MODIFICATION OF CATALYTIC PROPERTIES OF AMINE OXIDASES

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1. Introduction

Amine oxidases have attracted much interest in connection with studies of biogenic amines in the brain [1] under normal and pathological conditions [2] and in the development of new psychotropic drugs [3].

There are two main groups of amine oxidases [4]: monoamine oxidases (MAO) (EC 1.4.3.4) and diamine oxidases (DAO) (EC 1.4.3.6). MAO usually contain flavin components [5] and free SH groups [6, 7], but do not contain pyridoxal phosphate. MAO catalyze the oxidative deamination of biogenic monoamines; this reaction is inhibited by specific MAO inhibitors (pargyline, tranylcypromine) but not by carbonyl reagents. DAO belongs to the category of pyridoxal phosphate dependent enzymes [4]. The activity of DAO is inhibited by carbonyl reagents but not by MAO inhibitors [8]. No flavin components or free SH groups were found in preparations of DAO [4]. An important criterion for distinction between MAO and DAO is the fact, established by Blaschko [9, 10], that only MAO possess the ability to attack secondary amines (for example, *N*-methyl-β-phenylethylamine) with liberation of methylamine.

In this paper we report our findings which show that the catalytic properties of DAO and MAO are reversibly and qualitatively altered when highly purified preparations of these enzymes are treated with some reducing and oxidizing agents.

2. Experimental

DAO was isolated and purified from pig kidney cortex [11]. After filtration through a Biogel P-300 column the enzyme was homogeneous as evidenced by electrophoresis in polyacrylamide gel. MAO was isolated and purified [12] from beef liver mitochondria which was solubilized with the nonionic detergent Triton X-100. The enzyme was homogeneous as evidenced by polyacrylamide gel electrophoresis in the presence of 0.75% Triton X-100 (and in presence of 0.3% sodium dodecylsulphate).

Estimations of the content of SH groups in enzyme preparations were carried out by titration with 5,5′-dithiobis (2-nitrobenzoic acid) [13] or *p*-chloromercuribenzoate [14]. Both methods gave identical results.

Amine oxidase activity was assayed by measuring the rate of liberation of ammonia or methylamine (isothermic distillation in Conway units with subsequent Nesslerization) after incubation of enzymes with substrates in optimal conditions (concentration of substrates, pH values).

3. Results and discussion

Treatment of highly purified DAO (fig. 1) with H_2S under conditions which did not change the pH value of the samples, decreased the DAO activity (deamination of diamines or of histamine) and induced the ability to attack some monoamines (tyramine, tryptamine, serotonin) and the secondary amine *N*-methyl-β-phenylethylamine (this compound was kindly presented by Dr. H. Blaschko). These alterations

Table 1
Modification of enzymatic properties of DAO after treatment with H_2S.

Substrates	Concentrations (mM)	Liberation of NH_3 (or CH_3NH_2) (nmoles/mg protein/min)	
		Native DAO	Treated with H_2S
Putrescine·2HCl	10.0	380 ± 6 (12)	130 ± 10 (8)
Cadaverine·2HCl	10.0	510 ± 2 (5)	210 ± 1 (5)
Histamine·2HCl	0.5	310 ± 5 (5)	46 ± 8 (5)
N-Methyl-β-phenylethylamine·HCl	0.5	0 (6)	216 ± 4 (6)
Tyramine·HCl	0.1	0 (12)	54 ± 3 (10)
Tryptamine·HCl	1.0	0 (10)	111 ± 2 (10)
Serotonine creatine sulfate	5.0	0 (6)	58 ± 3 (3)

Samples contained 2–3 mg of DAO protein (purified about 2200-fold as compared with the homogenate of pig kidney) and 0.2 M potassium phosphate buffer (pH 7.6) to a total volume 10 ml. H_2S was passed through a capillary tube for 1 min (50–60 small bubbles). For estimation of enzymatic activity, aliquots (0.2 ml) were incubated 30 min at 37° with one of the substrates in 0.2 M.potassium phosphate buffer (pH 8.2) in a total volume of 2 ml. Average values ± standard error are shown. The number of experiments is shown in parentheses.

in enzymatic activity were accompanied by the appearance in DAO preparations of 6 SH groups per mole of enzyme (after treatment with H_2S the enzyme was passed through a Sephadex G-25 column to remove the excess of the reducing agent). Treatment with *o*-iodosobenzoate (0.1 mM), which oxidizes the SH groups, restored the DAO activity to the initial level, completely inhibited the ability to deaminate monoamines and decreased the content of SH groups in the modified enzyme to zero. These phenomena were readily reproduced when other reducing agents ($NaBH_4$, reduced glutathione) were used instead of H_2S [15].

Deamination of tryptamine by the modified DAO was accompanied by consumption of 1 mole of oxygen and formation of 1 mole of H_2O_2 per mole of NH_3 liberated. This reaction was completely inhibited by the specific MAO inhibitors iproniazid, pargyline or tranylcypromide (0.1 mM), but not by isoniazid (1.0 mM), phenylhydrazine or aminoguanidine (0.1 mM), which inhibit DAO activity [4].

Treatment of highly purified mitochondrial MAO (table 2) with oxidized oleic acid [16, 17] decreased the MAO activity (deamination of tyramine) and induced the ability to attack substrates of animal DAO (histamine, cadaverine) or plant DAO (lysine). These alterations in enzymatic activity were accompanied by a decrease in content of free SH groups in the MAO preparations from 8 to 3 per 10^5 of protein. Addition of reducing agents ($NaBH_4$ or sodium arsenite) partially restored the MAO activity, completely inhibited the ability to attack substrates of DAO and partially restored the content of SH groups to the initial level. The phenomena described above were readily reproduced when H_2O_2 was used instead of oxidized oleic acid or when samples were incubated under aerobic conditions in the presence of Cu^{2+}.

Previous work [18, 19] showed that similar phenomena (reversible qualitative alteration in catalytic properties accompanied by reversible oxidation of SH groups) occurred after incubation of highly purified bacterial tyramine oxidase with catalytic amounts of Cu^{2+} under aerobic conditions and after treatment with *o*-iodosobenzoate or ergosterol peroxide.

Deamination of histamine by the modified mitochondrial MAO was accompanied by consumption of 1 mole of oxygen and formation of 1 mole of H_2O_2 per mole of NH_3 liberated. This reaction was completely inhibited by hydroxylamine (0.1 mM) and partially (67%) by isoniazid (0.1 mM), but it was resistant to the powerful MAO inhibitors tranylcypromine or pargyline (0.1 mM).

The data obtained suggest that the substrate and inhibitor specificity of enzymes which possess quite different cofactors, may be altered qualitatively. Thus, treatment of DAO with reducing agents induced an ability to catalyze oxidative deamination of the sub-

Table 2
Modification of enzymatic properties of MAO after treatment with oxidized oleic acid.

Substrates	Concentrations (mM)	Liberation of NH_3 (nmoles/mg protein/min)	
		Native enzyme	Treated with oxidized oleic acid
Tyramine·HCl	3.2	882 ± 43 (12)	141 ± 12 (8)
Histamine·2HCl	25.0	0 (3)	97 ± 7 (8)
Cadaverine·2HCl	10.0	0 (4)	88 ± 4 (3)
L-Lysine·HCl	15.0	0 (3)	27 ± 1 (3)

Incubation mixtures contained 1–1.5 mg of protein of enzyme (purified about 250-fold as compared with beef liver homogenate) and 0.1 M potassium phosphate buffer (pH 7.4), tyramine (final concentration 3.2 mM; added for protection of active sites) and 10 mM oleic acid (previously bubbled with oxygen for 48 hr until the content of peroxides increased to 1 mmole of O_2 per g of acid 15). The mixtures (total volume 6.4 ml) were incubated for 2 hr at 37° in oxygen and then dialyzed for 48–52 hr at 4°, against three changes of 500-fold excess of 4 mM potassium phosphate buffer (pH 7.4) to remove oxidized oleic acid and tyramine. Control samples, subjected to all the steps of treatment described did not contain oxidized oleic acid. Assays of enzymatic activity were done with aliquots of the dialyzed enzyme preparations (0.09 mg of protein) and one of the substrates (in previously determined optimal concentrations) in 0.1 M potassium phosphate buffer, (pH 7.8) in a total volume of 2 ml. Incubation was carried out for 25 min at 37° in oxygen.

strates of MAO (including the secondary amine *N*-methyl-β-phenylmethylamine). These reactions were inhibited by specific MAO inhibitors. Similarly, treatment of MAO with oxidizing agents induced the ability to deaminate some substrates of DAO; these reactions were inhibited by inhibitors of DAO.

The role of coenzymes in these striking modifications of amine oxidases is, at the present time, unknown. We can only note that the alterations in catalytic properties were accompanied by reversible alterations in content of SH groups in the enzyme preparations.

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