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## Note

# Analysis of elastin peptide by reversed-phase high-performance liquid chromatography

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Elastin, the main component of elastic fibres, is insoluble in all solvents and is particularly resistant to hydrolysis by acids and bases and to proteolytic cleavage by mammalian enzymes.

It has a hydrophobic character and contains specific cross-linking amino acids, such as desmosine and lysinonorleucine. Studies on the structure and interactions of elastin have been carried out mainly on peptides obtained by partial hydrolysis of the native protein[1,2]. One of the chemical methods for the preparation of soluble peptides from "native" elastin is alkaline hydrolysis in aqueous alcohol[3], the mixture of soluble peptides obtained by this method being called kappa-elastin[2]. Hydrolysis for 3–8 h yields a high- (60,000–120,000) and a low-molecular-weight (10,000–16,000) peptide fraction.

After hydrolysis for 18–72 h, only low-molecular-weight (10,000–16,000) peptides are formed. The peptide mixture contains several groups of homologous compositions[3]. Some peptides were partially separated from these mixtures by isoelectric focusing and high-performance liquid chromatography (HPLC) on diol-bonded silica supports[4]. Two or three peptide fractions were obtained by the latter method from the low-molecular-weight hydrolysate. The separation and distribution of the material in the different fractions was strongly dependent on the concentration of the peptides injected on to the column.

These phenomena were attributed to hydrophobic interactions. Considering this phenomenon, and the composition of the kappa-elastin peptides, it appeared that methods involving hydrophobic interactions should be more effective for the separation of these substances than methods based on charge differences or on molecular size exclusion.

In this paper, we describe the separation of kappa-elastin peptides on a reversed-phase $C_{18}$ HPLC column.

## EXPERIMENTAL

### *Alkaline hydrolysis of elastin*

The hydrolysis of elastin, prepared by the Lansing *et al.* procedure[5] from bovine ligamentum nuchae, was performed with 80% aqueous ethanol containing 1 *N*

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potassium hydroxide for 4 and 72 h at 37°C[3]. The high- (60,000–120,000) and low-molecular-weight (10,000–16,000) fractions were separated on a Sephadex G-100 column as described previously[3].

*Chromatography*

HPLC was performed on a 10 $\mu$m $C_{18}$ $\mu$Bondapack column (30 × 0.4 cm I.D.) (Waters, Milford, MA, U.S.A.). Solvent delivery was carried out with a Gilson gradient system (Gilson, Villiers-le-Bel, France). The system was controlled by an Apple II microcomputer. The sample injector was a Rheodyne Model 7125. Detection was performed at either 206 or 280 nm with a M 1840 variable-wavelength detector–recorder (ISCO, Lincoln, NE, U.S.A.).

The best resolution was achieved with a gradient of 80% *n*-propanol (solvent B) in ammonium acetate $10^{-2}$ *M* (pH 7.8) (solvent A).

Kappa-elastin was dissolved in the primary eluting solution (6 mg/ml). A typical injection was 50 $\mu$l of this solution, but up to 0.5 ml can be injected on to the column.

The influence of sample concentration and eluent composition on the elastin peptide separation were investigated. The separated elastin peptide fractions were then subjected to amino acid analysis on a 3201 LKB Biocal analyser (LKB, Bromma, Sweden).

## RESULTS AND DISCUSSION

The usual eluent for protein separations by HPLC[6], an aqueous trifluoracetic acid–acetonitrile gradient adjusted to pH 2.2 with trifluoracetic acid, gave unsatisfactory results. The peptide mixture was resolved into only two distinct peaks, this resolution was not reproducible and a considerable amount of peptidic material was retained by the column.

It is known[2] that higher alcohols interfere with the hydrophobic interactions that occur between the molecules of solubilized elastin. Our earlier results[4] indicated, on the other hand, that the aggregation of the kappa-elastin molecules is diminished above pH 7. Therefore, we tested several gradient systems containing $10^{-2}$ *M* ammonium acetate or ammonium formate at pH 7.6–7.8 as primary solvents and isopropyl, *n*-propyl or *n*-butyl alcohol as secondary solvents. The best results were obtained with the ammonium acetate–*n*-propanol system described above. With this eluent, using the gradient indicated (Figs. 1 and 2), the low-molecular-weight kappa-elastin peptide mixture was separated into four main and several minor fractions.

The distribution pattern of the peaks is similar using continuous linear or stepwise gradients of solvent B. In the latter, the discontinuities after 10 and 20 min lead to an improved separation of peaks 4 and 5. In contrast to diol-modified silica columns[4], the distribution pattern and the relative intensities of the peaks are independent of the amount of sample injected.

Amino acid analysis of the separated peak material indicates that the individual peaks represent different polypeptide fractions. As the alkaline degradation of fibrous elastin yields a large number of peptides, the four major peaks probably do not represent homologous substances.

Peak 2, eluted at the beginning of the gradient (Fig. 1), is rich in polar amino

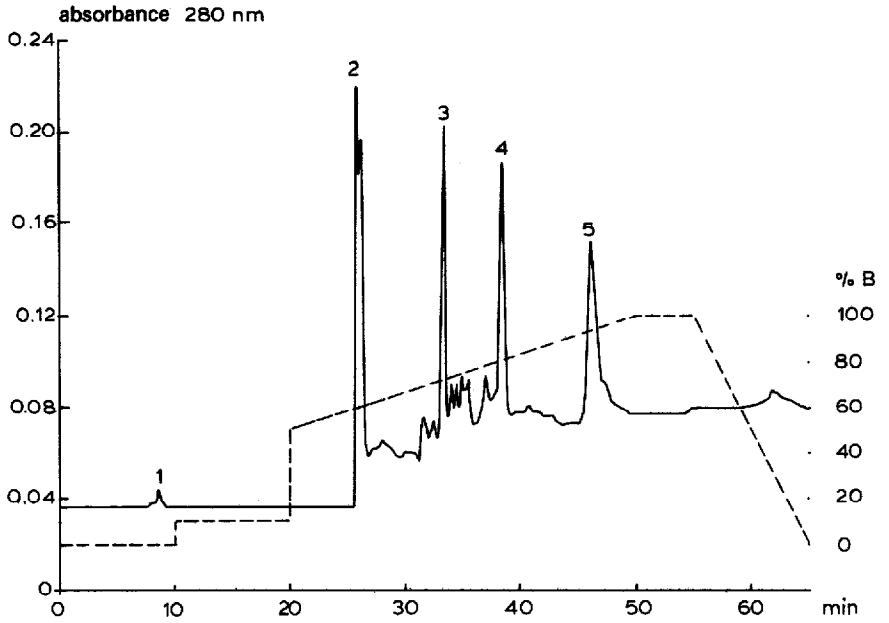


Fig. 1. HPLC separation of kappa-elastin peptides retarded on Sephadex G-100. Column: $C_{18}$ reversed-phase. Eluent: solvent A, 0.01 *M* ammonium acetate (pH 7.8); solvent B 80% *n*-propanol; complex gradient from 0 to 100% solvent B in 50 min. Flow-rate: 0.5 ml/min. Injection: 300 μg in 50 μl of solvent A.

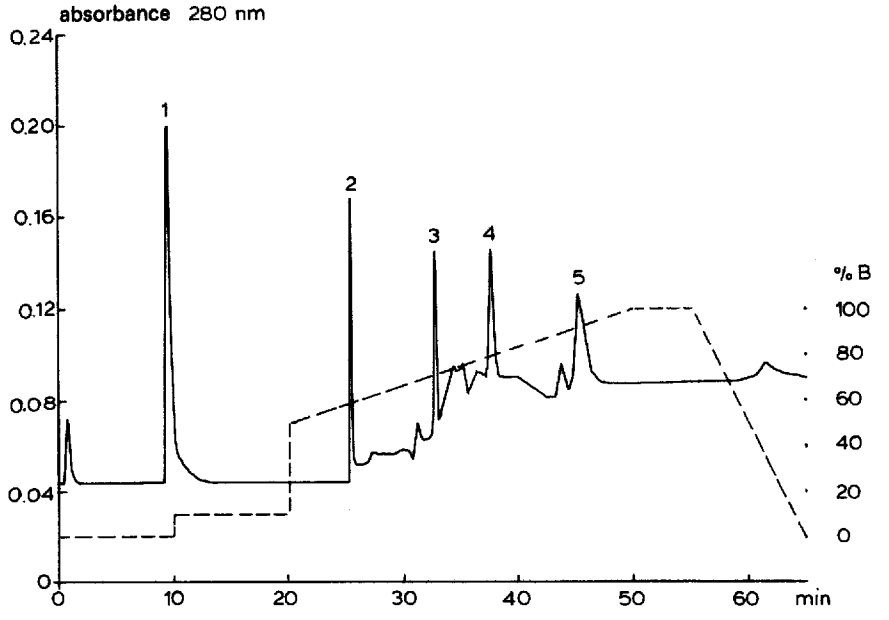


Fig. 2. HPLC separation of kappa-elastin peptides excluded from Sephadex G-100. Experimental conditions in Fig. 1.

acids and hydroxyproline (Hyp, Asp and Glu:31.1, 80.7 and 81.3 residues per 1000, respectively). It is relatively poor in glycine and alanine (98.5 and 125.5 residues per 1000, respectively), and contains desmosine and isodesmosine. Hence it seems to be a mixture of degradation products of collagen, structural glycoproteins and elastin.

Peaks 3, 4 and 5 correspond to peptides rich in non-polar amino acids. The glycine to alanine (Gly:Ala) ratios of these peptides are 1:1, 2.5:1 and 0.5:1, respectively. Peptides with similar Gly:Ala ratios were isolated by isoelectric focusing from the low-molecular-weight kappa-elastin peptides[3]. Hence these different polypeptide fractions seem to be derived from different well defined regions of the elastin molecule[3,7,8].

The bulk of the high-molecular-weight kappa-elastin fraction enriched in peptides of molecular weight 60,000–120,000 was eluted as a single peak (peak 1 in Fig. 2), before the elution of the low-molecular-weight peptides. From the presence of peaks 2, 3, 4 and 5 in this chromatogram, it can be concluded that the sample also contained low-molecular-weight peptides. A fraction of these peptides is strongly associated with the high-molecular-weight degradation products of elastin in aqueous solutions, and is excluded with them from the Sephadex G-100 column used for the separation of the two types of peptides[3].

It appears that reversed-phase HPLC on alkylated supports, with *n*-propanol-containing systems as eluents, is more suitable than diol-bonded silica for the separation of the main fractions of peptides obtained by chemical or enzymatic degradation of elastin.

It seems that comparative studies by reversed-phase HPLC of elastin peptides from different sources will be very useful for the investigation of pathological processes involving alteration of the elastin molecule[9].

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