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CONNECTIVE TISSUE AMINE OXIDASE

II. PURIFICATION AND PARTIAL CHARACTERIZATION OF LYSYL OXIDASE FROM CHICK AORTA

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SUMMARY

An amine oxidase, lysyl oxidase, which catalyzes the oxidation of peptidyl lysine has been isolated from immature chicken aortas. Although insoluble in salt buffers, the enzyme was extracted from the tissue with 4 M urea. A combination of DEAE-cellulose and affinity chromatography was used to purify the enzyme to a level 2400 times the concentration found in the tissue. The enzyme catalyzed the formation of tritiated water from [6-^{3}H]lysine-labeled proteins produced by embryonic aortas grown in organ culture. Soluble protein in the culture medium as well as protein in the insoluble tissue served as substrate(s). Saturation kinetics were observed with both sources of substrate. The enzyme activity was inhibited by β-aminopropionitrile, dithiothreitol and reagents that react with carbonyl groups. Sodium dodecylsulfate acrylamide gel electrophoresis of the purified enzyme revealed two major protein bands which had estimated molecular weights of 59 000 and 61 000. At this stage of purity the enzyme contained 0.14% copper. Mild acid treatment resulted in the loss of enzyme activity and dialysis of the partially inactivated enzyme against copper restored full activity. It is concluded that aortic lysyl oxidase is a copper metalloenzyme.

INTRODUCTION

An amine oxidase catalyzes the oxidation of peptidyllysines and hydroxylysines in soluble elastin and collagen to form α-aminoadipic-δ-semialdehyde (allysine) residues and the corresponding hydroxy derivative [1–3]. Allysine in elastin undergoes condensations to form cross-link compounds such as lysinonorleucine [4], aldol condensate [5] and the desmosines [6]. Similar Schiff base and aldol condensates are

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found in collagen but the products may be hydroxylated or involve histidine residues such as aldol-histidine and histidino-hydroxymerodesmosine [3, 7, 8].

The pathology of copper deficiency [9, 10] and lathyrism [11, 12], which involves a failure of cross-linking and maturation of elastin and collagen [13–17] implicates a copper-dependent amine oxidase which is sensitive to β-aminopropionitrile. Aortic tissue contains two benzylamine oxidases, one of which is soluble in salt buffers [18] and is closely related to the copper metalloenzyme found in plasma [19]. The other can be solubilized only by use of a detergent and its properties are similar to the mitochondrial monoamine oxidases [20]. Benzylamine oxidase activity is greatly depressed in the connective tissues of copper deficient animals and correlates inversely with intermolecular cross-linking of collagen [21]. However, benzylamine oxidase activity is not highly sensitive to β-aminopropionitrile [22].

An amine oxidase which is highly sensitive to β-aminopropionitrile and which catalyzes the oxidative deamination of peptidyllysine has been extracted from embryonic chick cartilage. This enzyme has been named lysyl oxidase [23] and its assay is based on the rate of release of tritiated water from aortic protein [23] or bone collagen labeled with [6-^{3}H]lysine [24]. Attempts to purify cartilage lysyl oxidase to homogeneity have so far been unsuccessful [25].

Reported here is a procedure for purifying a lysyl oxidase from immature chicken aortas. Lysyl oxidase activity was depressed in copper deficient aortas and the purified enzyme appears to be a copper metalloenzyme. A preliminary account of this study has been presented [26].

MATERIALS AND METHODS

Purification of buffers and solvents

Concentrated stock solutions of buffers (0.5 M) and urea (8 M) were purified by passage through columns of Chelex-100 (Biorad Laboratories). The phosphate buffer was further purified by extraction with dithizone in carbon tetrachloride (0.001 %, w/v), excess chelator being removed by continued extraction with carbon tetrachloride. Stock solutions of NaCl (3 M) were extracted with dithizone only. These solutions contained less than 0.01 ppm of copper. Laboratory glassware was washed with diluted (1:1) HNO_3 and rinsed thoroughly with deionized, distilled water before use. Urea solutions were passed through an anion exchange resin to remove isocyanate ion.

Enzyme source

Freshly dissected aortas from broiler age chickens were obtained from a processing plant, minced, frozen in liquid N_2 and ground to a fine powder in a Waring blendor. 1000 aortas yielded approx. 300 g (wet weight) of material. When stored at −15 °C the powdered tissue retained full enzyme activity for at least 4 months. All procedures involving extraction and purification of the enzyme were performed at 4 °C.

Substrates

^{3}H-Labeled aortic protein was prepared by organ cultures. Four minced aortas from 18-day-old chick embryos were suspended in 2.0 ml of lysine-free Waymouth's

medium and incubated in tubes under O_2–CO_2 (95:5, v/v) at 37 °C. The medium was supplemented with 20 μCi of DL-[6-^{3}H]lysine (New England Nuclear, spec. act. 20 Ci/mM), 50 μg of β-aminopropionitrile fumarate, 50 units of pencillin G, 50 μg streptomycin sulfate, and 50 μg ascorbic acid per ml. After 72 h the tissue was removed and the media dialyzed against distilled water at 4 °C, then boiled for 8 min to inactivate enzymes. Dialysis was continued against assay buffer (0.016 M potassium phosphate, 0.12 M NaCl, pH 7.7); the preparation was finally clarified by centrifugation (105 000 $\times$ g, 60 min) and designated as soluble substrate.

Insoluble substrate was prepared from the recovered aortic tissue as follows. The tissue was homogenized in 0.15 M NaCl, (0.25 ml per aorta) and collected by centrifugation (17 300 $\times$ g, 10 min). After two washings with 0.15 M NaCl the protein was suspended in water, boiled for 8 min and centrifuged. The pellet was washed twice with assay buffer and made up in buffer so that each ml contained $2.5 \cdot 10^6$ cpm.

Assay of lysyl oxidase

The assay was performed in a tube containing 1 ml of a solution with 16 μmoles potassium phosphate, pH 7.7, and 120 μmoles NaCl. The enzyme preparation and substrate were added as indicated and the tube agitated at 37 °C for 4 h. The tritiated water formed was isolated by vacuum distillation; 0.2-ml aliquots of the distillate were dissolved in 5.0 ml of scintillation fluid [27] and counted in a Packard liquid scintillation counter (Model 3003) under conditions which gave an efficiency of 26% for tritium. Activity is expressed as cpm released when the substrate contained 250 000 cpm unless otherwise noted. Values are corrected for substrate blanks.

Chromatography

DEAE-cellulose (Whatman Inc.) was twice cycled through washes of 2 M NaOH, 2 M HCl and water. In the chloride form it was adjusted to pH 7.7 with 0.5 M phosphate buffer then equilibrated against 0.016 M potassium phosphate, 0.12 M NaCl, pH 7.7. Columns of DEAE-cellulose were packed by gravity and washed overnight with starting buffer.

Preparation of affinity gel

The activated gel was prepared as described by Cuatrecasas and Anfinsen [36]. 30 ml of Sepharose-4B were mixed with an equal volume of the buffer described above and 8 g of CNBr; pH was maintained at 11.0 by dropwise addition of 2 M KOH. The CNBr-activated gel was washed with 50 ml of cold buffer, and 5 mg of protein from the aorta culture media added. After stirring overnight in the cold unbound protein was removed by washing with buffer. Experiments with radioactive protein showed that two-thirds of the added protein was bound to the Sepharose-4B.

Analytical measurements

Protein was determined by the method of Lowry et al. [28] employing a bovine serum albumin standard. Copper analyses were performed by atomic absorption spectrophotometry using a Perkin–Elmer, Model 303. The lower limit of detection was 0.01 ppm.

RESULTS

Purification procedure

The solubility properties of lysyl oxidase in chick aorta are presented in Table I. Salt buffer solubilized less than 10% of the lysyl oxidase activity but removed nearly

TABLE I

EXTRACTION OF LYSYL OXIDASE ACTIVITY FROM CHICK AORTA

Based on 50 g of tissue. The salt buffer was 0.10 M phosphate, 0.15 M NaCl, pH 7.7, and the urea solution 0.01 M phosphate, 0.015 M NaCl, 4.0 M urea, pH 8.4. Centrifuged at 65 000 × *g*, 4 °C, for 20 min after each extraction. N.D., none detectable.

Fraction	Total activity (cpm)	Recovery (%)
Salt buffer soluble		
1st, (150 ml)	42 720	6.1
2nd, (150 ml)	0	0
4 M urea soluble		
1st, (100 ml)	445 200	63.9
2nd, (100 ml)	118 000	16.9
3rd, (100 ml)	90 600	13.0
Residue	N.D.	N.D.

one-half of the solids. Extraction with 4 M urea solubilized essentially all of the remaining activity. Based on these observations the steps outlined in Table II were used to purify the enzyme.

(*a*) *Extraction.* 50 g of frozen aorta powder were suspended in 150 ml of buffer (0.1 M potassium phosphate, 0.15 M NaCl, pH 7.7) and homogenized for 90 s in a Model 45 Virtis homogenizer. After centrifugation (65 000 × *g*, 20 min) the red supernatant solution was discarded and the residue again homogenized in 150 ml of buffer. To the twice-extracted residue were added 100 ml of cold, 4 M urea in 0.01 M potassium phosphate, 0.015 M NaCl, final pH 8.4. The suspension was homogenized for 2 min at low speed and then stirred intermittently for 2 h. After centrifugation as

TABLE II

STEPS IN THE PURIFICATION OF LYSYL OXIDASE FROM AORTA

	Total activity (cpm)*	Total protein (mg)	Spec. act. (cpm/mg)	Yield (%)	Purification (×)
(a) Urea extact**	483 000	180	2 680	100	1.0
(b) DEAE eluate	374 000	25.6	14 610	77	5.5
(c) Affinity eluate	83 000	1.8	46 390	17	17.4

* Tritium released from 250 000 cpm of substrate during 4 h incubation at 37 °C.
** 50 g of aorta powder, extracted.

above the supernatant fraction was filtered through glass wool to trap lipid material. The residue was suspended in 50 ml of cold urea buffer, dispersed by homogenization, and centrifuged. The supernatant solutions were combined, filtered through 0.8 μm Millipore filters, and dialyzed against 4 l of Buffer A (0.016 M potassium phosphate, 0.12 M NaCl, pH 7.7) with three changes over a 2-day period. After dialysis the insoluble protein was removed by centrifugation (65 000 $\times$ g, 60 min).

(*b*) *Purification on DEAE-cellulose.* Dialyzed urea extract (130 ml) containing about 1 mg of protein per ml was passed through a 5.7 cm (diameter) $\times$ 3.8 cm DEAE-cellulose column which had been equilibrated against Buffer A. The loaded column was washed with Buffer A and then with a 100-ml solution of Buffer A fortified with 0.4 M NaCl to elute non-enzymic protein. The high salt buffer was followed by Buffer A until the $A_{275\ nm}$ of the effluent fell below 0.01. The enzyme was then eluted by the passage of 6 M urea in 0.015 M potassium phosphate, 0.2 M NaCl, pH 8.4. Elution continued until $A_{275\ nm}$ was no longer detectable in the eluate. The eluate was dialyzed extensively against Buffer A. Approx. 90% of the activity applied to the column was recovered; 1% was not adsorbed, 10–15% eluted with the salt buffer and 75–80% eluted with the urea solution. Best results were achieved by use of a column with a large diameter. Use of batchwise adsorption and elution yielded a low recovery and a less pure preparation.

(*c*) *Chromatography on affinity columns.* The dialyzed eluate from the DEAE column (200 ml) was adsorbed to a 1.9 cm $\times$ 1.6 cm affinity column. The column was washed with 25 ml of Buffer A followed by a solution containing 6 M urea and 0.2 M NaCl to elute the enzyme. This fraction was dialyzed and is the enzyme preparation whose properties are described in this paper. Recovery of activity from affinity chromatography amounted to 30–60% of that applied. The enzyme did not elute with high salt buffer and was not adsorbed by Sepharose gels which did not have bound protein.

The three step purification procedure led to an enzyme preparation which had approx. 20-fold higher specific activity than the original urea extract (Table II). The yield was nearly 20% with most of the loss occurring at the affinity chromatography step.

Polyacrylamide gel electrophoresis

The purified enzyme was analyzed by use of polyacrylamide gel electrophoresis. Two prominent slow moving bands which stained with amido black were observed when the separation was effected in the presence of 8 M urea (P-2 and P-3 in Fig. 1A). Faint bands appeared above and below these major bands. In the presence of sodium dodecylsulfate two major and several minor bands appeared (Fig. 1B). Slow moving, higher molecular weight components, probably collagen species, were detectable. Using proteins of known molecular weight as standards, the molecular weights for the two major components were estimated to be 59 000 and 61 000 (Fig. 2).

Although the electrophoretic analyses show lack of homogeneity in the enzyme preparation, it is estimated that 90% or more of the total protein occurred in the two major bands. These bands may be isozymes or the sodium dodecylsulfate and 8 M urea may have dissociated the enzyme into component parts. In the latter case, the two major bands would represent heterologous subunits of a larger oligomeric protein. No data are available to relate the observed protein bands with enzymatic activity.

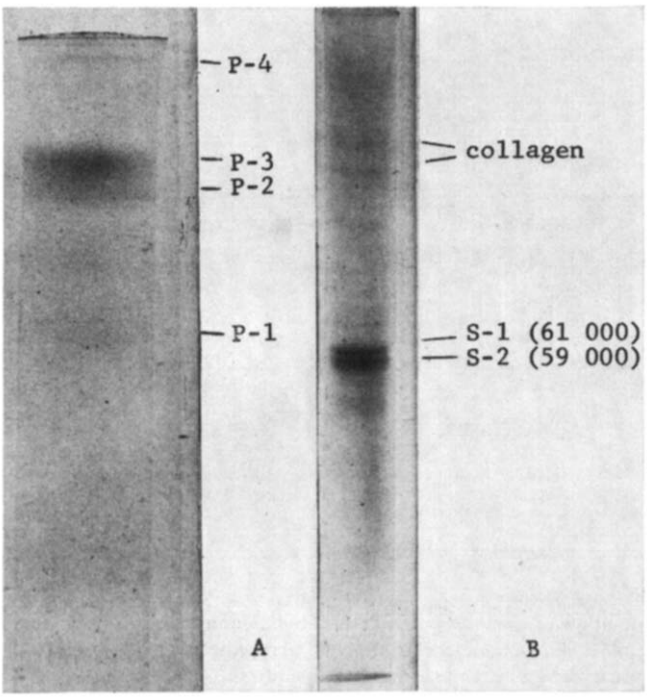

Fig. 1. Polyacrylamide electrophoretic analysis of chick aorta lysyl oxidase. Approx. 10 μg of protein with specific activity of 46 000 cpm per mg was used. The gels contained either 8 M urea (A) or sodium dodecylsulfate (B). The 8 M urea gels were prepared and run according to the method of Chrambach et al. [40]. For the sodium dodecylsulfate gels the discontinuous procedure of Neville [41] was followed. Gels were stained with 0.07% amido black in 7% acetic acid and excess stain removed by diffusion into 7% acetic acid. Estimated molecular weights shown in parentheses.

Substrates and kinetics

Purified aortic lysyl oxidase catalyzed the release of tritium from both the soluble and insoluble substrates prepared from embryonic aorta cultures (Table III). Release of tritium from both substrates was totally inhibited by 10^{-4} M β-aminopropionitrile and by boiling the enzyme for 8 min. As shown by the double reciprocal plots (Fig. 3), both the soluble and insoluble substrates showed saturation kinetics when variable amounts of labeled protein were added to a constant level of enzyme. In the case of the soluble substrate, doubling the quantity of enzyme increased V 2-fold with no change in K_m. Although not shown here, the quantity of tritiated water released in the assay with both soluble and insoluble substrates was linear with respect to time and enzyme concentration. However, the soluble substrate released more activity than the insoluble substrate when limiting concentrations (1–5 μg) of enzyme were used. This suggests that binding to the active site occurred more readily when a completely soluble system was employed.

Inhibitors

Further insight into the nature of the enzyme was afforded through the use of inhibitors. Table IV shows that carbonyl reagents at low concentrations (10 μM)

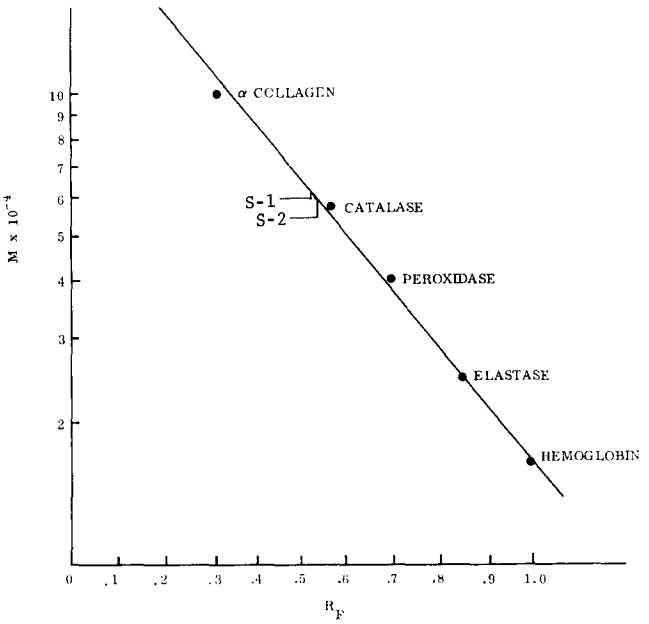

Fig. 2. Molecular weight (M) estimation of aortic lysyl oxidase(s) by sodium dodecylsulfate acrylamide gel electrophoresis. Proteins of known molecular weights were equilibrated by overnight dialysis against gel buffer and approx. 10 μg of each protein was added to the gels. The collagen was prepared from salt extracts of tendon [14]. The procedure of Neville was followed [41].

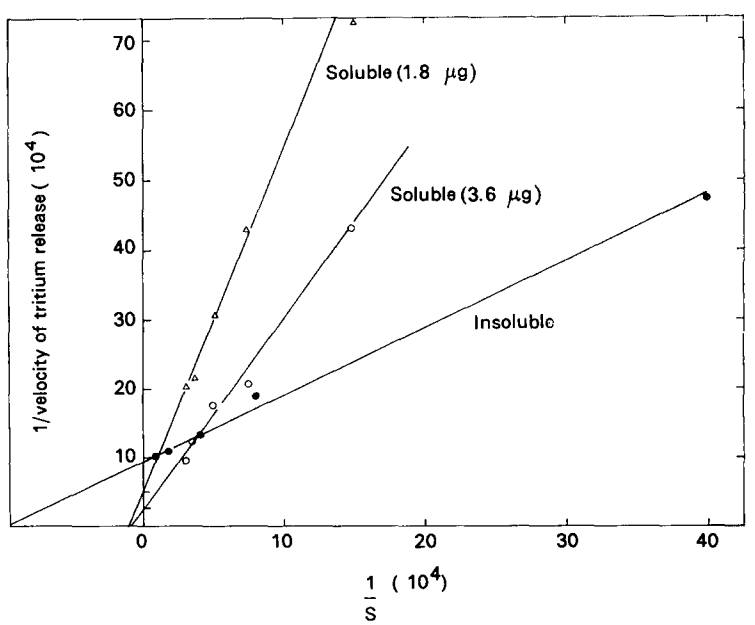

Fig. 3. Kinetics of lysyl oxidase with soluble and insoluble substrates. About 90 μg of enzyme per tube were used with the insoluble substrate and 1.8 and 3.6 μg with the soluble substrate. The double reciprocal plots involved a 50-fold range of substrate (25 000 cpm–1 250 000 cpm per ml). Velocity in cpm and substrate concentration in cpm per tube.

TABLE III

RELEASE OF TRITIATED WATER FROM [6-^{3}H]LYSINE-LABELED SOLUBLE AND INSOLUBLE AORTA PROTEIN

Additions	Efficacy of substrates*	
	Soluble (cpm)	Insoluble (cpm)
Experiment I**		
Substrate	61	106
Substrate + enzyme	3180	1800
Experiment II***		
Substrate	60	106
Substrate + β-aminopropionitrile fumarate†	65	—†††
Substrate + enzyme	610	780
Substrate + enzyme + β-aminopropionitrile fumarate	73	104
Substrate + enzyme + sodium fumarate††	—†††	740
Substrate + enzyme (boiled)	—†††	120

* The soluble substrate contained 340 000 cpm per tube; the insoluble protein 250 000 cpm.
** Enzyme preparation contained 76 μg of protein.
*** Approx. 11 μg of enzyme protein was used with the soluble and 22 μg with the insoluble substrate.
† β-Aminopropionitrile, 10^{-4} M.
†† Sodium fumarate concentration, 10^{-4} M.
††† Not determined.

effectively inhibited aortic lysyl oxidase. Unexpectedly, dithiothreitol also inhibited the enzyme. As shown in Fig. 4 inhibition by dithiothreitol was approx. 50% at 10^{-4} M and essentially complete at 10^{-2} M. It is doubtful whether thiolytic cleavage is involved in dithiothreitol inhibition since equivalent amounts of β-mercaptoethanol were only

TABLE IV

EFFECT OF INHIBITORS ON AORTA LYSYL OXIDASE ACTIVITY

Inhibitor	Concentration (M × 10^5)	Inhibition (%)
Carbonyl reagents		
Phenylhydrazine	1.0	100
Hydroxylamine	1.0	100
Semicarbazide	1.0	58
Sulfhydryl reagents		
Dithiothreitol	100.0	89
β-Mercaptoethanol	200.0	17
Chelators*		
Diethyldithiocarbamate	100.0	40
Cuprizone	1.0	9
EDTA	100.0	0

* In experiments involving chelating agents, the enzyme was dialyzed in the cold first against the indicated concentrations of chelators for 12 h and then against assay medium for an additional 12 h before assay. The other inhibitors were present during the assay.

weakly inhibitory (Table IV). Inhibition by selected chelators was determined after removal of excess reagent by dialysis. The results show that 10^{-3} M diethyldithiocarbamate decreased the activity by about one-half. Other chelators, such as cuprizone and EDTA were virtually without effect. The mechanism by which the sulfhydryl

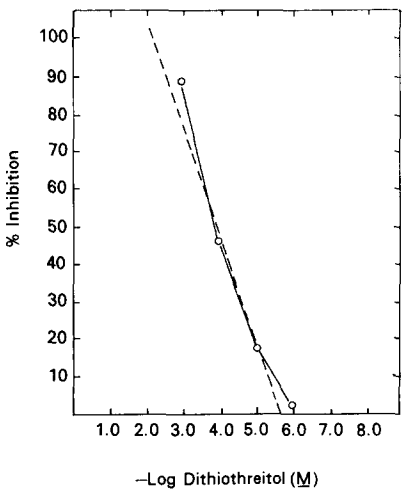

Fig. 4. Inhibition of aortic lysyl oxidase by dithiothreitol. Approx. 135 μg of enzyme was added to soluble substrate (400 000 cpm) containing the indicated amount of dithiothreitol. Percent inhibition based on blank corrected activity in absence of inhibitor.

compounds and chelating reagents effect inhibition is not established, but they may bind to an essential metal ion at the catalytic site or totally remove the metal ion. Whereas 10^{-4} M β-aminopropionitrile as the fumarate salt completely inhibits activity, sodium fumarate at this concentration was not significantly inhibitory (Table III).

Metal content of lysyl oxidase

Because of the pathology associated with copper deficiency, lysyl oxidase was predictably a copper-dependent enzyme. To establish the presence of copper in the protein, aliquots of the purified enzyme were dialyzed against metal-free phosphate buffer and copper was determined by atomic absorption analysis. An enzyme preparation which was 2500 times as active as aorta (70 320 cpm/mg protein) contained 0.14% of copper, i.e. 1 g atom per 45 000 g of enzyme. The copper to protein ratio increased linearly with increasing specific activity (Fig. 5).

The essentiality of copper for catalytic activity was also investigated. When the enzyme solution was adjusted to pH 5.1, either by addition of 10% acetic acid or dialysis against pH 5.1 buffer, a precipitate formed. This precipitate was solubilized in 6 M urea buffer and then dialyzed against Buffer A. Such mild acid treatment resulted in the loss of about one-half of the enzyme activity. Dialysis of the acid-treated, solubilized enzyme against a buffer containing Cu^{2+} and 10^{-5} M mercaptoacetate restored complete activity (Table V). Only copper restored activity; Zn^{2+} was

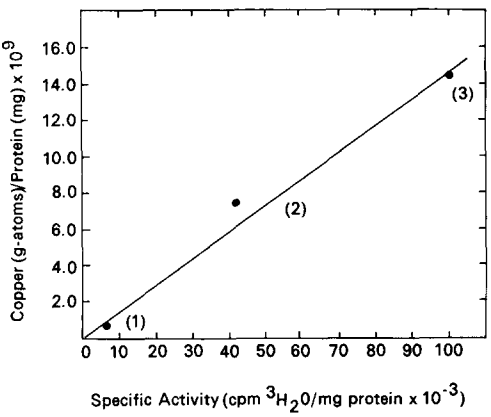

Fig. 5. Correlation of copper content with specific activity of one lysyl oxidase preparation at various stages of purification. Copper: protein ratios were determined for the extract [1], the DEAE eluate [2] and the eluate from affinity column [3].

slightly inhibitory and Fe^{2+} were without effect. These data establish the copper dependence of aortic lysyl oxidase and in fact show that it is a copper metalloenzyme. Whether or not copper is directly involved at the catalytic site is not known.

Dietary copper and lysyl oxidase

To determine whether or not the lysyl oxidase activity in aorta is dependent on dietary copper, groups of chicks were fed from hatching diets containing less than 1 ppm copper [9]. Control groups were fed a similar diet supplemented with 50 ppm Cu^{2+}. After 8 days aortas were pooled and urea extracts were examined for lysyl oxidase activity. Table VI shows that aortas from the deficient chicks contained hardly detectable activity, whereas the urea extracts of aortas from control groups showed

TABLE V

REACTIVATION OF ACID-TREATED LYSYL OXIDASE BY METAL IONS

Acid precipitation	Dialysis vs metal ion*	Activity (cpm/mg)	protein (%)
Experiment 1			
None	–	5 225	100
pH 5.1	–	2 666	51
pH 5.1	Cu^{2+}	4 164	80
Experiment 2			
None	–	15 561	100
pH 5.1	–	8 872	57
pH 5.1	Zn^{2+}	6 690	43
pH 5.1	Fe^{2+}	8 854	57
pH 5.1	Cu^{2+}	18 229	117

* Metal ions added to dialysis buffer as sulfate salts. Concentration in Exp. 1, 10^{-6} M; Exp. 2, 10^{-5} M.

TABLE VI

EFFECTS OF DIETARY COPPER DEFICIENCY ON LYSYL OXIDASE ACTIVITY IN CHICK AORTA

Group	Lysyl oxidase activity (cpm/mg protein in urea extract)	
	+Cu	−Cu
1	3438 (16)	9 (14)
2	3508 (16)	4 (16)
3	3153 (18)	9 (16)
4	2866 (19)	44 (16)
	3241 ± 147	16 ± 9

The numbers in parentheses refer to the number of pooled aortas.

activity slightly higher than that found in extracts from 2-month-old chickens. Neither the whole homogenates nor salt buffer extracts of the deficient tissue contained detectable enzyme activity. These data confirm the importance of copper for enzymatic function.

DISCUSSION

Aortic tissue from nearly mature chickens contains a lysyl oxidase, which unlike the one in embryonic chick cartilage, is not extracted by salt buffers. This property perhaps explains why lysyl oxidase has not been observed previously in tissue from more mature animals [25]. Despite its insolubility in the usual protein solvents the enzyme activity was efficiently extracted from aortic tissue with 4 M urea. Since urea disrupts hydrophobic bonding between proteins, the results suggest that the enzyme in immature chicken aorta is bound to insoluble proteins in the tissue. The lysyl oxidase found in embryonic chick cartilage and produced by fibroblasts grown in culture appears to be a soluble extracellular enzyme [29], whereas the lysyl oxidase in embryonic chick aorta has properties of an insoluble enzyme [30]. The location of lysyl oxidase in aortic tissue is unknown, but if it is extracellular, insolubility may be due to a strong association with hydrophobic proteins such as elastin.

Besides the value of high concentrations of urea for extraction of lysyl oxidase, the isolation procedure was materially facilitated by use of affinity chromatography. The affinity column procedure involved linking the soluble protein found in the culture medium to Sepharose. The rationale for use of this protein was based on the observation that protein(s) in the culture medium serve as substrate(s) for the enzyme. Affinity column purification has been used with good success to purify other enzymes from connective tissue [31].

At least one function of lysyl oxidase(s) is to catalyze the oxidative deamination of specific lysyl residues in soluble collagen and elastin molecules preliminary to the cross-linking process. Little is known about the mechanism of the reaction, its regulation or even the specific nature of the substrate. Embryonic chick aortas in culture synthesize a soluble protein which has an amino acid composition similar to that of

elastin [35]. In this study it has been observed that embryonic chick aortas synthesize and release soluble protein(s) which can function as substrate for lysyl oxidase. These proteins have not been characterized, but it is reasonable to believe that they are soluble precursors of collagen and/or elastin. Several laboratories have reported the isolation and described the structural properties of soluble collagen precursors in other culture systems [32–34].

Nutritional studies have provided information relative to the essential cofactors of lysyl oxidase. Copper deficiency results in severe pathology of the large arteries associated with extremely low levels of lysyl oxidase in the aorta. Chicks fed iproniazid show gross pathological symptoms similar to those of copper deficiency [37] and the benzylamine oxidase activity of the aorta is depressed. Iproniazid added to aorta organ cultures depresses cross-link formation and this effect is counteracted by pyridoxal phosphate [38]. Those amine oxidases which require pyridoxal phosphate as a cofactor are inhibited by carbonyl reagents. Since chick aorta lysyl oxidase is inhibited by several carbonyl reagents and cross-link formation is inhibited by iproniazid, it is probable that lysyl oxidase is dependent upon pyridoxal phosphate or a closely related compound.

A dietary deficiency of copper also lowers the activity of benzylamine oxidase in aorta [21]. It appears that only the soluble benzylamine oxidase in aorta is copper dependent (unpublished results). The structural relationship of lysyl oxidase and the soluble benzylamine oxidase of aortic tissue is not known but lysyl oxidase does not catalyze the oxidation of benzylamine. On the other hand soluble benzylamine oxidase catalyzes the oxidative deamination of lysine vasopressin [39].

Siegel et al. [25] observed that α,α'-dipyridyl at a relatively high concentration (2.5 mM) completely inactivates the lysyl oxidase of embryonic chick cartilage. Complete recovery was achieved when excess copper was incubated with the enzyme, but other ions, notably Fe^{2+}, were also active. After extensive purification aortic lysyl oxidase contains copper, the concentration increasing with purification. Removal of copper by mild acid treatment inactivates the enzyme, and activity is fully restored by dialysis against Cu^{2+}. These results strongly suggest that lysyl oxidase(s) is a copper metalloenzyme.

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