AMINO-TERMINAL SEQUENCE OF A LARGE NON-POLAR PEPTIDE FROM ELASTIN

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SUMMARY

A peptide of 6,700-8,000 daltons has been isolated from an ethanolic KOH digest of bovine ligamentum nuchae elastin by a combination of ion exchange chromatography and gel filtration techniques. The peptide is non-polar and is composed almost entirely of glycine, valine, and proline (or hydroxyproline) in the ratio 2:2:1. Edman degradation of the first 17 residues gave the sequence: NH_2-Gly-Phe-Pro-Gly-Val-Gly-Val-Pro-Gly-Val-Gly-Val-Pro-Gly-Val-Gly-Val. These results suggest that the peptide is made up of repeating pentapeptide units with the sequence: Pro-Gly-Val-Gly-Val.

INTRODUCTION

Elastin is one of the least polar proteins known. Digests of elastin have been found to include two general types of peptide chains: cross-linked regions rich in the desmosines and alanine, and non-polar regions composed mainly of glycine, valine and proline (1-3). Stretching of elastin fibers is believed to be due to the extensibility of these non-polar regions. An "oiled coil" model to account for this extensibility has been proposed by Gray et al (3) based on a study of tropoelastin. This report concerns the identification of a repeating pentapeptide sequence found in a large non-polar peptide from mature ligamentum nuchae elastin containing mainly glycine, valine and proline (4,5).

MATERIALS AND METHODS

Ligamentum nuchae elastin powder was digested by the ethanolic KOH procedure of Robert and Poullain (6). Five grams of elastin powder were mixed with 75 ml of 1N KOH in 80% ethanol at 37° for 18 hrs. After centrifugation, the supernatant was dialyzed overnight and lyophilized. One gram of the product was chromatographed on a column of SE-Sephadex C-50, as described earlier (4,5). The second peak obtained from this column contained non-polar peptide, and 150 mg of this material were further fractionated by gel filtration on a 2.5 x 200 cm column of Sephadex G-50, in 1% NH_4HCO_3. The non-polar peptide fraction of the elastin digest eluted in the first peak. This non-polar peptide material was further purified on 0.8 x 100 cm column of DEAE-Sephadex A-25 equilibrated and eluted with 0.01 M ammonium bicarbonate (Figure 1). The fractions comprising Peak II from this column were lyophilized and subjected to amino acid analysis (5).

Sequence analysis of 3 mg samples of the peptide was performed by manual Edman degradation (7) with modifications as described by Morgan et al (8). Hydrolysis of the phenylthiohydantoins, at each step, was accomplished by a modification of the method of Smithies (9). After conversion of the thiazolinones to phenylthiohydantoins, the PTH-amino acids were extracted with ethyl acetate, dried under a stream of nitrogen and redissolved in 100 µl of ethylene dichloride. Half of this solution was mixed with a known quantity of a PTH-norleucine internal standard, evaporated under vacuum in a hydrolysis tube and hydrolyzed in vacuo with 200 µl of hydroiodic acid at 125° C for 24 hrs. Following hydrolysis the acid was evaporated over NaOH. Amino acid analysis employed a single column procedure (8). All values for recovery of PTH-amino acids were corrected by recovery factors determined by hydrolysis of standard PTH-amino acids (Pierce Chem. Co.) under identical conditions. The remaining half of each sample was analyzed by thin layer chromatography and gas chromatography.

RESULTS AND DISCUSSION

The first two steps in the isolation of the peptide from the digest, namely the SE-Sephadex and G-50 column procedures, were described earlier (4,5). When the first, i.e., the largest molecular weight peptide fraction from the Sephadex G-50 column was further purified on the column of DEAE-Sephadex A-25, three peaks were obtained (Figure 1), of which the second represented the bulk of the material and was least polar in composition. The amino acid composition of this peptide at various stages of purification is given in Table I. The content of the desmosines and alanine decreased during purification which indicated removal of peptides liberated from the cross-linked regions of elastin.

Valine, glycine, proline and hydroxyproline increased during the purification. The molar ratio of glycine, valine and proline or hydroxyproline in the product is approximately 2:2:1. The hydroxyproline to proline ratio in the peptide is approximately three times higher than in elastin suggesting that hydroxyproline is concentrated in the non-polar region of the molecule. The minimum molecular weight of the peptide of Peak II calculated from the amino acid analysis, assuming one residue of phenylalanine is 6766 daltons. The molecular weight calculated from the average recovery of glycine and phenylalanine after Edman degradation is approximately 8,000 daltons. Therefore, the peptide can reasonably be assigned a weight of 6,700-8,000 daltons based on these two estimates.

The results of manual Edman degradation followed by hydrolysis and amino acid analysis of each residue are plotted in Figure 2. The main sequence present

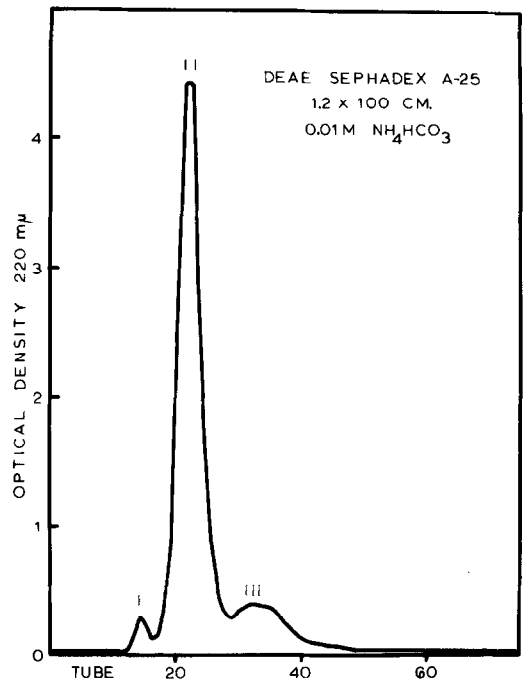

Figure 1. DEAE-Sephadex chromatography of 10 mg of the non-polar peptide material obtained from Sephadex G-50.

was found to be: Gly-Phe-Pro-Gly-Val-Gly-Val-Pro-Gly-Val-Gly-Val-Pro-Gly-Val-Gly-Val. Background levels at each step are also shown. The repeating peptide unit is Pro-Gly-Val-Gly-Val with phenylalanine present only at step 2. This peptide preparation was sequenced three times. The identifications of PTH-amino acids for this first two runs were made by thin layer chromatography and gas chromatography and on the third by "back" hydrolysis.

Although the primary structure of this peptide preparation exhibits some heterogeneity possibly due to the alkaline cleavage, the presence of a unique NH_2-terminal sequence was apparent when yields of residues cleaved by Edman degradation were quantitated. The pattern of yields at each step indicates a definite repeating pentapeptide sequence despite the presence of contaminating or overlapping residues.

The sequence and molecular weight of this peptide from mature bovine elastin closely resembles part of a large tryptic peptide (TI) isolated by

Table I

Amino Acid Analyses of the Non-polar Peptide at Various Stages of Purification

Amino Acids Residues/100	Elastin	SE-C50 (Fr II)	G-50 (Fr I)	DEAE A-25* (FR II)	
Lysine	0.5	0.1	0.0	0.0	
Histidine	0.04	0.0	0.0	0.0	
Arginine	0.5	0.0	0.0	0.0	
Hydroxyproline	0.9	3.7	3.5	3.2	(2.7)
Aspartic Acid	0.6	0.2	0.0	0.0	
Threonine	0.7	0.1	0.0	0.0	
Serine	0.8	0.2	0.2	0.2	
Glutamic Acid	1.4	0.5	0.1	0.0	
Proline	11.8	16.3	15.6	15.8	(13.2)
Glycine	35.4	38.7	41.3	38.3	(31.9)
Alanine	22.8	3.3	0.6	0.4	
Valine	12.6	34.8	37.4	40.7	(33.9)
Isoleucine	2.4	0.3	0.0	0.0	
Leucine	6.0	0.8	0.1	0.0	
Tyrosine	0.5	0.2	0.1	0	
Phenylalanine	2.9	1.0	0.9	1.2	(1.0)
Isodesmosine	0.1	0	0	0	
Desmosine	0.1	0	0	0	

*Numbers in parenthesis indicate estimated residues assuming one phenylalanine. These results are consistent with the proposed structure: Gly-Phe-(Pro-Gly-Val-Gly-Val)$_{16}$.

Gray et al (3) from porcine tropoelastin. Starting at position 13 of that sequence, the two primary structures are identical except for a deletion of a glycine at position 15 in the bovine peptide. This is probably a species difference. The molecular weight of 6,700-8,000 daltons assigned to the bovine peptide described in this paper is the same as the average chain mass (Mc) between desmosine "cross-link" regions predicted by Gray et al (3). Since the

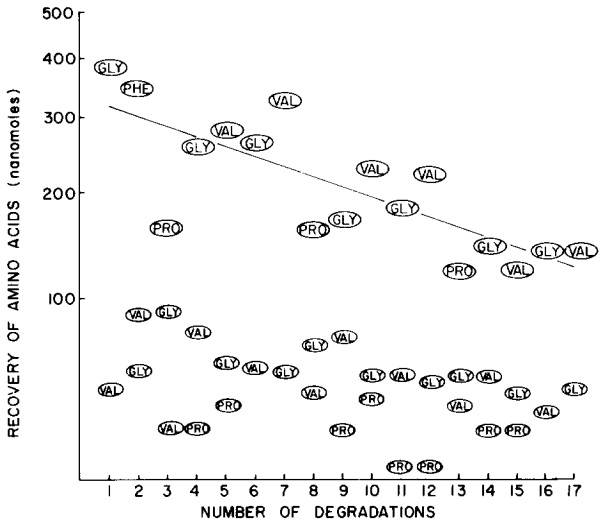

Figure 2. Yield of free amino acids recovered after HI hydrolysis of the PTH-amino acids produced by each step of manual Edman degradation (see text). Recoveries were corrected for destruction during hydrolysis. The line shown is the unweighted linear regression for the recovery of the major amino acids identified at each step (larger print) and the repetitive yield was 94%. At positions 3, 8 and 13 a small amount of hydroxyproline was detectable by thin layer chromatography. The partial hydroxylation of these proline residues contributes to the unexpectedly low recovery of proline at these positions.

bovine peptide is composed on a molar basis of 2 Gly: 2 Val: 1 Pro or Hyp, it is likely that the repeating pentapeptide unit of (-Pro-Gly-Val-Gly-Val)n extends to the COOH terminus. The finding of a repetitive sequence within mature elastin with a length of approximately 82 residues is consistent with predictions made from the earlier work with tropoelastin, an artificially induced, soluble form of elastin (3).

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