Properties of Rabbit Aorta Amine Oxidase (36127)

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Rucker and O'Dell (1) have recently reported that the source of plasma amine oxidase in the ruminant is connective tissue, but no information is available with regard to the source of plasma amine oxidase in other species. Since the physiological function of plasma amine oxidases is not known (1–3), such studies could further clarify the role of plasma amine oxidase in metabolism and were undertaken as reported below. The benzylamine oxidase from rabbit aorta was examined and compared to rabbit plasma amine oxidase. The amine oxidase from rabbit plasma had been previously well characterized (3). The similarities between rabbit aorta amine oxidase and the plasma enzyme support the hypothesis that connective tissue may be the source of amine oxidase in plasma.

Materials and Methods. Activity for amine oxidase was assayed using the methods of McEwen *et al.* (3) and Tabor *et al.* (5) with slight modification (1). When crude homogenates were used, assay mixtures consisted of 1 ml of enzyme preparation and 2 ml of 0.15 *M* phosphate buffer (pH 7.6) containing 10 μmoles of benzylamine. The preparations were incubated at 25° and the reaction was stopped by the addition of 0.1 ml of perchloric acid. The benzaldehyde which was formed during incubation was extracted with cyclohexane (3 ml) and measured at 242 nm (2). Soluble protein preparations with specific activities greater than 0.5 (Table I) could be measured directly. In this case, the production of benzaldehyde was determined (5) at 250 nm (ϵ, $1.20 \times 10^4\ M^{-1}\ cm^{-1}$).

Specific activity is defined as units per milligram of protein (6), where one unit is equal to the amount of enzyme that catalyzes a change in absorbance at 250 nm of 0.001 per min. Absorbance in cyclohexane at 242 nm is 4.2 times that in buffer when determined at 250 nm (2). For all other amine compounds, initial rates of H_2O_2 production were measured using an *o*-dianisidine–peroxidase assay system [Fig. 1, Ref. (3)]. Standard solutions of H_2O_2 were used to relate changes in absorbance at 440 nm to concentration. No changes in absorbance were observed without substrates or in the presence of other compounds added to reaction mixtures. All rates of benzaldehyde and H_2O_2 production were linear for at least 30 min and proportional to enzyme concentration.

Ten to 20 aortas from mature rabbits were used in the enzyme preparations. The aortas were removed and cleansed of adhering adi-

TABLE I. Purification of Rabbit Aorta Amine Oxidase.

Step	Volume (ml)	Units	Protein (mg)	Sp act	Yield (%)
1. Supernatant fraction[a] (15,000 *g*, 20 min)	150	270	660	0.41	100
2. $(NH_4)_2SO_4$ fractionation (0.65 satn)	50	250	225	1.10	93
3. $(NH_4)_2SO_4$ extraction (0.40 satn)	30	232	96	2.42	86
4. Calcium phosphate gel	8	152	28	5.45	56

[a] The initial homogenate contained 40 g of fresh rabbit aorta tissue plus 160 ml of 0.05 *M* sodium phosphate buffer (pH 7.6). The total activity in the homogenate equaled 1480 units of activity using benzylamine as substrate.

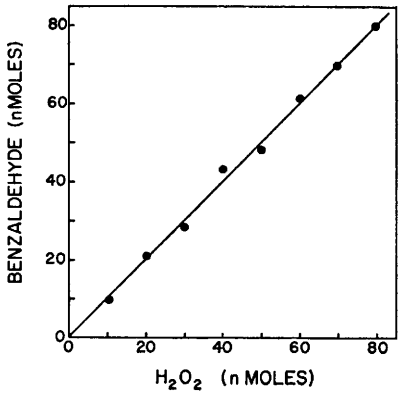

FIG. 1. The stoichiometry of H_2O_2 and benzaldehyde production from the oxidation of benzylamine: The reaction mixture (1.5 ml) contained 10 units of amine oxidase (sp. act 5.45), 150 μmoles of sodium phosphate buffer (pH 7.8), and 5 μmoles of benzylamine. The production of benzaldehyde was measured at 250 nm (4). The production of peroxide was measured at 440 nm by the addition of 220 μg of peroxidase (RZ >1) and 30 μg of *o*-dianisidine (3).

pose tissue. The aortas were then immersed in liquid nitrogen and ground to a fine powder using a stainless steel blender. The liquid nitrogen was evaporated; and 0.05 *M* sodium phosphate buffer (pH 7.4) was added to make a 20% (w/v) suspension of aortic tissue. This suspension was briefly rehomogenized and allowed to stand 6 to 8 hr (1). Then the material was centrifuged at 10,000*g* for 20 min. In a typical purification, the soluble fraction contained approximately 20% of the activity in the total homogenate. To this solution (3–4 mg of protein/ml), solid ammonium sulfate was added to 0.65 saturation. The precipitate which formed was separated by centrifugation; and the supernatant fraction was discarded. The precipitate was then extracted with a solution of ammonium sulfate at 0.4 saturation (pH 7.4). Proteins, soluble at this concentration of ammonium sulfate, were removed by centrifugation; and their solution was dialyzed exhaustively against 0.02 *M* sodium phosphate buffer (pH 7.4). To the dialyzed solution, a calcium phosphate gel suspension (5) was added at 6 mg of gel/mg of protein. Most of the enzyme activity (> 80%) adhered to the gel. The gel was collected by centrifugation (2000*g*, 15 min) and protein fractions were then obtained by elution with solutions of 0.05 *M* phosphate buffer, 0.1 *M* phosphate buffer, and 0.1 *M* phosphate plus 0.1% Triton (pH 7.6). Fractions with the greatest specific activities were obtained in the last step. All operations were performed at 4–8°. A summary of the purification is given in Table I.

Results and Discussion. Although most of the amine oxidase activity remained in particulate fractions, a substantial amount of the activity could be solubilized (Table I). The technique of ammonium sulfate extraction (step 4, Table I) significantly shortened the purification process. The enzyme was purified 20-fold with 50% yield. The rabbit aorta enzyme preparations, like those described for bovine aorta (1), lost activity at the rate of 30%/week. With preparations of specific activities equal to 4 or greater, peroxide production was equal to the production of benzaldehyde when benzylamine was used as substrate (Fig. 1). In this regard, the general stoichiometry was assumed to be that described by the equation, $R\text{–}CH_2\text{–}NH_2 + H_2O + O_2 \rightarrow R\text{–}CHO + NH_3 + H_2O_2$, *i.e.*, typical of other amine: O_2 oxidoreductases-deaminating (7, 8).

The enzyme from rabbit aorta catalyzed the oxidation of amine substrates (Table II) at approximately the same relative rates as those reported by McEwen *et al.* (3) for rabbit plasma amine oxidase. Like rabbit plasma, amine oxidase, benzylamine, tyramine, and tryptamine were actively oxidized. Histamine was oxidized at a distinctly lower rate. Butylamine was the most actively oxidized of the *n*-monoalkylamines tested (Fig. 3). Of the alkyldiamines tested, only 1, 8-diaminooctane was oxidized (Table II). Polyamines, such as spermine and spermidine, did not serve as substrates. The fact that putrescine (1,4-diaminobutane) and cadaverine (1,5-diaminopentane) were not substrates indicate that the preparations were free of diamine oxidase activity (8). In addition, the aorta enzyme preparations did not significantly oxidize *N*-methylbenzylamine, indicating that preparations were also essentially free of mitochondrial amine oxidase

TABLE II. Substrate Specificity of Rabbit Aorta Amine Oxidase.

Substrate	Relative rate[a]
Monoamines	
Benzylamine	1.00
2-Chlorobenzylamine	0.08
4-Chlorobenzylamine	0.28
Serotonin	0.03
Tryptamine	0.30
Tyramine	0.47
N-Methylbenzylamine	0.05
Diamines	
1,4-Diaminobutane	0.00
1,5-Diaminopentane	0.00
1,6-Diaminohexane	0.00
1,8-Diaminooctane	0.10
Histamine	0.19
Polyamines	
Spermine	0.00
Spermidine	0.00

[a] Five units of amine oxidase plus substrates were added to reaction mixtures (see Fig. 1). Rates of peroxide formation are relative to the rate observed when benzylamine is added as substrate all substrates were present in reaction mixtures at 1 m*M*.

which has specificity for secondary and tertiary amines (3, 7, 8).

Identical to the pH optimum reported for the rabbit plasma enzyme (3), the pH optimum for rabbit aorta amine oxidase was found to be pH 7.8–8.2 with benzylamine as substrate (Fig. 2). The Michaelis constant derived from Lineweaver-Burk plots [cited in Ref. (8)] for benzylamine at pH 7.8 was 0.09 m*M*. This value is in the range usually reported for most plasma and other connective tissue amine oxidases (1, 3, 5, 8–11). Furthermore, the enzyme appeared to be sensitive to various agents which attack carbonyl functions. Cyanide, hydroxylamine, semicarbazide, isoniazid, and iproniazid inhibited benzylamine oxidation (Table III). The majority of the amine oxidase activity in other connective tissues (1, 10, 11) and plasmas (7, 8, 12) has been found to be inhibited by carbonyl reagents. Particulate amine oxidases from liver and kidney are not carbonyl sensitive (8, 12, 13). In keeping with these reports the inhibitor data with respect to rabbit aorta amine oxidase add additional support to the general hypothesis that plasma and connective tissue amine oxidases are interrelated.

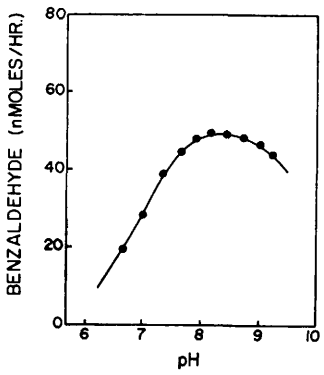

FIG. 2. Relationship of velocity of pH with benzylamine as substrate: Reaction mixtures (1.5 ml) contained 0.15 μmoles of benzylamine, 3 units of aorta amine oxidase, and 150 μmoles of phosphate buffer.

Summary. An amine oxidase from rabbit aorta has been purified 20-fold. The enzyme possesses a substrate specificity pattern very similar to that found in rabbit plasma. The enzyme actively catalyzes the oxidation of alkylamines, benzylamine, tyramine, and tryptamine. The pH optimum with respect to benzylamine oxidation is pH 7.9. The en-

TABLE III. Inhibition of Rabbit Aorta Amine Oxidase.[a]

Inhibitor	Concentration ($M \times 10^3$)	Inhibition (%)
Cyanide	0.1	48
	1.0	92
Hydroxylamine	0.1	25
	1.0	68
Semicarbazide	0.1	46
	1.0	92
Isoniazid	0.1	40
	1.0	64
Iproniazid	0.1	32
	1.0	75

[a] Assay mixtures (1.5 ml) contained 5 units of amine oxidase, 1.5 μmoles of benzylamine, 150 μmoles of sodium phosphate buffer (pH 7.6), and inhibitors as indicated. The enzyme was incubated 15 min in the presence of the inhibitor before addition of substrate.

zyme is inhibited by a variety of agents which are known to attack carbonyl functions.

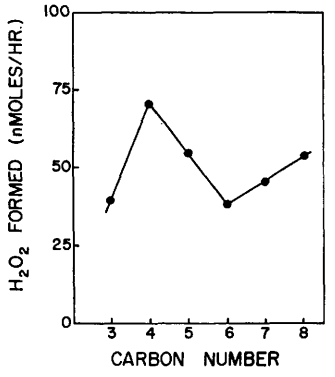

FIG. 3. Effect of chain length on the oxidation of *n*-alkylamines: Amine oxidase (5 units, sp act 5.45) was incubated at 25° in 1.5 ml of 0.1 *M* sodium phosphate buffer (pH 7.8), containing 200 μg of peroxidase, 30 μg of *o*-dianisidine, and substrates (1 m*M*).

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