Purification and Properties of an Amine Oxidase in Bovine Dental Pulp

Amine oxidase in connective tissue is supposed to participate in the cross-linking reaction of collagen and elastin[1]. The amine oxidase in connective tissue is assumed to be produced from fibroblasts. If connective tissue amine oxidase is responsible for the formation of both intramolecular and intermolecular cross-links of collagen[1], the enzyme may function both in the fibroblast and in the extracellular ground substance. Properties of amine oxidase in connective tissue such as skin[2], bone[3], and aorta[4] have been reported. Since dental pulp is a pure connective tissue, we have tried to isolate and characterize a connective tissue amine oxidase from bovine dental pulp[5].

Bovine dental pulp was obtained fresh packed in ice. The tissue could be stored at –20°C at least for several months without appreciable loss in the enzyme activity. Amine oxidase activity was measured spectrophotometrically by the formation of benzaldehyde from benzylamine[6,7]. Incubation mixture (final volume 1.5 ml) contained 75 µmoles of phosphate buffer (pH 7.4), 1.2 µmoles of benzylamine, and enzyme. Incubation was carried out for 60 min at 37°C under shaking in air. A unit of enzyme activity was defined as the amount of enzyme catalyzing a change in absorbance of 0.001 per min at 242 nm in cuvettes of 5 mm light path. 17 units in our assay are calculated to be 1 nmole of benzaldehyde from the reported molar absorptivity[6,7].

Bovine dental pulp was homogenized in 0.25 *M* sucrose and the subcellular fractions were separated by differential centrifugation. The specific activity of dental pulp homogenate was about 0.02 nmole of benzaldehyde formed/min/mg protein (37°C). The intracellular distribution of amine oxidase (% activity of homogenate) was: 55% in the soluble supernatant, 25% in microsomes, and 21% in mitochondria, respectively. Presence of about 50% of the total amine oxidase activity in the supernatant is characteristic for dental pulp.

Amine oxidase in the soluble supernatant from 240 g of bovine dental pulp was partially purified. Benzylamine was used as substrate throughout the purification. The purification procedure involved ammonium sulfate fractionation (40–80%), DEAE-cellulose chromatography and Sephadex G-200 chromatography. The purification was approximately 100-fold from the soluble supernatant with a yield of 30%. The most purified preparation had a specific activity of 2.2 nmoles of benzaldehyde formed/min/mg protein (37°C). Disc electrophoresis[8] of the purified enzyme showed 5 bands, only one of which (No. 2 band from the origin) had the enzyme activity. The enzyme purified by disc electrophoresis also deaminated kynuramine to form 4-hydroxyquinoline, when the activity was measured using kynuramine as substrate by the method of KRAML[9]. Optimum pH in *Tris*-HCl buffer was about 8 to 9. Apparent *Km* value toward benzylamine was 7×10^{-4} *M*. The molecular weight determined by Sephadex G-200 gel filtration by the method of WHITAKER[10] was approximately 170,000.

Effects of various affectors on the purified enzyme are shown in the Table. The activity was inhibited by isoniazid, and the inhibition recovered with addition of pyridoxal phosphate. Cuprizone, a copper-chelating agent, at 3×10^{-4} *M* completely inhibited the activity. The contents of pyridoxal phosphate and copper in the Sephadex eluate were increased about 10-fold from those in the ammonium sulfate fraction during the purification. These results suggest that the enzyme requires pyridoxal phosphate and copper.

p-Chloromercuribenzoate inhibited the enzyme activity, suggesting that sulfhydryl group(s) may be essential for the activity. β-Aminopropionitrile, which inhibits the cross-linking of collagen[1], inhibited the enzyme in competition to benzylamine. *Ki* value of β-aminopropionitrile was obtained as 2.2×10^{-4} *M*. The possibility that bovine dental pulp amine oxidase can deaminate peptide-bound lysine was suggested from the fact that lysine-vasopressin inhibited the enzyme activity.

These properties of dental pulp amine oxidase are similar to those of bone amine oxidase[3] and beef plasma amine oxidase[11,12]. A question is whether the dental pulp amine oxidase is synthesized locally by fibroblasts, or whether it is produced in some other tissues, transported by the blood, and stored in the dental pulp. Experiments for the comparison between dental pulp amine oxidase and plasma amine oxidase are in progress in our laboratory[13].

Zusammenfassung. In der löslichen Fraktion der Rinderzahnpulpa wurde die Aminoxydase gereinigt und die Enzymaktivität durch Isoniazid, Cuprizon, *p*-Chloromercuribenzoat, β-Aminoproprionitril und Lysin-Vasopressin gehemmt. Die Aminoxydase hat wahrscheinlich Kupfer und Pyridoxalphosphat als prosthetische Gruppen.

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Affectors of purified bovine dental pulp amine oxidase

Affector	*M*	Activity (%)
—(Control)		100*
Cu^{2+}	1×10^{-5}	73
Cuprizone	3×10^{-4}	0
Pyridoxal phosphate	1×10^{-5}	107
Isoniazid	4×10^{-5}	51
Isoniazid	4×10^{-5}	77
plus pyridoxal phosphate	1×10^{-4}	
Isoniazid	2×10^{-4}	19
Isoniazid	2×10^{-4}	58
plus pyridoxal phosphate	2×10^{-3}	
p-Chloromercuribenzoate	1×10^{-3}	0
β-Aminopropionitrile	2×10^{-4}	65
Lysine-vasopressin	6×10^{-4}	68

*The enzyme had a specific activity of 2.2 nmoles of benzaldehyde formed/min/mg protein (37°C).

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