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# Carbon-13 NMR Studies of Alpha-Elastin

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## ABSTRACT

NMR investigations of model protein of elastic fibre is presented. Detailed conformation of alpha-elastin polypeptide chain is discussed by comparison with the conformation of synthetic repeat peptides of elastin. Amino acid composition of alpha-elastin obtained from C-13 NMR spectra correlates with the results of sequencing of tropoelastin.

## INTRODUCTION

Elastin is a very important structure protein of connective tissue. The elastic fibre contribute essential resilient properties to ligaments, arterial wall, skin and lungs (5). Behaviour of elastic fibres under physiological and pathological conditions is significant because of the importance of the physical properties of elastin in such diseases as atherosclerosis and emphysema. Elastic fibre is the primary site of both calcification and lipid deposition in the vascular wall (9). Degradation of elastic fibres is reported in emphysema, atherosclerosis, skin diseases and in ageing of connective tissues. Peptides which are liberated during these degradation processes may exhibit biological effects either systemically or locally in the organism (3).

At present there are two prevalent views on the molecular structure of elastin. One is that elastin like rubber does not possess a definite secondary structure. The second view is that it does have a secondary structure. Although thermoelasticity studies of both natural elastin and synthetic elastin polypentapeptide indicate the elasticity to be entropic in origin, the molecular origin of entropy differences between stretched and relaxed polymer remains unclear (14).

Tropoelastin is a soluble precursor protein of elastin, it was identified and characterised in the 1960s through the work of Sandberg and Smith (7). Alpha-elastin is the solubilized product of partial hydrolysis of elastin in hot oxalic acid (4). Alpha-elastin is very similar to tropoelastin, it has the

same molecular weight and very similar physico-chemical properties. Tropoelastin has the amino acid composition of the alpha- elastin but it lacks the cross-links desmosine and isodesmosine. Instead, it has a high lysine content. Lysine is the precursor of the desmosine cross-links (2).

Tropoelastin contains repeating sequences: pentapeptide Val-Pro-Gly-Val-Gly, hexapeptide Val-Ala-Pro-Gly-Val-Gly, and tetrapeptide Val-Pro-Gly-Gly. The existence of repeating sequences suggests the existence of a specific secondary structure. It also opens the possibility of working with synthetic biopolymer model compounds of elastin.

Very important conformational studies on repeat pentamer, hexamer and their synthetic polymers were carried out by Urry and co-workers. Their work was based on the NMR investigations of repeat peptides of elastin (10,11,12).

Elastin is the insoluble protein and it is impossible to obtain standard high resolution NMR spectra of elastin. NMR spectra of alpha-elastin should be much more similar to spectra of tropoelastin then the spectra of repeat peptides of elastin and alpha-elastin is better model of tropoelastin then synthetic polypentapeptide. That is why the aim of this work was acquisition of carbon NMR spectra of alpha-elastin and comparison of these spectra with the spectra of synthetic polypentapeptide and polyhexapeptide of elastin.

**MATERIALS and METHODS**

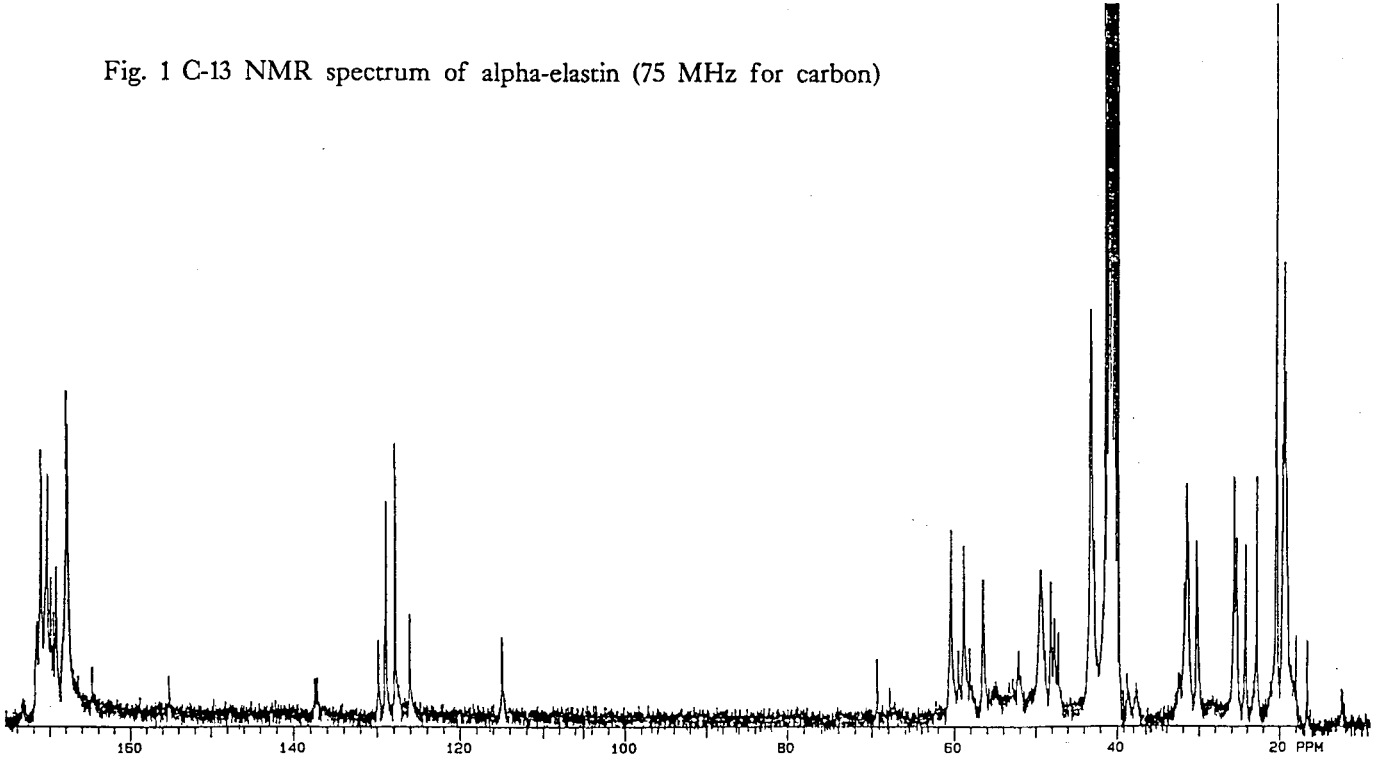
Elastin was obtained from fresh porcine aorta according to Robert (6), then partial hydrolysis of elastin in hot oxalic acid was performed (4). Gel filtration was done using 1 m long column filled with Sephadex G-75 in order to obtain fractions, the range of molecular weight of which being 65000-75000 daltons. Concentration of protein was measured using absorbancy in UV.

C-13 NMR spectra in dimethyl sulphoxide-d6 (DMSO) were recorded at a resonance frequency of 75 MHz on a Varian VXR-300 spectrometer using tetramethylsilane (TMS) as internal standard (temperature 30 $^{o}$C). The spectra were obtained with 35000 scans, 1.815 s acquisition time and 8.7 ms pulse width. For the quantitative measurements of C-13 NMR spectra pulse sequences giving decoupled spectra without NOE were used.

**RESULTS and DISCUSSION**

The carbon nuclear magnetic resonance spectrum of alpha-elastin is given in Figures 1,2,3,4. The chemical shifts of resonances are given in Table 1. The signals of Gly, Val, Pro were assigned by comparison with the spectra of the synthetic peptides and synthetic polypeptides of elastin (10,11,12). The

Fig. 1 C-13 NMR spectrum of alpha-elastin (75 MHz for carbon)



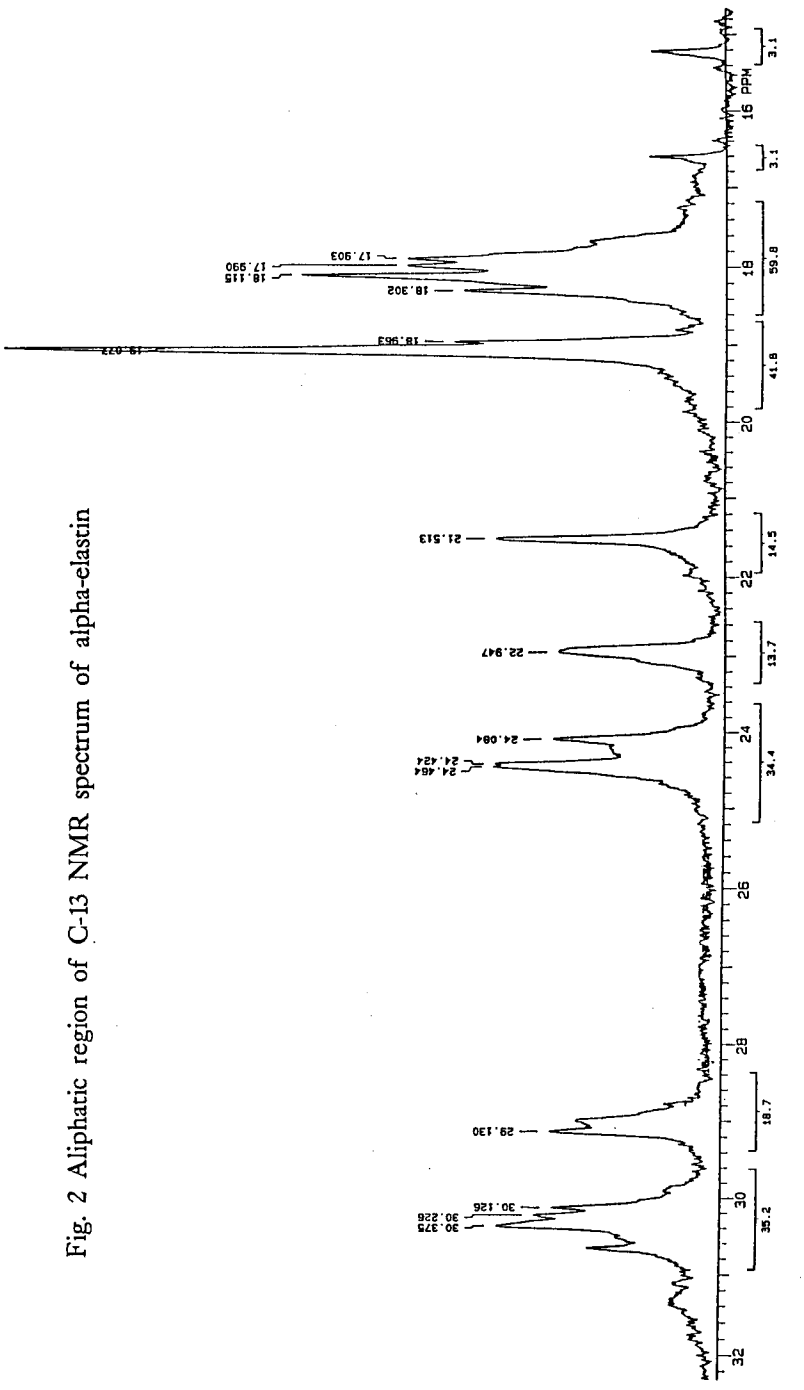
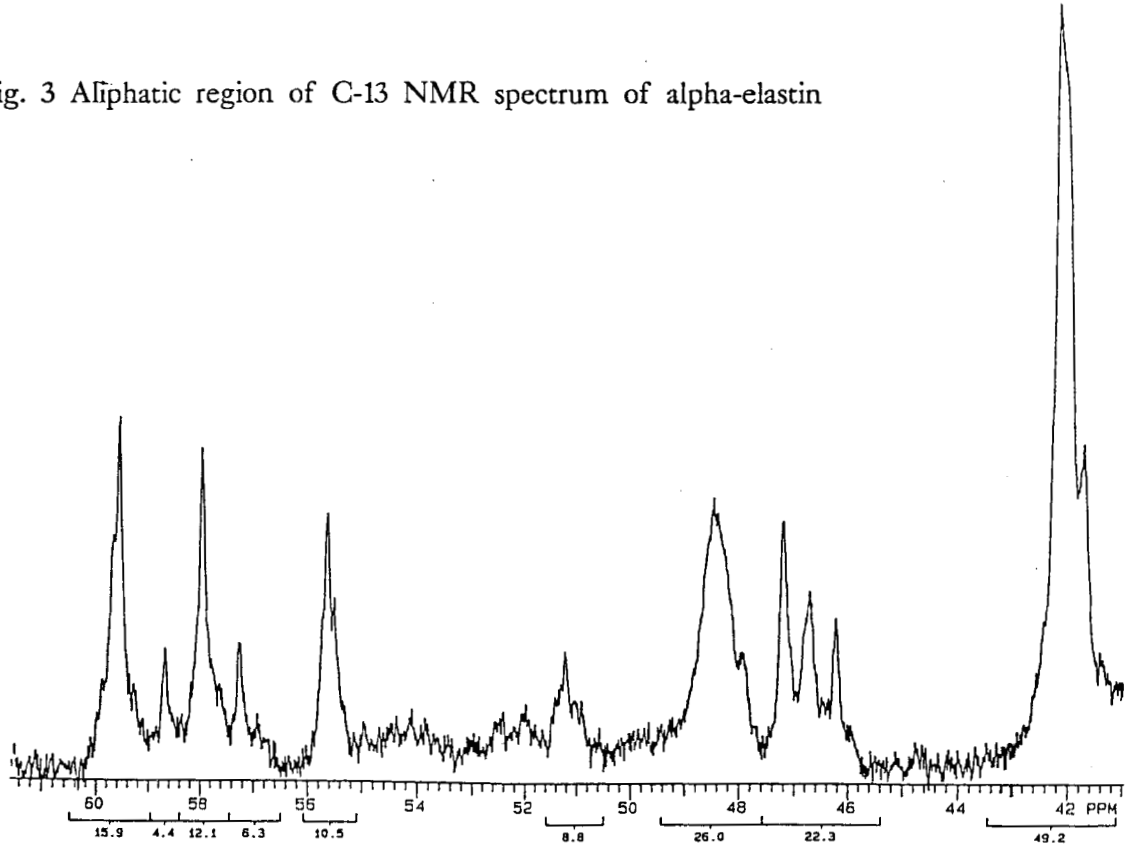



Fig. 2 Aliphatic region of C-13 NMR spectrum of alpha-elastin

Fig. 3 Aliphatic region of C-13 NMR spectrum of alpha-elastin



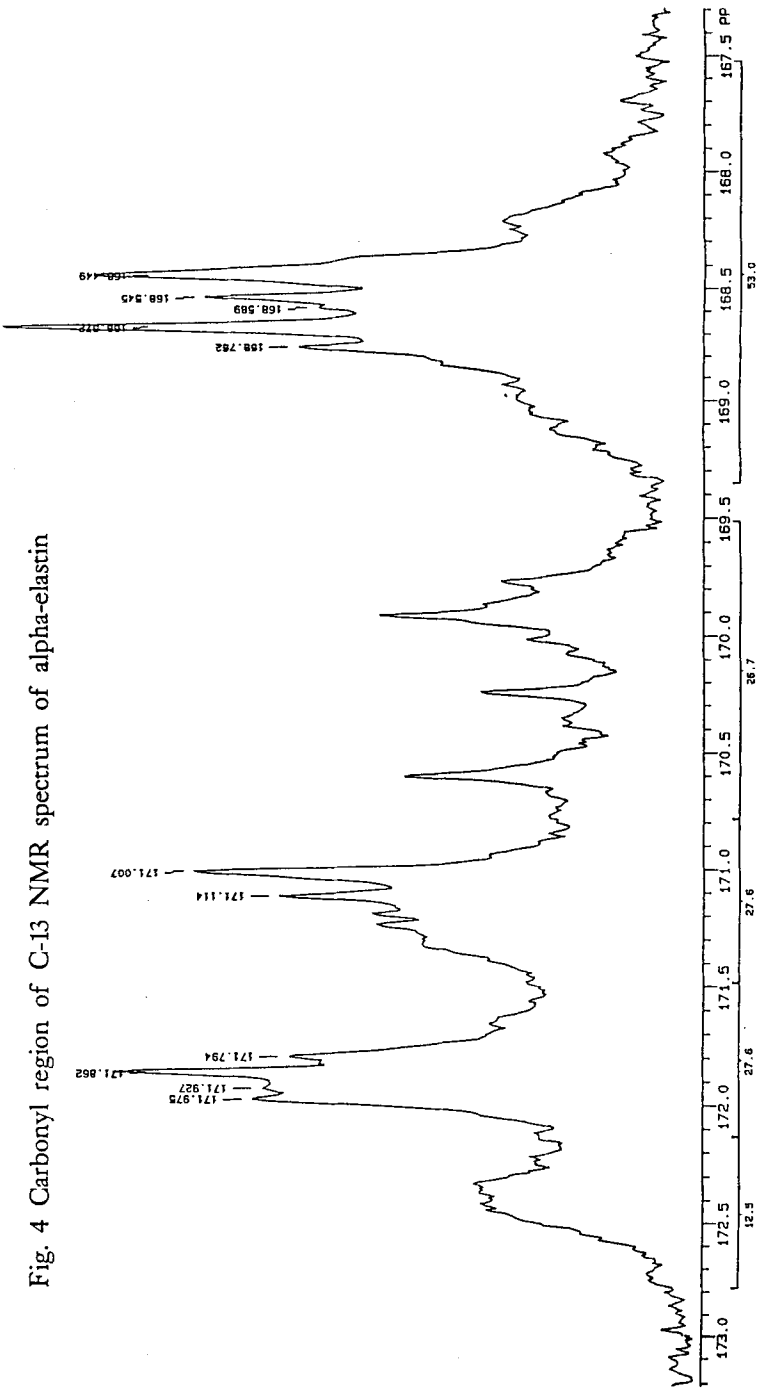



Fig. 4 Carbonyl region of C-13 NMR spectrum of alpha-elastin

lines of alanine, leucine, phenylalanine and tyrosine were assigned by comparison with chemical shifts of amino acids' carbons of random coil polypeptide chains (15).

| Chemical shifts of α-elastin carbons [ppm] | Assignment of α-elastin carbons | Chemical shifts of polypenta-peptide carbons [ppm] | Chemical shifts of random coil amino acids carbons [ppm] |
|---|---|---|---|
| 17.9-18.3 | Val γC | 18.3 | 17.9 |
| | Ala βC | - | 18.2 |
| 19.0 | Val γC | 19.03 | 19.0 |
| 21.5 | Leu δC | - | 21.6 |
| 22.95 | Leu δC | - | 23.0 |
| 24.1 | Leu δC | - | 24.0 |
| 24.4 | Pro γC | 24.41 | 24.4 |
| 29.1 | Pro βC | 29.22 | 29.0 |
| 30.1-30.6 | Val βC | 30.19 | 30.8 |
| DMSO | Leu βC | - | 41.0 |
| 42.1 | Gly αC | 41.98 | 42.1 |
| 46.0-47.2 | Pro δC | 47.18 | 45.8 |
| 48.0-49.0 | Ala αC | - | 48.0 |
| 51.0 | Leu αC | - | 50.5 |
| 55.6 | Val αC | 55.57 | - |
| 57.3 | Phe αC | - | 57.3 |
| | Tyr αC | - | 57.3 |
| 58.0 | Val αC | 57.86 | 60.7 |
| 59.7 | Pro αC | 59.51 | 61.6 |
| | AROMATIC | CARBONS | |
| 115.0 | Tyr C3,5 | - | 115.0 |
| 126.2 | Phe C4 | - | 126.1 |
| 127.9 | Phe C2,6 | - | 127.9 |
| | Tyr C1 | - | 127.9 |
| 129.0 | Phe C3,5 | - | 129.0 |
| 130.0 | Tyr C2,6 | - | 130.1 |
| 137.5 | Phe C1 | - | 137.8 |
| 155.8 | Tyr C2 | - | 155.5 |
| | CARBONYL | CARBONS | |
| 168.5-172.4 | | | |

Table 1. Chemical shifts of alpha-elastin carbons

Chemical shifts of Ala, Leu, Tyr and Phe are only little different from the chemical shifts of amino acids from random coil polypeptide chains. This may indicate that these amino acids are placed predominantly in the random coil regions of polypeptide chain.

Chemical shifts of Gly, Val, Pro are very similar to chemical shifts of amino acids of synthetic polypeptides of elastin. The upfield shift of one of Val αCH is due to the effects of the Pro residue (in the structure of pentapeptide and tetrapeptide of elastin). The signals of Pro $\beta CH_2$ and $\gamma CH_2$ show that only trans peptide groups of proline are encountered in polypeptide chains (cis forms have their signals shifted upfield for $\beta CH_2$ and downfield for $\gamma CH_2$ (15)).

Assignment of the lines of carbonyl carbons is very difficult because the signals are very crowded in this spectral region and the chemical shifts are affected by the primary structure of elastin. Probably the signal near 168.5 ppm derives from Gly carbonyl carbons.

Major differences between the spectra of alpha-elastin and synthetic polypentapeptides can be noticed near 46-47 ppm (signals from Pro $\delta CH_2$). This signal is splitted to three lines. This is probably caused by the different neighbourhood of proline in repeat peptides of elastin (Val for pentapeptide, Ala for hexapeptide and Val-Pro-Gly-Gly structure of tetrapeptide).

Proline is a very important amino acid for elastin structure. The torsion angles Φ are fixed by the covalent structure of proline (13). Proline is mainly situated in the place of polypeptide chain turn. Urry and co-workers showed that the prevalent structure of repeat peptides of elastin is the beta-turn. All three of repeating sequences contain -$Pro_2$-$Gly_3$- sequence which is important in forming a beta-turn (13). The beta-turn is a 10-atom hydrogen-bonded ring involving the C-O of residue-1 and the N-H of residue-4 with $Pro_2$ and $Gly_3$ at the corners of the beta-turn. Repeat peptides of elastin form beta-spirals. A beta-spiral is defined as a helical structure in which the beta-turn is the predominant structure. Polypentapeptide is responsible for the elastic properties of elastin. Hexapeptide is a more rigid structure with hydrogen bonding between repeats and with hydrophobic ridges which on hydrophobic association could result in the aligning and interlocking of chains. Hexapeptides repeat five times near the cross-linking regions (13). The latest work of Arad and Goodman show that repeating sequences of elastin show a combination of flexibility with conformational preferences. Several hydrogen-bonded structures occur and are in equilibrium while at the same time maintaining an overall folded structure. The absence of the beta turn hydrogen bond causes a change in the conformation that is more pronounced in the polymers (1).

C-13 NMR spectrum of alpha-elastin is very simple in comparison with the spectra of the protein of the same molecular weight. It is caused by the conservative amino acid composition of elastin. Tropoelastin has the

molecular weight of about 74000 daltons and contains 850 amino acid residues with following composition: 275 residues of glycine, 196 residues of alanine, 103 of proline, 110 of valine, 38 of leucine, 24 of phenylalanine, 12 of tyrosine, 38 of lysine. Arginine, aspartic acid, asparagine, treonine, serine, glutamine and glutamic acid constitute a total of 54 residues. These numbers are taken from sequencing of porcine elastin (7).

Quantitative analysis of C-13 NMR spectrum of alpha-elastin showed high similarity of the amino acid composition to the results of the tropoelastin sequencing. The results of the amino acid composition of alpha-elastin calculated from the carbon NMR spectrum are shown in Fig. 5.

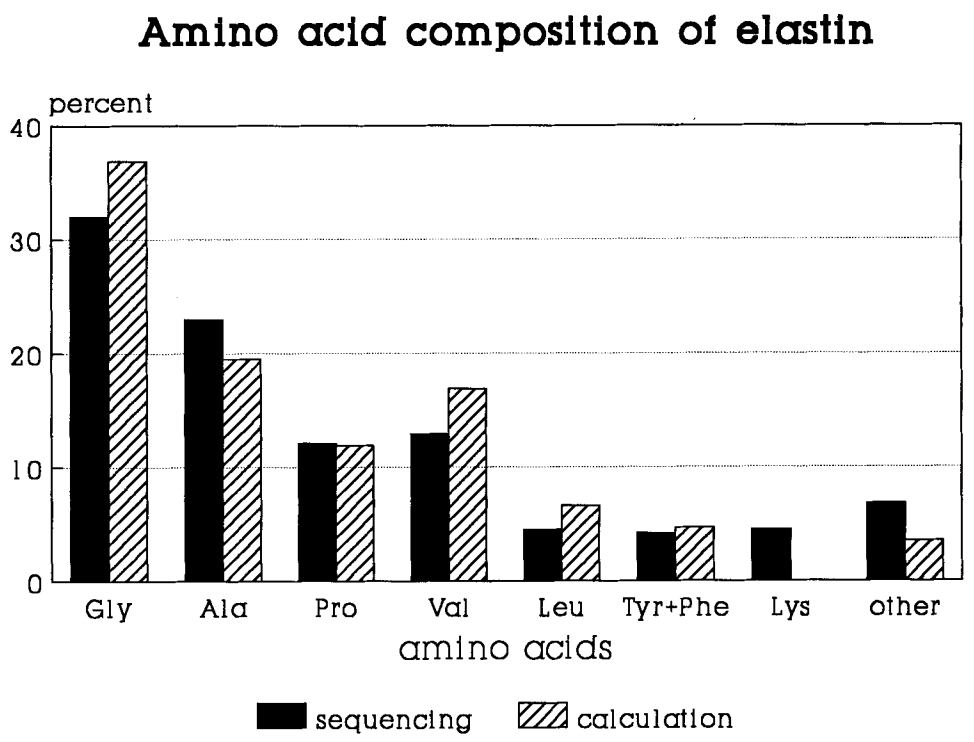



Figure 5

**CONCLUSIONS**

1) High resolution NMR spectra of alpha-elastin reveal the repeat peptide structure of alpha-elastin,

2) The amino acid composition of alpha-elastin obtained from quantitative analysis of C-13 NMR spectra correlates with the results of sequencing of tropoelastin.

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