THE AMINO ACID COMPOSITION OF ELASTIN IN ITS SOLUBLE AND INSOLUBLE STATE*

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Introduction

The protein elastin is highly insoluble in normal animals, being deposited in a cross-linked aggregated state (8). Its soluble noncross-linked form has yet to be isolated from normal elastic tissues.

When elastic tissues, such as aorta, nasal and ear cartilage, and nuchal ligament are autoclaved, the elastin is left behind as an insoluble residue (5). Subsequent extraction with hot alkali leads to some purification of the elastin (5), but it is doubtful that pure elastin is ever obtained. Small amounts of other proteins may remain firmly bound, perhaps covalently linked, to the elastin; in particular, ground substance protein and collagen, of which there are considerable amounts in the elastic tissues (2,4).

The soluble protein fraction obtained by neutral-salt extraction of elastic tissues from normal animals is mainly ground substance protein (4). Some collagen is also in this fraction, but very little, if any, elastin.

In animals raised on copper-deficient diets, however, it appears that the soluble protein fraction may contain substantial amounts of elastin in the noncross-linked form (13).

The amino acid compositions reported for soluble and insoluble elastic-tissue fractions in normal and copper-deficient animals are examined here. By making some reasonable assumptions about the protein components, we are able to extrapolate to a composition for pure elastin in its soluble and insoluble states.

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Sources of Data

The amino acid compositions examined pertain to bovine and porcine elastic tissue fractions. The bovine tissues are nuchal ligament (2,4,5,9,11), ear and nasal cartilage (5,9), and aorta (5) of normal cattle; the porcine tissues are aortas of normal and copper-deficient swine (13). The soluble fractions are obtained by neutral-salt extraction (4,9,13); the insoluble fractions by autoclaving (2,5). Although data are available for insoluble fractions

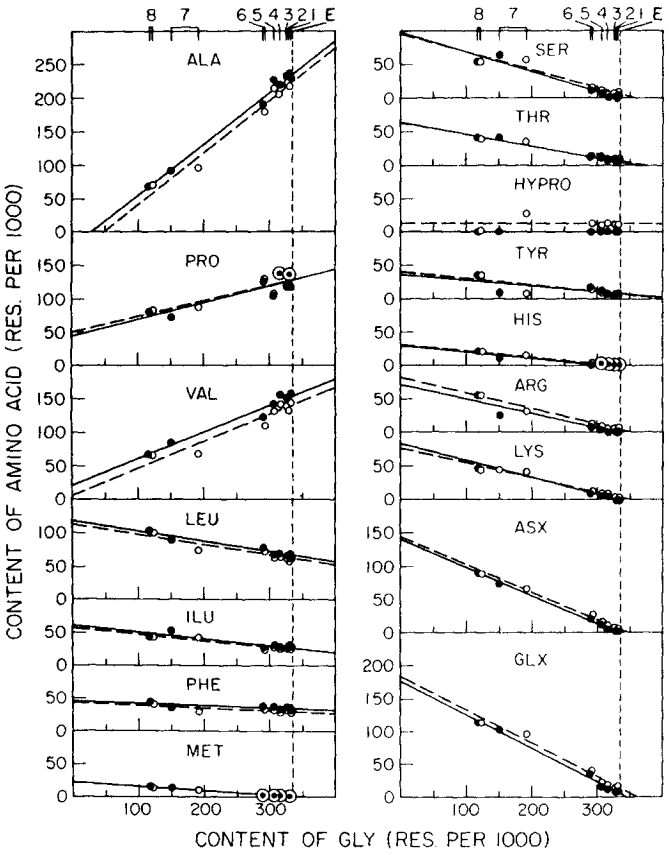

Fig. 1. Amino acid plots against gly, before correction for collagen (dotted lines and open dots), and after correction for collagen (solid lines and closed dots), for normal bovine elastic tissue fractions. 1,2,3, and 4 are insoluble fractions from nuchal ligament of 9-year old cow (11), 1-year old cow (11), adult cattle of unspecified age (5), and term-foetal calf (11), respectively. 5 and 6 are insoluble fractions from aorta and ear cartilage, respectively, of adult cattle of unspecified age (5). 7 is a soluble fraction from autoclaved bovine nuchal ligament (4); 8, the ground substance protein isolated from the soluble fraction of bovine nasal cartilage (9). The straight lines are fitted to the points by the least-squares method.

cleansed with hot alkali (1,5), these data are not used because the alkali treatment is destructive to some of the amino acids, particularly arginine.

Assumptions

Two assumptions are made about the protein components in the elastic tissues. First, we assume that only three components are present in significant amounts; namely, elastin, ground substance protein, and collagen. Second, we suppose that collagen is the only component containing hydroxyproline (hypro).

The amino acid contributions from collagen, corresponding to the content of hypro, are calculated using the composition of purified collagen from calf

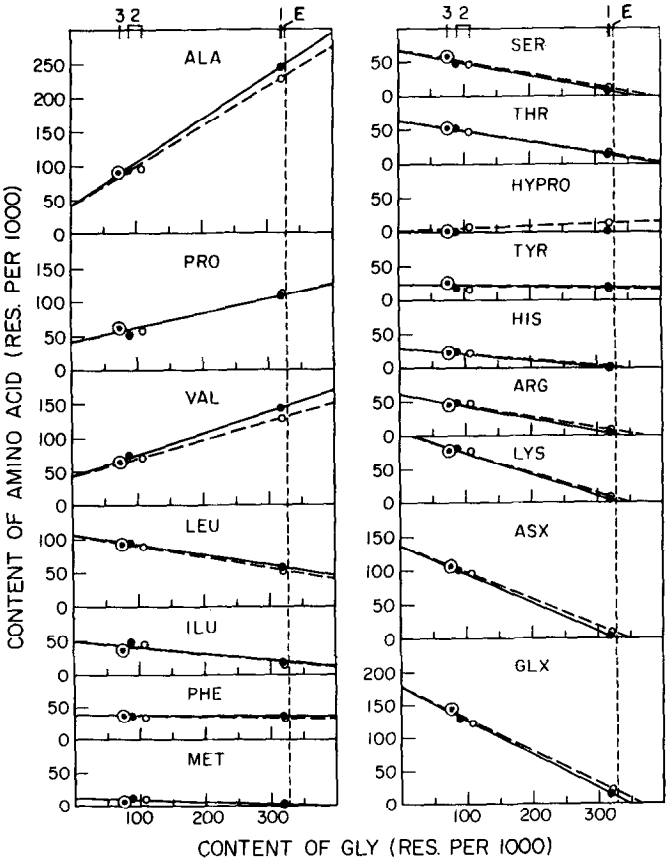

Fig. 2. Amino acid plots against gly, before correction for collagen (dotted lines and open dots), and after correction for collagen (solid lines and closed dots), for normal porcine aortic fractions. 1 is the insoluble fraction; 2 and 3 are the residue and supernatant, respectively, of the soluble fraction left standing in the cold (13), as a step in purification. The straight lines were fitted by least squares.

(6) and pig (3) skins. In correcting for collagen, we subtract these contributions from the observed amino acid contents in the fractions. The contents before and after correction for collagen are expressed in residues per 1000 total amino acid residues and plotted as described below.

The content of one of the amino acids showing a large net change, specifically gly, ala, or val, is plotted along the abscissa. Against this, along the ordinate, is then plotted the corresponding content for each of the other amino acids. When the data for all the fractions are so plotted, the points

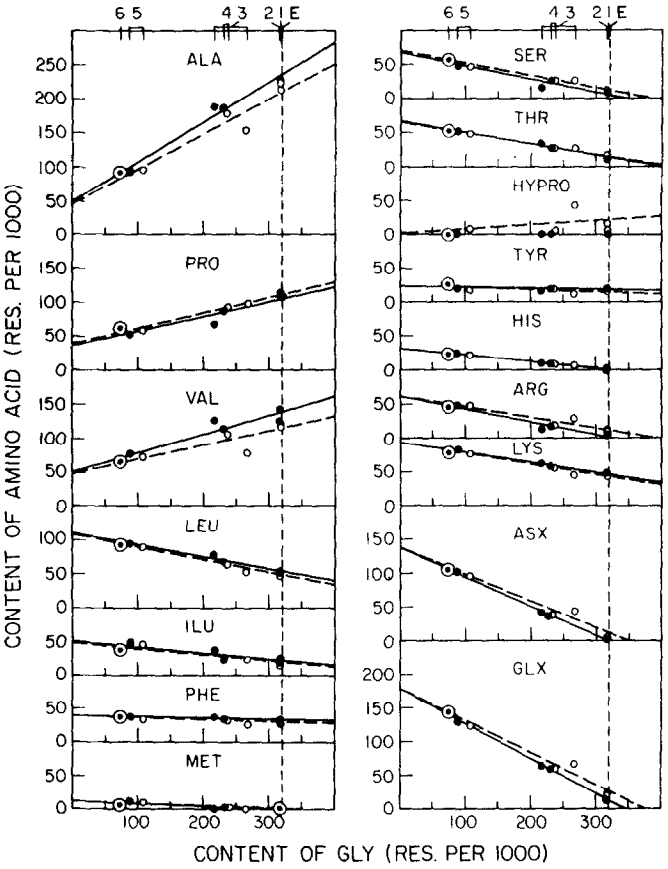

Fig. 3. Amino acid plots against gly, before correction for collagen (dotted lines and open dots), and after correction for collagen (solid lines and closed dots), for Cu-deficient porcine aortic fractions. 1 and 2 are residues from soluble fractions coacervated at room temperature (13); 3 and 4 are supernatant and residue, respectively, from soluble fraction left standing in the cold (13); 5 and 6 are residue and supernatant, respectively, from normal aortic soluble fraction left standing in the cold (same as 2 and 3, respectively, in Fig. 2). The straight lines are the least-squares fit.

for each amino acid should lie on a straight line if only elastin and ground substance protein are present. This is the line connecting pure elastin and pure ground substance protein. If collagen is present in the amounts calculated from hypro, there should be a better fit to a straight line after correction for collagen.

Results

The results are very similar whether one plots against gly, ala, or val. Here we described the results of plotting against gly before and after correction for collagen. To each set of points plotted, a straight line is fitted by least squares (Figs. 1,2,3). The standard deviation of the points from the line indicates the goodness of fit in each case (Table I). The average value of the standard deviation is smaller after correction for collagen than before (Table I).

Table I
Average Value of the Standard Deviation Between the Points and the Lines For the Sets of Plots in Figs. 1 to 3

	Average Standard Deviation in Residues per 1000 Residues	
Figure	Before Correction For Collagen	After Correction For Collagen
1	4.4	3.9
2	2.3	2.0
3	4.7	3.1

After correction for collagen, several of the lines intersect the gly axis at the same point, "E" (Figs. 1,2,3). The simplest interpretation is that "E" corresponds to pure elastin in which these amino acids are completely absent. Moving vertically along the dashed line at "E," we can read off the corresponding contents of other amino acids from the lines. In this manner we arrive at the compositions for pure elastin as shown in Table II.

Discussion

The computed compositions for soluble elastin in copper-deficient swine and insoluble elastin in normal swine are identical within the errors of amino

acid analysis, except for the content of lysine. The small difference in numbers noted between these two elastins (Table II) can be attributed to the lysine lost during the formation of cross-links. These have not been included in the tables. For the soluble elastin, there are 46 residues of lysine per

Table II
Amino Acid Contents Assigned to Pure Elastin

	Amino Acid Residues per 1000 Residues		
Amino Acid	Normal Bovine Insoluble Elastin	Normal Porcine Insoluble Elastin	Cu-Deficient Porcine Soluble Elastin
Gly	334	328	320
Ala	236	250	236
Pro	127	109	106
Val	154	147	138
Ilu	27	19	21
Leu	67	57	53
Phe	34	36	32
Met	0	1.1	0
Cys	0	0	0
Ser	0	7.2	5.3
Thr	5.7	13	13
Tyr	9.0	20	18
Try	0	0	0
His	0	0	0
Arg	0	1.3	0.4
Lys	0	2.1	46
Asx	0	0	0
Glx	5.9	10	12

Note: Each value is the average of the values determined from the plots against gly, ala, and val after correction for collagen. The determination is made at the point where the line for Asx intercepts the ordinate (the point labelled "E" in Figs. 1-3). The content of lysine is underlined to emphasize its much larger value in copper-deficient porcine elastin.

1000 residues of amino acid. With the insoluble material, the lysine content has dropped to two residues per 1000 residues. This difference in lysine content is in part due to the production of the cross-linking substances desmosine, isodesmosine, and lysinonorleucine. However, these known derivatives of lysine, which occur in insoluble elastin, account only for approximately one-half of the lysine present in the insoluble material. Recent reports (7,10) have indicated the presence of aldehydes in elastin derived from the enzymatic conversion of lysine to alpha-amino adipic-

semialdehyde. This aldehyde content of elastin accounts for about one-seventh of the total lysine in the noncross-linked soluble material. It would therefore seem that the remaining lysine residues are either lost during the conversion of soluble elastin to the insoluble fiber by some process of enzymatic peptide cleavage, or more likely, they are converted to cross-linking substances which have not as yet been identified. Some evidence has already been presented in favor of the latter argument (12).

The good agreement between the two computed compositions for soluble and insoluble porcine elastin (Table II), substantiates that the soluble elastin-like protein reported by Smith, et al. (13) is closely related to, if not actually, the elastin precursor (tropoelastin). Further, it can be said that the coacervated product reported by these workers is a fairly pure protein; the impurities being collagen and ground substance proteins present in not greater than 15% concentration.

Minimum molecular size of both bovine and porcine soluble elastin as indicated by amino acid composition is predicted to be about 350 residues or approximately 30,000 molecular polypeptide weight.

References

1. Cleary, E.G., L.B. Sandberg, and D.S. Jackson, Biochemistry and Physiology of Connective Tissue, P. Comte, editor, Societe Ormeco et Imprimerie du Sud-Est Lyons, France, 1966, p. 167.
2. Cleary, E.G., L.B. Sandberg, and D.S. Jackson, J. Cell Biol., 33 (1967) 469.
3. Eastoe, J.E., Biochem. J., 61 (1955) 589.
4. Gotte, L., Serafini-Fracassini, and V. Moret, J. Atheroscler. Res., 3 (1963) 244.
5. Gotte, L., P. Stern, D.F. Elsden, and S.M. Partridge, Biochem. J., 87 (1963) 344.
6. Heidrich, H.G. and L.K. Wynston, Z. Physiol. Chem., 342 (1965) 342.
7. Miller, E.J. and H.M. Fullmer, J. Exp. Med., 123 (1966) 1097.
8. Partridge, Fed. Proc., 25 (1966) 1023
9. Partridge, S.M., H.F. Davis, and G.S. Adair, Biochem. J., 79 (1961) 15.
10. Pinnell, S.R., G.R. Martin, and E.J. Miller, Science, 161 (1968) 475.
11. Sandberg, L.B., Ph.D. Thesis, Grad. Div. of the Univ. of Ore. Med. School, 1966.
12. Sandberg, L.B. and E.G. Cleary, Biochim. Biophys. Acta, 154 (1968) 411.
13. Smith, D.W., N. Weissman, and W.H. Carnes, Biochem. Biophys. Res. Commun., 31 (1968) 309.