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CONNECTIVE TISSUE AMINE OXIDASE

I. PURIFICATION OF BOVINE AORTA AMINE OXIDASE AND ITS COMPARISON WITH PLASMA AMINE OXIDASE*

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SUMMARY

1. Bovine plasma and aorta amine oxidase (amine:O_2 oxidoreductase (deaminating), EC 1.4.3.4) were purified approx. 60- and 170-fold, respectively. The aorta enzyme appeared to be non-mitochondrial, did not catalyze the oxidation of common short chain diamines and, in general, possessed properties previously described for plasma amine oxidase.

2. In the *n*-alkylamine series, CH_3–$(CH_2)_n$–CH_2–NH_2, the longer chain homologues were bound more tenaciously by both enzymes than the short chain homologues.

3. The aorta enzyme was inhibited by chelating agents and activity could be partially restored by addition of Cu^{2+}. Activity was lost when the aorta enzyme was incubated in the presence of cyanide, hydroxylamine, semicarbazide, isoniazid and iproniazid.

4. Preparations of amine oxidase from aorta catalyzed the oxidation of peptidyl lysine when lysine–vasopressin was used as substrate, but the plasma enzyme was inactive. The polyamines, spermine and spermidine, also served as substrates for the aorta enzyme and, relative to benzylamine, they were oxidized at the same rates as when the oxidation was catalyzed by the plasma amine oxidase.

INTRODUCTION

The function and site of action of plasma amine oxidase are not clear. Although the origin of this enzyme is unknown[1,2] it clearly is not directly related to the mitochondrial amine oxidases because of the difference in general properties and substrate specificities[3–7].

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Recently, amine oxidase activity has been found in various connective tissues[8–10]. This report describes the partial purification of an amine oxidase from bovine aorta which is similar in properties to bovine plasma amine oxidase[11,12]. The enzyme in aorta catalyzes the oxidation not only of long chain monoamines and benzylamine but also of spermine and spermidine. Uniquely, aorta preparations which are rich in amine oxidase activity catalyze the conversion of peptidyl lysine to peptidyl α-aminoadipic-δ-semialdehyde. The latter is the precursor of a family of compounds which stabilize and cross link both elastin and collagen[13–18]. A preliminary report of this work has been published[19].

MATERIALS AND METHODS

Materials

Beef blood was collected as described by YAMADA AND YASUNOBU[12] and segments of aorta from yearling cattle were obtained fresh and stored (0°) for periods no longer than 7 days before use. Calcium phosphate gel was prepared according to the methods of KEILIN AND HARTREE[20]. Benzylamine, methylamine, *n*-butylamine, *n*-pentylamine, *n*-hexylamine, *n*-heptylamine, and *n*-octylamine were purchased from Eastman Organic Chemicals and Aldrich Chemical Co. These amines were prepared as the hydrochlorides and recrystallized at least twice from absolute ethanol. Spermine, spermidine, putrescine, cadaverine, histamine, tyramine and tryptamine were purchased as the hydrochlorides from Sigma Chemical Co. Amine oxidase inhibitors, carbonyl reagents and chelating agents were obtained from other commercial sources that furnished evidence of chemical purity. Lyophilyzed horseradish peroxidase (EC 1.11.1.7), purity RZ > 1, catalase (EC 1.11.1.6), and *o*-dianisidine were purchased from Worthington Biochemical Corp. and synthetic lysine–vasopressin IV (70–100 I.U./mg) from Sigma Chemical Co. All inorganic salts were reagent grade. The purity of the distilled water is indicated by the fact that it contained less than 0.02 ppm copper.

Activity of amine preparations

In the standard assay amine oxidase activity was measured by the method of TABOR *et al.*[11] with slight modification. Reaction mixtures (3.0 ml) contained 3.33 mM benzylamine and 0.1 M sodium phosphate buffer (pH 7.4). One unit of activity was defined as the amount of enzyme that catalyzed at 25° a change in absorbance at 250 nm of 0.001 per min. Calculations for benzyaldehyde production were based on a molar absorptivity of $1.20 \cdot 10^4\ M^{-1} \cdot cm^{-1}$. Specific activity of the enzyme was expressed as units/mg of protein as determined by the method of FOLIN AND CIOCALTEU[21] using bovine serum albumin as the standard.

Initial rates of H_2O_2 production were measured using the *o*-dianisidine-peroxidase assay described by MCEWEN *et al.*[22]. Standard solutions of H_2O_2 were used to relate changes in $A_{440\ nm}$ to concentration. No changes in absorbance were observed without substrates or in the presence of other compounds added to reaction mixtures. The rates of H_2O_2 production for those amines that were oxidized as well as the production of benzaldehyde from benzylamine were linear for at least 30 min. Spectrophotometric measurements were made with a Cary spectrophotometer, Model 16K, and 1-cm cuvettes. Initial rates of oxygen consumption were measured with a Yellow

Springs Instrument oxygen monitor, Model 53. Ammonia production was measured by microdiffusion from an alkaline medium by a method adapted from BESSMAN AND BESSMAN[23].

Reduction of the reaction product from lysine–vasopressin and its isolation

Lysine–vasopressin was incubated in the presence of enzyme as described below and isolated from incubation mixtures by the procedure described by BLATT[24] using an Amicon ultra-filtration device equipped with a membrane having 40 000 mol. wt. cutoff point. The filtrate, which contained the lysine–vasopressin and only traces of contaminating proteins, was adjusted to pH 9.0 with 0.1 M NaOH. A mixture of solid $NaBH_4$ (189 mg) and NaB^3H_4 (10 μC/mg) was then added to 10 ml of the solution containing approx. 6 mg of lysine–vasopressin. This reaction mixture was allowed to react for 3 h, the pH being maintained constant by the addition of small amounts of 0.01 M HCl. The reaction was terminated by the addition of 0.1 M HCl. The reduced peptide was desalted by gel filtration on Sephadex G-25 (1 cm × 20 cm)[25], concentrated by means of a flash evaporator, and hydrolyzed in 2 M NaOH for 20 h at 110°. The hydrolyzate was analyzed for amino acids by use of a Beckman 116 analyzer[26] after drying three times by flash evaporation to remove exchangeable tritium. The radioactivity in the chromatographic fractions of the lysine–vasopressin hydrolyzate was determined with a Packard liquid scintillation counter employing BRAY'S[27] solution (15 ml) as the scintillation fluid (*cf.* ref. 18).

RESULTS

Purification of plasma amine oxidase

Fresh beef blood containing 1/6 vol. of citrate solution (8 g of citric acid and 26.7 g of sodium citrate · 5.5 H_2O per l) was centrifuged at 5000 × *g* for 20 min and 6 l of plasma were collected. $(NH_4)_2SO_4$ fractionation was performed as outlined by YAMADA AND YASUNOBU[12] and the resulting precipitate dialyzed extensively against 0.05 M sodium phosphate buffer (pH 7.4). All operations were performed at 0–5° unless otherwise specified.

The dialyzed solution (750 ml) was treated with calcium phosphate gel at a ratio of 1 mg of gel to 1 mg of protein. The gel was separated by centrifugation (2000 × *g* for 2 h) and discarded. The supernatant solution was then treated twice with a quantity of gel equal to 1/4 that used originally. Over 60% of the activity remained in the supernatant fraction and was precipitated by the addition of $(NH_4)_2SO_4$ to 0.55 satn. The precipitate, which could be stored for several weeks at −20° with little loss in activity, was in some cases further fractionated by gel filtration on columns of Sephadex G-200 (1.9 cm × 30 cm). The enzyme eluted from the column near catalase and in front of dimerized serum albumin. It possessed 15% of the specific activity reported for a homogeneous preparation of the enzyme[12]. As shown in Table I, the procedure resulted in a 60-fold purification.

Purification of aorta amine oxidase

Freshly collected aortas (1000 g) were freed of adhering adipose tissue and cut into small pieces. The pieces were then immersed in liquid nitrogen and ground to a fine powder in the presence of liquid nitrogen using a stainless steel Waring blender.

TABLE I

PURIFICATION OF AMINE OXIDASE FROM BOVINE AORTA AND PLASMA

Source	*Fractionation step*	*Total protein (mg)*	*Total activity (units)***	*Specific activity****	*Yield (%)*
Blood*	1. Plasma	552 000	540 000	0.98	100
	2. $(NH_4)_2SO_4$ (0.35–0.60 satn.)	57 000	515 000	8.96	93.4
	3. Calcium phosphate gel	26 300	378 000	14.8	68.5
	4. Sephadex G-200	2 800	169 500	60.5	30.7
Aorta	1. Extract†	39 100	12 500	0.32	100
	2. $(NH_4)_2SO_4$ (0.35–0.65 satn.)	8 200	10 600	1.29	85.0
	3. 1st calcium phosphate gel adsorption (Filtrate)	1 200	6 700	5.59	53.6
	4. 2nd calcium phosphate gel adsorption (Eluate)	134	2 300	17.2	18.4
	5. Sephadex G-200	34.2	1 900	55.6	15.2

* Starting material was equal to 13 l of blood.
** $\Delta 0.001A$/min at 250 nm with 10 μmoles of benzylamine as substrate in 3 ml at 25°.
*** Units/mg protein.
† A 3–4-fold purification of the original homogenate.

The liquid nitrogen was allowed to evaporate and sufficient 0.05 M sodium phosphate buffer (pH 7.4) was added to make a 20% (w/v) suspension of aortic tissue. The material was rehomogenized for 10 min at full speed in the blender and was allowed to stand in the refrigerator overnight. This suspension was then centrifuged at 15 000 $\times$ *g* for 60 min in a Spinco Model L ultracentrifuge. In a typical purification, the soluble fraction which contained approx. 20% of the activity in the total homogenate, was adjusted to 4–5 mg protein per ml by addition of buffer. Solid was added to 0.35 satn. and the precipitate that formed was discarded. $(NH_4)_2SO_4$ was added to the supernatant fraction to 0.65 satn. and the suspension allowed to stand for 6 h. Over 90% of the activity of the original extract could be collected by centrifugation (10 000 $\times$ *g* for 30 min). The supernatant fraction was discarded and buffer was added to the precipitate to give 15–20 mg of protein per ml. This fraction is relatively stable at −20° and could be stored for several weeks. The enzyme was dialyzed against three 10-vol. changes of 0.05 M phosphate buffer (pH 7.4) and the dialyzed solution suspended in calcium phosphate gel as described above for the purification of the plasma enzyme. Following initial treatment with the gel and centrifugation, the supernatant fraction was made 0.5 satd. with $(NH_4)_2SO_4$ and centrifuged (20 000 $\times$ *g* for 20 min). After resuspension in distilled water, a second gel treatment with 6 mg of gel per mg of protein adsorbed most of the activity. The gel was collected by centrifugation and the enzyme eluted with 0.20 M sodium phosphate buffer as described by McEwen *et al.*[22]. This step was repeated and those fractions with a specific activity greater than 15 were precipitated with $(NH_4)_2SO_4$ (0.65 satn.), collected by centrifugation, and suspended in 0.05 M sodium phosphate buffer. These fractions were either further purified by elution from columns of Sephadex G-200 or used directly after extensive dialysis against 0.05 M sodium phosphate buffer (pH 7.4).

The enzyme purified from aorta was markedly less stable than the one isolated

from plasma. After the initial calcium phosphate gel treatment, the preparation lost activity at the rate of 20–40% per week when stored at −20°. Furthermore, chromatography on DEAE- or CM-cellulose columns resulted in total loss of activity although nearly all of the protein could be eluted from the columns[12]. However, the enzyme from aorta, eluted from columns of Sephadex G-200 in a manner similar to plasma amine oxidase. As shown in Table I, a typical purification of the aorta enzyme resulted in a 170-fold enrichment of amine oxidase activity.

Enzyme properties

(I) Stoichiometry of amine oxidation by aorta amine oxidase

In general, the oxidation of amine substrates by the aorta enzyme is described by the equation:

$$R\text{–}CH_2\text{–}NH_2 + H_2O + O_2 \rightarrow RCHO + NH_3 + H_2O_2$$

The rates of oxygen consumption (catalase added), ammonia liberation, and H_2O_2 production were equal to the rate at which benzylamine was converted to benzaldehyde (Table II). In the case of spermidine and hexylamine, at least, oxygen consumption and H_2O_2 production exhibited the expected stoichiometry. All preparations contained some endogenous catalase activity, but its presence did not interfere with the determination of H_2O_2 by the coupled assay using peroxidase and *o*-dianisidine.

(II) General substrate specificity

The oxidases from the two sources, bovine aorta and plasma, catalyzed the oxidation of several amine substrates at essentially the same rates relative to benzylamine (Table III). These relative rates of oxidation of amines are similar to those reported earlier for the bovine plasma enzyme[11,12]. Furthermore the enzymes did not catalyze oxidation of the polyfunctional diamines, 1,4-diaminobutane (putrescine) and 1,5-diaminopentane (cadaverine). This observation provides evidence that the aorta preparations were not significantly contaminated with diamine oxidase activity. The absence of mitochondrial amine oxidase is demonstrated, at least in part, by the

TABLE II

STOICHIOMETRY OF O_2 UPTAKE AND PRODUCTION OF H_2O_2, NH_3 AND ALDEHYDE BY AORTA AMINE OXIDASE

The rate of oxygen uptake was measured by a Yellow Springs, Model 53, oxygen monitor in the presence of added catalase (20 units). Reaction mixtures contained 20 units of monoamine oxidase (specific activity, 31), 3.0 μmoles of amine substrate in 3.0 ml of 0.1 M sodium phosphate buffer (pH 7.4). When the rate of H_2O_2 production was measured, 400 μg of horseradish peroxidase and 60 μg of *o*-dianisidine were added to reaction mixtures that contained no added catalase. Ammonia production was determined in a separate experiment in which 31 units of monoamine oxidase were used.

Substrate	*O_2 uptake (gatoms/min × 10^9)*	*Products formed (moles/min × 10^9)*		
		Aldehyde	*H_2O_2*	*NH_3*
Benzylamine	4.9	5.0	4.8	—
Benzylamine	—	7.7	—	7.3
Hexylamine	6.9	—	7.0	—
Spermidine	8.3	—	8.2	—

TABLE III

RELATIVE RATES OF OXIDATION OF VARIOUS SUBSTRATES BY PLASMA AND AORTA AMINE OXIDASE

The reaction mixtures contained 3.0 μmoles of substrate, peroxidase reagent (see Table II), and 15 units of aorta amine oxidase (specific activity, 42) or 15 units of plasma amine oxidase (specific activity, 60), respectively, in 3.0 ml of 0.1 M sodium phosphate buffer (pH 7.4). Enzyme activity was determined spectrophotometrically by use of the peroxidase couple as described by McEwen *et al.*[22].

Substrate	*Relative rates*	
	*Plasma**	*Aorta*
Monoamines		
Benzylamine	1.0	1.0
Ethylamine	0.1	0.1
Butylamine	0.7	0.7
Hexylamine	1.0	1.4
Heptylamine	1.2	1.5
Octylamine	0.9	1.0
Tyramine	0.0	0.0
Tryptamine	0.0	0.0
ε-Aminocaproic acid	0.0	0.0
Diamines		
Putrescine	0.0	0.0
Cadaverine	0.0	0.0
Histamine**	0.0	0.0
Lysine	0.0	0.0
Polyamines		
Spermine	1.7	1.9
Spermidine	1.7	1.7
Peptidyl lysine		
Porcine vasopressin	0.0	0.2

* The relative rates are in agreement with the values given by Tabor *et al.*[11], but values for *n*-alkylamines are higher than those reported by Yamada and Yasunobu[12] with crystalline preparations of the enzyme. The rate for benzylamine was 15 nmoles/min per mg.

** Some preparations of plasma monoamine oxidase showed histaminase activity but at rates of 0.04 relative to benzylamine oxidation.

fact that tyramine and tryptamine were not oxidized[3]. Furthermore, the soluble aorta preparation actively catalyzed oxidation of the polyamines, spermine and spermidine, and at the same high relative rates as the plasma enzyme, whereas these amines are not good substrates for mitochondrial amine oxidase[41].

Significantly, the aorta enzyme oxidized peptidyl lysine as judged by the peroxidase coupled reaction when lysine–vasopressin (approx. 4 mg) was added to the reaction mixtures. H_2O_2 was produced at a rate equal to 20% of that obtained with benzylamine. Since the oxidative deamination of the ε-amino group of lysine in peptide linkage would result in the formation of peptidyl α-aminoadipic-δ-semialdehyde, the production of this compound was verified by incubation of 6 mg of lysine–vasopressin in the presence of 36 units of aorta amine oxidase (specific activity, 45) for 6 h in 5 ml of 0.05 M phosphate buffer (pH 7.4). The control was treated similarly except it was filtered through an ultramembrane immediately after the

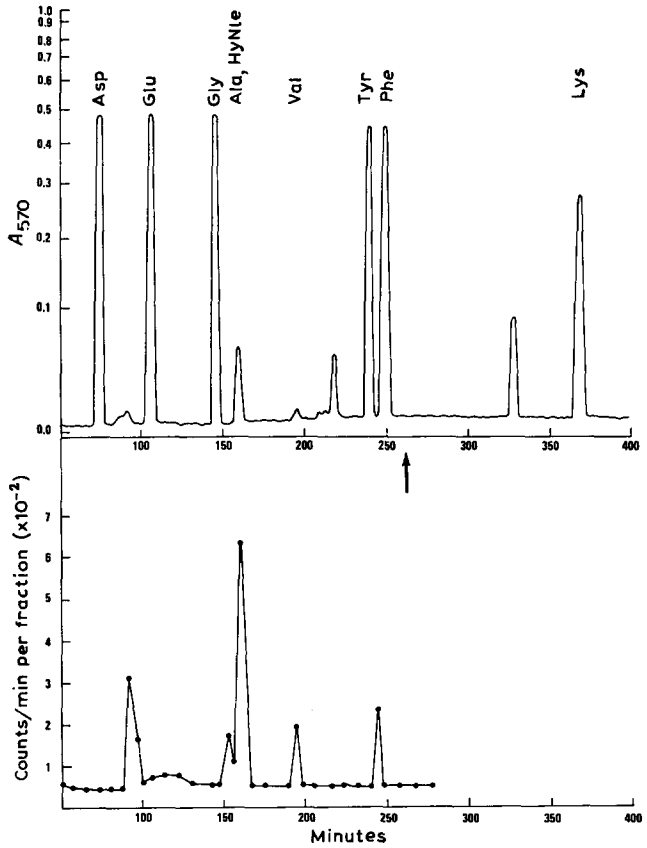

Fig. 1. Ninhydrin-positive and tritiated products from alkaline hydrolyzates of lysine–vasopressin after reduction with $NaBT_4$ and incubation in the presence of aorta amine oxidase followed by reduction with NaB^3H_4. Material corresponding to approx. 1 mg of vasopressin was applied to both the long (0.9 cm × 55 cm) and short (0.9 cm × 21 cm) columns of a Beckman 116 amino acid analyzer and developed as described by BEMSON *et al.*[26]. The arrow indicates the point of elution on the short column after the appearance of tyrosine and phenylalanine. The hydrolyzate was then rechromatographed on the long column and 5-ml fractions were collected and counted to determine tritium.

enzyme was added. When the components with molecular weights less than 40 000 were isolated from the catalyzed reaction mixture, reduced, hydrolyzed, and chromatographed (see MATERIALS AND METHODS), tritiated products were obtained as shown in Fig. 1. Ninhydrin-reactive material was found near the position of alanine. This material cochromatographed on the Dowex column with hydroxynorleucine to yield a single peak. Hydroxynorleucine is the expected reduction product of α-aminoadipic-δ-semialdehyde. Distribution of tritium in the hydrolyzed fractions indicates that most of the radioactivity (61%) appeared in the position typical of hydroxynorleucine. Of the remaining peaks, it is conceivable that the counts in the valine position of the chromatogram correspond to pipecolic acid, a condensation product which may be derived from hydroxynorleucine[18]. The counts in the area of tyrosine and between the peaks which correspond to aspartic and glutamic acids were assumed to be due to

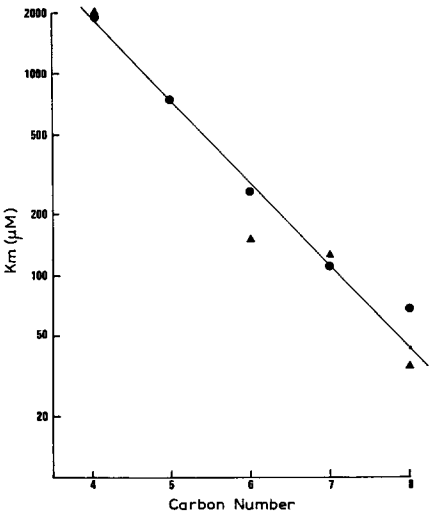

Fig. 2. Dependence of apparent Michaelis constants and inhibitor constants on the chain length of simple aliphatic amines. K_{app} values (●) and K_i values (▲) for *n*-alkylamines were derived from Lineweaver–Burk plots of initial rates of H_2O_2 production and benzaldehyde production, respectively. In the determination of K_{app} values, the reaction mixtures (3 ml) contained 25 units of monoamine oxidase (specific activity, 36); peroxidase reagent; and 300 μmoles sodium phosphate buffer (pH 7.4). Substrate concentrations were varied at least 2-fold above and 5-fold below the apparent K_m values. K_i values were determined by use of the standard assay system which contained 33 units of amine oxidase (specific activity, 52). Benzylamine was varied from 0.05 to 1 mM in the presence of the *n*-alkylamines indicated at 1 mM. All reactions were performed at 25°.

traces of tritium in tyrosine, serine and threonine that had not completely exchanged. The relative lysine content of the peptide was reduced 20–25% after enzymatic treatment compared to the control.

While the aorta enzyme actively oxidized peptidyl lysine, the plasma amine oxidase did not catalyze oxidation of this substrate. This appears to be the most significant difference in substrate specificity.

(III) The effect of carbon length on the binding of aliphatic amines

As indicated in Fig. 2 the apparent affinity of the aorta enzyme for *n*-alkylamines increased with chain length of *n*-alkylamines. Assuming that the Michaelis constant is the same as the equilibrium constant, calculation of the change in the apparent K_m and the inhibition constants (K_i) with each additional methylene residue in this series allows the conclusion that the standard free energy, ΔG, for apolar interactions at the active center of aorta amine oxidase is constant. The change in ΔG per methylene residue was estimated using the following equation[4,31]:

$$\Delta\Delta G = 2.3\ RT\ (\log K_m\ (1) - \log K_m(2))$$

where $\Delta\Delta G$ is the change in free energy per alkane methylene residue, R is the universal gas constant, T is the temperature (°K) and $K_m(1)$ and $K_m(2)$ are apparent Michaelis constants calculated for the *n*-alkylamines that differ by one methylene group. The change in the apparent Michaelis constants was assumed to reflect primarily the association due to methylene binding. The contribution from the binding

of amine functions of the various alkyl homologues was ignored since the ionization constants of the *n*-alkylamines in the range from 4 to 8 carbons in length do not vary significantly (pK_a 10.61 to pK_a 10.66). From the values in Fig. 2, $\Delta\Delta G$ was calculated to be approx −0.6 kcal/mole (see DISCUSSION). No anomalous behavior (substrate activation or inhibition) was observed within the range of substrate concentrations described in Fig. 2.

Binding of *n*-alkylamines by plasma amine oxidase was similar to that described for the aorta enzyme. Butyl-, hexyl-, and octylamine were oxidized at rates of 134, 196, and 168 nmoles/h, respectively, at 1 mM and at 14, 70, and 140 nmoles/h at 0.05 mM when 13 units of the plasma enzyme (specific activity, 15) were used. The relatively higher oxidation rates for octylamine compared to butylamine at the lower substrate concentration suggests that the apparent binding constant of the plasma enzyme for shorter chain primary amines is considerably greater than that for longer chain homologues (*cf.* ref. 2, 4, 21, 28).

(IV) Effect of pH on the rate of catalytic oxidation of benzylamine

The effect of pH on the reaction velocity was investigated over the pH range 6.8–9.0. Both plasma and aorta amine oxidase catalyzed the oxidation of benzylamine maximally at pH 7.6–7.8 (Fig. 3). Under these conditions the enzymes remained relatively stable and there was no appreciable change in pH during the course of the

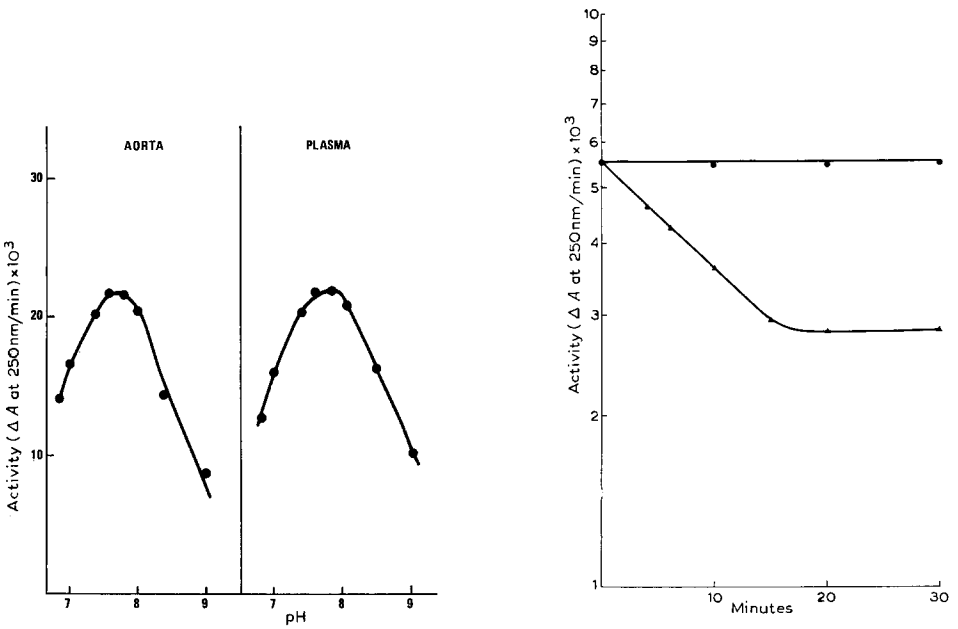

Fig. 3. Effect of pH on bovine plasma and aorta amine oxidase activity. Reaction mixtures contained 1 mM benzylamine and 20 units of enzyme in 0.1 M sodium phosphate buffer at the pH indicated and initial rates were measured at 25°.

Fig. 4. Time dependent inhibition by iproniazid. Preincubation reaction mixtures containing 5.5 units of enzyme in 2.0 ml of 0.05 M sodium phosphate buffer (pH 7.6) with (▲) and without (●) $5 \cdot 10^{-5}$ M iproniazid were maintained at 25° for the time indicated. Following this pre-incubation, 1 ml of 10 mM benzylamine in buffer was added to each reaction mixture and initial rates were determined.

reaction. The Michaelis constant for preparations of the aorta enzyme at pH 7.6 was $6.5 \cdot 10^{-4}$ M, a value somewhat lower than that observed for the preparation of plasma amine oxidase ($1.4 \cdot 10^{-3}$ M).

(V) Inhibitors of amine oxidation

Carbonyl reagents are potent inhibitors of bovine plasma amine oxidase. The data presented in Table IV indicate that the amine oxidase of bovine aorta is completely inhibited by semicarbazide and hydroxylamine at 100 μM. The classical amine oxidase inhibitors[33], isoniazid and iproniazid, inhibit aorta amine oxidase preparations to the same degree as that described previously for bovine plasma amine oxidase[34]. Inhibition with iproniazid was time dependent and appeared to follow pseudo-first-order kinetics until maximum inhibition occurred (Fig. 4).

TABLE IV

EFFECT OF INHIBITORS ON AORTA AMINE OXIDASE

The enzyme was assayed using benzylamine (1 mM) as substrate in 0.1 M sodium phosphate buffer (pH 7.4) at 25°. The inhibitors were preincubated in the presence of enzyme for the indicated time before addition of substrate (see Fig. 4). In the chelation studies, the control preparations were dialyzed against buffer (see text) for the same period.

Inhibitor	*Pre-incubation conditions*	*Concn. ($M \times 10^5$)*	*Inhibition (%)*
NaCN	10 min	100	82
Hydroxylamine	30 min	10	100
Semicarbazide	30 min	10	100
Isoniazid	30 min	10	100
Iproniazid	30 min	10	75
Ethylenediamine tetraacetate	Dialysis (12 h)	100	1
Sodium diethyl dithiocarbamate	Dialysis (12 h)	50	45
Cuprizone	Dialysis (12 h)	5	68

It is well established that bovine plasma amine oxidase is a copper-dependent enzyme[34]. The aorta enzyme (specific activity, 11) was dialyzed for 12 h against various chelating agents at the concentrations given in Table IV. The enzyme was then further dialyzed against three 20-vol. changes of 0.05 M phosphate buffer (pH 7.4). Control preparations were dialyzed in the same manner against buffer without chelating agents. After dialysis, various metals (Ni, Fe, Co, Mn, Cu, Mg) were preincubated with enzymes ($3.33 \cdot 10^{-6}$ M) before addition of the substrate. Of these, only copper significantly stimulated activity. After dialysis, 1 mg of the control aorta preparation in a reaction mixture oxidized benzylamine at the rate of 2.50 nmoles/min. Addition of copper had little effect on this rate, but increased the oxidation of preparations dialyzed against cuprizone from 0.28 to 1.37 nmoles/min and preparations dialyzed against diethyldithiocarbamate from 1.38 to 2.25 nmoles/min.

DISCUSSION

An amine oxidase has been partially purified from bovine aorta extracts approx. 150-fold. Using benzylamine as substrate, the specific activity was 1/8 that of

crystalline amine oxidase isolated from bovine plasma[12]. In general, the properties of the aorta oxidase are similar to those of plasma amine oxidase. Both oxidases catalyzed the oxidative deamination of simple aliphatic amines, benzylamine, and the polyamines, spermidine and spermine, at essentially the same relative rates and behave similarly when subjected to gel filtration. In addition, both enzymes catalyze the oxidation of benzylamine at a pH optimum (7.6–7.8) which is significantly lower than that demonstrated[4] for bovine mitochondrial amine oxidase (pH 9.2–9.4).

The active center of bovine aorta and probably plasma amine oxidase contains binding sites for both the amine function and hydrophobic moieties of the substrates. In the homologous series, CH_3–$(CH_2)_n$–CH_2–NH_2, longer chain amines are bound more tenaciously than short chain amines. From the estimated substrate and inhibitor constants of *n*-alkylamines, the determination of $\Delta\Delta G$ for the simple change in dispersion energy for methylene residues (−0.6 kcal/mole) indicates that the binding of hydrocarbon side chain of *n*-alkylamines is well within the range for simple protein–alkane interactions (−0.36 to −0.95 kcal/mole)[31,32]. Similarly, the binding of *n*-alkylamines by other amine oxidases increases with the length of the carbon chain of *n*-alkylamines[4,22,28,29].

The inability of various amine oxidases to catalyze the oxidation of short-chain diamines may be related to the nature of such hydrophobic regions. Aliphatic diamines and ω-amino acids act as substrates for some monoamine oxidases when the length of the carbon chain is at least 8–10 methylene groups[30,35]. With respect to the oxidation of lysine, 1,4-diaminobutane, 1,5-diaminopentane and ε-aminocaproic acid by the aorta enzyme, a hydrophobic binding site adjacent to the amine binding site may interact with the terminally charged functional groups of these compounds. The "transformation" of mitochondrial monoamine oxidase into an enzyme resembling diamine oxidases described by GORKIN *et al.*[36] may also be due to alterations in hydrophobic binding areas.

Aorta amine oxidase is inhibited by chelating agents which have a high affinity for copper and by reagents that are very sensitive to carbonyl groups. Similar experiments with bovine plasma amine oxidase have been used as indirect evidence that copper and pyridoxal phosphate serve as cofactors for this enzyme. With respect to the aorta enzyme, it is significant that copper partially restores activity after pretreatment with cuprizone or diethyldithiocarbamate.

The origin of plasma amine oxidase has not been established. The fact that bovine plasma aorta amine oxidase resembles aorta amine oxidase more than it does other tissue amine oxidases strongly suggests that connective tissue is the source of the plasma enzyme. The definitive physiological role of the connective tissue enzyme, which may be related to the oxidation of peptidyl lysine, is yet to be fully established. Although the aorta enzyme catalyzes the oxidation of peptidyl lysine, plasma amine oxidase does not appear to catalyze this reaction which is critical to connective tissue integrity. This discrepancy does not preclude the possibility that plasma amine oxidase represents an altered[36] or a multiple form[39,40] of the connective tissue enzyme. Even if the oxidation of peptidyl lysine is catalyzed by another oxidase found in the aorta preparations, the fact that a plasma-like amine oxidase is present in connective tissue is of interest in as much as it appears to be non-mitochondrial and catalyzes the oxidation and deamination of mono- and polyalkylamines, but not the oxidation of the common biogenic amines.

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