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PURIFICATION AND PROPERTIES OF AN AMINE OXIDASE IN BOVINE DENTAL PULP AND ITS COMPARISON WITH SERUM AMINE OXIDASE

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SUMMARY

An amine oxidase (EC 1.4.3.4) was purified approx. 115-fold from the soluble fraction of bovine dental pulp (which is a pure connective tissue), by $(NH_4)_2SO_4$ fractionation (40–80% saturation), and DEAE-cellulose and Sephadex G-200 chromatographies. Bovine serum amine oxidase was also purified to the same extent, and the properties of both amine oxidases were compared. The dental pulp amine oxidase had an optimum pH between 8 and 9 and an approximate mol. wt. of 170 000. The dental pulp enzyme was inhibited by cuprizone, *p*-chloromercuribenzoate, iproniazid, β-aminopropionitrile, and lysine–vasopressin. The inhibition by iproniazid was reversed by the addition of pyridoxal phosphate. The dental pulp enzyme oxidized preferentially polyamines (spermine and spermidine), benzylamine, and peptidyllysine such as lysine–vasopressin and prolyllysylglycinamide, but did not oxidize collagen and elastin substrates. The properties of bovine serum amine oxidase were similar to those of dental pulp amine oxidase, but the serum enzyme was almost inactive towards lysine–vasopressin.

INTRODUCTION

Amine oxidase (EC 1.4.3.4) activity has been found in various connective tissues, such as bone [1], aorta [2–4], skin [5], and dental pulp [6, 7]. The properties of these amine oxidases are similar to those of plasma amine oxidase [8–11], and they deaminate benzylamine, and polyamines such as spermidine and spermine. However, the relationship between amine oxidases in connective tissues and plasma amine oxidase is unknown. The amine oxidase in the aorta also deaminated peptidyllysine in lysine–vasopressin [4], indicating the possibility of deamination of peptidyllysine in collagen or elastin, to produce the peptidyl α-aminoadipic-δ-semialdehyde which is supposed to participate in the cross-linking reaction of collagen or elastin [12].

On the other hand, Martin et al. [13–16] discovered a new amine oxidase in cartilage, lysyl oxidase, which deaminates peptidyllysine in collagen or elastin. This enzyme is supposed to participate specifically in the cross-linking formation of

collagen and elastin and may not deaminate benzylamine. Plasma amine oxidase did not oxidize peptidyllysine in collagen and elastin substrates [16].

In order to know the similarity and dissimilarity between amine oxidases in connective tissues, plasma oxidase, and lysyl oxidase in cartilage, we have purified an amine oxidase from bovine dental pulp which is a pure connective tissue, and the enzyme properties have been examined. Bovine serum amine oxidase was also purified for comparison. Their substrate specificities have been compared using benzylamine, synthetic peptidyllysine substrates, and natural collagen or elastin substrates. The results indicate that the dental pulp amine oxidase is similar to but different from serum amine oxidase [8–11], but may be a distinct enzyme from lysyl oxidase in cartilage [13–15].

MATERIALS AND METHODS

Lysine-vasopressin (synthetic) was obtained from Sigma. Prolyllysylglycinamide was kindly synthesized by Dr S. Sakakibara and his collaborators in the Protein Research Foundation (Osaka). The [6-^{3}H]lysylelastin substrate was kindly supplied by Dr G. R. Martin (National Institute of Dental Research, Bethesda). The [6-^{3}H]lysylcollagen substrate was prepared from the calvaria of 17-day-old chick embryos in our laboratory by Dr K. Shimozato, using the method of Siegel and Martin [15].

Bovine dental pulp and bovine blood were obtained fresh from the slaughterhouse. The dental pulp was stored frozen at −20 °C. The serum was separated and also stored at −20 °C.

Benzylamine was used as substrate throughout the enzyme purification, and the enzyme activity was measured spectrophotometrically by the formation of benzaldehyde from benzylamine [10, 11], as described before [6]. The incubation mixture (final volume 1.5 ml) contained: phosphate buffer (pH 7.4), 75 μmoles; benzylamine, 1.2 μmoles; and enzyme (0.1–0.2 munit). Incubation was carried out for 60 min at 37 °C under shaking in air. As a blank, enzyme was omitted during the incubation, and added after the incubation. 1 munit of enzyme activity was defined as the amount of enzyme catalyzing the formation of 1 nmole of benzaldehyde per min at 37 °C. The benzaldehyde formed was calculated based on the molar absorptivity of benzaldehyde ($1.2 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$), as described by McEwen [11]. A change in absorbance of 0.017 per min at 242 nm in cuvettes of 10-mm light path was calculated to be equivalent to 1 nmole of benzaldehyde formed.

When the substrate specificity was examined using various substrates, the enzyme activity was measured spectrofluorimetrically by the formation of H_2O_2 according to the method of Guilbault et al. [17]. The incubation mixture contained: 0.3 M phosphate buffer (pH 7.4), 0.9 ml; horseradish peroxidase (1 mg/ml), 0.2 ml; *p*-hydroxyphenylacetic acid (2.5 mg/ml), 0.1 ml; 8 mM substrate, 0.3 ml; enzyme (about 2 munits); and water to 3.0 ml. The enzyme was omitted for the blank. Incubation was carried out for 10–60 min at 37 °C and the fluorescence of the condensation product of *p*-hydroxyphenylacetic acid was assayed at 414 nm with the excitation at 317 nm.

When kynuramine was used as substrate the fluorescence of the product 4-hydroxyquinoline, was determined, as described before [18]. The incubation mixture

contained: 0.4 M phosphate buffer (pH 7.4), 0.25 ml; 1 mM kynuramine, 0.25 ml; enzyme (about 0.03 munit); and water to 1.5 ml. Incubation was carried out at 37 °C for 60 min.

When [6-^{3}H]lysylelastin and collagen substrates were used, the ^{3}H released following the deamination of peptidyllysine was isolated by vacuum distillation and counted in a liquid scintillation counter, as described by Siegel et al. [14]. The incubation mixture contained: the labeled elastin or collagen substrate, 0.7 ml (600 000 cpm); 0.3 M phosphate buffer (pH 7.4), 0.3 ml; the enzyme (4.8 munits either for dental pulp or serum amine oxidase); one drop of toluene; and water to 1.5 ml. For the blank incubation, 100 μg of β-aminopropionitrile were added. Incubation was generally carried out at 37 °C for 16 h.

The approximate molecular weight was determined by gel filtration on Sephadex G-200 according to the method of Whitaker [19]. The molecular weights of ovalbumin, bovine serum albumin, γ-globulin, and apoferritin were taken as 45 000, 67 000, 160 000 and 480 000, respectively.

Disc electrophoresis was carried out as described by Davis [20]. After electrophoresis, the protein on one gel was located by staining with Amidoschwartz. The enzyme activity on the other gel was located on the gel histochemically by the method of Glenner et al. [21].

Protein was measured by the method of Lowry et al. [22], using bovine serum albumin as standard. Pyridoxal phosphate was assayed fluorimetrically by the method of Adams [23]. Cu concentration was assayed by using an atomic absorption spectrophotometer (Hitachi-Perkin Elmer 303).

Subcellular fractionation of bovine dental pulp was carried out by differential centrifugation, as described previously [24]. The homogenate was filtered through gauze to remove the fibres.

RESULTS

Purification of an amine oxidase from the soluble fraction of bovine dental pulp

Studies on the intracellular distribution of benzylamine oxidase activity in bovine dental pulp indicated about 55% of the amine oxidase activity to be in the soluble fraction [6, 7]. This soluble amine oxidase was purified. Bovine serum was also used as the starting material for the purification.

Step 1. Extraction. Bovine dental pulp (80–240 g) was homogenized in 9 vol. of 0.25 M sucrose using an Ultra-Turrax homogenizer. The soluble supernatant fraction was isolated after centrifuging at 100 000 $\times$ *g* for 60 min.

Step 2. $(NH_4)_2SO_4$ fractionation. Solid $(NH_4)_2SO_4$ was added to the soluble fraction of the dental pulp to 40% saturation. After mixing for 30 min, the solution was centrifuged at 10 000 $\times$ *g* for 20 min. The supernatant was removed and the precipitate discarded. To the supernatant, solid $(NH_4)_2SO_4$ was added to 80% saturation. After mixing for 30 min, the solution was centrifuged at 10 000 $\times$ *g* for 20 min. The supernatant was discarded, and the precipitate dissolved in 20 mM phosphate buffer, pH 7.4. The solution was dialyzed against a large volume of the same buffer.

Step 3. DEAE-cellulose chromatography. The enzyme at Step 2 was passed through a column (2 cm $\times$ 45 cm) of DEAE-cellulose equilibrated previously with 20 mM phosphate buffer (pH 7.4). Stepwise elution by increasing concentrations of

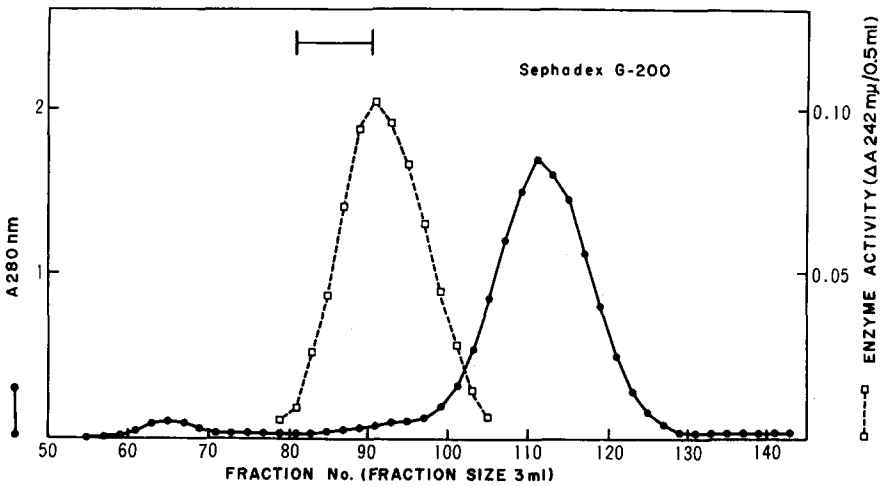

Fig. 1. Chromatography of bovine dental pulp amine oxidase on Sephadex G-200. The conditions are described in Results. |—|, fractions pooled.

phosphate buffer (20 mM, 50 mM, 75 mM, and 100 mM) was carried out. The enzyme activity was eluted with 75 mM and 100 mM buffers. The active fractions were combined.

Step 4. Sephadex G-200 chromatography. The enzyme at Step 3 was concentrated by ultrafiltration. The enzyme solution was passed through a column (2.5 cm × 87 cm) of Sephadex G-200 equilibrated with 75 mM phosphate buffer (pH 7.4). Elution was carried out using the same buffer at a flow rate of 10 ml/h, and fractions of 3 ml each were collected. As shown in Fig. 1, the enzyme activity was eluted before the main protein peak, and most of the contaminating protein was removed. The first half portions of the active fractions were combined. The specific activities of the purified bovine dental pulp amine oxidase samples were different, depending upon the specific activities of the starting bovine dental pulp. The example in Table I shows an ideal preparation.

TABLE I

PURIFICATION OF AMINE OXIDASES FROM BOVINE DENTAL PULP AND BOVINE SERUM

Source	Step	Protein (mg)	Total activity (munits)	Specific activity (munits/mg)	Yield (%)	Purification
Dental pulp*	Soluble fraction	2816	165	0.06	100	1
	$(NH_4)_2SO_4$ (40–80%)	1567	140	0.09	85	1.5
	DEAE-cellulose	399	82	0.21	50	2.5
	Sephadex G-200	4.9	32	6.54	20	112
Blood**	Serum	1718	365	0.21	100	1
	$(NH_4)_2SO_4$ (40–80%)	797	278	0.35	76	1.7
	DEAE-cellulose	177	184	1.04	50	5.0
	Sephadex G-200	1.9	45	23.7	12	113

* Bovine dental pulp, 80 g.
** Bovine serum, 21.9 ml.

Bovine serum amine oxidase was purified in a similar way for comparison. The results are also shown in Table I.

Properties of purified amine oxidase in bovine dental pulp

The purity of the purified amine oxidase from bovine dental pulp was examined by polyacrylamide disc electrophoresis. As shown in Fig. 2, four protein bands were observed. When the amine oxidase activity on the gel was stained histochemically by the method of Glenner et al. [21] using tryptamine as substrate, only the gel segment coinciding with the second protein band showed the most intense staining for the enzyme activity, and the other three protein bands showed faint staining. The other gel was cut into 5-mm segments, and each gel segment was homogenized and extracted with 20 mM phosphate buffer, pH 7.4, and the extract was used as enzyme for the assay of amine oxidase activity using benzylamine, Pro–Lys–Gly–NH_2, tryptamine, and kynuramine as substrate. As shown in Fig. 2, only the gel segment coinciding

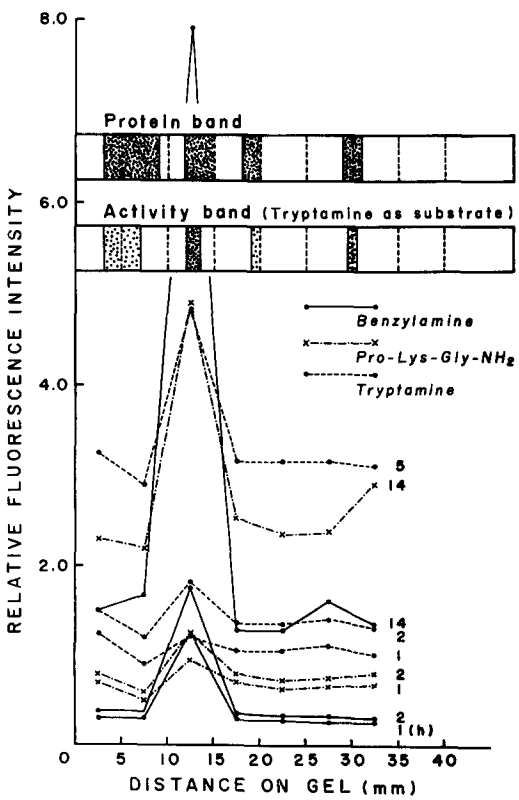

Fig. 2. Disc electrophoresis of an amine oxidase preparation (spec. act. 6.54 munits/mg; 150 μg) from bovine dental pulp. Protein was located by staining with Amidoschwartz. The enzyme activity was located by incubating the gel with tryptamine by the method of Glenner et al. [21]. The other gel was cut into 5-mm segments, and the extract from each gel segment was served as enzyme to measure the oxidation of benzylamine, prolyl–lysyl–glycinamide, and tryptamine fluorimetrically by the method of Guilbault et al. [17], as described in Materials and Methods. The numbers indicate the incubation time (h).

with the second protein band showed the enzyme activity towards these substrates. The purity of the enzyme was estimated to be about 10 % based on disc electrophoresis.

Disc electrophoresis using serum amine oxidase was also carried out. Four protein bands were observed in similar positions as in the case of dental pulp amine oxidase. Only one band, which coincided with the second protein band, was found to have the tryptamine-oxidation activity in the histochemical method [21]. When the other gel was cut into segments to extract the enzyme with the buffer, only the extract from the gel segment coinciding with the second protein band showed the enzyme activity towards benzylamine, tryptamine, and kynuramine, but only about half of the activity of the corresponding dental pulp amine oxidase was observed towards Pro–Lys–Gly-NH_2.

The approximate molecular weight estimated by gel filtration on Sephadex G-200 was 170 000. Serum enzyme was eluted slightly earlier than the dental pulp enzyme, and the molecular weight was estimated to be 220 000.

The pH–activity curve showed a broad peak between 8 and 9. The maximum activity was observed at pH 8.3 (Fig. 3).

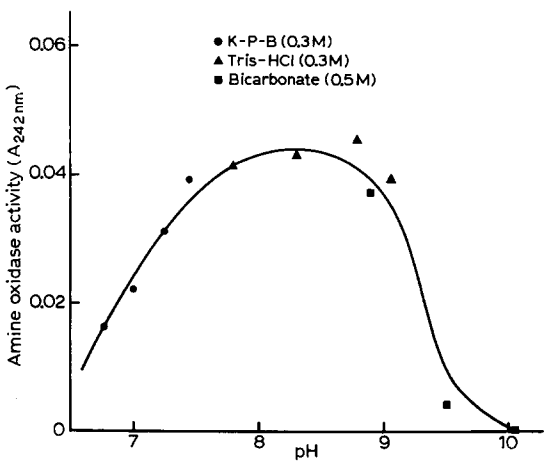

Fig. 3. pH–activity curve of partially purified amine oxidase from bovine dental pulp. The specific activity was 6.54 munits/mg, and 0.07 munit per tube was used for the assay.

The K_m value towards benzylamine for the dental pulp enzyme obtained from a Lineweaver–Burk plot was 1.2 mM (Fig. 4), whereas that for serum enzyme was 1.3 mM.

β-Aminopropionitrile, which inhibits the cross-linking formation of collagen in vivo, inhibited the dental pulp enzyme activity competitively, with benzylamine as substrate (Fig. 4). Lysine–vasopressin ($6 \cdot 10^{-4}$ M), also inhibited the dental pulp enzyme about 32 %.

Effects of various affectors on the benzylamine oxidase activity of dental pulp and serum enzymes are shown in Table II. Essentially similar inhibitory patterns are observed for both dental pulp and serum enzymes. Various copper-chelating agents inhibited both enzymes. *p*-Chloromercuribenzoate was also strongly inhibitory,

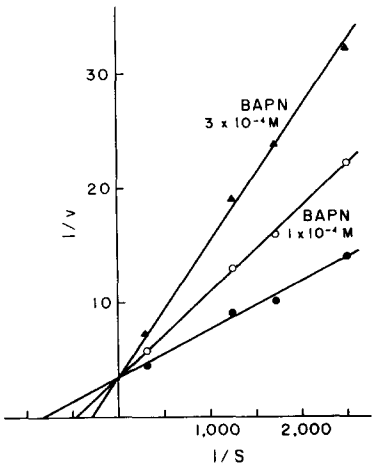

Fig. 4. Inhibition of partially purified dental pulp amine oxidase by β-aminopropionitrile (BAPN). ●—●, control; ○—○, 0.1 mM; and ▲—▲, 0.3 mM. Velocity (V) was expressed by $\Delta A_{242\ nm} \times 1000$. The specific activity of the enzyme was 6.54 munits/mg, and 0.24 munit per tube was used for the assay.

TABLE II

AFFECTORS OF AMINE OXIDASES FROM BOVINE DENTAL PULP AND BOVINE SERUM

Benzylamine was used as substrate. The specific activity of dental pulp enzyme was 6.54 munits/mg, and that of serum enzyme 23.7 munits/mg. Each time, 0.15 munit of the enzyme was used for the assay.

Affector	Concn (M)	Activity (% of the control)	
		Dental pulp enzyme	Serum enzyme
Control	—	100	100
Cuprizone	$3 \cdot 10^{-4}$	0	0
KCN	$1 \cdot 10^{-2}$	20	21
Penicillamine	$5 \cdot 10^{-3}$	64	62
p-Chloromercuribenzoate	$1 \cdot 10^{-3}$	0	0
Isoniazid	$4 \cdot 10^{-5}$	51	59
β-Aminopropionitrile	$2 \cdot 10^{-3}$	19	21
Iproniazid	$1 \cdot 10^{-4}$	69	71
Pargyline*	$1 \cdot 10^{-3}$	0	0
JB-516**	$6 \cdot 10^{-6}$	4	5
Marplan***	$7 \cdot 10^{-6}$	20	26

* Pargyline, *N*-methyl-*N*-2-propynylbenzylamine.
** JB-516, 1-phenyl-2-hydrazinopropane.
*** Marplan, 5-methyl-3-isoxazolecarboxylic acid-2-benzylhydrazide.

TABLE III

CONTENTS OF COPPER AND PYRIDOXAL PHOSPHATE IN THE AMINE OXIDASE PREPARATIONS FROM BOVINE DENTAL PULP

Purification step	Specific activity (munits/mg protein)	Cu (ng/mg protein)	Pyridoxal phosphate (nmole/mg protein)
$(NH_4)_2SO_4$	0.05	30.3	0.013
DEAE-cellulose	0.13	37.5	0.030
Sephadex G-200	2.80	642	0.070

suggesting the presence of essential SH group(s). Iproniazid and isoniazid were inhibitory for both enzymes, and the inhibition could be recovered by the addition of pyridoxal phosphate. In one experiment, iproniazid inhibited the dental pulp enzyme by 36% at 0.1 mM, and this inhibition was completely reversed by 1 mM of pyridoxal phosphate. This result suggests the presence of pyridoxal phosphate. Various monoamine oxidase inhibitors inhibited both enzymes to similar extents.

The contents of copper and pyridoxal phosphate were assayed with enzyme preparations during the purification. As shown in Table III, copper concentrations increased simultaneously with the increase of the specific activity, but pyridoxal phosphate concentrations increased only to a small extent.

TABLE IV

SUBSTRATE SPECIFICITY OF AMINE OXIDASES FROM BOVINE DENTAL PULP AND BOVINE SERUM

The enzyme activity was assayed fluorimetrically by the formation of H_2O_2 [17] as described in Materials and Methods. The specific activity of dental pulp enzyme was 6.54 munits/mg, and that of serum enzyme 23.7 munits/mg.

Substrate	Activity (munits)			
	Dental pulp enzyme		Serum enzyme	
	$8 \cdot 10^{-4}$ M	$1 \cdot 10^{-5}$ M	$8 \cdot 10^{-4}$ M	$1 \cdot 10^{-5}$ M
Benzylamine	1.96	0.09	2.29	0.14
Tyramine	0.53	0.02	0.67	0.04
Metanephrine	0.00	0.00	0.05	0.00
Normetanephrine	0.08	0.00	0.23	0.00
Octopamine	0.00	0.00	0.00	0.00
Tryptamine	0.14	0.00	0.17	0.00
Kynuramine	0.07	0.36	0.01	0.31
Histamine	0.09	0.12	0.07	0.00
Cadaverine	0.23	0.10	0.04	0.02
Spermine	5.87	2.00	4.33	1.47
Spermidine	5.65	0.43	4.20	0.36
Lysine	0.00	0.00	0.00	0.00
Glucosamine	0.00	0.00	0.00	0.00
Galactosamine	0.00	0.00	0.00	0.00
Lysine–vasopressin	0.11	—	0.01	—
Pro–Lys–Gly-NH_2	0.11	—	0.06	—

Substrate specificity of purified amine oxidase in bovine dental pulp

Oxidation of various amines by purified amine oxidases from bovine dental pulp and from bovine serum has been examined. As shown in Table IV, substrate specificities of both dental pulp enzyme and serum enzyme are similar. Among various amines, polyamines such as spermine and spermidine, and benzylamine were good substrates. Substrate inhibition was observed with kynuramine at concentrations higher than 10 μM. The apparent K_m value of kynuramine was 3.1 μM.

Free lysine was not oxidized at all, but lysyl peptides were oxidized. When lysine–vasopressin was used as substrate, the dental pulp amine oxidase had a much higher activity than the plasma amine oxidase. When Pro–Lys–Gly-NH_2, which is a tripeptide and a sequence taken from lysine–vasopressin, was used as substrate, the serum enzyme had about half of the activity of the dental pulp enzyme.

Oxidation of peptidyl lysine in [6-^{3}H]lysylelastin and in [6-^{3}H]lysylcollagen substrates was examined by the method of Siegel and Martin [15]. As shown in Table V, chick cartilage lysyl oxidase prepared by the method of Siegel and Martin [15] oxidized both substrates, but neither the dental pulp enzyme nor the serum enzyme oxidized the elastin or collagen substrate. In order to exclude the possibility that the lack of activity for collagen or elastin substrate was not due to the presence of inhibitors in the dental pulp amine oxidase, a mix experiment was carried out. Both dental

TABLE V

ACTIVITY OF DENTAL PULP AMINE OXIDASE AND SERUM AMINE OXIDASE ON COLLAGEN AND ELASTIN

The enzyme activity was assayed by measuring the ^{3}H released following the deamination of peptidyllysine from [6-^{3}H]lysylcollagen or elastin substrate [14], as described in Materials and Methods. Incubations were carried out at 37 °C for 16 h except in Nos 1–4 for elastin (21 h). Bovine dental pulp amine oxidase had a specific activity for benzylamine of 6.54 munits/mg, and bovine serum amine oxidase had a specific activity of 23.7 munits/mg. The amount of each enzyme used was 4.8 munits per incubation. 600 000 cpm of the labeled collagen or elastin substrate were added per incubation except in Nos 3 and 4 for elastin (1 200 000 cpm).

Sample No.	Incubation	Lysyl oxidase activity (cpm)	
		Collagen	Elastin
1	Bovine dental pulp amine oxidase	184	2716
2	Bovine dental pulp amine oxidase + β-Aminopropionitrile (100 μg)	216	2443
3	Bovine serum amine oxidase	182	4117
4	Bovine serum amine oxidase + β-Aminopropionitrile (100 μg)	269	3971
5	Chick cartilage lysyl oxidase (5.3 mg protein)	1338	3914
6	Chick cartilage lysyl oxidase (5.3 mg protein) + β-Aminopropionitrile (100 μg)	340	2748
7	Chick cartilage lysyl oxidase (5.3 mg protein) + Bovine dental pulp amine oxidase	1305	–
8	Chick cartilage lysyl oxidase (5.3 mg protein) + Bovine dental pulp amine oxidase + β-Aminopropionitrile (100 μg)	340	–

pulp amine oxidase and lysyl oxidase were mixed together with collagen substrates. As shown in Table V (Nos 5–8), an essentially similar activity was obtained with chick lysyl oxidase either in the absence or presence of dental pulp amine oxidase. Therefore, it is concluded that the dental pulp amine oxidase preparation had no endogenous inhibitor for lysyl oxidase activity.

DISCUSSION

Bovine dental pulp, which is a pure connective tissue, was found to contain about 60% of the amine oxidase activity in the soluble fraction. Among various connective tissues, about 20% of the enzyme activity in aorta [14] and about 15% of that in bone were reported to be localized in the soluble fraction. The soluble amine oxidase in connective tissues is assumed to have some relation to plasma amine oxidase, but the relationship between both enzymes is not clear. This amine oxidase in bovine dental pulp has been partially purified approx. 113-fold.

The partially purified amine oxidase appears to contain copper. Copper-chelating agents inhibited the enzyme, and the copper content was increased during the purification. The inhibition by iproniazid and the reversal of the inhibition by pyridoxal phosphate suggest the presence of pyridoxal phosphate in the enzyme. However, pyridoxal phosphate content increased only slightly during the purification. Isoniazid behaved in a similar way to iproniazid, but isoniazid reacts readily with pyridoxal, which in effect reduces the inhibitor concentration. It should be also remembered that, as reported by McEwen et al. [25, 26] and Rucker et al. [27], hydrazides can also act as substrate analogs for the benzylamine-type oxidase. The dental pulp enzyme may require SH group(s), since *p*-chloromercuribenzoate was strongly inhibitory.

The dental pulp enzyme has been compared to serum amine oxidase. The properties of both enzymes are quite similar. However, one significant difference was the substrate specificity towards a peptidyllysyl substrate. The dental pulp enzyme oxidized lysine–vasopressin about ten times faster than serum amine oxidase. Rucker and O'Dell [4] also reported that the properties of an amine oxidase from the soluble fraction of aorta were very similar to those of plasma amine oxidase [4], and that only the preparations of amine oxidase from aorta catalyzed the oxidation of peptidyl-lysine. However, in the present study, serum amine oxidase was found to catalyze the oxidation of Pro–Lys–Gly-NH_2 to a lesser extent than the dental pulp enzyme. These results suggest that the dental pulp amine oxidase may be similar to, but distinct from, serum amine oxidase.

Since the properties of the dental pulp amine oxidase are very similar to those of serum amine oxidase except the substrate specificity, it is possible that serum enzyme represents an altered form [28] of the dental pulp enzyme.

Since four bands (one main band and three minor bands) of tryptamine-oxidase activity were found in the acrylamide gel electrophoresis study of dental pulp enzyme (Fig. 2), any difference in the substrate specificities between the dental pulp and serum amine oxidases (Table IV) may have been due to the presence of multiple forms or other amine oxidases in the dental pulp enzyme preparation. Serum amine oxidase preparation showed only one band of tryptamine-oxidase activity in the gel electrophoresis. Also, the serum amine oxidase preparation may have contained trace

amounts of a diamine oxidase, since cadaverine was oxidized to a small extent.

The cross-linking formation of collagen and elastin is assumed to be catalyzed by an amine oxidase which oxidizes the peptidyllysine [12]. A new amine oxidase specific for peptidyllysine in collagen and elastin has been described by Martin et al. [13, 16] and named as lysyl oxidase. The dental pulp amine oxidase appears to be a distinct enzyme from lysyl oxidase. Although lysine–vasopressin could be oxidized by dental pulp amine oxidase, neither collagen nor elastin substrate could be oxidized by the dental pulp enzyme or the serum enzyme (Table V). The inability of plasma amine oxidase on collagen and elastin substrates had been reported by Siegel et al. [16].

The lack of activity for collagen and elastin substrates in the dental pulp amine oxidase is not due to the presence of inhibitors, since the mix experiment with dental pulp amine oxidase and lysyl oxidase, mixed together with collagen substrate, revealed essentially the same activity as the experiment with lysyl oxidase alone.

An explanation for the lack of activity of the bovine dental pulp amine oxidase for collagen and elastin substrates could be species specificity. We have shown that a cartilage lysyl oxidase preparation of rat also had activity for collagen substrate from chick, indicating that a mammalian lysyl oxidase works on the chick collagen substrate. Siegel et al. [16] have also reported that pig cartilage lysyl oxidase can oxidize chick elastin substrate. However, it is necessary to prepare a bovine lysyl oxidase and to demonstrate that it is active on the chick substrates. Such a bovine lysyl oxidase would be prepared from the cartilage of fetal cattle. This remains to be further investigated.

Another possibility is that the dental pulp preparations may contain some lysyl oxidase as a contaminant, thus allowing the partial oxidation of small peptidyl lysine substrates such as lysine–vasopressin or Pro–Lys–Gly-NH_2. Measurable amounts of lysyl oxidase activity could be detected if small and highly soluble peptidyllysine substrates are present at high concentrations, even though the lysyl oxidase activity towards collagen or elastin substrate is very low.

The fact that the dental pulp enzyme can oxidize prolyl–lysyl–glycinamide or lysine–vasopressin, but not collagen or elastin suggests that the use of natural collagen and elastin substrates as established by Martin et al. [13], may be necessary for the study of lysyl oxidase which directly relates to the collagen and elastin biosyntheses.

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