CHROM. 6152

Amino acid analysis of elastin — a rapid method

Work in this laboratory has involved the routine amino acid analyses of fractions derived from elastin, and as a result, a method capable of detecting desmosine, isodesmosine, α-amino adipic acid and hydroxyproline in addition to the more usual amino acids was desirable. The analysis of desmosine and isodesmosine has been described[1-3], but involves a separate run, so a system allowing their separation on the same run as the acidic and neutral amino acids was developed.

Experimental

A Beckman amino acid analyser system was used with a column (69 × 0.9 cm) packed to a height of 52 cm with Beckman Custom research resin Type AA 15. The buffers used, 0.2 *N* sodium citrate, pH 3.25, and 0.38 *N* sodium citrate, pH 4.26, were prepared according to the Beckman manual. Flow rates were 70 ml/h for the buffer and 35 ml/h for the ninhydrin.

The column was equilibrated with 0.2 *N* sodium citrate buffer and eluted with this for 2 h at a column temperature of 31°. This permits the separation of hydroxyproline and α-amino adipic acid. The buffer change to 0.38 *N* sodium citrate was accompanied by a temperature change to 50°.

The system was calibrated with the amino acid calibration mixture from Beckman Instrument Inc., hydroxyproline from Nutritional Biochemicals Corporation, α-amino adipic acid (DL-2-aminohexanedioic acid) from the J. T. Baker Chemical Co. and desmosine and isodesmosine prepared by the method of ANWAR[1]

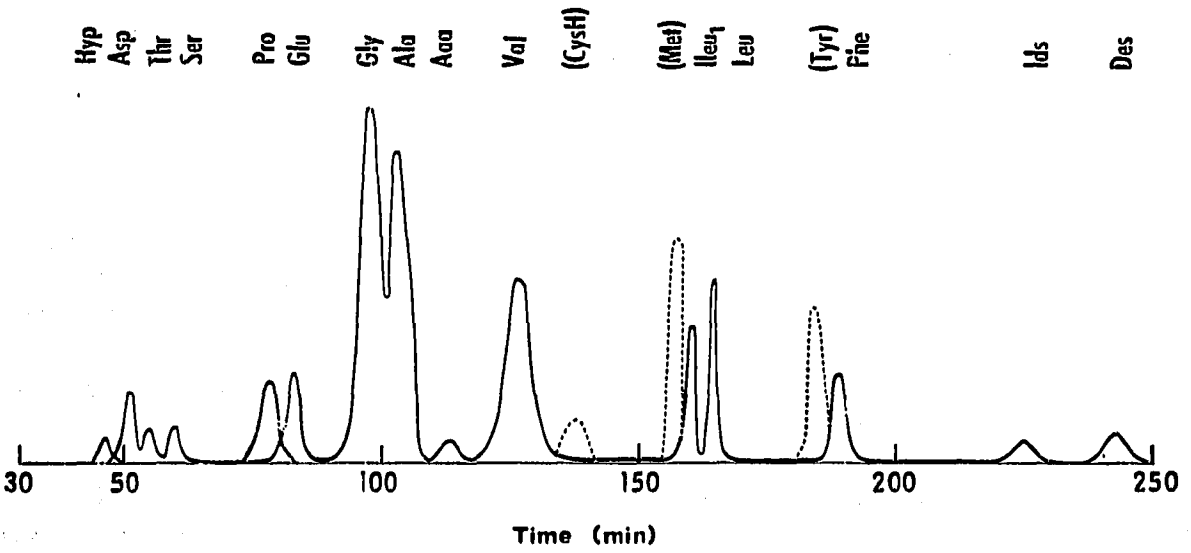

Fig. 1. Chromatogram from the hydrolysate of an elastin derivative, with the positions of cysteine, methionine and tyrosine, taken from a standard run, also shown. Aaa, α-amino adipic acid; Ids, isodesmosine; Des, desmosine.

Results and discussion

The amino acids elute as shown in the elution diagram taken from the results obtained with the hydrolysate of an elastin derivative. (Fig. 1). The positions of cysteine, methionine and tyrosine, taken from a standard run are also shown. Isodesmosine and desmosine elute as shown at 225 min and 243 min, respectively. The pH and temperature of the first buffer are critical if good separations are to be achieved. The acidic amino acids and cysteine appear particularly sensitive, for example higher values of pH or temperature will cause glutamic acid to move under proline, while at lower values of either parameter, aspartic acid and threonine will not be adequately resolved. In our experience the correct pH value is most satisfactorily achieved by a trial-and-error process with standard runs. This system has proved to be a very satisfactory one, and has been in use in this laboratory for over a year.

This work was supported by a Medical Research Council of Canada grant to Dr. R. A. ANWAR, a Canadian Heart Foundation Fellowship (G.D.K.) and an Ontario Graduate Fellowship (G.E.G.). Dr. ANWAR's advice and encouragement is gratefully acknowledged.

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Received May 15th, 1972