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SOME EFFECTS OF NON-IONIC DETERGENT TRITON X-100 ON RAT LIVER MONOAMINE OXIDASE

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Abstract—The non-ionic detergent Triton X-100, an agent used to solubilize mitochondrial membrane monoamine oxidase (EC 1.4.3.4, MAO), has been shown to inhibit markedly MAO activity. The inhibition was non-competitive in nature. Triton X-100 changed the susceptibility of MAO toward clorgyline, a specific type A MAO inhibitor, and deprenyl, a type B inhibitor. Its effect on the temperature dependence of the initial velocity revealed that the transition temperatures for *p*-tyramine and serotonin (22°) and β-phenylethylamine (16° and 27°) were not changed. The stability of the MAO decreased considerably, however, in the presence of Triton X-100, and its inactivation was particularly pronounced somewhat higher temperatures.

Monoamine oxidase (EC 1.4.3.4, MAO) is localized on the outer membrane of the mitchondria [1]. It is difficult to solubilize unless relatively vigorous procedures, such as repeated freezing and thawing, prolonged sonication [2, 3], or detergents, are used [2]. Triton X-100 is the most common non-ionic detergent used in the solubilization process [4], but it can change the lipid microenvironment of the enzyme and it appears to bind strongly to the protein and to modify the properties of the enzyme [5–7]. It has been noted that Triton X-100 exhibits inhibitory effects on MAO activity [8]. The nature of this inhibition, however, is not yet clear. In this report the mechanism of the Triton X-100 inhibitor effect on MAO is described.

MATERIALS AND METHODS

Materials. Male Wistar rats (150–200 g) were used. *para*-[1-^{14}C]Tyramine (*p*-TA), β-[ethyl-^{14}C]phenylethylamine (PEA), 5-[2-^{14}C]hydroxytryptamine (5-HT) and [benzylmethylene-^{3}H]pargyline were purchased from the New England Nuclear Corp. (Boston, MA). Deprenyl was a gift from Prof. J. Knoll, Semmelweis University, Budapest. Clorgyline was provided by May & Baker, Ltd., Dagenham, U.K. All other chemicals were of analytical grade.

Assay of MAO activity. The enzyme activity was determined radioenzymatically as described previously [9]. The enzymes (protein concentration, 19–35 μg/assay) were incubated at 37° for 30 min in the presence of radioactive substrates (0.1 μCi) that had been diluted with unlabeled substrates a yield a final concentration of 1×10^{-4} M for tyramine and 5-hydroxytyramine and 5×10^{-5} M for β-phenylethylamine in a final volume of 200 μl of 0.05 M phosphate buffer (pH 7.5). The reaction was terminated by adding 250 μl of 2 M citric acid. The acid products formed were extracted into 1 ml of toluene–ethylacetate (1:1, v/v), and 600 μl of the extract was transferred to a counting vial containing 10 ml Omnifluor counting fluid (NEN, Boston, MA) and assayed by liquid scintillation spectrometry (Searle, Mark III). Blank values were obtained by including pargyline (5×10^{-3} M) in the incubation mixtures under identical experimental conditions.

Protein was determined by the method of Lowry *et al.* [10] using crystalline bovine serum albumin as a standard.

Preparation of mitochondrial MAO. Freshly dissected rat livers were rinsed with chilled saline, cut into small pieces, and homogenized immediately in ice-cold 0.32 M sucrose in 0.01 M phosphate buffer (pH 7.5). Mitochondria were obtained by differential centrifugation as described previously [11]. Mitochondrial membrane fragments were prepared by lysing the mitochondria in chilled, distilled water followed by centrifugation at 105,000 *g* for 30 min. The membrane preparations were then washed by suspension in chilled, distilled water and recentrifuged.

Effect of temperature on MAO activity. The effect of temperature on MAO activity was determined over a temperature range of 6–41° with the increments being 3–4°. The enzyme was preincubated for 2 min at a specified temperature. Enzyme reactions were initiated by adding the substrates and terminated after 10 min of incubation by adding 2 N citric acid.

Binding of [^{3}H]pargyline. Rat liver mitochondrial membrane fragments (7–15 mg), either treated or untreated with Triton X-100, were incubated with 10.4 nmoles [^{3}H]pargyline (0.5 μCi) at 37° for 60 min in 0.01 M phosphate buffer (pH 7.5). The [^{3}H]pargyline–MAO adduct was then precipitated by centrifugation at 105,000 *g* for 30 min. The pellets were homogenized in chilled, distilled water, recentrifuged, and washed twice more using the same procedure. The final pellets were solubilized in ACS counting fluid (Amersham Co., Arlington Height,

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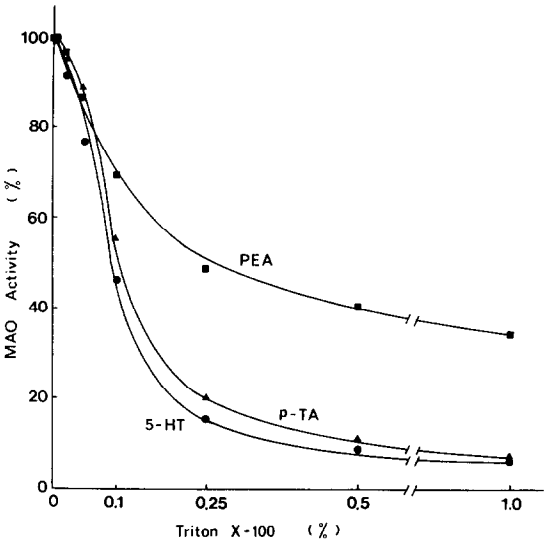

Fig. 1. Effect of Triton X-100 on rat liver monamine oxidase. MAO activity toward *p*-tyramine (*p*-TA), serotonin (5-HT), and β-phenylethylamine (PEA) was assayed over a range of Triton X-100 concentrations, as indicated. The specific activities of MAO in the control reactions were 2.3, 1.3, and 4.5 nmoles·mg^{-1}·min^{-1} for *p*-TA, 5-HT, and PEA respectively.

IL), and the associated radioactivity was counted. Non-specific binding was determined by preincubating the mitochondrial preparation with 1×10^{-3} M nonlabeled pargyline before addition of the tritium-labeled pargyline.

RESULTS AND DISCUSSION

The rat liver MAO activity toward different substrates, i.e. serotonin (type A substrate), β-phenylethylamine (type B substrate), and *p*-tyramine (type A and B substrate), was measured in the absence and presence of Triton X-100 at various concentrations. As indicated in Fig. 1, MAO activity was strongly inhibited by Triton X-100. The inhibition was substrate selective, since the oxidation of β-phenylethylamine (IC_{50}, 0.25%) was somewhat less affected by Triton X-100 than was serotonin (IC_{50}, 0.1%) or *p*-tyramine (IC_{50}, 0.12%). A complete inhibition of the MAO activity could not be achieved even at a concentration of Triton X-100 of 5%.

Lineweaver–Burk plots revealed that the inhibition was typically non-competitive with respect to the oxidations of all three substrates (Fig. 2).

The effect of Triton X-100 on the susceptibility of the rat liver MAO to the specific inhibitors [12, 13] deprenyl (type B MAO inhibitor) and clorgyline (type A inhibitor) during the oxidation of serotonin and β-phenylethylamine was examined. As indicated in Fig. 3, in the presence of the detergent, a decrease in the susceptibility of the MAO to clorgyline in the oxidation of serotonin, as well as to deprenyl in the oxidation of β-phenylethylamine, was observed. No conversion from type A MAO activity to type B activity or vice versa was observed.

A number of membrane-bound enzymes have

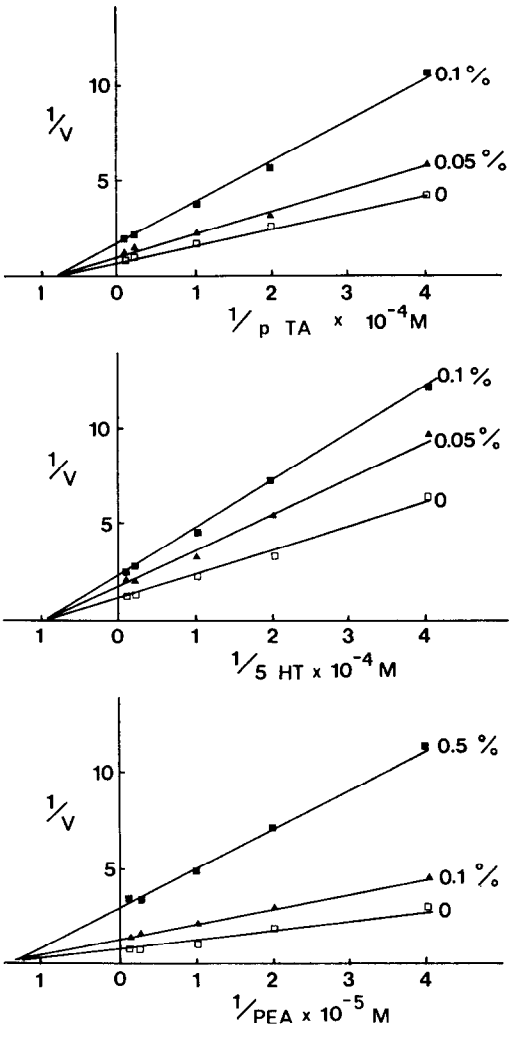

Fig. 2. Lineweaver–Burk plots of rat liver monamine oxidase activity. MAO activity toward *p*-tyramine, serotonin, and β-phenylethylamine was measure in the absence (□——□) and presence of 0.1% (■——■) and 0.05% (▲——▲) Triton X-100. The K_m values obtained were 1.35×10^{-4} M, 1.15×10^{-4} M, and 6.7×10^{-6} M for *p*-tyramine, serotonin, and β-phenylethylamine respectively.

been found to be influenced by the lipid environment that surrounds them; this is reflected by the appearance of transition temperatures in Arrhenius plots. Since it has been suggested that lipids are of considerable importance with respect to the properties of MAO [8, 14–16], it was thought that an Arrhenius plot of the enzyme activity might reveal some information regarding the effect of Triton X-100, which profoundly modifies the lipid environment surrounding mitochondrial MAO. Figure 4 is an Arrhenius plot of mitochondrial MAO activity in the absence and presence of Triton X-100 with *p*-tyramine, 5-hydroxytryptamine, and β-phenylethylamine as substrates. Changes in slope at 22° were observed when serotonin and tyramine were employed as substrates. In contrast, the oxidation of β-phenylethylamine exhibited transition temperatures at 16 and 27°. In

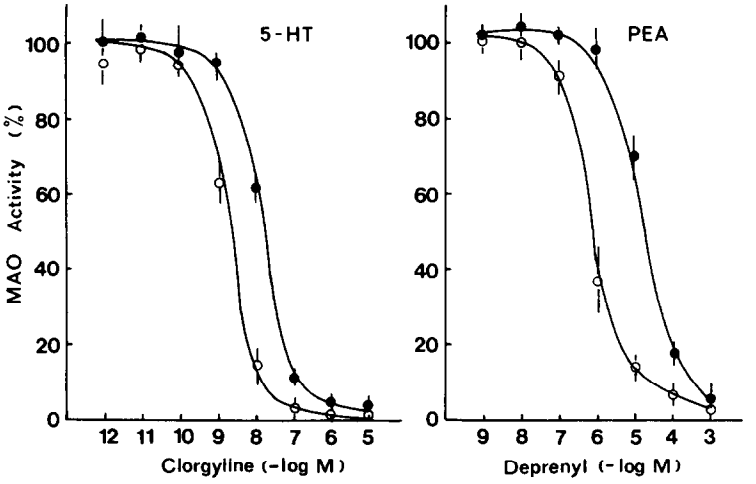

Fig. 3. Effect of Triton X-100 on the susceptibility of rat liver monoamine oxidase to the specific inhibitors clorgyline and deprenyl. The inhibition of MAO was measured in the absence (○——○) and presence (●——●) of 0.2% Triton X-100. The specific activities of MAO in the control reactions were 2.3, 1.1, and 5.7 nmoles·mg^{-1}·min^{-1} for *p*-TA, 5-HT, and PEA respectively. Each value is the mean ± S.D. of triplicate determination.

the presence of 0.25% Triton X-100, the transition temperatures (22° for serotonin and tyramine and 16° for β-phenylethylamine) were apparently not altered. MAO activity, however, decreased at higher temperatures (i.e. above 40°); this may indicate that MAO inactivation occurs under these conditions.

Because MAO in intact mitochondria is usually reasonably stable at temperatures below 50° [7, 17], the effect of Triton X-100 on this temperature stability was investigated. Mitochondrial MAO incubated at 45° in the presence of 0.2% Triton X-100 was much less stable than in the absence of the detergent (Fig. 5). It was concluded that the inhibitory effect of Triton X-100 was primarily due to a change in the stability of the enzyme, perhaps resulting from an induced conformational change in the lipid–protein interaction in the vicinity of the MAO or to an intrinsic alteration in the enzyme protein itself. The inhibition was temperature dependent. At lower temperatures (i.e. less than 10°) Triton

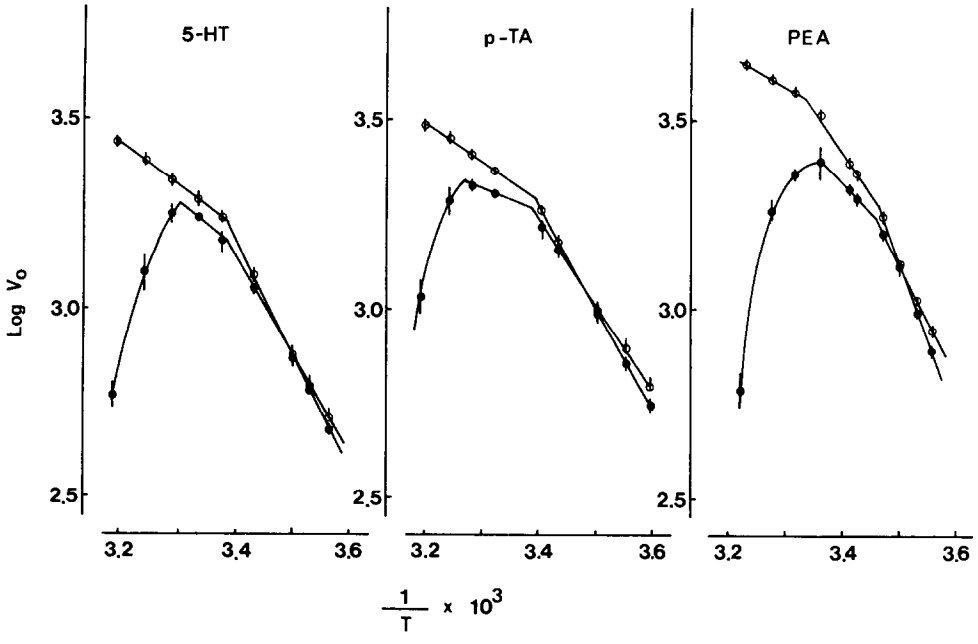

Fig. 4. Arrhenius plot of rat liver monoamine oxidase activity against the reciprocal absolute temperature. MAO activity toward β-phenylethylamine, serotonin, and *p*-tyramine was measured in the absence (○——○) and presence (●——●) of 0.25% Triton X-100. Each value is the mean ± S.D. of triplicate determinations.

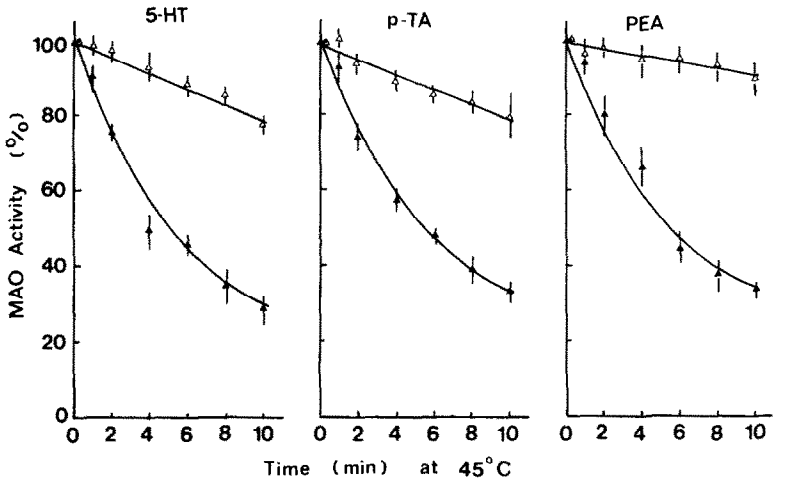

Fig. 5. Effect of Triton X-100 on the thermostability of rat liver monamine oxidase with respect to various substrates. The enzyme was incubated at 45° in the absence (△——△) and presence (▲——▲) of 0.2% Triton X-100. The reaction tubes were transferred at different time intervals to a water bath and allowed to equilibrate for 2 min at 37°. Substrates were then added, and the tubes incubated for a further 10 min at 37°. The specific activities of MAO in the control reactions were 1.5, 0.7, and 2.9 nmoles·mg^{-1}·min^{-1} for *p*-TA, 5-HT, and PEA respectively. Each value is the mean ± S.D. of triplicate determinations.

X-100 did not inhibit MAO activity. The detergent, therefore, could be used as a solubilizing agent for the purification of MAO providing that the purification procedure was carried out at low temperature and the Triton X-100 was removed at a later stage. The inclusion of agents that usually stabilize enzyme activity, such as dithiothreitol (1×10^{-2} M), glycerol (20%) or bovine serum albumin (5%), failed to protect MAO from the Triton X-100-induced inactivation.

The effect of Triton X-100 on the enzyme was so severe that the enzyme lost much of its ability to bind tritium-labeled pargyline in the presence of the detergent (Table 1). Pargyline is a highly specific irreversible MAO inhibitor, which forms stoichiometrically a covalent adduct with the flavine moiety at the active site of the enzyme [18]. The loss of this [^{3}H]pargyline binding activity indicates that the nature of the non-competitive inhibition, namely the decrease in V_{max} and no change of K_m, was due mainly to a reduction in the active enzyme, but not to a dissociation of enzyme–substrate (EA) or enzyme–substrate–Triton X-100 (EAI) complex.

It is interesting that MAO activity has also been

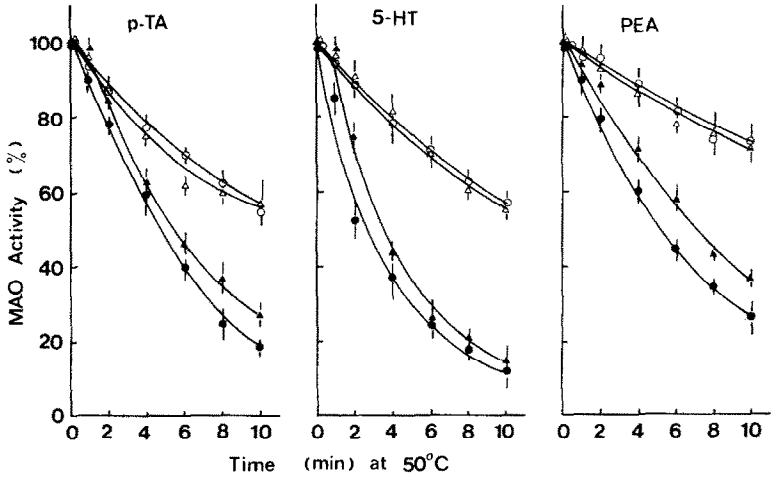

Fig. 6. Effect of ionic strength on the thermostability of rat liver monoamine oxidase with respect to various substrates. The enzyme was incubated at 50° in 0.02 M, (pH 7.5) phosphate buffer (○——○) containing 0.01 M dithiothreitol (△——△) and in 0.2 M (pH 7.5) phosphate buffer (●——●) containing 0.01 M dithiothreitol(▲——▲). The assay conditions are described under Fig. 5. The specific activities of MAO in the control reactions were 2.0, 1.1, and 4.1 nmoles·mg^{-1}·min^{-1} for *p*-TA, 5-HT, and PEA respectively. Each value is the mean ± S.D. of triplicate determinations.

Table 1. Effect of Triton X-100 on binding of [^{3}H]pargyline to rat liver monoamine oxidase*

	Binding of [^{3}H]pargyline (%)	MAO activity (%)
No Triton X-100	100	100
Triton X-100		
0.2%	43	55
0.5%	24	18

* The assay procedure is described in Materials and Methods. Results obtained are mean values of two experiments.

observed to be drastically reduced in the presence of buffers with high ionic strength [8]. MAO is much less stable in 0.2 M phosphate buffer (pH 7.5) than in 0.02 M phosphate buffer at the same pH value (Fig. 6). It is apparent, therefore, that MAO becomes inactivated in a lipophobic polar environment (i.e. high buffer concentrations) as well as in a non-polar lipophilic environment (i.e. Triton X-100). This perhaps indicates that the MAO molecules exist in an intricate conformation and that any slight modification to this conformation inactivates the MAO irreversibly. Although there is as yet no direct evidence to indicate that any modification of the surrounding micro-environment will affect the multiplicity of MAO (i.e. changes to type A from type B and vice versa), properties such as thermostabilities, kinetic parameters, and sensitivities toward inhibitors are changed. With respect to amine oxidation, it seems likely that the environment around MAO is as important as the enzyme itself.

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