Isolation of Salt-Soluble Elastin from Ligamentum Nuchae of Copper-Deficient Calf

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This paper describes the isolation and amino acid analysis of un-cross-linked elastin obtained by neutral salt extraction from the ligamentum nuchae of a calf fed from birth to 9 months on a diet low in copper.

The preceding paper (Sykes & Partridge, 1974) describes the isolation by improved methods of a homogeneous salt-soluble elastin from lathyrctic chicks. During the course of that work we were fortunate to receive from Dr. C. F. Mills, Rowett Research Institute, Aberdeen, U.K., the gift of tissues from Friesian calves fed on a copper-deficient semi-synthetic diet based on dried skim milk, urea, starch and cellulose with vitamins A, D and E and supplements of all known essential elements other than copper. The copper content of this diet was 1 µg/g dry wt. Corresponding tissues were provided from five control animals on the same diet to which was added initially 7.5 and later 15 µg of copper/g. These treatments were maintained for 260–280 days during which time the plasma amine oxidase activity of deficient animals fell to 10–20% of that of controls. Deficient animals also suffered a significant decline in liver cytochrome oxidase activity, plasma caeruloplasmin activity and copper contents of blood and liver (C. F. Mills & A. C. Dalgarno, unpublished work).

Experimental

Aortas and ligaments were rapidly dissected *post mortem*, plunged into liquid N₂ and subsequently stored at –30°C before analysis. Salt-soluble elastin was prepared by extraction of aortas or ligaments with 0.02M-sodium phosphate buffer, pH 7.2, containing 0.5M-NaCl at +5°C (Smith *et al.*, 1972). β-Aminopropionitrile fumarate (0.05%, w/v) was added to the extraction buffer as a precaution against oxidation and cross-linking *in vitro*. The extract was adjusted to pH 4.0 with 4M-acetic acid to precipitate salt-soluble collagen. The soluble elastin was then precipitated from the supernatant by coacervation at 37°C and pH 8.0 after raising the salt concentration to 1.0M and was further purified by resolution at +5°C and repeating the coacervation step. The yield of soluble elastin was negligibly small from the aortas

of all the animals, both copper-deficient and control. Soluble elastin was also absent from the ligaments of the control animals, but was readily isolated from the ligamentum nuchae of one of the animals on the copper-deficient diet. This animal had the lowest plasma amine oxidase activity of the group (0.015 unit/ml of plasma; copper-supplemented control range 0.14–0.16 unit). The ligament (300 g wet wt.) yielded 32 mg of purified soluble elastin.

Results and Discussion

Amino acid analysis

Samples of the purified protein were hydrolysed in 6M-HCl in sealed tubes at 108°C for various times. Amino acid analyses were carried out by using a Locarte amino acid analyser. The amino acid composition (Table 1) is closely similar to insoluble elastin from bovine ligamentum nuchae, except for the absence of desmosine cross-links and the higher lysine content. As with the salt-soluble elastin from lathyrctic chicks described in the preceding paper the lysine content of the soluble protein (49 residues/1000 residues) is greater than the sum of the lysine and quarter-desmosine residues found for fibrous calf elastin, which together amount to about 24 residues/1000 residues. The unaccounted proportion of lysine residues is less than was observed with chick aorta elastins but the reason for the discrepancy may be similar and may be related to the formation of coloured or fluorescent compounds which do not appear as discrete peaks under the condition of amino acid analysis. In the present work no special techniques for the quantification of amino acids present in small amounts have been attempted, and the estimates for all the amino acids have been expressed as residues/1000 residues smoothed to the nearest integer. Work with the aim of establishing stoichiometric relationships and a minimum molecular weight for an elastin repeating unit is in progress. In the present experiments, the lysine content of insoluble elastin purified from the ligament of copper-deficient

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Table 1. Amino acid composition (residues/1000 residues) of elastins from ligamentum nuchae from control and copper-deficient calves

Amino acid	Amino acid composition (residue/1000 residues)		
	Copper-deficient animal		Control animal
	Insoluble elastin	Salt-soluble elastin	Insoluble elastin
Hydroxyproline	8	8	8
Aspartic acid	7	6	5
Threonine	8	8	8
Serine	8	9	9
Glutamic acid	14	15	16
Proline	106	92	108
Glycine	319	316	327
Alanine	230	220	220
Cystine	0	0	0
Valine	154	147	154
Methionine	0	0	0
Isoleucine	26	20	24
Leucine	63	55	65
Tyrosine	6	6	8
Phenylalanine	30	32	30
Isodesmosine	2	0	2
Desmosine	2	0	2
Histidine	Trace	Trace	Trace
Hydroxylysine	0	0	0
Lysine	9	49	8
Arginine	5	5	4
Total	997	988	998

animals was close to the value obtained with the copper-supplemented controls. This contrasts with the considerably higher lysine content of insoluble elastin from other tissues in which cross-linking is sufficiently inhibited for accumulation of salt-soluble elastin to occur (Starcher *et al.*, 1964; Sykes & Partridge, 1972).

Coacervation temperature and isoelectric point

A sample of the purified protein dissolved in 10mM-acetic acid-10mM- Na_2HPO_4 was adjusted to pH 11.0 with 10M-NaOH. This solution was titrated with 6M-HCl to give the pH range required, withdrawing samples into melting-point tubes at intervals of about 0.5pH unit. Further samples were prepared in the same buffer system, but with NaCl added to give final ionic strengths of 0.1 and 1.0 with respect to Na^+ . The melting-point tubes containing the samples of soluble elastin at the appropriate pH and ionic strength were then sealed and placed in a constant-temperature water bath. The variation of coacervation temperature with pH and ionic strength, deter-

Fig. 1. Variation with pH of the coacervation temperature of salt-soluble elastin from a copper-deficient calf

For details see the text. $\square$, $I = 0.01$; $\triangle$, $I = 0.10$; $\circ$, $I = 1.0$.

mined as in the preceding paper (Sykes & Partridge, 1974), is shown in Fig. 1.

Salt-soluble elastin was focused on an LKB preparative isoelectric-focusing column (LKB, Uppsala, Sweden) by using the pH8-10 ampholine. Under these conditions the protein exhibited cationic behaviour and focused at the steeper part of the gradient, indicating an isoelectric point of approx. 10.0.

Comparison of the coacervation curves of salt-soluble elastin (Fig. 1) with those obtained by Partridge *et al.* (1955) for oxalic acid-solubilized α -elastin from bovine ligament shows a greater influence of ionic strength on coacervation temperature for the salt-soluble elastin from the same tissue. The coacervation minimum is shifted from pH 5.2, for oxalic acid-soluble elastin, to pH 10.0 for salt-soluble elastin. This shift indicates a change of isoelectric point and is to be expected since salt-soluble elastin contains an elevated lysine content compared with α -elastin.

Although comparison of the amino acid analysis of the soluble elastin with cross-linked elastin from the

same tissue and species suggests a high degree of chemical homogeneity, insufficient material was available to obtain conclusive evidence of the homogeneity of the hydrodynamic particle in solutions of buffer salts or to obtain a reliable estimate of molecular weight.

It may be significant that, although salt-soluble elastin was found in the ligamentum nuchae, the aorta of the same animal contained no detectable amounts of the protein. This was unexpected and suggests that the ligaments may be more susceptible than aorta to low dietary copper and could prove a rich source of soluble elastin under conditions where the yield from aorta is low.

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