Structural and Catalytic Properties of Copper in Lysyl Oxidase*

(Received for publication, February 6, 1990)

Stephen N. Gacheru, Philip C. Trackman, Manzoor A. Shah, Coleen Y. O’Gara, Peter Spacciapoli, Frederick T. Greenaway, and Herbert M. Kagan†

From the Department of Biochemistry, Boston University School of Medicine, Boston, Massachusetts 02118 and the Department of Chemistry, Clark University, Worcester, Massachusetts 01610

The spectral and catalytic properties of the copper cofactor in highly purified bovine aortic lysyl oxidase have been examined. As isolated, various preparations of purified lysyl oxidase are associated with 5–9 loosely bound copper atoms per molecule of enzyme which are removed by dialysis against EDTA. The enzyme also contains 0.09 ± 0.10 g atom of tightly bound copper per 32-kDa monomer which is not removed by this treatment. The copper-free apoenzyme, prepared by dialysis of lysyl oxidase against α,α′-dipyridyl in 6 M urea, catalyzed neither the oxidative turnover of amine substrates nor the anaerobic production of aldehyde at levels stoichiometric with enzyme active site content, thus contrasting with the ping pong metalloenzyme. Moreover, the spectrum of the apoenzyme was not measurably perturbed upon anaerobic incubation with n-butylamine, while difference absorption bands were generated at 250 and 308 nm in the spectrum of the metalloenzyme incubated under the same conditions. A difference absorption band also developed at 300–310 nm upon anaerobic incubation of pyrroloquinoline quinone, the putative carbonyl cofactor of lysyl oxidase, with n-butylamine. Full restoration of catalytic activity occurred upon the reconstitution of the apoenzyme with 1 g atom of copper/32-kDa monomer, whereas identical treatment of the apoenzyme with divalent salts of zinc, cobalt, iron, mercury, magnesium, or cadmium failed to restore catalytic activity. The EPR spectrum of copper in lysyl oxidase is typical of the tetragonally distorted, octahedrally coordinated Cu(II) sites observed in other amine oxidases and indicates coordination by at least three nitrogen ligands. The single copper atom in the lysyl oxidase monomer is thus essential at least for the catalytic and possibly for the structural integrity of this protein.

Lysyl oxidase is a copper metalloenzyme which initiates covalent cross-linkage formation in elastin and collagen by oxidizing peptidyl lysine in these proteins to aminoadipic semialdehyde (1, 2). Removal of copper from preparations of lysyl oxidase of chick cartilage (3) or chick aorta (4) results in the loss of enzyme activity as does the exposure of the metalloenzyme to copper chelating agents (3–5). Nutritional studies have also indicated that copper deficiency reduces cross-linkage formation in vivo and thus leads to faulty connective tissue formation (6–8). Such evidence points to an essential catalytic and/or structural role for copper in this enzyme. Lysyl oxidase also contains PQQ or a PQQ-like molecule as a second cofactor at its active site (9, 10) which likely acts as a reactive carbonyl mediating electron passage from the amine substrate to the molecular oxygen substrate during catalysis.

The ability to catalyze the oxidative deamination of primary amines as well as the presence of both copper (11) and a PQQ-like carbonyl are properties shared by lysyl oxidase (9, 10), diamine oxidase (12), and plasma amine oxidase (13). While there has been considerable investigative effort directed at the copper cofactor in diamine and plasma amine oxidase, very little information is available about the nature or the role of copper in lysyl oxidase. Using highly purified bovine aorta lysyl oxidase, we describe herein the quantitative relationship between enzyme-bound copper and the expression of catalytic activity, the specificity of the catalytic reaction for the metal ion species at the active site, and the EPR parameters of the active site copper.

MATERIALS AND METHODS

Enzyme Purification and Assay—Lysyl oxidase was purified to apparent homogeneity from bovine aorta as described previously (14). Purity was assessed by the presence of a single band migrating at a position equivalent to 32 kDa by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (15). The purified preparations of lysyl oxidase consist of copurified mixtures of four ionic variants (14, 16), each of which has a molecular weight of 32,000 in sodium dodecyl sulfate. These variants exhibit a high degree of structural similarity as seen by peptide mapping of proteolytic digests of each. Moreover, substrate specificities, inhibition profiles, and steady-state kinetic parameters of each of the variants appear to be virtually the same, consistent with the operation of the same catalytic mechanism for each of these enzyme forms (16, 17).

Lysyl oxidase was assayed against an insoluble elastin substrate prepared from chick embryo aortae which had been pulsed in organ culture with [4,5-3H]lysine, as described (18). Enzyme assays included 125,000 cpm of the elastin substrate in 0.1 M sodium borate, 0.15 M sodium chloride at pH 8.0 in a total volume of 750 μl and were incubated for 2 h at 37 °C. Tritiated water formed during the incubation was isolated by vacuum distillation and quantified by liquid scintillation spectrometry of 0.5-ml aliquots of the distillates. All activities were corrected for enzyme-free controls and were within the linear range of this assay (100–1,200 cpm released per 2 h). One enzyme unit was defined as 1 cpm of 3H released by enzyme action in 2 h. Specific activities of the purified enzyme preparations used here varied from 600,000 to 900,000 units mg⁻¹. Functional active site content was quantified by comparing the specific activity of each enzyme preparation against the elastin substrate to the theoretical, maximum value of 4 × 10⁶ units mg⁻¹ previously estimated as the

* This research was supported by National Institutes of Health Grant AI13880 (to H. M. K.) and by American Heart Association, Massachusetts Affiliate, Inc., Grant 13-505-878, and National Institutes of Health Grant R15 GM42104 (to F. T. G.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Dept. of Biochemistry, Boston University School of Medicine, Boston, MA 02118.
value for the fully functional enzyme (19).

The enzyme was also assayed against n-hexylamine as the substrate for a peroxidase-coupled fluorescence method at 55 °C (20). The reaction mixtures contained 40 μg of horseradish peroxidase, 2.5 mM n-hexylamine, 0.7 mM homovanillic acid, 1.2 mM urea, 0.02 mM potassium phosphate, 0.02 M sodium borate buffer, at pH 8.1 in a total volume of 2 ml. Lysyl oxidase (2-4 μg) was added to initiate the reaction and fluorescence was continuously monitored as an excitation wavelength of 515 nm and an emission wavelength of 425 nm. Initial rates of substrate oxidation were assessed from the slopes of the tracings of fluorescence versus time, between 20 s and 3 min after the addition of enzyme. The enzyme-dependent production of hydrogen peroxide was quantified by reference to standard plots relating nanomoles of hydrogen peroxide to fluorescence units.

Removal and Addition of Metal Ions—Copper was removed from the enzyme by dialysis at 4 °C against 10 mM 2,2'-bipyridyl, 6 mM urea, 16 mM potassium phosphate, pH 7.8, followed by dialysis against 16 mM potassium phosphate to remove the urea and bipyridyl. All buffers used in the preparation or subsequent treatment of apolysyl oxidase were prepared from analytical grade reagents and distilled, deionized water. Metal ion contaminants were removed by extracting stock solutions three times with 0.1 volume of 10 mM disopropylthiocarbamate in CCl₄ (21). The extracted buffers were then freed of traces of CCl₄ by gentle aeration under a vacuum for 30 min. Glassware was treated with 20% nitric acid and then thoroughly rinsed with distilled, deionized water. Dialysis tubing was pretreated as described (22) in metal-free distilled water. The copper contents of the metallo- and apoenzyme and of solutions used for metal-free or specific metal ion conditions were assessed with a Varian model AA 10 atomic absorption spectrophotometer equipped with a graphite furnace and autosampler. Calibration curves were prepared using copper sulfate solutions containing the same concentrations of all components as the solution of enzyme with the exception of the enzyme protein. The metalloenzyme was dialyzed at 4 °C against 16 mM potassium phosphate, pH 7.8, in the presence or absence of 10 mM disodium EDTA as specified, and then against metal-free 16 mM potassium phosphate buffer to remove EDTA if necessary, in preparation for analyses of metal ion tightly bound to lysyl oxidase.

Apolysyl oxidase was reconstituted with incremental amounts of copper to assess the quantitative relationship between enzyme activity and bound copper. Aliquots (0.5 ml; 700 μg ml⁻¹) of apolysyl oxidase were incubated in triplicate with varying molar ratios of CuCl₂ in 16 mM potassium phosphate, pH 7.8, at 4 °C for 20 h with gentle agitation on an inclined rotating platform. A set of aliquots of the metalloenzyme was incubated in parallel in this buffer not supplemented with copper to serve as controls for the active enzyme activity and copper content. Each incubated aliquot was then dialyzed against 16 mM potassium phosphate, pH 7.8, containing 10 mM EDTA at 4 °C for 6 h and then against 16 mM potassium phosphate for an additional 18 h. The dialyzed enzymes were assayed for enzyme activity against elainin and for tightly bound copper as described. The metalloenzyme obtained by using metal ions to restore enzyme activity in the apoenzyme was also assessed. Aliquots of apolysyl oxidase (0.5 ml; 700 μg ml⁻¹) were individually dialyzed against 4 liters of 16 mM potassium phosphate, pH 7.8, supplemented with 0.1 mM concentrations of CuCl₂, ZnCl₂, Co(NO₃)₂, FeCl₂, HgCl₂, MgCl₂, or CdCl₂, at 4 °C for 20 h. The dialyzed enzymes were then assayed for lysyl oxidase activity.

X-band EPR spectra were obtained using a Varian E-9 spectrometer operating at 9.2 GHz with 100 kHz modulation. Temperatures between 100 and 300 K were obtained by using a stream of cooled nitrogen and a quartz dewar. The magnetic field was calibrated with a Magnicon gausmeter, and the frequency was measured by a Hewlett-Packard frequency counter. EPR spectra were accumulated with an Apple II Plus computer and a data acquisition program and interface obtained from Computerware Interface Technology, Inc., Wilmington, DE. One to four spectra were obtained and averaged for each sample, and then base-line spectra, obtained of solutions containing only buffer and appropriate concentrations of urea, were subtracted.

Copper concentrations were determined from the EPR spectra by comparison with spectra of standard solutions of copper sulfate in a 50/50 (v/v) water/glycerol solution. First moments of EPR spectra of enzyme and standard solutions were compared, after correction for differences in g value, using standard procedures previously discussed (23). Spectral data were transferred to either an IBM PC or a Digital VAX-11/150 computer and were double-integrated to provide a second measure of copper concentration. Copper(II) concentrations in enzyme samples were also determined after denaturation by first adding p-chloromercuribenzoate to the enzyme solutions to prevent reduction of Cu(II) by sulfhydryl groups and then heating the solutions to 95 °C.

The effect of the presence or absence of enzyme-bound copper on the first half reaction of lysyl oxidase-catalyzed amine oxidation was assessed spectrophotometrically with a Hewlett-Packard diode array spectrophotometer, with temperatures controlled by circulating water through the cuvette holder. Anaysis ensured the formation of p-hydroxybenzaldehyde upon the incubation of 30 μg of apo- or copper-reconstituted apolysyl oxidase (native enzyme specific activity: 880,000; 0.21 μmol of functional active site/30 μg of enzyme) with p-hydroxybenzylamine. Reaction mixtures were composed under metal-free conditions and included 2 mM p-hydroxybenzylamine in 16 mM potassium phosphate, pH 7.8. For anaerobic assays, the buffered substrate solution was contained in the main chamber and the enzyme was contained in the side-arm of an anaerobic cuvette. After alternately evacuating and flushing the cuvette with nitrogen several times, anaerobic reactions were initiated by inverting the cuvette to mix the contents of the side arm with the buffered substrate solution. The production of p-hydroxybenzaldehyde was followed by the change in absorption at 332 nm, using 19,330 M⁻¹·cm⁻¹ as the molar extinction coefficient. This value was experimentally established in these studies using authentic p-hydroxybenzaldehyde under the same solvent and temperature conditions used in these assays.

RESULTS

Copper Content of Lysyl Oxidase—Table I lists the copper contents of purified preparations of lysyl oxidase. As shown, there is approximately 1 tightly bound copper atom per 32-kDa monomer of lysyl oxidase as determined by atomic absorption spectroscopy. Tightly bound copper was also quantified from the EPR spectra, yielding an average of 0.92 ± 0.11 g atoms of Cu/enzyme monomer, in reasonable agreement with the results of atomic absorption spectroscopy. As described under “Materials and Methods,” tightly bound copper is defined as the fraction of bound copper which was not removed by dialysis of the purified enzyme against 10 mM EDTA. The ratio of copper associated with the enzyme was 7.3 ± 2.1 mol/mmol if this treatment was omitted. Nevertheless, the purified enzyme which had been dialyzed against 10 mM EDTA, as described, retained 94 ± 5% of the catalytic activity against the elastin or monoamine substrate of non-dialyzed enzyme. These results are consistent with the presence in the isolated enzyme of several moles of loosely bound copper which do not appear to be involved in the expression of enzyme activity, in addition to the 1 mol of tightly bound copper.

Preparation and Properties of the Apenzyme—Initial efforts at removing the tightly bound copper from the EDTA-dialyzed enzyme employed the method of Suzuki et al. (24) according to which the enzyme was reduced with 5 mM sodium dithionite for 30–60 min at 4 °C until catalytic activity was lost in an effort to reduce tightly bound Cu²⁺ to Cu⁺. The enzyme was then dialyzed against 10 mM NaCN in 16 mM potassium phosphate, pH 7.8, under anaerobic conditions.

<table>
<thead>
<tr>
<th>Enzyme preparation</th>
<th>Cu/enzyme (Cu atoms/32 kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.95</td>
</tr>
<tr>
<td>2</td>
<td>0.85</td>
</tr>
<tr>
<td>3</td>
<td>1.06</td>
</tr>
<tr>
<td>4</td>
<td>0.99</td>
</tr>
<tr>
<td>5</td>
<td>1.10</td>
</tr>
</tbody>
</table>

Average:
0.99 ± 0.1
continuously purging the external dialysate with high purity nitrogen. Cyanide was then removed by further dialysis against metal-free 16 mM potassium phosphate, pH 7.8. While this method had succeeded in preparing metal-free bovine serum amine oxidase (24), the same was not the case in the present study. Various preparations of lysyl oxidase treated in this fashion lost a maximum of 20% of tightly bound copper while the enzyme activity was decreased by as much as 50%. Although the basis of the greater loss of activity was not investigated in this study, this may have resulted from adventitious reduction of disulfide bonds in lysyl oxidase, the structure of which is stabilized by 3 disulfides per molecule (14). Efforts were then directed at removing the metal ion by dialyzing the EDTA-treated metalloenzyme against 16 mM potassium phosphate, pH 7.8, supplemented with 10 mM 2,2'-bipyridyl, a chelating agent with affinity for copper. These efforts succeeded in removing only 40-50% of the tightly bound copper. The inhibitory effect of 2,2'-bipyridyl on enzyme activity was then explored by preincubating the enzyme in phosphate-buffered solutions containing various concentrations of this chelator in the presence or absence of 1.2 M urea. As shown (Fig. 1), the presence of urea during the exposure to the chelating agent greatly enhances the susceptibility of lysyl oxidase to inhibition by this agent. Thus, the metal-free enzyme was successfully prepared by the procedure described under "Materials and Methods" involving the dialysis of the metalloenzyme against this bipyridyl phosphate buffer further supplemented with 6 M urea and then dialyzed against phosphate buffer to remove urea and the chelating agent. The resulting enzyme lacked measurable levels of copper as assessed by atomic absorption spectrometry and was fully inactive against elastin or n-hexylamine substrates.

While removal of copper results in the loss of enzyme activity, consistent with a cofactor role for this metal ion, it was also of interest to assess the effect of metal removal on the first half-reaction of this ping pong enzyme (25). The catalytic reaction can be considered to be the sum of two component reactions, as shown (25):

\[
\text{RCH}_2\text{NH}_2 + \text{Enz}_{\text{Cu}} \rightarrow \text{RCHO} + \text{Enz}-\text{NH}_2(\text{reduced}) \quad (1)
\]

\[
\text{Enz}-\text{NH}_2(\text{reduced}) + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{Enz}_{\text{Cu}} + \text{NH}_3 + \text{H}_2\text{O}_2. \quad (2)
\]

The amine is oxidized to the aldehyde in the first half-reaction converting the enzyme to a two electron-reduced, presumably aminated derivative in a process that does not depend on O\textsubscript{2}. The reduced, aminated enzyme is reoxidized by O\textsubscript{2} which is then released as H\textsubscript{2}O\textsubscript{2}. Steady-state kinetic analyses indicate that ammonia is released last from the enzyme (25), presumably by hydrolysis of the resulting imino enzyme. Support for this hypothesis derives in part from the prior demonstration that the amine is oxidized to the aldehyde at a level stoichiometric with enzyme active sites in the absence of oxygen (25). As shown in Fig. 2, the copper-free enzyme does not catalyze the formation of p-hydroxybenzaldehyde in the presence or absence of oxygen even at levels stoichiometric with the active sites present in the native metalloenzyme. However, the copper-reconstituted apoenzyme regains the ability to catalytically oxidize p-hydroxybenzaldehyde (Fig. 2).

Absorption Spectra—It was of interest to assess whether interactions might exist between amine substrates and apolysyl oxidase which, though noncatalytic, might be detectable by other means. Toward that end, absorption spectra of the native and copper-depleted enzymes as well as of PQQ were recorded under anaerobic conditions in the absence or presence of n-butylamine, a productive substrate of lysyl oxidase (20). As shown (Fig. 3), the spectrum of the native enzyme in the absence of the amine substrate contains bands at 270-280 nm while the corresponding spectrum of the apoenzyme exhibits slightly more distinct features at 270-285 nm as well as a shoulder at 305 nm. Otherwise, the spectra of both enzymes are apparently featureless at wavelengths up to 600 nm. The bands that are seen presumably stem from aromatic amino acids and/or the carbonyl cofactor and appear more resolved in the apoenzyme. These spectra appeared to be unchanged under anaerobic or aerobic conditions in the absence of the amine substrate. Anaerobic addition of a nitrogen-purged solution of n-butylamine to a final concentration of 10 mM resulted in distinct changes in the spectrum of the native enzyme but no significant changes to the spectrum of the apoenzyme. The difference spectrum induced upon the anaerobic addition of n-butylamine to the native enzyme displays positive bands at 300-305 nm and at 250 nm, with the former band occurring in the same spectral region as that of authentic PQQ. Additions of n-butylamine under aerobic conditions did not perturb the spectrum of the native or of the copper-depleted enzyme perceptibly (not shown).

Reconstitution Studies—The quantitative relationship between the copper content and the enzyme activity was assessed by reconstituting the apoenzyme with molar incre-
FIG. 3. Effect of n-butylamine on the absorption spectra of native and copper-depleted lysyl oxidase under anaerobic conditions. Top, copper-depleted apolysyl oxidase (ApoLO) in the absence (—) and presence (-----) of 10 mM n-butylamine. Bottom, spectra of native lysyl oxidase (CuLO) recorded in the absence (—) and at 1 min (-----) and 5 min and thereafter (-----) following the anaerobic addition of 10 mM n-butylamine. Inset, difference spectra of [(native lysyl oxidase ± n-butylamine) — (native lysyl oxidase)] (----); and of [(PQQ + n-butylamine) — (PQQ)] (-----). Spectra were obtained of enzyme or PQQ solutions at 25 °C in 16 mM potassium phosphate, pH 7.8. The apo and native enzyme concentrations were each 30 µg ml⁻¹. The concentration of PQQ was 10 µM.


The apoenzyme was also incubated with other divalent metal ions to assess the relative specificity of lysyl oxidase for copper as its metal ion cofactor. As shown in Table II, reconstitution of the apoenzyme with Cu²⁺ restored essentially full activity against elastin while corresponding treatment of the apoenzyme with 0.1 mM concentrations of divalent zinc, cobalt, iron, mercury, magnesium, or cadmium as described did not restore enzyme activity significantly. The same results were also obtained if the apoenzyme was reconstituted with 2 molar equivalents of these metals in phosphate-buffered stock solution just prior to addition of the enzyme to the assay, thus omitting the dialysis of the enzyme-metal ion mixtures prior to assay. Corresponding assays of these various preparations against the n-hexylamine substrate gave the same results (not shown).

EPR Spectra—Representative X-band EPR spectra of frozen solutions of lysyl oxidase at 110 K. Microwave power, 20 milliwatts. Spectra A and B, the enzyme was dialyzed against 10 mM EDTA followed by extensive dialysis against buffer. Spectrum C, not dialyzed against EDTA. Modulation amplitude: A, 5 G; B, 32 G; C, 32 G. Spectra have been normalized to equal heights. The relative gain settings are: A, 6.4; B, 1.0; C, 0.5. The actual gain for B was 6,300.

The spectra of the EDTA-dialyzed enzyme (Fig. 5) have g\textsubscript{II} > g\textsubscript{+} typical of the tetragonally distorted, octahedrally coordinated Cu(II) sites observed in other amine oxidases (11). Thus, all enzyme preparations were first dialyzed against 10 mM EDTA, 16 M potassium phosphate, to remove adventitious copper prior to EPR spectroscopy.

The spectra of the EDTA-dialyzed enzyme (Fig. 5) have g\textsubscript{II} > g\textsubscript{+}, typical of the tetragonally distorted, octahedrally coordinated Cu(II) sites observed in other amine oxidases (11). Thus, the copper in lysyl oxidase is Type 2 copper (27). The values observed, g\textsubscript{II} = 2.285 ± 0.0005 and A\textsubscript{Cu} = 0.0153 ±
resistant to irreversible denaturation by urea, and, in fact, are the subjects of continued investigation. Differ in these enzymes, possibly due to differences in protein conformation. Lysyl oxidase of several different tissues is significantly different line shape in the perpendicular was the finding that the removal of copper from lysyl oxidase study. Critical to the development of the present investigation high concentration of urea. As noted, we were unable to the carbonyl cofactor does not become reduced and, conceivably, might not react at all with the substrate in the absence of the copper ion may be directly involved in the binding of the residue which participates in the first half-reaction. In that amine substrate and/or in the orientation of the carbonyl as a general base in lysyl oxidase catalysis (34) and it is of some interest, therefore, that histidine imidazole nitrogens as the ultimate electron acceptor. Although the consecutive transfer of electrons should produce a superoxide intermediate, superoxide dismutase does not affect the rate of lysyl oxidase-catalyzed amine oxidation (29). However, this may simply reflect the steric inaccessibility of a putative O2 intermediate. An alternative role for copper is suggested by studies with plasma amine oxidase (30,31) which raise the possibility that the metal ion simply provides a binding site for molecular oxygen with passage of electrons from the reduced enzyme directly to the bound oxygen without an intermediary electron transport role for copper. If such were the only role(s) for copper in lysyl oxidase, it might have been expected that metal-depleted lysyl oxidase would catalyze the formation of a stoichiometric quantity of p-hydroxybenzaldehyde anaerobically or aerobically. Since that did not happen, additional possibilities must be considered. Clearly, copper might play an important structural role as it appears to do in plasma amine oxidase (32). It is also possible that a water ligand of enzyme-bound copper may be the water of hydrolysis for the cleavage of a tautomerized carbanion in which the oxidized amine is linked to the carbonyl cofactor, thus releasing the aldehyde and yielding an aminated, reduced form of the PQQ-like cofactor at the active site, presumably identical or analogues to the aminophenol formed from authentic PQQ upon its anaerobic reduction by amines (33). Indeed, it is relevant to note that the absorption band generated in the spectrum of the metalloenzyme and in that of free PQQ at 300-305 nm to note that the absorption band generated in the spectrum of the metalloenzyme and in that of free PQQ at 300-305 nm indicates that the copper cofactor. The copper cofactor is clearly essential to the expression of the first half-reaction and thus of the net catalytic reaction.

Other possible roles for copper can be envisioned. Thus, the metal ion may be directly involved in the binding of the amine substrate and/or in the orientation of the carbonyl cofactor for its optimal interaction with the amine substrate. It is also possible that the copper ion influences a functional residue which participates in the first half-reaction. In that regard, an active site histidine has recently been implicated as a general base in lysyl oxidase catalysis (34) and it is of some interest, therefore, that histidine imidazole nitrogens appear to be involved in the binding of copper to plasma amine oxidase and diamine oxidase (35). Such possibilities are the subjects of continued investigation.
As noted, the EPR spectra of the resting enzyme at neutral pH resembled those of protein-bound Cu++ . The spectra changed as the pH was lowered, consistent with an alteration of the ligand field. Moreover, the lack of reversibility of the EPR spectral features upon restoration of the sample to neutral pH is consistent with our unpublished observations that the activity of bovine aortic lysyl oxidase is irreversibly reduced upon exposure to pH 6.5 or below. This suggests a role for the bound copper in the stabilization of protein structure.

Previous studies have addressed the essentiality of copper in lysyl oxidase catalysis, although the methodologies, results, physical properties, and state of purity of the enzyme preparations used in those instances differed significantly from the present investigation. Thus, isolation and characterization of the enzyme-bound copper have been reported for an enzyme purified from bovine lung which exhibited lysyl oxidase activity against an elastin substrate and which consisted of 53,000 (major)- and 28,000 (minor)-Da protein subunits (36). However, this enzyme was remarkably resistant to β-amino-2-proprionitrile, with an (exapropal) 50% of the activity which was then restorable at neutral pH but not by Co++ or Fe++ (4), with the latter specificity consistent with the present results. This preparation exhibited two bands in sodium dodecyl sulfate at 59-kDa and 61-kDa which collectively contained 0.14% copper or 1 g atom/45 kDa (4), thus differing significantly from the bovine aortic enzyme. A saline-soluble enzyme activity was partially purified from chick embryo cartilage in the absence of urea (3). Although metal ion content was not measured, removal of metal by chelation in the absence of urea resulted in essentially complete loss of activity which could then be completely restored by Cu+++ and partially restored by Co++ and Fe++ salts (3). The copper content and the EPR spectra of enzyme-bound copper have been reported for an enzyme purified from bovine lung which exhibited lysyl oxidase activity against an elastin substrate and which consisted of 53,000 (major)- and 28,000 (minor)-Da protein subunits (36). However, this enzyme was remarkably resistant to β-amino-2-proprionitrile, with an extrapolated IC50 at 400 μM versus the more typical range of 10-50 μM (2) and thus is quite atypical of lysyl oxidase purified from several different connective tissues (2). These reports thus describe enzymes whose apparent properties differ significantly from the well-characterized, highly purified bovine aortic enzyme. Direct comparisons of the catalytic and molecular properties of highly purified preparations of these various enzymes are required to adequately resolve these apparent differences. The results of the present study thus represent the first documentation of key catalytic and physical-chemical properties of the copper cofactor in a pure preparation of lysyl oxidase with the solubility, molecular weight, and sensitivity to β-amino-2-proprionitrile which are characteristic of lysyl oxidase isolated from a variety of connective tissues of various species (16, 20, 37-39).

Note Added in Proof—It has been very recently reported that the carbonyl cofactor in bovine serum amine oxidase is not PQQ but is a residue of 2,4,5-trihydroxyphenylalanine (6-hydroxydopa), apparently derived from a tyrosine residue within the primary structure of the enzyme (Janes, S. M., Mu, D., Wemmer, D., Smith, A. J., Kaur, S., Malby, D., Burlingame, A. L., and Kliman, J. P. (1990) Science 246, 981-987). The oxidized form of this compound can exist as an o-quinone, and thus its chemical reactivity toward amines, hydrazines, and other carbonyl reagents would be expected to be quite similar to that seen with PQQ. The identity of the carbonyl cofactor in lysyl oxidase is being reinvestigated in light of this report.

REFERENCES

Structural and catalytic properties of copper in lysyl oxidase.
S N Gacheru, P C Trackman, M A Shah, C Y O’Gara, P Spacciapoli, F T Greenaway and H M Kagan


Access the most updated version of this article at http://www.jbc.org/content/265/31/19022

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/265/31/19022.full.html#ref-list-1