INHIBITION OF CARP LIVER MITOCHONDRIAL MONOAMINE OXIDASE BY SOME COMMONLY-USED DETERGENTS

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Abstract—The inhibitory effects of some detergents commonly used in biochemical research on carp liver mitochondrial monoamine oxidase were examined. Sodium dodecylsulfate, octyl-β-D-glucopyranoside, sodium cholate and Triton X-100 at relatively low concentrations caused strong dose-dependent inhibition of the activity towards tyramine, but digitonin caused only weak inhibition. Sodium dodecylsulfate, octyl-β-D-glucopyranoside and sodium cholate caused almost complete inhibition of activity in the concentration ranges tested. The extent of inhibition by Triton X-100 was greater after preincubation at 37°C for 30 min than that without preincubation, but with or without preincubation, the inhibition was not substrate-selective and was not complete at a relatively high concentration (2%) of Triton X-100. Without preincubation, the mode of inhibition by Triton X-100 was competitive and reversible with respect to the oxidations of 5-HT, tyramine and PEA, but after preincubation (37°C for 30 min), it became non-competitive and irreversible, depending on the concentration of detergent used. These findings suggest that it had different actions on the enzyme depending on preincubation. Triton X-100 also slightly changed enzyme sensitivity towards clorgyline and deprenyl, regardless of the preincubation time or the substrate used. Some possible mechanisms of the inhibitory effect of Triton X-100 are discussed.

On the basis of different sensitivities to the inhibitors clorgyline and deprenyl, mitochondrial monoamine oxidase [MAO, amine: O2 oxidoreductase (deaminating) EC 1.4.3.4] is classified into two forms, MAO-A and MAO-B. MAO-A is very sensitive to clorgyline and less sensitive to deprenyl, whereas the reverse is true for MAO-B (1–3).

Many tissues of various species examined so far have been shown to contain both forms of MAO in different proportions or only one of these two forms of MAO (4, 5). However, our recent studies on carp (Cyprinus carpio) liver mitochondria suggest the existence of a single form of MAO with the properties of both forms of mammalian MAO, but differing from both in showing the same sensitivity to clorgyline and deprenyl (6).

This MAO may be a classical mitochondrial MAO, presumably with FAD as its prosthetic group, since we also observed time-dependent change in its mode of inhibition, which was first reversible and then irreversible, like that of mammalian MAO by clorgyline and deprenyl (6).

The inhibitory effect of Triton X-100 on MAO activity was noticed during solubilization of carp liver mitochondrial MAO with this non-ionic detergent (7). In the present study, we examined the inhibitory effects of various commonly used detergents on carp liver mitochondrial MAO. Special attention was paid to the inhibitory effects of Triton X-100.
Materials and Methods

**Chemicals:** Clorgyline hydrochloride and 1-deprenyl hydrochloride were kindly provided by May & Baker, Ltd., U.K., and Prof. J. Knoll, Semmelweis University of Medicine, Budapest, Hungary, respectively. $[^{14}C]-5$-hydroxytryptamine bioxalate (5-HT, 44 mCi/mmol), $[^{14}C]-\beta$-phenylethylamine hydrochloride (PEA, 48.25 mCi/mmol) and $[^{14}C]$-tyramine hydrochloride (56.2 mCi/mmol) were purchased from New England Nuclear, Boston, MA, U.S.A. Triton X-100 was obtained from Nakarai Pure Chemicals, Osaka, Japan; sodium dodecylsulfate (SDS) from Tokyo-Kasei Kogyo Co., Tokyo, Japan; octyl-$\beta$-D-glucopyranoside (octyl-$\beta$-glucoside) from Calbiochem-Behring Corp., La Jolla, CA, U.S.A.; sodium cholate from Sigma Chemical Co., St. Louis, MO, U.S.A. and digitonin from Wako Chemical Industry, Ltd., Tokyo, Japan. All other chemicals used were of the highest grade available commercially.

**Carp liver mitochondrial preparation:** The livers of carp weighing 900–1,200 g were rapidly removed, blotted on filter paper, weighed and cut into small pieces. Mitochondria in the heavy mitochondrial fractions were isolated by differential centrifugation as described previously (6). Briefly, the livers were promptly homogenized in 9 vol. of sucrose-buffer solution (0.25 M sucrose, 10 mM phosphate buffer, pH 7.4) with a Waring blender and then a Teflon homogenizer. The homogenate was first centrifuged at 600×g for 10 min to remove nuclei and cell debris, and the resulting pellet was resuspended in about 3–4 vol. of sucrose-buffer solution and centrifuged as before. The supernatants obtained by the two centrifugations were combined and centrifuged at 8,500×g for 20 min. The resulting pellet was washed by suspension in sucrose-buffer and centrifuged as before. It was then resuspended in sucrose-buffer as the mitochondrial preparation and used immediately or stored frozen in aliquots until use.

**Assay of MAO activity:** MAO activity was assayed radiochemically, as described previously (8), with $[^{14}C]$-tyramine, $[^{14}C]$-5-HT or $[^{14}C]$-PEA diluted with the respective unlabeled amine as substrate at 37°C and pH 8.0 for 10 min. The standard assay mixture contained 20 µl of mitochondrial suspension (0.5 mg/ml of protein), 20 µl of 0.1 M phosphate buffer, pH 8.0, 30 µl of distilled water and 10 µl of bovine serum albumin (BSA) solution (final concentration, 1 mg/ml) to stabilize the enzyme (6). The reaction was started by adding 20 µl of substrate solution. The final substrate concentrations used were approx. the same as their $K_m$ values (0.15 mM for tyramine, 0.05 mM for 5-HT and PEA) estimated previously (6). Enzyme activity was found to be proportional to the incubation time and the amount of enzyme preparation.

When the effects of various detergents on MAO were determined, 20 µl of an appropriate concentration of the test detergent was added to the standard reaction mixture, and the amount of water added was reduced to 10 µl.

When clorgyline and deprenyl were used to determine the susceptibility of MAO to these inhibitors after treatment with Triton X-100, mitochondria were first treated with an appropriate concentration of detergent and then preincubated with one of the MAO inhibitors at 37°C for 30 min before adding the substrate to estimate the remaining activity.

Protein concentrations were determined by a modified biuret method (9) with BSA as the standard.

Part of this work has previously been reported elsewhere (10).

**Results**

Carp liver mitochondrial MAO activity
towards tyramine, a substrate for both forms of MAO, was measured in the presence and absence of different concentrations of SDS, Triton X-100, octyl-$\beta$-glucoside, sodium cholate or digitonin with or without preincubation at 37°C for 30 min. Preliminary experiments on the extraction of tyramine metabolites produced in the enzyme reaction were performed with these detergents at a concentration of 2%, except in the case of SDS which was used at 0.2%. Two identical enzyme assay mixtures were prepared and incubated for 10 min as described under Materials and Methods. Just before stopping the reaction with 3N HCl, the test detergent was added to one mixture and the same volume of water was added to the other, and the metabolites formed in the two mixtures were extracted and compared. The level of radioactivity in the mixture with detergent was always similar to that in the respective control, recovery being 92.0–99.5%, indicating that these detergents did not affect extraction of metabolites; this was also the case with 5-HT or PEA as substrate.

As shown in Fig. 1, even without preincubation, SDS, Triton X-100, octyl-$\beta$-glucoside and sodium cholate strongly inhibited MAO activity with IC50 values of about 0.02, 0.14, 1.2 and 0.7%, respectively. All these detergents except Triton X-100 caused nearly complete inhibition of the activity in the concentration ranges used. After preincubation, the extents of inhibition by these detergents were higher (IC50 values, about 0.012, 0.08, 0.5 and 0.6%, respectively). Digitonin caused only weak inhibition at the highest concentration used, 2%; with and without preincubation for 30 min, it caused only about 50% and 30%
inhibition, respectively (Fig. 1).

As with tyramine as substrate, the inhibitory effects of Triton X-100 on MAO activity towards 5-HT, a substrate for MAO-A, and towards PEA, a substrate for MAO-B, were also observed with or without preincubation at 37°C for 30 min (data not shown), confirming our previous observation of the inhibitory effect of Triton X-100 on oxidations of these two substrates (7). With these two substrates, inhibition also depended on the dose and preincubation-time, giving IC50 values of about 0.05% with both 5-HT and PEA and both about 0.1%, respectively, with or without preincubation for 30 min at 37°C. Moreover, the extents of inhibition with these three substrates were similar.

The time course of inhibition by Triton X-100 of carp liver mitochondrial MAO during preincubation at 37°C was determined with 5-HT, tyramine and PEA as substrates. As shown in Fig. 2, slight inhibition was observed without preincubation, and with increase in the preincubation time, the extents of inhibition increased to a maximum after 30–40 min preincubation with 5-HT and PEA as substrates: even after preincubation for 90 min, inhibition was incomplete with either substrate. This was also the case with tyramine as substrate. In these experiments, there was no remarkable difference in the extents of inhibition with any of the three substrates used.

Analysis of Lineweaver-Burk plots revealed that without preincubation, the modes of inhibition of activities towards 5-HT and PEA were both competitive with respect to the substrates (Fig. 3). However, on preincubation at 37°C for 30 min, inhibition became non-competitive with both substrates. This was also found with tyramine as substrate.

The effects of Triton X-100 on the susceptibility of carp liver mitochondrial MAO to clorgyline, a specific MAO-A inhibitor, and deprenyl, a specific MAO-B inhibitor, were examined with 5-HT and PEA as substrates.

![Fig. 2. Time course of inhibition of MAO activity in carp liver mitochondria by Triton X-100. MAO activity in carp liver mitochondria (0.01 mg protein) was determined with 5-HT (0.05 mM, solid line) or PEA (0.05 mM, dotted line) as substrate after the various preincubation times indicated at 37°C with 0.02% (○) or 0.07% (●) Triton X-100. The specific MAO activities with the two substrates were similar to those in Fig. 1. Points are means of two duplicate determinations, expressed as percentage of the activity in the absence of Triton X-100.](image-url)
Fig. 3. Lineweaver-Burk plots of inhibition of 5-HT and PEA oxidations by Triton X-100. The oxidations of 5-HT (upper) and PEA (lower) were determined in the absence (○–○) or presence of 0.02% (●–●) and 0.07% (□–□) Triton X-100. For the two left panels, the oxidations of 5-HT and PEA were determined without preincubation with Triton X-100. For the two right panels, the oxidations were determined with preincubation with Triton X-100 at 37°C for 30 min. Ordinates: 1/initial velocity, expressed as 1/dpm 10⁻⁴ and abscissae: 1/mM substrate. Points are means of duplicate determinations.

Fig. 4. Effect of Triton X-100 on the susceptibility of carp liver mitochondrial MAO to clorgyline. The oxidations of 5-HT (left) and PEA (right) were determined in the absence (○–○) and presence of 0.02% Triton X-100 with (●–●) or without (□–□) preincubation at 37°C for 30 min. For determination of the susceptibility to clorgyline, mitochondria (0.01 mg protein) pretreated with Triton X-100 were further preincubated with clorgyline at 37°C for another 30 min before adding the substrate to estimate activity. Values obtained with the detergent-treated mitochondria were expressed as percentages of the inhibition of activity in the absence of clorgyline. The control preparation was pretreated like the detergent-treated preparation, but in the absence of detergent, and then further preincubated at 37°C for 30 min with different concentrations of clorgyline. Points are means of two duplicate determinations.
with or without preincubation with detergent at 37°C for 30 min. The results obtained with clorgyline are shown in Fig. 4. After preincubation, a slight decrease in susceptibility of MAO to clorgyline, compared with that in untreated mitochondria, was observed with either 5-HT or PEA as substrate. A decrease was also the case without preincubation: with and without preincubation, an almost identical slight decrease in susceptibility was observed with deprenyl as inhibitor (data not shown). Thus the extents of decrease in responses to these inhibitors were similar, with or without preincubation, regardless of the inhibitor or substrate used.

For analysis of the reversibility of inhibition by Triton X-100 by Ackermann-Potter plots (11), the MAO activities in various amounts of untreated carp liver mitochondria and of detergent-treated preparation without preincubation were determined. The plots of remaining activities towards 5-HT and PEA were straight, but with a reduced slope compared with those of the control, and both lines passed through the origin, indicating reversible inhibition (Fig. 5) (11). Almost straight lines, intercepting the abscissa to the right of the origin, were obtained with mitochondria preincubated with the detergent at 37°C for 30 min (Fig. 5). However, these lines were not exactly parallel to those of the controls, indicating partially irreversible inhibition, and thus pseudo-irreversible inhibition of MAO activity (11-13). For further investigation of whether inhibition by Triton X-100 at higher concentrations (0.4 and 1.0%) was reversible or irreversible, mitochondria were diluted 10 times with sucrose-buffer after preincubation at 37°C for 30 min. After treatment at either concentration of detergent, no increase of activity towards tyramine was obtained on dilution (87.3±13.8 and 89.4% inhibition of MAO activity by 0.4 and 1.0% Triton X-100, before dilution, and 88.8±9.4 and 88.4% inhibition, after dilution, respectively), indicating that the inhibition was completely irreversible.

**Discussion**

The presence of two different functional forms of mitochondrial MAO, presumably differing in molecular weight (14-17) and physiological functions (3) are usually reported.
Our earlier studies showed the existence of a single form of mitochondrial MAO in carp liver. Although able to oxidize various amine substrates of both mammalian MAO-A and MAO-B, it appeared to be distinct from these two forms of MAO because it showed the same sensitivities to the inhibitors clorgyline and deprenyl (6, 7). These findings suggested that this MAO might be useful in studies on the genesis of the two forms of mammalian MAO (6, 7).

In the present work, we investigated the inhibitory effects of some detergents on carp liver mitochondrial MAO. These detergents have been used to solubilize membrane proteins by breaking down lipid-protein associations; among them, Triton X-100 is the most commonly used for solubilizing membrane-bound enzymes because of its mild effect on protein conformation. However, recently, this detergent has been reported to cause differential inhibition of mammalian mitochondrial MAO-A and MAO-B in different preparations, depending on the conditions used (18-20). Thus, in this study, using carp liver MAO as a model enzyme preparation, we concentrated on the precise mechanisms of the inhibitory effect of Triton X-100.

As shown here, at relatively low concentrations, all these detergents, except digitonin, inhibited mitochondrial MAO activity in carp liver. Thus, caution is recommended in using these detergents to solubilize membrane-bound MAO from various mitochondria.

Among the detergents tested, SDS was the most inhibitory, probably because of its denaturing action. Although Triton X-100 was less inhibitory, it inhibited the activity with three substrates at relatively low concentrations of about 0.02%. Binding of the detergent to membrane components would decrease the concentration in the reaction mixture and, therefore, would lead to an apparent increase in the critical micelle concentration (about 0.016% in the case of Triton X-100) (21, 22). Assuming that the amount of the monomeric form of Triton X-100 bound to proteins is 0.2 mg/mg of protein (23), under these experimental conditions (total amount of protein in the reaction mixture =0.03 mg: 0.01 mg of mitochondrial protein plus 0.02 mg of BSA), the apparent critical micelle concentration of Triton X-100 would be about 0.054%, very close to the concentration (about 0.02%) for inhibition of carp liver MAO. Thus, enzyme inhibition is probably due to the micelle form of Triton X-100 rather than to the monomeric form.

Analysis of the mode of inhibition revealed that without preincubation, Triton X-100 competitively and reversibly inhibited oxidations of all three substrates tested, but after preincubation (37°C for 30 min), the inhibition became non-competitive and partially irreversible. Achee and Gabay (20) reported that Triton X-100 inhibited 5-HT oxidation (MAO-A) more than PEA oxidation (MAO-B) in bovine brain mitochondria. Moreover, they found that it caused competitive inhibition with MAO-A, varying from that with MAO-B after a short preincubation time (10 min at 37°C). These evidence suggested that it inhibits the two forms of mammalian MAO differentially. Studies on the effect of Triton X-100 on rat liver mitochondria suggested changes in not only the mode of inhibition for both forms of MAO, but also in the extent of inhibition of MAO-B, which increased to almost the same level as that of MAO-A during preincubation (30 min at 37°C) with Triton X-100 (18).

From these results and the present findings, we conclude that carp liver MAO is homogeneous, but a tissue containing both MAO-A and MAO-B will be more preferentially inhibited towards MAO-A with addition of Triton X-100. In the first phase of the reaction, it inhibits MAO-A competitively and reversibly; but with increasing preincubation time,
its inhibition becomes gradually irreversible and non-competitive for both forms of MAO, finally resulting in similar extents of inhibition of the two forms of MAO. As with rat liver MAO (18), the rate of change in the mode of inhibition by Triton X-100 probably depends on the preincubation temperature as well as the ratio of enzyme to detergent. At low ratios, irreversible and presumably non-competitive inactivation is probably more rapid than when less detergent is present, judging from the pseudo-irreversible inhibition detected at lower concentrations by Ackermann-Potter plots; at higher concentrations, completely irreversible inhibition occurs, as shown by the “dilution” experiments in this study. This competitive inhibition by Triton X-100 without preincubation is understandable in as much as this detergent is lipophilic, so may have high affinity for membrane lipids, including lipids around the active site of the enzyme. Mammalian MAO-A was found to be much more dependent on membrane lipids than MAO-B (24–26), and carp liver MAO is also dependent on lipids (7). This dependence would largely explain the competitive inhibition of MAO-A in mammalian tissues and of carp liver MAO in the first phase of the reaction with Triton X-100. With increase in the preincubation time, some lipids essential for activity and also some of the membrane-bound enzyme molecules may be solubilized, so that the detergent at this stage displays different inhibitory effects from those in the initial stage. This latter possibility is supported by the finding that partial removal of membrane phospholipids may render MAO more sensitive to inhibition by detergent (27).

However, neither of the above explains the lowered susceptibility of carp liver MAO to clorgyline and deprenyl after treatment with Triton X-100 (Fig. 4). This might be explained by the finding that changes in the lipid environment around the enzyme in the membranes, which would probably be caused by Triton X-100 in this study, influence the affinities of these ligands for the active site of the enzyme (28–30). Another suggestion is that these ligands do not readily penetrate to the active site of the enzyme in detergent-protein or detergent-lipid-protein complexes (21, 22) formed on treatment with Triton X-100.

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