STUDIES ON MONOAMINE OXIDASE (REPORT 13)
ISOLATION OF TWO DIFFERENT TYPES OF MONOAMINE OXIDASE FROM BEEF LIVER MITOCHONDRIA

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Studies on the subcellular distribution of monoamine oxidase (MAO) [EC 1.4.3.4. monoamine: oxygen oxidoreductase (deaminating)] have shown that the enzymic activity is mainly associated with the mitochondrial fraction (1-5). However, recently appreciable activities have been found in the other particulate fractions (6-8), and in the soluble fraction (9). There have been numerous reports on the role of MAO in metabolism and its possible importance in control of amine levels in the brain.

From results on the substrate specificities and effect of temperature on mitochondrial MAO, Oswald and Strittmatter (8) postulated that various tissues may contain several kinds of MAO, or different groups of enzymes. Gorkin et al. (10-12) confirmed indirectly the “multiplicity” of mitochondrial and partially purified MAO in experiments on differences in inhibitor sensitivities and types of inhibition.

Fujimaki (13) separated two distinct spots of MAO activity from preparation of rat liver and brain by electrophoresis. Watanabe et al. (14) also reported that during starvation the pattern of changing in MAO activity in rat liver differed from that in brain.

MAO has only been partially purified because it is difficult to solubilize it from mitochondria, but recently mitochondrial MAO has been purified to various extents in several laboratories (15-20).

Preliminary observations in our laboratory showed that partially purified mitochondrial MAO from beef liver, which had been solubilized with sodium cholate could be separated into two fractions by precipitation with ammonium sulfate. These were named MAO-1 and MAO-2, respectively (21).

The present work was the different enzymic properties of the two enzymes, including their substrate specificities, pH optima and Michaelis constants and the effects of various metal ions, chelating agents, and heat treatment on them.

METHODS

Measurement of MAO activity

MAO activity was determined with tyramine hydrochloride as substrate at 38°C using the standard Warburg’s manometric method or an oxygen electrode method as described previously (22). In the manometric method, the reaction mixture, containing the enzyme solution, 0.1 M Tris-HCl buffer, pH 8.0, distilled water and substrate solution in a total
volume of 3.0 ml, was equilibrated with oxygen gas for 10 minutes. A small filter paper soaked in 3 M KOH was placed in the inner well to absorb any carbon dioxide evolved. The reaction was started by tipping the substrate solution from the side arm into the main well of the Warburg vessel. Oxygen consumption was followed for 60 minutes, starting 10 minutes after addition of substrate. Enzymic oxidation of substrate usually proceeded linearly during the periods of observations. Corrections were made for non-enzymic oxidation of the substrate and oxygen absorption by the enzyme in the absence of substrate. All determinations were done in duplicate.

In the other method of assay of MAO activity, oxygen consumption was determined using a Clark's oxygen electrode. In this method, the volume of the reaction mixture was also 3.0 ml and the mixture was kept at 38°C. The rate of oxygen consumption was determined during the first few minutes after addition of substrates and corrections were made the same as in the manometric method.

Enzymic activity is expressed as \( Q_{O_2} \), which is defined as the amount of oxygen consumption (\( \mu l \)) per hour per mg dry weight of the enzymic preparations. Unless otherwise stated, 0.1 M Tris-HCl buffer at pH 8.0 was used.

**Measurements of other enzymic activities**

Cytochrome oxidase in the mitochondria and MAO-1 and MAO-2 preparations was determined by a modification of the method of Eichel et al. (23), succinic dehydrogenase by the method of Schneider and Potter (24) and catalase by the method of George (25) using an oxygen electrode.

**RESULTS**

**Isolation of MAO-1 and MAO-2**

Beef liver was used as a starting material for isolation of both enzymes. Fresh beef liver was obtained from a slaughter house and promptly frozen and stored until use. The tissue was cut into small pieces, weighed and washed with 0.25 M sucrose, adjusted at pH 8.0 with 0.01 M Tris-HCl buffer. Then, it was homogenized with 3 volumes of chilled 0.25 M sucrose containing 0.01 M Tris-HCl buffer, at pH 8.0, using a Potter-Elvehjem glass homogenizer, to obtain a 25% (W/V) homogenate.

All operations were performed in a cold room (0-4°C) unless otherwise specified. Beef liver mitochondria were isolated by a modification of the method of Schneider and Hogeboom (26). The mitochondria were suspended in 2 volumes of 0.1 M Tris-HCl buffer and gently rehomogenized for 2 minutes in a Potter-Elvehjem glass homogenizer.

Then, an equal volume of 2% (W/V) sodium cholate in the same buffer was gently added to the mitochondrial preparation with stirring, to give a final concentration of sodium cholate of 1%. A final concentration of 1% sodium cholate was optimal for solubilization of mitochondrial MAO as we reported previously (27). The mixture was allowed to stand for 30 minutes in a cold room. And then, centrifuged at 50,000 \( \times \) g for 30 minutes and the precipitate was discarded. The clear and yellowish brown supernatant fluid contained about 60% of the total MAO activity of the mitochondria. After saturated ammonium sulfate
solution in 0.1 M Tris-CHI buffer was added drop-wise with stirring to give 30 to 40% saturation, the mixture was centrifuged at 10,000 x g for 10 minutes. The supernatant was carefully removed by decantation into a chilled beaker and the precipitate was dissolved in 3 volumes of chilled 0.1 M Tris-HCl buffer. Further saturated ammonium sulfate solution was added to the supernatant solution with gentle stirring to give 50% saturation and then, the solution was centrifuged at 10,000 x g for 10 minutes. The supernatant was decanted off and the precipitate was dissolved in about 3 volumes of chilled 0.1 M Tris-HCl buffer. Both solutions were dialyzed overnight against 300 volumes of 0.001 M Tris-HCl buffer, pH 8.0 with 2 changes of the outer fluid. The enzyme precipitated at 30 to 40% saturation of ammonium sulfate and 40 to 50% saturation were named MAO-1 and MAO-2, respectively.

Fig. 1. Isolation of MAO-1 and MAO-2 from beef liver mitochondria.

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**Fig. 2.** Ammonium sulfate fractionation of solubilized mitochondrial MAO from beef liver. Solid line : % of total solubilized MAO activity in each fraction. Dotted line : % of dry weight of solubilized enzymes. Activity was determined manometrically with tyramine hydrochloride (1 x 10^-5 M) as substrate at pH 8.0 and 38°C.
After dialysis, insoluble materials in the preparations were removed by centrifugation at 10,000 x g for 10 minutes. The resulting preparations of MAO-1 and MAO-2 both contained 20–30 mg dry weight of protein per ml of solution. The specific activities of MAO-1 and MAO-2, respectively, were about 6- and 10-fold that of homogenate. The procedures for isolation of MAO-1 and MAO-2 are summarized in Figs. 1 and 2.

The rates of oxygen consumption catalyzed by MAO-1 and MAO-2 with tyramine hydrochloride as substrate were linearly to the amount of enzyme added.

On storage in the frozen state at −20°C MAO-2 lost about 20% of its initial activity in a few days, but then its activity remained constant for up to 15 days. MAO-1 was more stable than MAO-2.

**pS maxima**

Oxygen consumption could not be measured manometrically with either enzyme when the substrate concentration was below $1 \times 10^{-4}$ M. Accordingly, to determine the pS maxima, substrates were used at concentration of from $1 \times 10^{-4}$ M to $1 \times 10^{-1}$ M. Results were summarized in Table 1. With both enzymes the activity-pS curves were a typical bell-shape for enzymic oxidation of all the substrates listed in Table 1 except tyramine and 5-hydroxytryptamine (5-HT). The rates of oxidation of tyramine and 5-HT were the most rapid and increased almost linearly with increase in the concentration of substrate. Maximal activity was obtained with $1 \times 10^{-1}$ M tyramine and $1 \times 10^{-3}$ M 5-HT, and both gave typical S-shaped curves with both enzymes.

| Substrate         | Neg. log. mol. conc. of substrate |
|-------------------|-----------------------------------|
| Tyramine          | Enzyme | MAO-1 | MAO-2 |
| β-Phenylethylamine| 2.5     | 2.5    |       |
| Dopamine          | 2.3     | 2.5    |       |
| Tryptamine        | 2.0     | 2.0    |       |
| 5-HT              | 2.0     | 2.0    |       |
| Benzylamine       | 1.5     | 1.5    |       |
| iso-Propylamine   | 2.0     | 1.0    |       |
| mono-Ethylamine   | 2.5     | 2.5    |       |
| nor-Amylamine     | 2.7     | 2.5    |       |

Both enzymes oxidized aliphatic amines, such as iso-propylamine, ethylamine and n-amylamine much less rapidly than aromatic amines.

MAO-2 showed considerable higher activity than MAO-1 with all the substrates examined.

The maximal QO₂ values of MAO-2, with tyramine, β-phenylethylamine, dopamine, tryptamine, 5-HT, benzylamine, iso-propylamine, ethylamine and n-amylamine as substrate, were 27.0, 13.9, 12.1, 8.2, 19.4, 17.0, 8.2, 1.3, and 6.3, respectively. Those of MAO-1 with
these substrates were 18.0, 13.9, 6.1, 8.2, 12.9, 11.0, 6.3, 1.3, and 6.2, respectively (Table 1). As shown in Table 1, when dopamine, iso-propylamine or n-amylamine was used as substrate, the pS maximum was slightly different from that of MAO-2.

Diamines such as cadaverine, histamine, putrescine, ethylenediamine and agmatine were not oxidized by either enzyme.

**pH optima**

The effect of pH on the rates of oxidation of various substrates by MAO-1 and MAO-2 were investigated manometrically. Phosphate buffer (0.1 M KH$_2$PO$_4$-0.1 M Na$_2$HPO$_4$) was used from pH 5.5 to 7.0, Tris-HCl buffer from pH 7.0 to 8.5, Clark-Lub's buffer (0.1 M NaOH-0.1 M boric acid plus 0.1 M KCl) from pH 8.5 to 9.5, and Kolthoff's buffer (0.1 M NaCO$_3$-0.1 M sodium borate) from pH 10.0 to 11.0 were used in this experiment. As shown in Table 2, the pH optima of MAO-1 and MAO-2 were between 7.1 and 8.5. When tyramine, dopamine or tryptamine was used as substrate, the pH-activity curves for MAO-1 and MAO-2 were almost identical in shape. However, when β-phenylethylamine or 5-HT was used as substrate the pH optima of MAO-1 and MAO-2 were slightly different. Thus, the pH optima of MAO-1 and MAO-2, respectively, were 7.1 and 8.0 with β-phenylethylamine as substrate and 8.5 and 7.9 with 5-HT (Table 2).

| Substrate          | MAO-1 | MAO-2 |
|--------------------|-------|-------|
| Tyramine           | 8.0   | 8.0   |
| β-Phenylethylamine | 7.1   | 8.0   |
| Dopamine           | ≥8.0  | ≥8.0  |
| Tryptamine         | 8.2   | 8.2   |
| 5-HT               | 8.5   | 7.9   |

**Table 3. Km values of MAO-1 and MAO-2.** Km values were determined from Lineweaver-Burk's double reciprocal plots of activities measured at 38°C.

| Substrate            | MAO-1     | MAO-2     |
|----------------------|-----------|-----------|
| Tyramine             | 1.2 × 10^{-2} M | 3.0 × 10^{-2} M |
| β-Phenylethylamine   | 3.3 × 10^{-2} M | 2.7 × 10^{-2} M |
| Tryptamine           | 7.1 × 10^{-3} M | 6.7 × 10^{-3} M |
| 5-HT                 | 7.7 × 10^{-3} M | 3.2 × 10^{-2} M |

**Michaelis constants of MAO-1 and MAO-2**

The Michaelis constants of MAO-1 and MAO-2 were determined from Lineweaver-Burk double reciprocal plots of values obtained manometrically.

The Km values of the two enzymes with various substrates are summarized in Table 3. The Km values of MAO-2 were 1.2 × 10^{-2} M for tyramine, 3.3 × 10^{-2} M for β-phenylethylamine, 7.1 × 10^{-3} M for tryptamine and 7.7 × 10^{-3} M for 5-HT while those of MAO-2 were 3 × 10^{-2} M, 2.7 × 10^{-2} M, 6.7 × 10^{-3} M and 3.2 × 10^{-3} M respectively with these substrates.
Thus, the $K_m$ values of MAO-1 and MAO-2 for tyramine and 5-HT are very different while those for $\beta$-phenylethylamine and tryptamine, respectively, are similar (Table 3).

**Effect of metal ions**

Table 4 shows the effects of various metal ions at concentrations of $1 \times 10^{-8}$ M to $1 \times 10^{-2}$ M on the activities of MAO-1 and MAO-2 with tyramine hydrochloride as substrate. At high concentration most of the metal ions tested inhibited both enzymes.

Sulfhydryl reagents such as HgCl$_2$ and AgNO$_3$ were the most potent inhibitors. As shown in Table 4, HgCl$_2$ or AgNO$_3$ at the concentration of $1 \times 10^{-2}$ M or $1 \times 10^{-4}$ M completely inhibited both enzymes, but at a concentration of $1 \times 10^{-5}$ M neither inhibited either enzyme.

At high concentration, Zn$^{++}$, Cu$^{++}$ and Cd$^{++}$ were also more inhibitory. Concentrations of $1 \times 10^{-3}$ M and $1 \times 10^{-4}$ M Fe$^{++]$ and Fe$^{+++}$ had no effect on either enzyme (Table 4). Cu$^+$ and Cu$^{++}$ completely inhibited both enzymes when added at high concentration, but caused little inhibition when added low concentration. As shown in Table 4, MAO-1 and MAO-2 were affected slightly different by Zn$^{++}$, Co$^{++}$, Pb$^{++}$, Mn$^{++}$ and Cd$^{++}$.

**Table 4. Effects of various metal ions on MAO-1 and MAO-2 activities.** MAO activity was determined manometrically at pH 8.0 and 38°C with tyramine hydrochloride ($1 \times 10^{-3}$ M) as substrate. Activity is expressed as a percentage of the control activity.

| Conc. (M)    | MAO-1 | MAO-2 |
|--------------|-------|-------|
| AgNO$_3$     | $1 \times 10^{-4}$ | 1.6   | 1.2   |
| CuCl         | $1 \times 10^{-3}$ | 3.0   | 6.0   |
| Zn(NO$_3$)$_2$ | $1 \times 10^{-4}$ | 54.4  | 37.6  |
| FeSO$_4$     | $1 \times 10^{-4}$ | 98.1  | 100.9 |
| Co(NO$_3$)$_2$ | $1 \times 10^{-3}$ | 65.8  | 76.1  |
| Ni(NO$_3$)$_2$ | $1 \times 10^{-4}$ | 51.8  | 54.6  |
| Cu(NO$_3$)$_2$ | $1 \times 10^{-3}$ | 3.2   | 1.9   |
| Pb(NO$_3$)$_2$ | $1 \times 10^{-4}$ | 81.9  | 93.5  |
| MnSO$_4$     | $1 \times 10^{-2}$ | 87.2  | 97.9  |
| HgCl$_2$     | $1 \times 10^{-1}$ | 0.9   | 0.5   |
| CdSO$_4$     | $1 \times 10^{-4}$ | 63.8  | 39.9  |
| AlCl$_3$     | $1 \times 10^{-4}$ | 103.2 | 96.1  |
| Fe(NO$_3$)$_2$ | $1 \times 10^{-4}$ | 102.7 | 97.7  |

**Effect of various metal chelating agents**

Table 5 shows that o-phenanthroline and sodium diethyldithiocarbamate (DDC) strongly inhibited MAO-1 and MAO-2 at concentrations of $1 \times 10^{-4}$ M and $1 \times 10^{-3}$ M (Table 5). However, the degrees of inhibition of MAO-1 and MAO-2 by o-phenanthroline and DDC were different. Thus, o-phenanthroline at a concentration of $1 \times 10^{-4}$ M caused 77% inhibition of MAO-1 and only 45% inhibition of MAO-2.

At a concentration of $1 \times 10^{-3}$ M 8-hydroxyquinoline inhibited both enzymes while at the same concentration of EDTA and KCN were not inhibitory.
TABLE 5. Effects of metal chelating agents on MAO-1 and MAO-2 activities. Activities were determined manometrically at pH 8.0 and 38°C. Activity is expressed as a percentage of the control activity. Tyramine hydrochloride (1 x 10^{-3} M) was used as substrate.

| Chelator      | Conc. (M) | MAO-1 | MAO-2 |
|---------------|-----------|-------|-------|
| 8-Hydroxyquinoline | 1 x 10^{-2} | 99.2  | 87.3  |
| o-Phenanthroline     | 1 x 10^{-4} | 23.0  | 54.3  |
| EDTA                    | 1 x 10^{-3} | 105.2 | 104.6 |
| KCN                     | 1 x 10^{-3} | 101.5 | 98.6  |
| Cuprizone               | 1 x 10^{-3} | 94.0  | 93.2  |
| DDC                     | 1 x 10^{-3} | 45.6  | 60.5  |

Temp. : 38°C  pH : 8.0  Sub. : Tyramine 1 x 10^{-3} M

**Thermal inactivation**

Preparations of both enzymes in Erlenmeyer flasks with loosely fitting stopper were heated for various periods at various temperatures in 0.1 M Tris-HCl buffer, pH 8.0 in a constant temperature metabolic shaker with stirring. At intervals flasks were removed and quickly chilled in iced water. Residual activity was determined with tyramine hydrochloride as substrate at 38°C using an oxygen electrode.

The heat-inactivation curves of MAO-1 and MAO-2 obtained by heating the preparations at various temperatures for 5 minutes, differed as shown in Fig. 3. Thus, at 45°C MAO-1 lost approximately 20% of its activity while MAO-2 was not inactivated by the treatment of the same temperature, and at 50°C, MAO-1 lost about 70% of its activity while MAO-2 lost only 10%.

MAO-1 was completely inactivated by heating at 55°C for 5 minutes while MAO-2 completely inactivated by heating at 60°C for 5 minutes (Fig. 3). From Fig. 3, the tempera-
ture for 50% inactivation were estimated as approximately 48.0°C for MAO-1 and 54.5°C for MAO-2.

The degrees of inactivation of the enzymes on incubation at 42.0°C for 10 to 120 minutes were also determined. As shown in Fig. 4, MAO-2 was not affected by incubation at 42.0°C for 120 minutes while MAO-1 activity decreased gradually to about 50% of the initial activity.

Fig. 5 shows that the inactivation curves of the two enzymes also differed at 50.0°C. But, at 50.0°C, MAO-1 was completely inactivated after 20 minutes, while MAO-2 activity decreased to a constant level of about 20% of the initial activity.

**Fig. 4.** Thermal inactivation of MAO-1 and MAO-2 at 42°C. Samples were incubated at 42°C for the times indicated before measuring activity. Open circles: MAO-1 activity. Solid points: MAO-2 activity.

**Fig. 5.** Thermal inactivation of MAO-1 and MAO-2 at 50°C. Samples were incubated at 50°C for the times indicated before measuring activity. Open circles: MAO-1 activity. Solid points: MAO-2 activity.
Other enzymic activities in the MAO-1 and MAO-2 preparations

About 4.3% of the total cytochrome oxidase activity was recovered in the MAO-1 preparation and 5.1% of that in the MAO-2 preparation. The specific activity of cytochrome oxidase in the MAO-1 preparation was the same as that in the mitochondrial preparation while that in the MAO-2 preparation was about 3 times more than in mitochondria.

The total and specific activities of succinic dehydrogenase in the two preparations were negligible. About 5.3% and 34.2% of the total catalase activity was recovered in the MAO-1 and MAO-2 preparations, respectively. The specific activity of catalase in the MAO-1 preparation was about the same as that of the mitochondrial preparation while that of the MAO-2 preparation was 18 times more.

DISCUSSION

In a preliminary report (21), we suggested that beef liver mitochondria may contain two different types of MAO with different enzymic properties, we named these enzymes MAO-1 and MAO-2. The properties of the two enzymes described in this report seem comparable to those of the enzymes in mitochondria from various other animals.

There are many recent reports (28-30) on multiple forms of mitochondrial MAO. As shown in this paper, MAO-1 and MAO-2 from beef liver mitochondria differed in many enzymic properties, such as their pH maxima and pH optima, and the effects of metal chelating agents and heat treatment on them.

Many authors (16, 31) have reported that mitochondrial MAO in various tissues is a metal-dependent enzyme and is inhibited by metal chelating agents. It is found that chelating agents, such as o-phenanthroline and DDC strongly inhibited MAO-1 and MAO-2. o-Phenanthroline, a specific chelating agent for iron caused the most inhibition of both enzymes while DDC, a specific chelator of cupric ion, was only inhibitory at high concentration. Nara et al. (18) have demonstrated that beef liver MAO contained copper but not significant amount of other metals known to be present in oxidases except for a small amount of iron. Cupric ion was also found in partially purified preparations of MAO from rat liver (17), and pig brain (32), but the enzyme in rat liver contained more iron than copper. Moreover, Tipton (32) suggested that the enzyme in pig brain did not require copper for activity. o-phenanthroline strongly inhibited MAO-1 and MAO-2 and the degrees of inhibition of MAO-1 and MAO-2 caused by different chelating agents differed. These results suggest that partially purified preparations of MAO-1 and MAO-2 may also require iron for activity. But, addition of Cu⁺ and Cu²⁺ at concentrations of 1 x 10⁻⁴ M to 1 x 10⁻² M did not affect their activities.

There are many reports indicating that mitochondrial MAO is a sulfhydryl enzyme (20, 33-36), and the sulfhydryl groups are believed to be involved in the reaction site. In agreement with this, it is found that MAO-1 and MAO-2 were inhibited by sulfhydryl agents, such as AgNO₃, HgCl₂ and CdSO₄. AgNO₃ and HgCl₂ inhibited the two enzymes to similar extents. However, a concentration of 1 x 10⁻⁴ M and 1 x 10⁻⁵ M CdSO₄ inhibited MAO-1 and MAO-2 to different extents. AgNO₃ and HgCl₂ at concentrations of 1 x 10⁻⁴ M, completely inhibited these enzymes while they had little affected at a concentration of less than
I $10^{-5}$ M. The "all or none" type of inhibition caused by HgCl$_2$ and AgNO$_3$ can be explained by supposing that sulfhydryl groups are required for the conformational stability of the enzyme rather than for activity, as suggested by Gomes et al. (37).

MAO-1 and MAO-2 were both strongly inhibited by Cu$^+$ and Cu$^{++}$, but not by Fe$^{++}$ and Fe$^{+++}$. The extents of inhibition of the two enzymes differed both with Cu$^+$ and with Cu$^{++}$. These results indicate that MAO-1 and MAO-2 differ in sensitivity to chelating agents and metal ions.

The presence of two MAO's rather than a single enzyme in rat brain mitochondria was also reported by Johnston (38) and Tipton (39). They reported a biphasic curve of inhibition by M & B 9302, a new and potent inhibitor of MAO. MAO-2 was completely inhibited by pheniprazine at a concentration of $10^{-4}$ M while a concentration of more than $10^{-2}$ M was necessary for complete inhibition of MAO-1 (40). A similar result was reported by Squires and Lassen (41), who suggested that existence of two forms of enzyme in mitochondria.

The Km values of MAO-1 and MAO-2 differed both for tyramine and for 5-HT. In general, the values obtained in these experiments were slightly larger than those reported by other investigators (8, 42). But the Km values of MAO-1 for tyramine agreed with that of pig brain MAO reported by Tipton (20). Moreover, the Km values of MAO may vary both in different species and also indifferent organs of the same species (8).

MAO-1 and MAO-2 showed different thermal inactivation curves with tyramine hydrochloride as substrate. This suggests that the two enzymes differ in heat stability.

In this work two different types of mitochondrial MAO were isolated by ammonium sulfate fractionation after treatment with sodium cholate. The most probable explanation of the results is that the two enzymes are probably an isozyme.

**SUMMARY**

Two different types of MAO, such as MAO-1 and MAO-2 were isolated from beef liver mitochondria by a procedure involving solubilization with sodium cholate and fractionation with ammonium sulfate. The properties of partially purified preparations of these two enzymes were studied with the following results.

1. MAO-2 had higher activity than MAO-1 with tyramine, dopamine, 5-hydroxytryptamine, benzyamine, or isopropylamine as substrate. Aromatic amines were the best substrates of both enzymes. The substrate specificity of both enzymes were the same as that of mitochondrial MAO.

2. The pH optima of MAO-1 and MAO-2 were between 7.1 and 8.5, and with $\beta$-phenylethylamine or 5-hydroxytryptamine as substrate, those of MAO-1 was slightly different from those of MAO-2.

3. The Km values of the two enzymes differed both for tyramine and 5-hydroxytryptamine.

4. Most of metal ions and metal chelating agents tested inhibited both enzymes when added at high concentration.
o-Phenanthroline and sodium diethyldithiocarbamate, which were the most inhibitory, showed different pattern of inhibition of the two enzymes and o-phenanthroline was the more inhibitory.

5. The temperature-inactivation curves of MAO-1 and MAO-2 differed.

From their different enzymic properties, MAO-1 and MAO-2 seemed to be an isozyme.

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