SYNTHESIS OF SOLUBLE ELASTIN BY AORTIC MEDIAL CELLS IN CULTURE*

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SUMMARY

Incubation of a long term culture of cells derived from newborn pig aortic media with [^{3}H]proline and [^{3}H]valine yielded a labeled protein that was isolated and purified with salt-soluble elastin from copper deficient pig aorta as carrier. The labeled protein was soluble in strong neutral salt solution in the cold. It coacervated reversibly at 37° and migrated in polyacrylamide gel electrophoresis in 6M urea with the carrier protein. The valine, proline and hydroxyproline of the newly synthesized protein had high radioactivity and the ratio of activities of hydroxyproline:proline were essentially the same (1:11) as the molar ratios of these amino acids in the carrier protein (1:10.3), indicating their identity.

INTRODUCTION

Elastic tissue consists of two distinct structural components, a microfibrillar acidic protein (1) and an amorphous, extensively cross-linked protein, elastin (2). The biogenesis of elastic tissue by cells of the arterial media has been inferred by anatomical and autoradiographic evidence (3). The formation of microfibrils in explants of aorta in culture has been shown by electron microscopy, but evidence of the formation of elastin has been inadequate (4,5).

Theoretical considerations require the cellular synthesis and secretion of a soluble precursor of elastin (6). A soluble elastin has been isolated from the aortas of copper-deficient swine and characterized (7,8,9). A similar soluble elastin has been reported in the aortas of copper-deficient (10) and lathyritic chicks (11). The biosynthesis of this soluble elastin by normal pig aortic tissue *in vitro* has been recently demonstrated (12) and it is presumed, but still not proven, to be a precursor of the insoluble protein.

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With a view to studying the cellular synthesis of intermediates and the formation of cross-links in elastin, a long term culture of aortic medial cells has been established. The biosynthesis of a protein with distinctive properties of soluble elastin by these cells in culture has now been demonstrated by the isolation of a radioactively labeled component using purified soluble elastin as a carrier protein.

MATERIALS AND METHODS

The culture was initiated from cells isolated from normal newborn pig aortic media by incubation with pure collagenase and elastase. It had been maintained for approximately two years on Eagle's minimal essential medium containing 10% fetal bovine serum, penicillin and streptomycin in Falcon plastic flasks under 10% CO_2 in air when these experiments were carried out.

In Experiment I a 9 day old monolayered culture was incubated in a protein-free, modified Eagle's minimal essential medium lacking valine and supplemented with 75 µg/ml of ascorbic acid, to which was added 8 µCi/ml of L-[2,3-^{3}H]valine and L-[5-^{3}H]proline (Fig. 1). The culture was incubated for 24 hours in the presence of penicillin and streptomycin in Falcon flasks gassed with 10% CO_2 in air. All the following operations were performed at 4° unless otherwise stated. The cells were harvested, homogenized and extracted overnight in 0.5M NaCl (pH 7.2) with 0.02M Na_2HPO_4. The supernatant was separated (90,000 x g) and dialysed exhaustively against the same buffer. The dialysed cell extract was desalted on a Bio-Gel P-2 column (2.5 x 35 cm) and eluted with 0.5M NaCl (pH 7.2). The dialysed culture medium was concentrated by filtration (UM 2 Amicon membrane) and similarly desalted on a P-2 column. Effluent protein fractions were monitored for absorbance at 280 nm and for radioactivity in a scintillation counter.

The labeled proteins were combined with a clarified salt extract of copper-deficient pig aorta and soluble elastin was prepared by coacervation at 37° after precipitation of collagen as described earlier (9). Collagen was purified according to the method of Piez, et al (13). Amino acid analy-

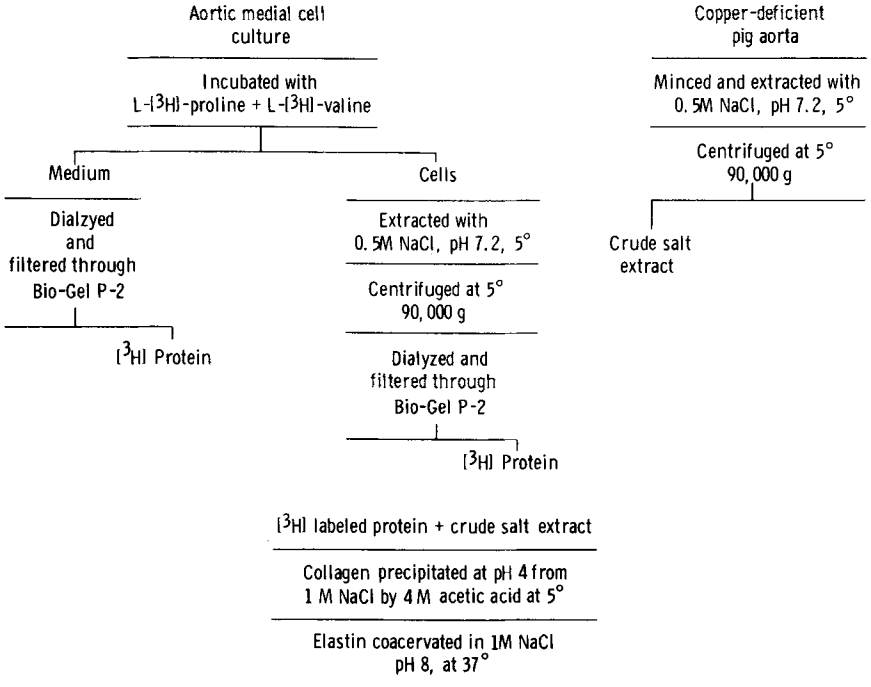

Figure 1: Preparation of labeled and carrier proteins and purification of soluble elastin.

ses were performed on a Beckman model 120B. Protein was determined by the Lowry method using α-elastin (14) as standard. Disc electrophoresis was performed on 7.5% acrylamide gels at pH 4.5 (15) in the presence of 6M urea (7). Soluble elastin was coacervated within the gels by equilibrating them with 1M NaCl (pH 8) at 37° (12). The protein in the gel slices was solubilized with H_2O_2 at 55°, the excess H_2O_2 was removed with catalase, and the radioactivity was counted in xylene-based scintillation fluid (12).

In Experiment II the 9 day old culture was incubated for 24 hours in the same modified medium to which was added 8 μCi/ml [^{3}H]valine alone. Homogenization and extraction were carried out in the presence of 0.5% 2-mercaptoethanol. Collagen was precipitated from the salt extract, containing 5 μg pepstatin[1]/g of aorta used in order to inhibit proteolysis at low pH (12,16).

[1] Pepstatin was kindly supplied by Dr. H. Umezawa, Microbial Chemistry Research Foundation and Institute of Microbial Chemistry, Tokyo.

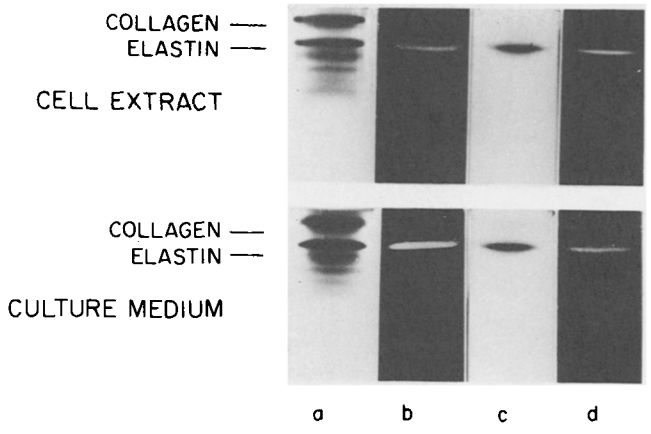

Figure 2: Disc electrophoresis of Bio-Gel P-2 filtrate of labeled culture medium and cell extract, mixed with crude salt extract of aorta (a, b), and of the purified soluble elastin prepared therefrom (c, d). The elastin band is stained with Coomassie blue (a, c) and is revealed by dark field illumination (b, d) after coacervation in the gel at 37°. The relative mobility of this band was 0.07± 0.004 under conditions of the experiment.

RESULTS

Disc electrophoresis patterns of crude salt extracts and purified soluble elastin are shown in Fig. 2. Soluble collagen (α1 and α2) and elastin bands (Fig. 2a) were identified by their mobilities (17). Coacervation in the gels at 37° showed a single light-scattering band by dark field illumination (Fig. 2b,d) that corresponds to the stained elastin band (Fig. 2a,c). All the radioactivity was confined to this band (Fig. 3). The ratio of activities of hydroxyproline:proline (1:11) in the newly synthesized protein is identical to their molar ratios (1:10.3), within limits of experimental error (Fig. 4). Table I shows the recovery of the soluble proteins and their radioactivity in Experiment II. The specific activity of the collagen exceeds that of the elastin and is higher in the cells than in the medium. A very small insoluble residue with high specific activity (1,277,000 cpm/mg) was obtained even after repeated autoclaving of the cells. The fraction solubilized by autoclaving also had high radioactivity (550,000 cpm/mg).

DISCUSSION

Circumstantial evidence that the smooth muscle cell of the aortic media

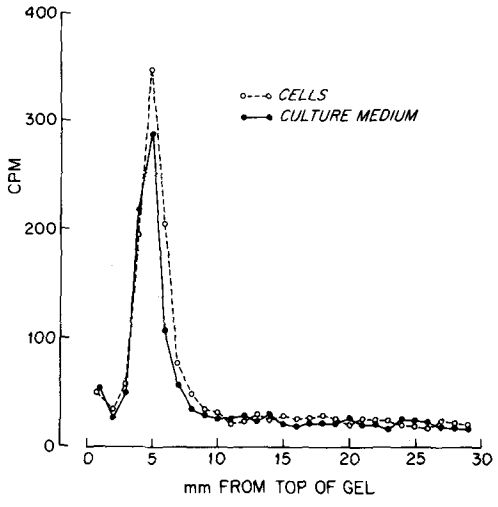

Figure 3: Radioactivity of gel slices after electrophoresis of soluble elastin isolated from mixture of crude aortic extract with culture medium and cells of labeled culture. The single peak of radioactivity corresponds to the elastin band in Figure 3.

is multipotential, synthesizing elastin and collagen as well as microfibrils, is strong. However, the fibrous forms of these proteins have not been identified with certainty in primary cultures of aortic media of guinea pigs (4) or monkeys (5). In the long-term culture of pig aortic media used here, we have also failed to identify periodic collagen fibrils or elastin by electron microscopy although abundant microfibrils are formed, and segment long spacing crystallites of collagen have been precipitated from the culture medium (M.L. Hart, unpublished).

The experiments reported here demonstrate the synthesis in an aortic medial cell culture of a protein identified as soluble elastin by its reversible coacervation, electrophoretic mobility and hydroxyproline:proline ratio. The synthesis of collagen is indicated by the precipitation of the valine label with the cold carrier collagen, although no further characterization of the labeled protein has been carried out as yet.

The high radioactivity of the insoluble residue after autoclaving suggests the synthesis of insoluble elastin also. The amount was too small to anal-

TABLE I: Distribution of radioactivity among the soluble carrier proteins mixed with the [^{3}H] valine-labeled culture.

Protein Fraction	Recovery (mg protein/g wet weight)		Radioactivity Total cpm x 10^{-2}		Radioactivity cpm/mg protein	
	Medium	Cells	Medium	Cells	Medium	Cells
Collagen	22.10	20.93	10,100	15,100	45,700	83,900
Elastin	6.48	7.88	2,200	1,500	33,900	38,000
Other	42.89	37.39	11,900	10,700	26,000	28,600

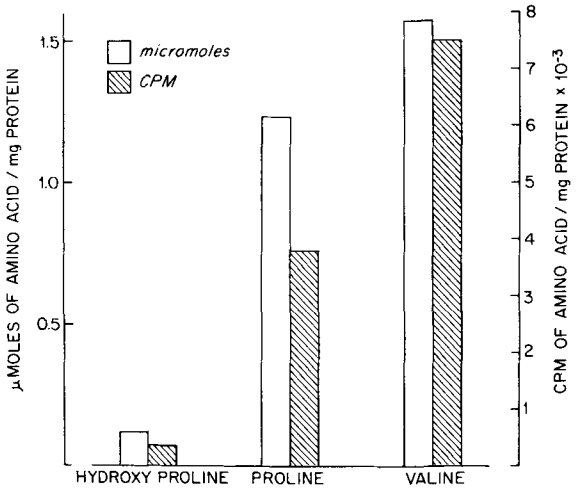

Figure 4: Specific activities of labeled amino acids in soluble elastin isolated from mixture of aortic extract with culture medium after incubation with L-[2,3-^{3}H] valine and L-[5-^{3}H] proline. The ratio of hydroxyproline:proline in the labeled protein (1:11) is similar to the molar ratio (1:10.3) of these amino acids in the soluble elastin.

yze. This recalls the binding of labeled valine to the insoluble elastin of newborn pig aorta in short term incubation (12) and again suggests the rapid cross-linkage of soluble elastin to the insoluble in the aortic medial cell systems. The nature of the linkages is under investigation.

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REFERENCES

1. Ross, R., and Bornstein, P. (1969) J. Cell Biol. 40, 366-381.
2. Partridge, S.M. (1962) Adv. Protein Chem. 17, 227-267.
3. Ross,R. (1973) J. Histochem. Cytochem. 21, 199-208.
4. Ross, R. (1971) J. Cell Biol. 50, 172-186.
5. Fisher-Dzoga, K., Jones, R.M., Vesselinovitch, D., and Wissler, R.W. (1973) Exper. Mol. Pathol. 18, 162-176.
6. Partridge, S.M. (1969) Gerontolgia 15, 85-100.
7. Smith, D.W., Weissman, N. and Carnes, W.H. (1968) Biochim. Biophys. Res. Commun. 31, 309-315.

8. Sandberg, L.B., Weissman, N. and Smith, D.W. (1969) Biochemistry 8, 2940-2945.
9. Smith, D.W., Brown, D.M., and Carnes, W.H. (1972) J. Biol. Chem. 247, 2427-2432.
10. Rucker, R.B. and Goettlich-Riemann, W. (1972) J. Nutr. 102, 563-570.
11. Sykes, B.C. and Partridge, S.M. (1972) Biochem. J. 130, 1171-1172.
12. Smith, D.W., and Carnes, W.H. (1973) J. Biol. Chem. 248, 8157-8161.
13. Piez, K.A., Eigner, E.A., and Lewis, M.S. (1963) Biochem. 2, 58-67.
14. Partridge, S.M., David, H.F., and Adair, G.S. (1955) Biochem. J. 61, 11-21.
15. Gabriel, O. (1971) Methods in Enzymology, pp. 565-585, Academic Press, New York.
16. Umezawa, H., Aoyagi, T., Morishima, H., Matsuzaki, M., Hamada, M., and Takenchi, T. (1970) J. Antibiot. 23, 259-262.
17. Rodbard, D., and Chrambach, A. (1971) Anal. Biochem. 40, 95-134.