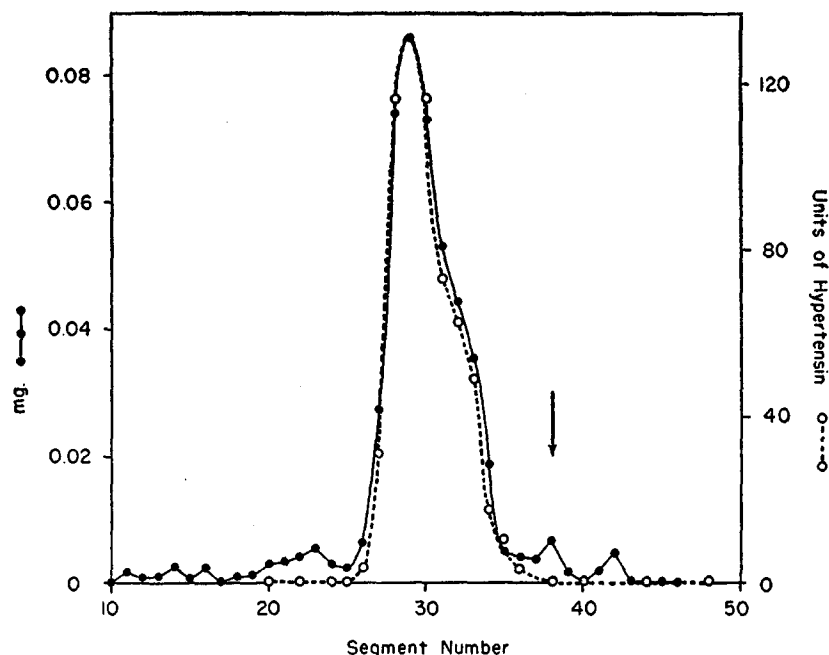


FIG. 4. Correlation between hypertensin activity and Folin tyrosine color in electrophoretic fractions.

Potato starch was washed in barbiturate buffer (pH 9.3, $\Gamma/20.05$) and then was formed into a pad $33 \times 7.5 \times 0.5$ cm. supported on an enclosed plate with the temperature controlled at 2°C . A sample of hypertensin I weighing 0.74 mg. and containing 833 Goldblatt units of pressor activity was fractionated for 12.5 hours under a potential difference of 300 volts. At the end of this period the pad was cut into 66 strips 0.5 cm. in width and eluted for determination of protein content and pressor activity. The quantity of protein was expressed in equivalents of horse serum giving equal color after reaction with a modified Folin phenol reagent (10).

Two experiments gave similar results. As can be seen in Fig. 4, which shows the details of one experiment, the pressor activity and the amount of protein migrated together. In this figure the arrow indicates the true origin corrected for electroosmotic flow. The recovery of activity amounted to 78 per cent of that contained in the original sample.

DISCUSSION

The preparation of hypertensin I used in this study was obtained by means of countercurrent distribution and was demonstrated to be a single component by this method and by electrophoresis in a starch medium at a pH of 9.3. This was also the case when the electrophoresis was performed at a pH of 4.2 in an acetate buffer for a period of 12 hours, and also in a second experiment, for a period of 24 hours. Further evidence of the essential purity of the product is gained from the amino acid analysis. The nine different amino acids are in approximately whole number molar proportion and the good recovery of nitrogen would seem to preclude the presence of other amino acids. However the absence of tryptophan and of ammonia from the original material must be verified by other methods.

We have therefore been unable to confirm the recent findings of Kuether and Haney (11) who described the preparation of an unstable hypertensin containing 14 amino acids with a specific pressor activity ten times that of the present product.

SUMMARY

A preparation of hypertensin I was purified by countercurrent distribution and was shown to migrate as a single component in starch blocks at pH 9.3 and 4.2. It had an isoelectric point of 7.7. Quantitative analysis by ion exchange column chromatography showed eight amino acids in approximately unimolar proportion: aspartic, proline, valine, isoleucine, leucine, tyrosine, phenylalanine, and arginine. There were in addition two moles of histidine.

BIBLIOGRAPHY

1. Kahn, J. R., Skeggs, L. T., Shumway, N. P., and Wisenbaugh, P. E., *J. Exp. Med.*, 1952, **95**, 523.
2. Skeggs, L. T., Marsh, W. H., Kahn, J. R., and Shumway, N. P., *J. Exp. Med.*, 1954, **99**, 275.
3. Skeggs, L. T., Marsh, W. H., Kahn, J. R., and Shumway, N. P., *J. Exp. Med.*, 1954, **100**, 363.
4. Craig, L. C., *Methods Med. Research*, 1952, **5**, 3.
5. Goldblatt, H., *The Renal Origin of Hypertension*, Springfield, Illinois, Charles C. Thomas, 1948.
6. Moore, S., and Stein, W. H., *J. Biol. Chem.*, 1951, **192**, 663.
7. Kunkel, H. G., Taylor, S. P., and du Vigneaud, V., *J. Biol. Chem.*, 1953, **200**, 559.
8. Durrum, E. L., *J. Am. Chem. Soc.*, 1950, **72**, 2943.
9. Skeggs, L. T., Kahn, J. R., Marsh, W. H., *Lab. Inv.*, 1953, **2**, 109.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, 1951, **193**, 265.
11. Kuether, C. A., and Haney, M. E., *Science*, 1955, **121**, 65.