CHICK TROPOELASTIN

ISOLATION AND PARTIAL CHEMICAL CHARACTERIZATION

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SUMMARY. A method is described for the purification of tropoelastin from the aortas of copper-deficient chicks. The final product was suitable for sequencing. The first 35 N-terminal residues are presented. The N-terminal sequence is similar in many respects to the N-terminal sequence of porcine tropoelastin. This segment probably represents one of the extendable regions in mature elastin and contains no lysine.

A native soluble elastin, often referred to as tropoelastin, has been isolated from the aortas of copper-deficient swine (1-3) and chicks fed diets either deficient in copper or containing lathrygens (3-6). During the last five to six years, considerable insight regarding the metabolism and structure of this protein has been obtained. Data pertaining to amino acid sequences in porcine tropoelastin have been published (7). From this and other information various models related to the structure of elastin have been proposed (7-10). In the present report, it is shown that tropoelastin can be isolated from chick aorta in a form suitable for physicochemical studies.

MATERIALS AND METHODS

Materials

For the purification of chick tropoelastin the thoractic aortas (cleansed of adventitial tissue) from White Leghorn Chicks were used varying in age from one to six weeks. All reagents were reagent grade and of the highest quality commercially available.

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Purification of Chick Tropoelastin

Day-old chicks were fed a copper-deficient diet based on skim milk for 15 days. The assessment of copper-deficiency was based on criteria previously reported (11). Signs of dissecting anurysms were observed in approximately 25% of the 500 birds used for each preparation. The aortas were removed by surgical excision, quickly cleansed, and frozen in liquid nitrogen. The frozen pieces were then milled through a 20-mesh screen using a Wiley Mill. The mill was chilled throughout this process with liquid nitrogen. Thirty to 35 grams of frozen milled tissue are usually obtained from 400-500 birds.

This tissue was then homogenized in a Sorvall homogenizer at full speed using 300 ml of 0.5 M acetic acid containing 1 mM EDTA, 0.5 mM N-ethylmaleimide and 0.5 mM phenylmethylsulfonyl fluoride. Three serial extractions were performed (4°). Following each extraction (4 hours), the homogenate was centrifuged at 20,000 X g for 45 min. and the pellet was resuspended in one-half the volume of the previous extraction. The three supernatant solutions (approximately 500 ml) were combined, filtered and then solid ammonium sulfate was added slowly to 50% saturation. After standing (12 hours), precipitated protein was collected by centrifugation (20,000 X g; 30 min.). This precipitate was then suspended in a solution of 0.5 M ammonium acetate previously adjusted to pH 5.0 with concentrated acetic acid (approximately 25 mg per ml). After dialysis against the same solution, a 1.5 volume of n-propanol was added dropwise at 4° with constant stirring. This addition was followed by a 2.5 volume of n-butanol, and the resulting precipitate was separated by means of a filtering flask. Protein soluble in the aqueous alcohol mixture was then evaporated to near dryness using a rotary flask evaporator. The residue which consists primarily of tropoelastin and salts was washed on a filtering flask with 50 ml of chloroform. The final rubber-like precipitate was then suspended in 0.2 M pyridine acetate buffer (pH 5.0) and dialyzed exhaustively against the same solution.

To prepare the protein samples for gel filtration chromatography the dialyzed solution was first lyophilized. Approximately 150 mg of material were usually obtained as protein. Although the material (95% elastin-like protein based on amino acid composition) could be stored at this point, the ease of subsequent dissolution for chromatography was somewhat related to the storage conditions. The protein was not easily redissolved into weak acid solutions or physiological buffers if stored for longer than several months.

Chromatography on a column (2.5 X 50 cm) of Sephadex G-150 appeared to be sufficient to obtain a relatively homogeneous preparation of tropoelastin. Usually 40-50 mg of the lyophilized protein was dissolved in 3 mls of 0.2 M pyridine acetate buffer (pH 5.0) and was applied to a column previously equilibrated with the same buffer. Protein in the eluted fractions was determined manually by the method of Lowry et al. (12). The procedure as outlined is similar to that described by Sandberg et al. (13), but with some modification.

Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis was performed using methods previously outlined (14). Sodium dodecyl sulfate was incorporated into gels. Two hundred μgram samples were applied to each gel. Tropoelastin and other proteins (albumin, ovalbumin, myosin) which were used as molecular weight markers were visualized by staining with Coomossie Blue.

SEQUENCING METHODOLOGY

Sequence determinations: About 8-9 mg of purified tropoelastin and its 4-sulfophenylthiocarbamyl derivative (15) were analyzed in the Beckman Model 890 Protein Sequencer (16). The protein double cleavage program was used and the phenylthiohydantoin derivatives of the amino acids were analyzed by gas chromatography (17), and by amino acid analyses of acid hydrolyzates (18). The COOH-terminal amino acid of tropoelastin was determined by the hydrazinolysis procedure described by Bradbury (19).

RESULTS AND DISCUSSION

A typical elution pattern from a column of Sephadex G-150 is shown in Figure 1. The major peak of protein eluting near the void volume (fractions 25-32) appeared to represent one major component of approximately 72,000 daltons based on mobility in polyacrylamide gel containing sodium dodecyl sulfate. Approximately 50 to 60 percent of the total protein applied on the column was usually recovered in this fraction. This amounted a final yield of 2-3 mg of relatively pure tropoelastin per gram of the wet aorta used as starting material. End group analysis and the fact that this material was suitable for sequencing confirmed the high degree of homogeneity.

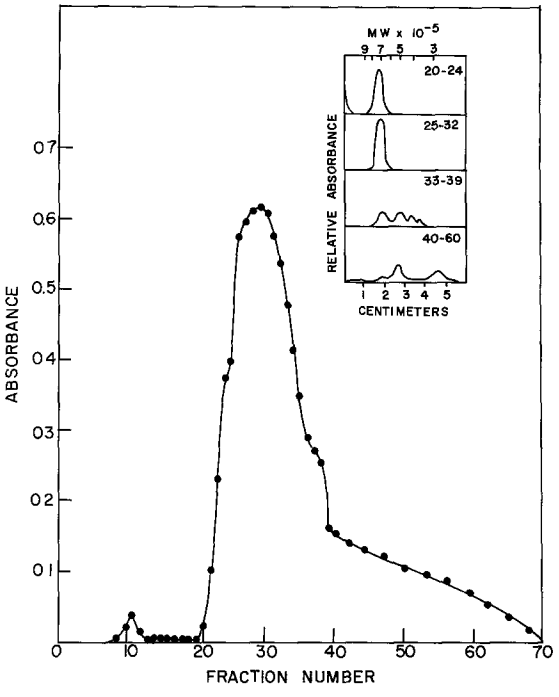

Figure 1: Elution of chick aorta tropoelastin by gel chromatography (Sephadex G-150) in a 2.5 x 50 cm column. Three milliliter fractions were collected. Protein was estimated in 0.1 ml aliquots by the method of Lowry et al. (12). The small inserts represent scans from polyacrylamide gels after staining (14). Two hundred µgram samples taken from various pooled fractions were applied to the gels. The log scale at the top corresponds to the migration of standard proteins of known molecular weight.

Electrophoretic patterns of various fractions obtained after chromatography are shown in the small insert (Figure 1). Some of the material applied appeared to migrate as components ranging in size from approximately 27,000 to 52,000 daltons (fractions 33-39 and 40-60). Although the lower molecular weight components possessed amino acid compositions similar to that for tropoelastin,

TABLE I

Automatic Protein Sequencer Results Obtained with Chick Aorta Tropoelastin

Step No.	Pth-Amino acid	Yield	Identification Method	
1	Glycine	100	GC	HYD
2	Glycine	94	GC	HYD
3	Valine	92	GC	HYD
4	Proline	86	GC	HYD
5	Glycine	82	GC	HYD
6	Alanine	81	GC	HYD
7	Isoleucine	79	GC	HYD
8	Proline	75	GC	HYD
9	Glycine	73	GC	HYD
10	Glycine	71	GC	HYD
11	Glycine	68	GC	HYD
12	Valine	66	GC	HYD
13	Proline	62	GC	HYD
14	Glycine	58	GC	HYD
15	Glycine	56	GC	HYD
16	Glycine	53	GC	HYD
17	Phenylalanine	47	GC	HYD
18	Phenylalanine	46	GC	HYD
19	Proline	44	GC	HYD
20	Glycine	41	GC	HYD
21	Alanine	40	GC	HYD
22	Glycine	37	GC	HYD
23	Valine	35	GC	HYD
24	Glycine	34	GC	HYD
25	Glycine	32	GC	HYD
26	Leucine	31	GC	HYD
27	Glycine	28	GC	HYD
28	Alanine	28	GC	HYD
29	Glycine	26	GC	HYD
30	Leucine	26	GC	HYD
31	Glycine	24	GC	HYD
32	Alanine	24	GC	HYD
33	Glycine	22	GC	HYD
34	Leucine	21	GC	HYD
35	Glycine	20	GC	HYD

these components were assumed to represent degradation products.

Some of the protein fractions obtained from the experiment summarized in Figure 1 were analyzed in the Protein Sequencer. Only the pooled fractions from tubes 25-32 were of sufficient purity to yield meaningful sequence data. The results obtained with this fraction are summarized in Table I. At each step of Edman degradation, one main amino acid phenylthiohydantoin was detected, but a 10-20% background of other amino acid phenylthiohydantoins was observed as reported for pig tropoelastin (7). The first 35 residues from the NH_2-terminal end of the chick tropoelastin were determined on the 4-sulfophenylthiocarbamyl derivative. The COOH-terminal amino acid of the chick tropoelastin was determined to be glycine by the hydrazinolysis procedure of Bradbury (19) and the yield of glycine was 80%. The NH_2-terminal sequences of the chick and pig aorta tropoelastin (7) are compared in Figure 2. The NH_2-terminal sequences of the two different tropoelastin are quite similar but an insertion of a glycine residue at position 9 in the chick tropoelastin or a deletion of glycine in the pig tropoelastin at this position was noted. In addition, a valine/isoleucine, valine/glycine and leucine/valine changes were noted at positions 7, 16, and 23. No lysine is found in this portion and very little alanine. In porcine elastin, most of the

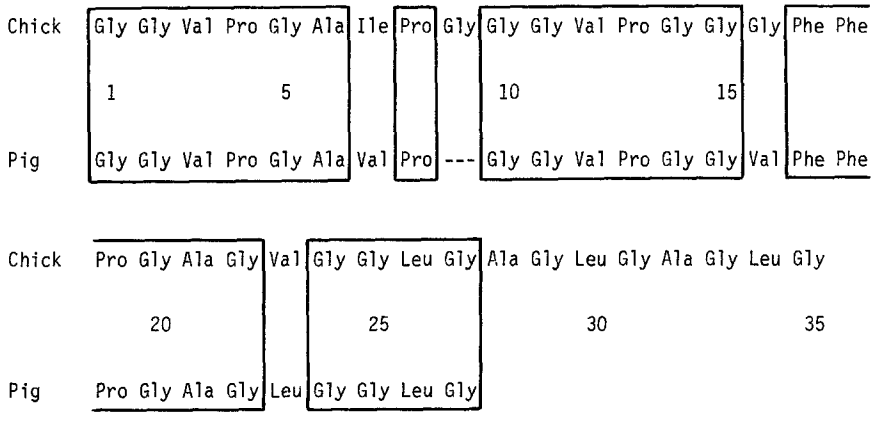

Figure 2: A comparison of the NH_2-terminal sequences of the chick and pig aorta tropoelastin. Identical residues of the two tropoelastins are blocked off.

alanine and lysine appears to be found in the crosslinking regions of the molecule (7). The N-terminal portion as sequenced probably represents one of the extensible portions of elastin.

In summary, aorta tropoelastin can be isolated from copper-deficient chicks. However, it is not a simple matter and great precautions must be taken to prevent proteolysis as well as care in handling the fresh tissue and short extraction periods are essential. Since sequence analysis is one of the best methods for checking the purity of an isolated protein and since meaningful sequence data were obtained here, the method of isolation of chick tropoelastin reported should yield tropoelastin of sufficient studies for most physicochemical studies but it must be kept in mind that 10-20% impurity is still detectable.

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