# INHIBITION OF DOPAMINE β-MONO-OXYGENASE BY NON-IONIC DETERGENTS OF THE TRITON X-SERIES

Ole TERLAND, Erik SLINDE, Tore SKOTLAND and Torgeir FLATMARK
*Department of Biochemistry, University of Bergen, N-5000 Bergen, Norway*

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## 1. Introduction

Since the discovery that dopamine β-mono-oxygenase (3,4-dihydroxyphenylethylamine, ascorbate-oxygen oxidoreductase (β-hydroxylating), EC 1.14.17.1) is distributed equally between the soluble matrix phase and the inner membrane phase of the adrenal chromaffin granules [1], the non-ionic detergent Triton X-100 has been frequently used for the assay of the latent forms of this enzyme [1–5]. In some cases Triton X-100 has been reported not to affect the enzymic activity [1,5], whereas others have found a rather high degree of inhibition by this detergent [2,4]. During our studies on latent dopamine β-mono-oxygenase activity in the bovine adrenal chromaffin granules, we have observed that Triton X-100 markedly inhibits the enzymic activity of matrix preparations [6]. In order to get a more clear idea of the mechanism of this inhibition and to find the most useful detergent for the assay of latent enzyme activity the effect of a series of related non-ionic detergents have been studied using highly purified dopamine β-mono-oxygenase.

## 2. Materials and methods

### 2.1. *Purification of dopamine β-mono-oxygenase*

Dopamine β-mono-oxygenase was isolated from whole bovine adrenal medulla as described [7], except that Triton X-100 was omitted in the extraction procedure. The specific activity of the enzyme preparation used was 9.7 $\mu$mol·min$^{-1}$·mg protein$^{-1}$ in the ascorbate/tyramine assay [7].

*Abbreviations:* HLB hydrophile-lipophile balance, CMC critical micelle concentration, *n* number of polyethylene units

### 2.2. *Analytical methods*

Dopamine β-mono-oxygenase was assayed by a direct spectrophotometric method, using dopamine both as the substrate to be hydroxylated and as the electron donor [8]. The dopaminequinone thus formed [8] was not reduced to dopamine by NADH as in the assay of Craine et al. [9], but was allowed to be converted into dopaminochrome [10]. The formation of dopaminochrome was followed by measuring the increase in absorbance at 480 nm [10] using the extinction coefficient $\epsilon$(mM$^{-1}$·cm$^{-1}$) = 3.5 [10]. The formation of dopaminochrome was linear with time for about 4 min and the rates given are all initial rates. The formation of dopaminochrome was also linear with the enzyme concentration as long as the rate was less than 3 nmol·min$^{-1}$. The amount of enzyme used in a single assay was always adjusted to give a rate within the linear range. The assay system was: 20 mM PIPES (piperazine-*N*,*N*′-bis[2-ethanesulfonic acid]) buffer, pH 7.0 containing 400 mM KCl [8], 2 mM dopamine and sufficient amount of enzyme; the temperature was 25°C. Detergents or isolated detergent monomers were present at concentrations given in the legends to figures and tables.

Purified dopamine β-mono-oxygenase was estimated from the absorbance at 280 nm, using the absorption coefficient $A^{1\%}_{280\,nm}$ = 9.0 [11]. Protein was determined using bovine serum albumin as a standard [12].



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### 2.3. *Fractionation of detergents*

The different monomers of Triton X-100 were separated by thin-layer chromatography on 0.2 mm silica-gel using water-saturated ethyl-methyl ketone as the solvent [13] and visualized by iodine vapour [13]. The gel material containing the different Triton species was scraped off and the detergent monomers were eluted from the gel by methanol, evaporated to dryness and redissolved in water. The number of ethylene-oxide units ($n$-value) in the monomers was determined by clouding [14], the critical micelle concentration was determined spectrophotometrically [15], and the hydrophile–lipophile balance was calculated as described in the literature [16].

### 2.4. *Chemicals and buffers*

Solutions were made up of deionized water. All chemicals were of analytical grade. The detergents used were obtained from the Sigma Chemical Co., USA except for a single batch of Triton X-100 which was obtained from Serva Feinbiochemica, Germany.

## 3. Results

All the detergents of the 4 (1,1,3,3-tetramethylbutyl)phenylethyleneoxide (Triton X) series tested, except the water-insoluble species, inhibited the catalytic activity of the highly purified dopamine β-mono-oxygenase (table 1). The most widely used detergents of this series, i.e., Triton X-114 ($\bar{n}$ = 7–8), Triton X-100 ($\bar{n}$ = 9–10), and Triton X-102 ($\bar{n}$ = 12–13) were the most potent inhibitors (table 1), whereas the more hydrophilic species with an average hydrophile–lipophile balance, $\overline{HLB} > 15$ were less effective. It should be pointed out that the inhibition also varied from one commercial preparation to the other of a single detergent (fig.1 and table 1). Whereas Triton X-100 obtained from Serva inhibited the activity by approx. 50%, three different batches of Triton X-100 obtained from Sigma revealed a higher degree of inhibition, i.e., 60%, 62% and 66%, respectively. In either case, the inhibition increased when the detergent concentration was increased until the critical micelle concentration (CMC) was reached (fig.1).

The monomeric species of Triton X-100 were separated by thin-layer chromatography (fig.2), and the species with $n$ = 9 ethyleneoxide units and HLB = 13.2 was isolated on a preparative scale. From figs 1 and 2 and table 1 it is seen that this species had no inhibitory effect on the activity of purified dopamine β-mono-oxygenase. Thus, the inhibitory compound migrated on thin-layer chromatography together with the Triton monomers of 7 and 8 ethyleneoxide units (fig.2).

A kinetic plot of $S/v$ versus $S$ (where $S$ is dopamine concentration and $v$ is the amount of dopaminochrome formed per unit of time) gave parallel lines at 0, 0.01 and 0.2% (w/w) of Triton X-100 with no

Table 1
Effect of different non-ionic detergents on the enzymic activity of dopamime β-mono-oxygenase

| Detergent[a] | Activity (%)[b] |
|---|---|
| Control (no detergent) | 100 |
| Triton X-35 (7.8) | 100 |
| Triton X-45 (10.4) | 86 |
| Triton X-114 (12.4) | 40 |
| Triton X-100 (13.5) | |
| Serva Feinbiochemica | 50 |
| Sigma, lot No. 33C-2930 | 34 |
| Sigma, lot No. 100C-3130 | 40 |
| Sigma, lot No. 100C-3130-1 | 38 |
| Triton X-102 (14.6) | 49 |
| Triton X-165 (15.8) | 76 |
| Triton X-305 (17.3) | 79 |
| Triton X-405 (17.9) | 83 |
| Triton X-monomer, $n$ = 9 (13.2)[c] | 100 |
| Triton WR-1339 | 94 |
| Triton N-101 (13.4) | 77 |
| Tween-20 (16.7) | 100 |
| Brij 58 (15.7) | 100 |

[a] The numbers given in parentheses represent the value of hydrophile–lipophile balance which is either calculated (the purified monomers) or obtained from Rohm and Haas Company

[b] $n$ = 3

[c] $n$ represents the number of ethylenoxide units

The detergents Triton X-35 and Triton X-45, which are poorly water soluble, were added to the assay medium at a concentration of 0.02% (w/w), mixed and left for 24 h at 20°C for phase separation. The aqueous phase was used as the assay medium. All other detergents were present at a concentration of 0.02% (w/w). The specific activity of the enzyme was 351 nmol·min$^{-1}$·mg protein$^{-1}$ in the absence of added detergents and represents the 100%-value; all other activities are given in percent of this value.

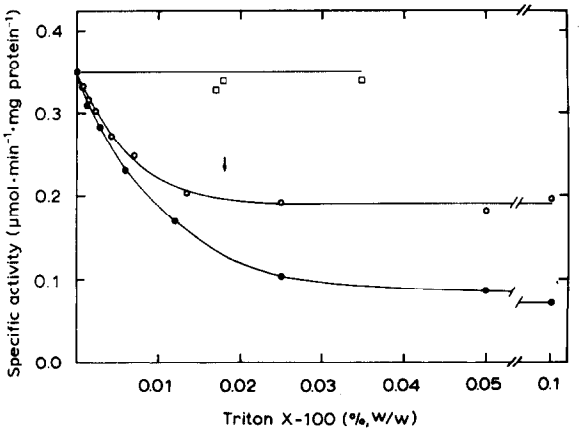


Fig.1. Effect of Triton X-100 and the purified monomer ($n$ = 9) on the activity of dopamine β-mono-oxygenase. The enzyme was assayed in the presence of different concentrations of Triton X-100 from Serva (○) and Sigma, lot No. 33C-2930 (●) or Triton monomer, $n$ = 9 (□). The arrow indicates the critical micelle concentration of Triton X-100 in the assay medium. For experimental details, see Materials and methods section.

measurable change in $V$, but the $K$-value (dopamine) was increased from 5 mM in the absence of Triton X-100 to 17 mM in the presence of 0.2% (w/w) Triton X-100 (Sigma, lot No. 33C-2930).

In the absence of Triton X-100 30 mM fumarate and 400 mM chloride increased the activity of dopamine β-mono-oxygenase to approx. the same degree, i.e., from 33 (no anion added) to 344 and 351 nmol·min$^{-1}$·mg protein$^{-1}$, respectively, but the inhibition by 0.02% (w/w) Triton X-100 was slightly lower with fumarate (i.e., 48%) as compared to chloride (i.e., 65%) as the anion activator.

A marked inhibition of the dopamine β-mono-oxygenase activity by Triton X-100 was also observed when the activity was measured in the ascorbate/tyramine assay systems[7]. Approximately 40% inhibition was observed at 0.92% (w/w) Triton X-100 (Sigma, lot No. 33C-2930) when the substrate concentrations were 1 mM for both tyramine and ascorbate and 0.5 M acetate was used as the activat-

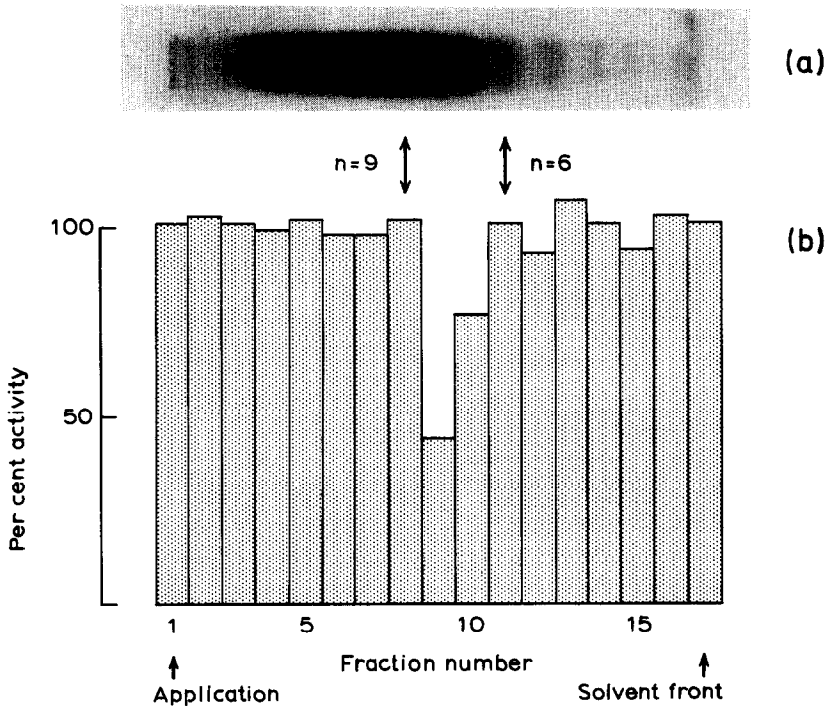


Fig.2. (a) Photography of the iodine spots on thin-layer chromatogram of the Triton X-100 monomers. (b) The distribution of the dopamine β-mono-oxygenase inhibitor(s) on a thin-layer chromatogram (a). Bands of the silica gel (8.5 mm wide) were removed and extracted with methanol, evaporated to dryness and redissolved in the standard assay buffer of the enzyme. The activity measured in the presence of the extracted band is expressed in percent of that measured in the standard assay buffer only. i.e., 351 nmol·min$^{-1}$·mg protein$^{-1}$.

ing anion. Maximal inhibition was also in this case observed at the CMC-value of the detergent.

## 4. Discussion

Triton X-100 has been widely used for the solubilization of the membrane-enclosed form of dopamine β-mono-oxygenase in various biological materials [1–5] since it is generally considered to maintain proteins in their native state [17]. The experiments reported in the present study have demonstrated that a purified monomer of Triton X-100 with a hydrophile–lipophile balance which reveals maximal efficiency in disrupting and dispersing components of biological membranes [18,19] i.e., HLB = 13.2 and $n = 9$, do not affect the activity of dopamine β-mono-oxygenase (fig.1). On the other hand, commercial preparations of Triton X-100 are contaminated to a variable extent by a potent inhibitor of the enzyme (table 1). Different brands appear to contain variable concentrations of the inhibitor (e.g., compare the Serva product and the Sigma products). In this context it is interesting to note that whereas some authors have found 80% inhibition of purified dopamine β-mono-oxygenase by Triton X-100 [2] others have reported no inhibition at all [1,5]. This discrepancy may be due to the absence of the inhibitor in certain commercial preparations, e.g., those used by the latter authors [1,5]. Since maximal inhibition is reached at a detergent concentration around the CMC-value (fig.1) and most workers use detergent concentrations above this value, differences in detergent concentration can not explain the variable degree of inhibition reported in the literature [1,2,4,5].

The chemical nature of the inhibitor and the mechanism of inhibition is not yet known. No evidence has been obtained that, e.g., Triton X-100 induces a change in the quaternary structure of the enzyme [Terland, O., unpublished data] as recently reported for $(Na^+–K^+)$ATPase [17]. Trace metals [17] do not seem to be involved in the inhibition of dopamine β-mono-oxygenase since the inhibitory component(s) appears to have rather hydrophobic properties based on the mobility on thin-layer chromatography (fig.2). Furthermore, our kinetic data indicate that the inhibition is of a competitive type and a submaximal concentration of the substrate (2 mM dopamine) was therefore used in our standard assay procedure in order to clearly demonstrate the inhibition. The competitive type of the inhibition may also explain why Triton X-100 has been reported to inhibit dopamine β-mono-oxygenase activity almost completely when low concentrations of tyramine is used as the substrate to be hydroxylated [4]. The present study has also revealed that the percentage degree of inhibition of the enzyme by commercial preparations of Triton X-100 varies with the chemical nature of the anion activator (fumarate versus chloride).

The inhibition by Triton X-100 is not unique to dopamine β-mono-oxygenase. Thus, the rotenone-sensitive mitochondrial NADH dehydrogenase is inhibited to approx. 80% by 0.005% (w/w) Triton X-100 [20], and the *sn*-glycerol-3-phosphate acyltransferase of *Escherichia coli* [21] is almost completely inhibited by Triton X-100. Other non-ionic detergents, e.g., Lubrol, are also known to inhibit certain enzymic activities [22]. The mechanism of all these inhibitions [20–22] are not yet well characterized and it is not known if they can be avoided by using purified detergent monomers, as is the case for dopamine β-mono-oxygenase. However, such monomers should be used in future studies on the membrane-enclosed form of this enzyme.

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