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Note

Procedure for the automatic analysis of all amino acids in elastin hydrolyzates on a routine basis

Although the polyfunctional amino acids present in elastin (isodesmosine, desmosine and lysinonorleucine) can be separated chromatographically from all of the other amino acids that are normally found in protein hydrolyzates, the chromatographic procedures used to resolve them have required buffers other than those used in standard amino acid analysis[1–5]. These different chromatographic conditions are inconvenient when analyzing elastin hydrolyzates on a routine basis.

A simple modification of the standard amino acid analysis procedure, which resolves the polyfunctional amino acids together with all of the other amino acids that are normally present in an elastin hydrolyzate, is described in this paper.

Methods

Bovine *ligamentum nuchae* elastin was prepared using the procedure of PARTRIDGE *et al.*[6]. Amino acid analyses were performed in constant-boiling HCl at 110° for 22 h.

Desmosine, isodesmosine and lysinonorleucine were used as standards. A standard procedure with a taped programme for the multisample fully automated JLC-5AH amino acid analyzer was used, which required the use of a short column (15 × 0.8 cm) and a long column (70 × 0.8 cm), both packed with JLC-R-2 resin. The basic amino acids (excluding desmosine, isodesmosine, merodesmosine and lysinonorleucine) were resolved on the short column by using 0.35 *N* sodium citrate buffer of pH 5.28 (171.5 g of $Na_3C_6H_5O_7 \cdot 2H_2O$, 32.5 ml of concentrated HCl, 0.5 ml of *n*-caprylic acid and water to 5000 ml). The acidic and neutral amino acids were separated on the long column by using a stepwise buffer gradient starting with 0.2 *N* sodium citrate of pH 3.30 (98.5 g of $Na_3C_6H_5O_7 \cdot 2H_2O$, 61.5 ml of concentrated HCl, 0.5 ml of *n*-caprylic acid, 25 ml of thiodiglycol, 400 ml of methyl alcohol and water to 5000 ml) and changing to 0.2 *N* sodium citrate of pH 4.25 (98.5 g of $Na_3C_6H_5O_7 \cdot 2H_2O$, 42 ml of concentrated HCl, 0.5 ml of *n*-caprylic acid, 25 ml of thiodiglycol and water to 5000 ml) after 230 min.

After the phenylalanine, the last amino acid expected from the long column, the long column was eluted with 0.35 *N* sodium citrate of pH 5.28, normally used for the small column in order to resolve isodesmosine, desmosine, merodesmosine and lysinonorleucine very easily.

Results and discussion

By using this simple modification, all of the amino acids in elastin hydrolyzate can be completely separated in about 7 h, which is only 90 min more than the time required for a routine analysis with the standard automatic JLC-5AH programme without modifying the buffer or chromatographic conditions.

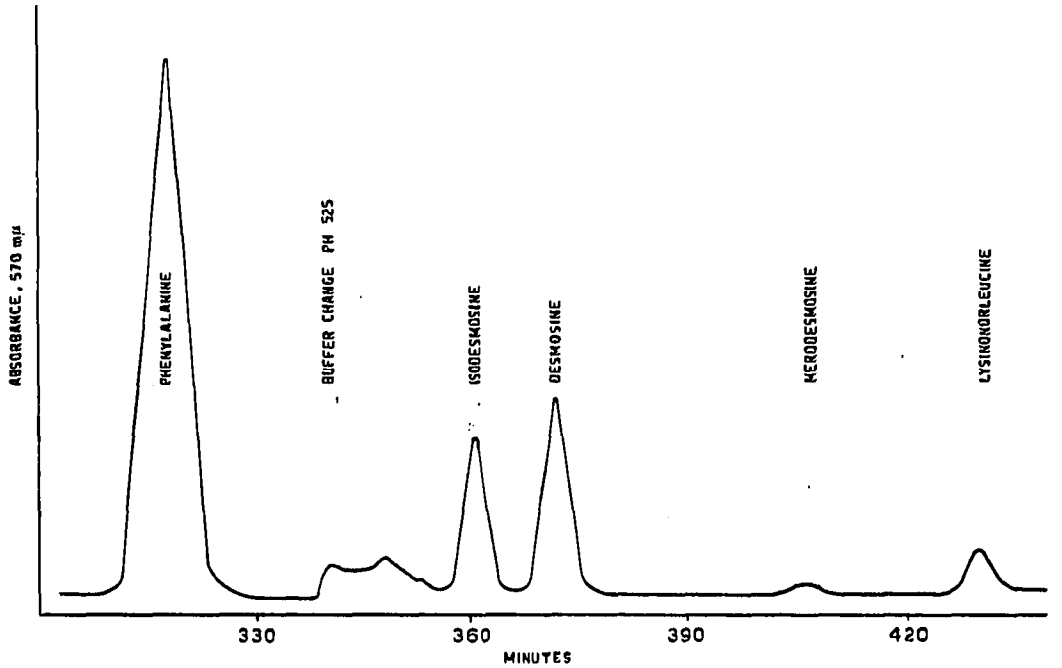

Fig. 1. Part of an amino acid chromatogram of a sample (0.5 mg) of adult bovine ligament elastin showing the separation of polyfunctional amino acids.

Fig. 1 shows a portion of the chromatogram of the elastin hydrolyzate in 6 *N* HCl.

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