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SOME PROPERTIES OF MONOAMINE OXIDASE IN CARP HEART

MASATO YAMAMOTO, SHINICHI KOBAYASHI and KATSUJI OGUCHI
Department of Pharmacology, School of Medicine, Showa University, Shinagawaku, Tokyo 142, Japan

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Abstract—1. Studies using clorgyline, deprenyl and semicarbazide as inhibitors showed that carp heart homogenate contained a new type of monoamine oxidase (MAO) and a clorgyline- and deprenyl-resistant amine oxidase (CRAO).

2. The deamination of monoamines by carp heart MAO proceeded in two steps by a double-displacement (ping-pong) mechanism.

3. The K_m values of the MAO for oxygen (K_0 values) with tyramine, 5-hydroxytryptamine (5-HT) and β-phenylethylamine (PEA) as substrates were identical (59 μM).

INTRODUCTION

In 1968, Johnston proposed that there are two forms of monoamine oxidase (MAO; monoamine: O_2 oxidoreductase (deaminating); EC 1.4.3.4) in rat brain, type-A and type-B, based on results of *in vitro* studies with the irreversible inhibitor clorgyline (Johnston, 1968). Type-A MAO is highly sensitive to clorgyline, while type-B MAO is only inhibited in the presence of high concentrations of this reagent. In contrast to clorgyline, deprenyl inhibits type-B MAO at lower concentrations than it does type-A MAO (Knoll *et al.*, 1965; Knoll and Magyar, 1972; Neff and Yang, 1974). The proportions of these two forms of MAO activity vary widely in different tissues of many mammalian species (Houslay *et al.*, 1976) and thus, the substrate specificities of the two forms of the enzyme vary in different tissues (Fowler *et al.*, 1978). However, in spite of extensive studies, it is still unclear whether these two forms of MAO represent two different proteins (Cawthon *et al.*, 1981) or the same enzyme protein with a different composition of attached membrane lipids (Tipton *et al.*, 1973; Tipton and Corte, 1979).

In addition to MAO, it is now clear that in certain tissues in man and animals (Lyles and Callingham, 1975; Lewinsohn *et al.*, 1978; Norqvist *et al.*, 1981; Clarke *et al.*, 1982) there is an amine oxidase that is resistant to concentrations of clorgyline and deprenyl sufficient to inhibit both types of MAO activity completely; in addition, it is sensitive to inhibition by carbonyl reagents such as semicarbazide. This amine oxidase was named clorgyline-resistant amine oxidase (CRAO) (Lyles and Callingham, 1975).

An interesting finding is that carp (*Cyprinus carpio*) liver mitochondria contain a single MAO, apparently not belonging to either type-A or type-B, since it is equally sensitive to inhibition by clorgyline and deprenyl (Kinemuchi *et al.*, 1983). In the present work, we studied the enzymic properties of MAO and CRAO of carp heart using specific inhibitors.

MATERIALS AND METHODS

Enzyme preparation

Fresh ventricular muscles from carp (*Cyprinus carpio*) heart were cut into small pieces. The tissue was then homogenized with 4 vol. of ice-cold 0.01 M phosphate buffer (pH 8.0) which contained 0.25 M sucrose in a Waring blender and a Potter-type glass homogenizer. The homogenate was centrifuged for 10 min at 600 *g* and 4°C, and the supernatant fraction obtained was stored at −20°C until used in assay. It was used as the carp heart enzyme preparation at a protein concentration of 4.3 mg/ml.

Assay of MAO and CRAO activities

The enzyme solution was pre-incubated with 10^{-3} M semicarbazide or clorgyline for 30 min at 37.0°C and the resulting preparations were used as MAO and CRAO, respectively.

MAO and CRAO activities were determined radiochemically by the method of Fowler *et al.* (1979) with [^{14}C]-labelled amines as substrates. Incubation was performed at pH 8.0 and 37.0°C for 20 min. In this assay, enzyme activity was found to be linearly proportional to the amount of protein and to the time. Radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer and expressed as disintegrations per minute (dpm). The substrate concentrations used in this study were approximately the K_m values of the untreated enzyme; namely, 50 μM 5-hydroxytryptamine (5-HT) and β-phenylethylamine (PEA) and 100 μM tyramine and benzylamine. In inhibition studies with clorgyline, deprenyl and semicarbazide, reactions were carried out after preincubation for 30 min at 37.0°C. Previously, the presence of bovine serum albumin (BSA) was reported to stabilize the mitochondrial enzyme in carp liver (Kinemuchi *et al.*, 1983), but amine oxidase activity has been detected in commercial preparations of BSA (Al-Naji and Clarke, 1983). Preliminary tests in the present work showed that the enzyme preparation used was thermostable without BSA. Therefore, no BSA was added to the reaction medium.

Protein concentration

Protein concentration was determined by the modified biuret method (Yonetani, 1961) using BSA as a standard.

Chemicals

[^{14}C]Tyramine HCl (50 mCi/mmol), [^{14}C]5-HT (51.5 mCi/

Table 1. K_m values of carp heart amine oxidase

	Km value (μM)	
	Untreated	Treated with 10^{-3} M semicarbazide (MAO)
Tyramine	126.3 ± 6.0	111.7 ± 21.0
5-HT	47.0 ± 6.9	45.0 ± 7.1
Tryptamine	23.6 ± 2.0	22.7 ± 3.3
PEA	46.9 ± 6.8	85.0 ± 9.2
Benzylamine	104.7 ± 13.5	400.3 ± 15.5

K_m values were determined from Lineweaver–Burk plots at six substrate concentrations assayed in duplicate with a single enzyme source prepared from fresh hearts of more than ten carp. All values are means ± SD ($N = 3$ in each experiment). For each assay (final volume 100 μl), 0.086 mg of carp heart protein was used. The assay method is described in the Materials and Methods.

mmol) and [^{14}C]PEA HCl (48.4 mCi/mmol) were purchased from New England Nuclear, Boston, Massachusetts, U.S.A. [^{14}C]Benzylamine hydrochloride (60.6 mCi/mmol) was purchased from Amersham International, Arlington Heights, Illinois, U.S.A. Deprenyl and clorgyline were gifts from Dr K. Magyar, Department of Pharmacology, Semmelweis University of Medicine, Budapest, Hungary, and from May and Baker Ltd, Dagenham, U.K., respectively. All other chemicals used in this study were of the highest grade available commercially.

RESULTS

K_m *values of carp heart amine oxidase*

The Michaelis–Menten kinetic constants (K_m values) of the untreated enzyme from carp heart and the enzyme treated with 10^{-3} M semicarbazide (MAO) were determined from Lineweaver–Burk plots, using tyramine, 5-HT, tryptamine, PEA and benzylamine as substrates. The results are summarized in Table 1. Little difference was observed between the K_m values of the untreated enzyme and the semicarbazide treated enzyme, using tyramine, 5-HT and tryptamine as substrates. When PEA and benzylamine were used as substrates, however, a considerable difference was found between the K_m values of these two enzymes.

Inhibition of amine oxidase activity in carp heart by clorgyline and deprenyl

The effects of clorgyline and deprenyl on amine oxidase activity of carp heart with tyramine, 5-HT, PEA and benzylamine as substrates are shown in Fig. 1.

When tyramine and 5-HT were used as substrates, both inhibitors (10^{-4}–10^{-5} M) caused complete inhibition of amine oxidase activity. With PEA and benzylamine as substrates, these two inhibitors, at the highest concentration tested (10^{-3} M), caused about 90% and 30% inhibition of amine oxidase activity, respectively, and after pre-incubation with 10^{-3} M semicarbazide, the remaining activities were completely inhibited with 10^{-5} M clorgyline or deprenyl (data not shown). The curves for inhibition by clor-

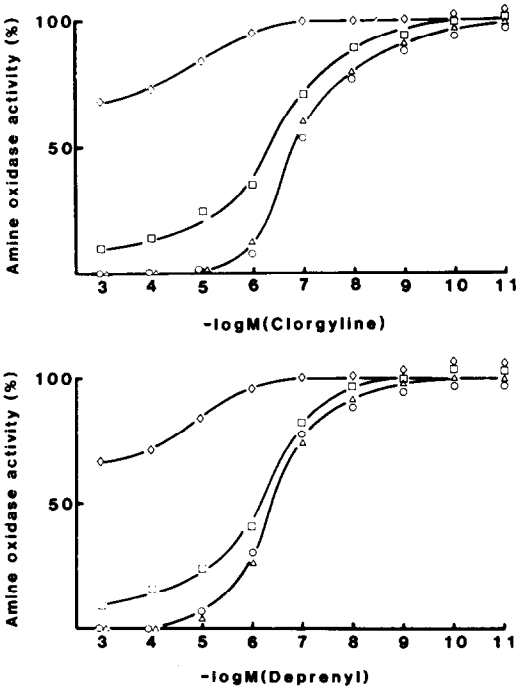

Fig. 1. Inhibition of amine oxidase activity in carp heart with tyramine, 5-HT, PEA and benzylamine as substrates by various concentrations of clorgyline (upper) and deprenyl (lower). Reactions were carried out at 37.0°C for 20 min after treatment with inhibitors at 37.0°C for 30 min. Substrate: (—○—) [^{14}C]tyramine (100 μM, 4 μCi/μmol); (—△—) [^{14}C]5-HT (50 μM, 8 μCi/μmol); (—□—) [^{14}C]PEA (50 μM, 8 μCi/μmol); (—◇—) [^{14}C]benzylamine (100 μM, 4 μCi/μmol).

Table 2. Specific activities of carp heart amine oxidase

	Untreated	Treated with 10^{-3} M clorgyline (CRAO)	Treated with 10^{-3} M semicarbazide (MAO)
Tyramine	47.0 ± 1.7	0.5 ± 0.1	44.7 ± 1.1
5-HT	37.6 ± 2.2	0	32.8 ± 0.5
Tryptamine	32.1 ± 1.1	0.4 ± 0.2	30.6 ± 1.2
PEA	17.4 ± 0.8	1.4 ± 0.2	11.5 ± 0.9
Benzylamine	2.9 ± 0.2	1.1 ± 0.1	0.5 ± 0.3
Histamine	0	0	0
Cadaverine	0	0	0

Specific activities of untreated enzyme and enzyme treated with 10^{-3} M clogyline (CRAO) or semicarbazide (MAO) were determined with [^{14}C]tyramine (100 μM, 4 μCi/μmol), [^{14}C]5-HT (50 μM, 8 μCi/μmol), [^{14}C]tryptamine (25 μM, 16 μCi/μmol),[^{14}C]PEA (50 μM, 8 μCi/μmol), [^{14}C]benzylamine (25 μM, 16 μCi/μmol), [^{14}C]histamine (100 μM, 4 μCi/μmol) and [^{14}C]cadaverine (100 μM, 4 μCi/μmol). Substrate concentrations were approximately the K_m values of the untreated enzyme. Activities are expressed as nmol/mg protein/hr. Values are as means ± SD (N = 6). Reactions were carried out at 37.0°C for 20 min after pre-treatment with inhibitors at 37.0°C for 30 min. The assay method is described in the Materials and Methods.

gyline and deprenyl with tyramine and 5-HT as substrates were both single-sigmoidal and were almost identical. Using PEA and benzylamine as substrates, the inhibition curves with clorgyline and deprenyl, after pre-incubation with 10^{-3} M semicarbazide, were also single-sigmoidal and very similar to those with tyramine and 5-HT as substrates. Thus there was no difference in the sensitivities to clorgyline and deprenyl, and similar single-sigmoidal curves for inhibition by both inhibitors were obtained with these four substrates.

Specific activities

The specific activities of MAO and CRAO in carp heart are shown in Table 2. The remaining activities after treatments with 10^{-3} M clorgyline and semicarbazide were those of CRAO and MAO, respectively.

High MAO activities were observed with tyramine, 5-HT and tryptamine as substrates and low CRAO activities were observed with PEA and benzylamine as substrates under the experimental conditions shown in the legend in Table 2.

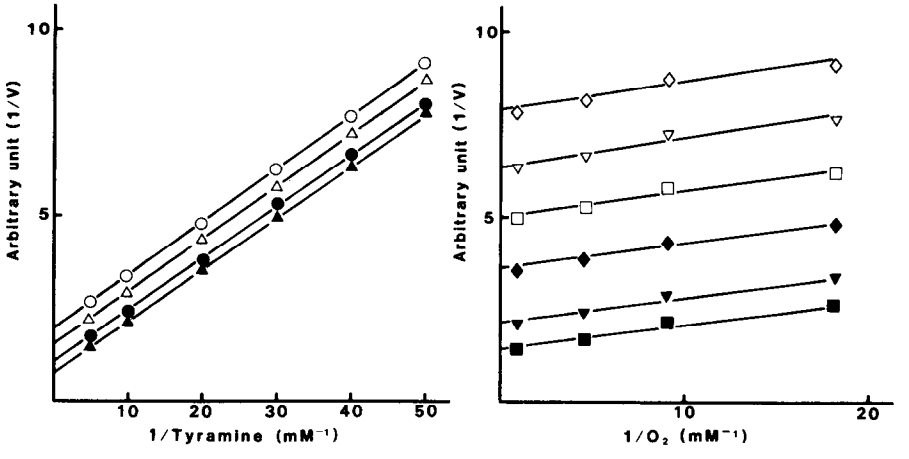

Fig. 2. Lineweaver–Burk plots of carp heart MAO activities at various oxygen (left) and tyramine (right) concentrations. The left figure shows Lineweaver–Burk plots of MAO in carp heart against tyramine concentration at various fixed concentrations of oxygen. The tyramine concentrations were 20–200 μM. The concentrations of oxygen were (—○—) 55μM (5%); (—△—) 110 μM (10%); (—●—) 217 μM (20%) (—▲—) 1085 μM (100%). The right figure shows Lineweaver–Burk plots of MAO in carp heart against oxygen concentration at various fixed concentrations of tyramine. The oxygen concentrations were 55–1085 μM. The concentrations of tyramine were (—◇—) 20 μM; (—▽—) 25 μM; (—□—) 33 μM; (—◆—) 50 μM; (—▼—) 100 μM; (—■—) 200 μM.

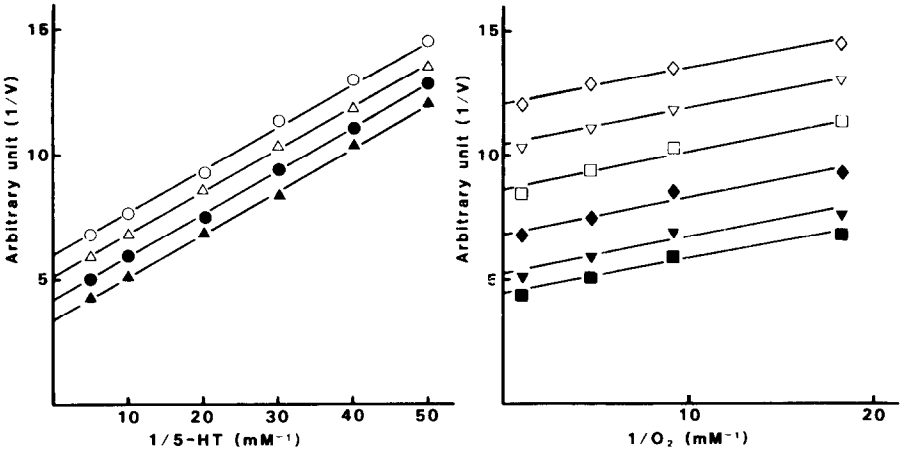

Fig. 3. Lineweaver–Burk plots of carp heart MAO activities at various oxygen (left) and 5-HT concentrations. The left figure shows Lineweaver–Burk plots of MAO in carp heart against 5-HT concentration at various fixed concentrations of oxygen. The 5-HT concentrations were 20–200 μM. The concentrations of oxygen were (—○—) 55 μM (5%); (—△—) 110 μM (10%); (—●—) 217 μM (20%); (—▲—) 1085 μM (100%). The right figure shows Lineweaver–Burk plots of MAO in carp heart against oxygen concentration at various fixed concentrations of 5-HT. The oxygen concentrations were 55–1085 μM. The concentrations of 5-HT were (—◇—) 20 μM; (—▽—) 25 μM; (—□—) 33 μM; (—◆—) 50 μM; (—▼—) 100 μM; (—■—) 200 μM.

Kinetic investigations

Lineweaver–Burk plots were made of carp heart MAO activities at various oxygen and substrate concentrations. As shown on the left side of Figs 2–4, when concentrations of oxygen of 55–1085 μM were used, parallel lines were obtained and an increase in the V_{max} of MAO in carp heart was parallel to increase in oxygen concentration. As shown on the right of Figs 2–4, using various concentrations (20–200 μM) of tyramine, 5-HT and PEA as substrates, parallel lines were also obtained and the V_{max} of the enzyme increased in parallel with an increase in concentration of each substrate.

The K_m values of the MAO for oxygen (K_0 values) with tyramine, 5-HT and PEA as substrates were calculated from secondary plots (Houslay and Tipton, 1973) and are shown in Fig. 5. All the K_0 values were found to be 59 μm.

DISCUSSION

There are many reports on the enzymic properties of MAO from the heart of different species such as chick (Fowler and Callingham, 1977; Suzuki *et al.*, 1980), rat (Corte and Callingham, 1977; Lyles and Callingham, 1979), golden hamster (Edwards and

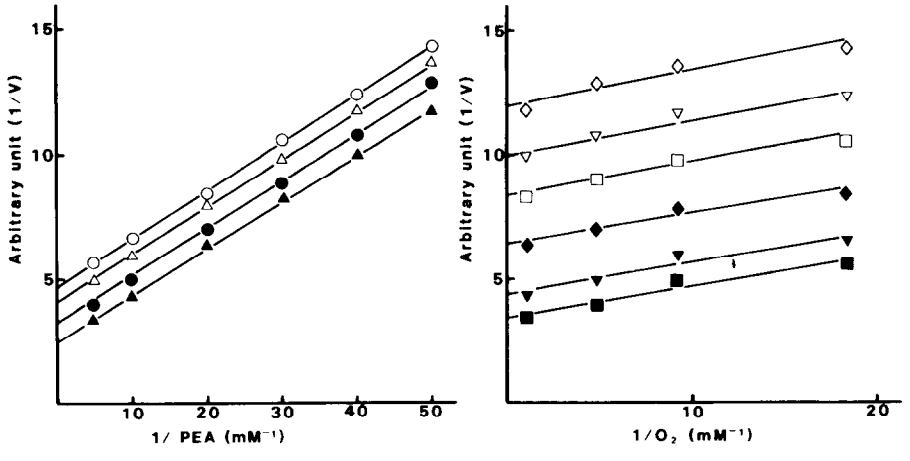

Fig. 4. Lineweaver–Burk plots of carp heart MAO activities at various oxygen (left) and PEA (right) concentrations. The left figure shows Lineweaver–Burk plots of MAO in carp heart against PEA concentration at various fixed concentrations of oxygen. The PEA concentrations were 20–200 μM. The concentrations of oxygen were (—○—) 55 μM (5%); (—△—) 110 μM (10%); (—●—) 217 μM (20%); (—▲—) 1085 μM (100%). The right figure shows Lineweaver–Burk plots of MAO in carp heart against oxygen concentration at various fixed concentrations of PEA. The oxygen concentrations were 55–1085 μM. The concentrations of PEA were (—◇—) 20 μM; (—▽—) 25 μM; (—□—) 33 μM; (—◆—) 50 μM; (—▼—) 100 μM; (—■—) 200 μM.

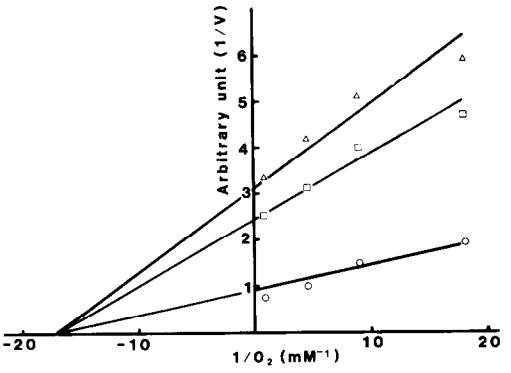

Fig. 5. K_m values of carp heart MAO for oxygen (K_0 values) with tyramine, 5-HT and PEA. K_0 values were all calculated as 59 μM from secondary intercept replots (Houslay and Tipton, 1973) of data in Figs 2–4 with oxygen concentrations of 55–1085 μM. Substrates: (—○—) tyramine; (—△—) 5-HT; (—□—) PEA.

Malsbury, 1978), pig (Lyles and Greenawalt, 1978), bovine (Mantle *et al.*, 1976) and man (Parkinson and Callingham, 1979; Dial and Clarke, 1979).

In the present study, we examined the enzymic properties of a unique type of MAO, distinct from type-A and type-B, from carp heart using clorgyline and deprenyl, and determined the K_m values of the enzyme for several substrates and oxygen.

Clorgyline is thought to be a preferential and irreversible inhibitor of type-A MAO, while deprenyl is a preferential inhibitor of type-B MAO. Both inhibitors seem to bind covalently to the cofactor, flavin adenine dinucleotide (FAD) (Erwin and Hellerman, 1967; Salach and Detmer, 1979). Semicarbazide is thought to inhibit CRAO selectively, leaving MAO substantially unaffected (Lewinsohn *et al.*, 1978). CRAO is not a flavoprotein, but is copper-dependent and may use pyridoxal as a cofactor (Blaschko, 1974).

Carp heart MAO shows no difference in sensitivities to clorgyline and deprenyl, giving similar single-sigmoidal curves with tyramine, 5-HT, PEA and benzylamine as substrates. Taking the established characteristics of the two types of MAO into account, these single-sigmoidal curves indicate the existence of a single form of MAO in carp heart. Moreover, our finding that this enzyme has identical sensitivities to the two types of MAO inhibitor with every substrate used in this study indicates that this single form of MAO is distinct from both type-A and type-B MAO. These results are very similar to those on carp liver (Kinemuchi *et al.*, 1983). Unfortunately, there are few reports on MAO in various organs of other species of fish. Three studies (Hall *et al.*, 1982a, b, c) have demonstrated that brain homogenates of teleosts such as goldfish (*Carassius auratus*), trout (*Salmo gardneri*) and perch (*Perca flavescens*) contain predominantly a type-A-like enzyme, and that MAO in goldfish displayed changes in functional activity in response to change in environmental temperature. In this study, the carp used were obtained commercially during the winter when the water temperature was 8–12°C.

Inhibition studies with semicarbazide after pre-incubation with 10^{-5} M clorgyline or deprenyl indicated the existence of low CRAO activity which could oxidize PEA and benzylamine. Similar CRAO activity has been found in heart tissues of other animals (Fowler and Callingham, 1977; Corte and Callingham, 1977; Edwards and Malsbury, 1978; Lyles and Greenawalt, 1978).

The K_m values for carp liver MAO with tyramine, 5-HT and PEA as substrates were reported to be 132, 51.3 and 40.2 μM, respectively (Kinemuchi *et al.*, 1983). Little difference was observed between the K_m values of carp heart and carp liver MAO with tyramine and 5-HT as substrates.

Previously, reactions catalyzed by MAO from different species were reported to proceed by a double displacement (ping–pong) mechanism (Fischer *et al.*, 1968; Tipton, 1968; Oi *et al.*, 1970; Houslay and Tipton, 1973).

In the present study, the test designed by Cleland (1963) to differentiate the ping–pong mechanism from other possible mechanisms was applied to carp heart MAO. The initial rates of monoamine oxidation were determined at various monoamine concentrations and at a series of fixed oxygen concentrations, and series of parallel lines were obtained. Similar parallel reciprocal plots were obtained when the oxygen concentration was varied at a series of fixed monoamine concentrations. These results indicate that the deamination of monoamines by carp heart MAO also proceeded in two steps by a ping–pong mechanism.

The K_0 values for tyramine, 5-HT and PEA were all found to be 59 μM. Fowler and Callingham (1978) demonstrated two forms of MAO activity in rat liver, tentatively called MAO-1 and MAO-2, on the basis of the difference in their K_0 values. These forms were unrelated to MAO-A and MAO-B. However, carp heart MAO cannot be present in two forms, judging from its sensitivity to inhibitors and its affinity to oxygen. The K_0 values of carp heart MAO (59 μM) were lower than those of rat brain (303 μM) (Oreland and Fowler, 1980) and frog brain (116 μM) with tyramine as substrate (Kobayashi *et al.*, 1981). These kinetic data suggest that the affinities of flavoprotein MAO for oxygen with tyramine, 5-HT and PEA are higher in carp heart than in rat or frog brain.

It is concluded from the present results that carp heart MAO is a single form of MAO, distinct from type-A MAO and type-B MAO, with the properties of both forms of mammalian MAO. It seems very similar to carp liver mitochondrial MAO. Carp heart MAO may have a higher affinity for oxygen than MAO of mammals.

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