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K. MONOAMINE OXIDASES

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The term "monoamine oxidase" (MAO) (EC 1.4.3.4) is used to designate a group of enzymes catalyzing the oxidative deamination of tyramine, tryptamine, serotonin, noradrenaline, dopamine and other monoamines (52). The enzymes are mainly bound with mitochondria (9, 52) and do not exhibit sensitivity toward carbonyl reagents. These properties, as well as characteristic patterns of substrate specificity (9) and of sensitivity toward "MAO inhibitors" (57, 59) enable one to distinguish MAO from both diamine oxidase (EC 1.4.3.6) and spermine oxidase. The latter enzyme occurs mainly, but not exclusively (see 38), in the blood plasma of ruminants (10), it does not attack the biogenic monoamines mentioned above, and it is not inhibited by MAO inhibitors. Its inclusion into the group of monoamine oxidases does not seem to be warranted (see 41).

To estimate the activity of MAO, new methods have been developed, based on measurements of radioactivity in deamination products of labeled monoamines (41, 54). The possibilities offered by this approach are exemplified by the discovery of tyraminase in human blood plasma (41). Its activity is inhibited both *in vitro* (41) and *in vivo* (31) by a number of MAO inhibitors, including β-phenylethylhydrazine (nardil) and trans-2-phenylcyclopropylamine (parnate), while the carbonyl reagents and aminoguanidine do not influence the activity (41). Modern radiometric (41, 54), fluorometric (36), and spectrophotometric (51) procedures for measurement of MAO activity may be used for direct estimation of enzymatic activity, for example, in samples of human intestinal mucosa (see 35). When only a single monoamine is used as a substrate for estimation of MAO activity, some valuable information is missed. The restricted availability of various labeled amines accounts for the fact that other methods (13) are still widely used in studies of the deamination of monoamines.

Reinvestigation of the intracellular distribution of MAO in rat liver cells showed that mitochondria contain from 70% (5) to 76.7% (40) of the total MAO in the homogenate. The succinate oxidase in mitochondria accounts for 82.2% (40) of the amount of this enzyme system in the homogenate. The small difference in intracellular distribution between this typical mitochondrial enzyme system and MAO confirms the fact that at least in rat liver cells MAO is concentrated mainly in mitochondria. The activity of mitochondrial MAO reflects to a certain degree the state of mitochondria. Swelling of rat liver mitochondria (caused by various factors) stimulates the oxidation of tyramine about 10-fold (1). It was shown (34) that unsaturated fatty acids participate in regulation of swelling of mitochondria. We have found (25, 45) that sodium oleate in relatively low concentrations (10^{-4} M) inhibits the deamination of tyramine by rat liver mitochondrial MAO but does not influence the deamination of benzyl-

amine. Experiments with the mitochondria of liver (27, 49) and brain (44) show that MAO is tightly connected with insoluble structures of the mitochondrial membranes. Sonication in an atmosphere of hydrogen converts the membranes into fragments of about 50 to 200 Å in diameter without affecting the enzymatic activity (49). These particles, containing the major part of mitochondrial MAO, may be sedimented at 105,000 × *g* within 2 hr (49) and purified by DEAE-cellulose column chromatography (29), by density gradient electrophoresis, or by gel filtration through Sephadex G-200. So far all attempts to separate MAO from insoluble structures have failed. Partially purified MAO was prepared (3, 15, 18) by treatment of the mitochondrial membranes with detergents and subsequent fractionation by ammonium sulphate or adsorption on various modifications of calcium phosphate gel.

The possibility was shown (17, 18) of partial separation of rat liver mitochondrial amine oxidases attacking *m*-nitro-*p*-hydroxybenzylamine (22) and *p*-nitrophenylethylamine (58). In recent experiments with density gradient electrophoresis of mitochondrial sonicates treated by urea or 8-hydroxyquinoline, we have confirmed and extended these data. One can now speculate that the particles of mitochondrial membranes may dissociate and the subfragments formed may acquire a substrate specificity distinct from that of parent particles (the relative rate of oxidation of the benzylamine derivative increases). These results are in accordance with the hypothesis of "multiplicity" of MAO (see 18), which suggests the possibility of more or less selective inhibition of deamination of various monoamines by different inhibitors. Several compounds (including some hydrazine derivatives, harmine, synthetic tricyclic dyes and many others) exhibit this interesting property both *in vitro* and *in vivo* (see 20, 23, 48). Harmine (11) is 1000 times more potent as an inhibitor of the deamination of serotonin than of tyramine or tryptamine.

All the concepts of catalytic sites of mitochondrial MAO are based on indirect evidence (see 6, 20). SH-groups (33), flavin (53) and metal ions (26, 46) may be considered in this connection.

Inhibition of MAO activity by mercaptide-forming reagents (see 33) was confirmed in experiments with partially purified preparations (3, 17, 18). It was found that in presence of 1.2×10^{-5} M *p*-chloromercuribenzoate the oxidation of benzylamine is inhibited by 50% (3). The deamination of tyramine and *p*-nitrophenylethylamine by rat liver mitochondrial MAO purified by column chromatography on brushite (17, 18) is strongly inhibited by 10^{-5} M *p*-chloromercuribenzoate (30). There is a distinct increase in sensitivity of purified preparations of MAO, as compared with the solubilized preparations not subjected to column chromatography, toward the inhibitory effect of *p*-chloromercuribenzoate or Ag ions (50). On the basis of a very attractive hypothesis by Belleau and Moran (6) that pargyline (N-methyl-N-benzylpropynylamine) inhibits MAO by acting on SH-groups, we expected that the purified preparations of MAO would be more sensitive towards pargyline as well, but this was found not to be the case (50). Also the content of SH-groups determined by amperometric titration in MAO preparations did not change after complete inhibition of enzymatic activity by pargyline (50).

Regeneration of MAO activity *in vivo* after introduction of iproniazid was found to be retarded in conditions of riboflavin deficiency (53). The restoration of dopamine oxidation in these experiments was slower than that of serotonin oxidation (53). The hypothetical flavin component of MAO was considered as a possible primary site of action of harmala alkaloids (6) and the possibility of charge transfer complex formation between the flavin and harmine was discussed. The selective inhibition by proflavin of deamination of various monoamines (23) probably should not be ascribed to the effect of proflavin on flavin component of MAO.

In continuation of our studies on inhibition of MAO by chelating agents (26), we have found (46) that 2-hydroxyquinoline competitively and reversibly inhibits the deamination of tyramine and benzylamine (I_{50} values are 2×10^{-4} and 2×10^{-3} M, respectively). 8-Hydroxyquinoline inhibits the oxidation of tyramine in the same manner, but it does not inhibit the oxidation of benzylamine. 4-Hydroxyquinoline, the isomer completely devoid of the ability to chelate metals, does not influence the deamination of either benzylamine or tyramine. Addition of metal ions (Co^{++}, Ni^{++}, Zn^{++}, Fe^{++}) either deinhibits or prevents, completely or partially, the 8-hydroxyquinoline effect on deamination of tyramine. The only metal possessing the deinhibiting or preventive activity in similar experiments with 2-hydroxyquinoline as inhibitor and benzylamine as a substrate was Co^{++}. None of the metals tested could deinhibit or prevent the effect of 2-hydroxyquinoline on the deamination of tyramine (46).

With partially purified MAO it was found (3) that 1,10-phenanthroline (2.10^{-3} M) inhibits the oxidation of kynuramine. This result is in accordance with our data on the effect of this chelating agent on oxidation of tyramine by rat liver and brain mitochondria (26). The recently confirmed (41) fact that EDTA in similar concentrations does not inhibit MAO (21, 26) need not be considered as contradictory, since EDTA, which forms highly stable complexes with metal ions in aqueous solutions, was found to be a relatively weak chelating agent in systems with protein-bound metals (50a).

The question arises whether the metals participating in MAO activity are involved in the inhibitory action of MAO inhibitors? When tyramine is used as the substrate, the irreversible inhibition of MAO activity by iproniazid can be prevented by pretreatment of the enzyme with the chelating agents cysteamine (24) or 8-hydroxyquinoline (28), which are reversible inhibitors of MAO. With tyramine as the substrate, the irreversible inhibition produced by pargyline could be prevented by pretreatment of the enzyme with 8-hydroxyquinoline, but not with harmine (50). It is interesting to note that when serotonin was used as a substrate for estimation of the residual MAO activity (by measuring the ammonia liberated), quite different results were obtained: pretreatment of MAO by either 8-hydroxyquinoline or harmine increased the inhibitory action of pargyline. Metal ions may be also involved in supporting the structure of particles containing the mitochondrial MAO (49).

The problem of a physiological role of MAO has been extensively discussed in several publications (*e.g.*, 2, 32, 56) but is far from being clarified. The "protective" role (9) of enzymatic deamination of biogenic monoamines in organism

is supported by recent clinical (14, 16) and experimental (8, 39) findings on the effects caused by monoamines introduced in conditions of almost complete blockade of oxidative deamination by one of the MAO inhibitors (parnate, pargyline or nardil). At the same time data are accumulating which are compatible with the idea (37) that the role of MAO in organism is not confined to inactivation of biogenic monoamines. Products of enzymatic deamination of monoamines were shown to alter significantly the patterns of carbohydrate metabolism (4, 42) at least in some tissues. The still unexplained effects of MAO inhibitors on carbohydrate metabolism *in vivo* (12, 43) are probably due to the interference of the inhibitors with some unknown function of MAO. The correlation of radioprotective activity of indolylalkylamines with their ability to be attacked by MAO, the suppression of radioprotection by pretreatment of animals with MAO inhibitors (60), and the stimulation of acetoin synthesis in liver homogenates by products of tyramine oxidation (7) may mean that MAO catalyzes the formation of physiologically active compounds. Further investigations on the nature of structure-bound MAO may clarify this and other still unexplored aspects of the metabolism of biogenic monoamines.

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