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EFFECT OF TRITON X-100 ON THE ACTIVITY AND
SOLUBILIZATION OF RAT LIVER MITOCHONDRIAL
PHOSPHATIDYLSERINE DECARBOXYLASE*

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It was shown that, among ionic and non-ionic detergents tested, only Triton X-100 was able to stimulate the activity of rat liver phosphatidylserine decarboxylase, whereas other detergents were without effect or were inhibitory.

The solubilization procedure of phosphatidylserine decarboxylase from mitochondrial membranes with Triton X-100 was elaborated.

The dependence of the solubilized decarboxylase on the Triton X-100 to phosphatidylserine ratio and the inhibitory effect of Triton X-100 at its molar ratio to phospholipid higher than 5.6 was observed.

No divalent cation requirement and no dependence of the ionic strength for the solubilized enzyme were observed. Kinetic parameters were determined.

Decarboxylation of phosphatidylserine is one of the pathways of phosphatidylserine formation in animal cells [1]. The reaction proceeds in mitochondria [2, 3] to which the lipid substrate has to be transported from the endoplasmic reticulum where it is synthesized [4, 5]. A mediatary role of cytosolic non-specific phospholipid transfer protein(s) in this process is generally accepted [6-8].

Inside the mitochondrion, phosphatidylserine decarboxylase (EC 4.1.1.65) is located in the inner membrane in liver [9, 10] and brain [11]. As established for rat liver mitochondria, the active centre of the enzyme is available for its substrate from the outer surface of the membrane [10].

Although the activity of the brain [11, 12] and liver [2, 13] mitochondrial phosphatidylserine decarboxylase has also been investigated in the presence

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of detergents, neither systematic studies nor solubilization of the enzyme have been achieved. This is in contrast to bacterial enzyme that has been purified with Triton X-100 almost to homogeneity [14].

In the present paper, the effect of various ionic and nonionic detergents on the activity of rat liver mitochondrial phosphatidylserine decarboxylase and conditions for its release from the membrane are investigated. In addition, some properties of the solubilized enzyme are described.

METHODS

Unlabelled phosphatidylserine from beef brain cortex was purified by a combination of extraction method [15] and column chromatography on DEAE-cellulose according to Rouser et al. [16]. Labelled phosphatidylserine was obtained by incubation of rat liver microsomes with DL-[1-\textsuperscript{14}C]serine as described by Bjerve [4] followed by lipid extraction by the method of Bligh & Dyer [17] and the same purification procedure as for unlabelled material.

Liposomes were prepared from the mixture of unlabelled and labelled phosphatidylserine by 4 x 30 s sonication of dry lipid material in 250 mM sucrose, 10 mM Tris/HCl, 1 mM EDTA, pH 7.4 using Techpan UDM-10 (Warsaw, Poland) sonicator working at maximum output.

Decarboxylation of phosphatidylserine was followed by measuring the production of \textsuperscript{14}CO\textsubscript{2} [2, 13] using test tubes sealed with flexible rubber stoppers equipped with hanging polypropylene cups containing a strip of Whatman 3 MM filter paper soaked with 150 \textmu l phenylethylamine. The incubation medium contained 100 mM phosphate buffer, pH 7.4 or 250 mM sucrose, 10 mM Tris/HCl or phosphate buffer, pH 7.4 and 10 mM mercaptoethanol, mitochondria or mitochondrial membranes (1.5 mg protein/ml) and liposomes 400 nmol of phospholipid phosphorus and about 20000 d.p.m. of \textsuperscript{14}C phosphatidylserine labelled in the carboxyl carbon of the serine moiety. Total volume was 1 ml. Where indicated rat liver cytoplasmic fraction and/or Triton X-100 were added. Incubation was carried out under constant shaking for 60 min at 37°C. At the end of incubation, 0.25 ml of 6 M HClO\textsubscript{4} was added. After the reaction was stopped, shaking was continued for further 30 min in order to bind the liberated CO\textsubscript{2}. Thereafter, the polypropylene cups were transferred into scintillation counting vials and counted for radioactivity.

Mitochondria, microsomes and the cytoplasmic fraction from rat liver were isolated by the conventional procedure [18]. The cytoplasmic fraction was made free of lipoproteins by sedimenting them at pH 5.1 [19].

The purity of phosphatidylserine preparations was checked by thin-layer
chromatography using two-dimensional system [20]. Phospholipid phosphorus was determined as described by Rouser et al. [20].

Protein was determined according to Lowry et al. [21] after its precipitation with 5% trichloroacetic acid in the presence of 2.5% sodium deoxycholate. Bovine serum albumin served as standard. Only in the case of fresh intact mitochondria the biuret method of Gornall et al. [22] was applied.

RESULTS

Freezing and thawing of mitochondria did not change the activity of phosphatidylserine decarboxylase. Such treatment followed by 105,000 × g centrifugation can be useful in removing soluble mitochondrial proteins and permitting to obtain an enzyme-enriched preparation. All studies described in this paper, (except those of Fig. 3), are performed on the preparation that was subjected to this procedure. As shown in Fig. 1, Triton X-100 was the only one among various ionic and nonionic detergents used which stimulated the decarboxylation of phosphatidylserine in mitochondrial membranes. Mitochondrial membranes treated with 5.4 mM Triton X-100, i.e. the concentration that gave the maximum activity, were further analysed after centrifugation for 1 h at 105,000 × g. The enzyme activity was found in the

![Graph](image)

Fig. 1. Effect of various detergents on the activity of phosphatidylserine decarboxylase in mitochondrial membranes. The incubation medium contained 250 mM sucrose, 1 mM EDTA, 10 mM Tris/HCl, pH 7.4, 1.5 mg mitochondrial protein, 0.2 mM [1-14C]phosphatidylserine (specific radioactivity 37000 d.p.m./µmol). The total volume was 2 ml and incubation time 60 min. The data from two independent experiments. Additions: ▼, none; ▲, sodium dodecylsulfate; △, sodium cholate; ■, sodium taurocholate; ▽, cetyltrimethyl bromide; ○, Tween 80; □ octylglucoside; ●, Triton X-100.
supernatant fraction (Fig. 2) indicating solubilization of the enzyme from the inner mitochondrial membrane. The solubilization medium contained 1 mM EDTA and 10 mM mercaptoethanol. Both these components were necessary to obtain the highest activity and removal any of them resulted in a decrease of the supernatant phosphatidylserine decarboxylase activity by about 10-20%.

The effect of Triton X-100 was also checked on whole mitochondria in the medium containing 100 mM phosphate. It was observed (Fig. 3) that a significant activity was obtained without addition of Triton X-100 in the presence of rat liver cytoplasmic fraction, which indicates that in its absence transfer of phosphatidylserine may be a limiting factor. With increasing
concentrations of the detergent in the presence as well as in the absence of the cytoplasmic fraction, the decarboxylation activity decreased up to 0.31 mM Triton X-100. It increased again, reaching at 1.55 mM of Triton X-100,

![Graph](image-url)

Fig. 3. Effect of Triton X-100 on phosphatidylserine decarboxylase activity in rat liver mitochondria in the presence and absence of the cytoplasmic fraction. The incubation medium contained 125 mM sucrose, 100 mM phosphate buffer, pH 7.4, 1 mM EDTA, 10 mM mercaptoethanol, 0.4 mM \([1^-{\text{C}}]\)phosphatidylserine (specific radioactivity 32,000 d.p.m./µmol) and 1.5 mg of mitochondrial protein. The total volume was 1 ml and incubation time 60 min; O, 7.5 mg protein of the cytoplasmic fraction added; ●, cytoplasmic fraction absent.

![Graphs](image-url)

Fig. 4. Dependence of the activity of solubilized phosphatidylserine decarboxylase on the time of incubation, substrate concentration and protein content. The assay of phosphatidylserine decarboxylase activity was performed in the medium consisting of 100 mM phosphate buffer, pH 7.4, 1 mM EDTA, 10 mM mercaptoethanol, 10% glycerol, and 25 mM sucrose in total volume of 1 ml. In (A) and (B) 0.44 mg solubilized mitochondrial protein and in (A) and (C) 0.4 mM \([1^-{\text{C}}]\)phosphatidylserine (specific radioactivity 48,000 d.p.m./µmol) was used. In (B) and (C) the concentration of phosphatidylserine and protein varied, as indicated; the incubation time for (B) and (C) was 60 min. Each point in this Figure is the mean of two incubations.
the level observed in the presence of the cytoplasmic fraction without Triton X-100. At such high concentration of Triton X-100 the activity was independent of the presence or absence of the cytoplasmic fraction.

![Graph 1](image1.png)

**Fig. 5.** Dependence of the activity of the solubilized phosphatidylserine decarboxylase on Triton X-100 concentration in the presence and absence of the cytoplasmic fraction. Solubilized mitochondrial membrane proteins (0.16 mg) was incubated with 0.16 mM [1-14C] phosphatidylserine (specific radioactivity 49,500 d.p.m./μmol) in the medium containing 250 mM sucrose, 10 mM phosphate, pH 7.4, 0.1 mM EDTA, 10 mM mercaptoethanol in the absence (●) and presence (○) of the cytoplasmic fraction (10 mg protein) and various concentrations of Triton X-100. Total volume was 2.5 ml and incubation time 60 min.

![Graph 2](image2.png)

**Fig. 6.** Effect of pH variation on the activity of solubilized phosphatidylserine decarboxylase. Each incubation medium contained 100 mM phosphate adjusted to the required pH value, 10 mM mercaptoethanol, 1 mM EDTA, 1.55 mM Triton X-100, 25 mM sucrose, 10% glycerol, 0.4 mM [1-14C]phosphatidylserine (specific radioactivity 40,000 d.p.m./μmol) and solubilized membrane proteins (0.33 mg). Total volume was 1 ml and the incubation time 60 min. Each point is the mean of two incubations.
Table 1

Dependence of phosphatidylserine decarboxylase activity on the molar ratio
Triton X-100 to phosphatidylserine

Incubation conditions as in the legend to Fig. 5, except that solubilized membrane proteins were added in the amount of 0.29 mg per sample

<table>
<thead>
<tr>
<th>Triton X-100/ phosphatidylserine (molar ratio)</th>
<th>Activity of phosphatidylserine decarboxylase (nmol $^{14}$CO$_2$/mg protein per h) with 0.16 mM phosphatidylserine</th>
<th>0.32 mM phosphatidylserine</th>
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<tr>
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<td>75.7</td>
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With the enzyme solubilized in the presence of 1.55 mM Triton X-100 the decarboxylation of phosphatidylserine proceeded nearly linearly within 60 min with 0.4 mM lipid substrate (Fig. 4A). The rate of decarboxylation was dependent on the concentration of lipid substrate (Fig. 4B). An apparent $K_m$ value of 67 µM was calculated from the double reciprocal plot (insert of Fig. 4B). The $V_{max}$ value was 77 nmol $^{14}$CO$_2$ per mg protein per hour. The linearity of the reaction was shown for protein concentration up to 0.25 mg/ml. The lowering of Triton X-100 concentration in the incubation medium restore the requirement for the presence of the cytoplasmic transfer protein for full decarboxylase activity (Fig. 5). One of possible explanations could be formation of protein aggregates. If so, they must have been small since they were not sedimented on centrifugation at 105 000 x $g$ for 1 h in the medium containing 0.15 mM Triton X-100. On the other hand, concentrations of Triton X-100 higher than 1.5 mM were inhibitory. This inhibitory effect was related to the ratio of Triton X-100 to phosphatidylserine. As shown in Table 1, maximum activity of decarboxylase, independently of phosphatidylserine concentration, was expressed at Triton to phospholipid molar ratio of 5.6.

The solubilized enzyme was maximally active in slightly acidic medium and the activity decreased with increasing pH (Fig. 6). The solubilized phosphatidylserine decarboxylase did not require any divalent cations for its activity. As shown in Fig. 7, concentration of divalent cations higher than in the millimolar range were inhibitory. The solubilized enzyme was not dependent either on the ionic strength (not shown). As in earlier studies of other
authors [11, 23, 24], the activity of phosphatidylserine decarboxylase was inhibited by hydroxylamine. In our experimental conditions with 0.18 mg of solubilized mitochondrial membrane protein, hydroxylamine as low as 1 μM concentration gave a 50% inhibition of phosphatidylserine decarboxylase activity.

DISCUSSION

Earlier studies have shown that Triton X-100 stimulates the activity of phosphatidylserine decarboxylase in rat liver [2] and brain mitochondria [12] and in a particulate fraction from Tetrahymena pyriformis [23, 25]. Other detergents as Cutsccum and sodium taurocholate also stimulated decarboxylase of calf brain mitochondria [11]. Warner & Dennis have shown that phosphatidylserine decarboxylase of particulate fraction from Tetrahymena is mildly stimulated at certain Triton X-100 to substrate ratio and therefore concluded that the enzyme acts on phosphatidylserine present in either bilayer form or mixed micelles. The inhibitory effect of Triton X-100 at molar ratio to phospholipid higher than 8:1 they explained by dilution of the phospholipid substrate at the micellar surface. The same authors, studying the effect of Triton X-100 on the purified enzyme from Escherichia coli, have shown maximum activity of the decarboxylase at molar ratio of Triton X-100 to phosphatidylserine of about 6 and inhibition at the
higher ratio [27]. The conclusion drawn from these studies is that phosphatidylserine in the bilayer is not accessible to the solubilized form of the enzyme while this phospholipid incorporated into mixed micelles is accessible.

In our investigations with the solubilized enzyme we have also observed a dependence of the phosphatidylserine decarboxylation on Triton X-100 to phospholipid ratio and an inhibitory effect of Triton X-100 at its molar ratio to phospholipid higher than 5.6 (Table 1). Although the source of the enzyme was different, the explanation given above can be applied to our data as well. However, such explanation must assume that the detergent itself does not affect the enzyme structure. The inhibitory effect of low concentrations of Triton X-100 found for whole mitochondria (Fig. 3) could be due to the inappropriate detergent/phospholipid ratio or to a direct effect of the detergent on the mitochondrial membrane.

Solubilization of phosphatidylserine decarboxylase from the mitochondrial membrane allowed us to study some of its properties, e.g. pH optimum or cation requirement that could not be determined under conditions in which the transfer of the phospholipid substrate to the enzyme in the membrane was a limiting factor. As shown in Fig. 7, solubilized rat liver mitochondrial phosphatidylserine decarboxylase had no absolute metal requirement. In this respect it differs from the membrane-bound enzyme which has been found to be activated by manganese ions [13]. Percy et al. [11] have also found that phosphatidylserine decarboxylase of calf brain mitochondria measured in the presence of sodium taurocholate is partially inhibited by 4 mM Mn\(^{2+}\), Mg\(^{2+}\) or Ca\(^{2+}\). According to the data of McMurray [28], Mn\(^{2+}\) stimulates mitochondrial decarboxylation of phosphatidylserine added in the form of liposomes but not when the microsomal membranes are used as a donor of substrate. In the presence of Triton X-100, which by itself stimulates decarboxylation with both forms of substrate, he observed that the action of Mn\(^{2+}\) is more inhibitory with microsomes; this indicates that Mn\(^{2+}\) affects the substrate rather than the enzyme. No requirement for divalent cations, shown in case of the solubilized enzyme, supports our earlier suggestion [13] that these cations act by facilitating the accessibility of negatively charged liposomes to the enzyme located in the negatively charged mitochondrial membrane.

The data of other authors using bacterial and protozoan material [14, 26, 29] and of our previous study [13] have shown that the decarboxylase can act in a broad pH range. Similar pH dependence has also been obtained for the solubilized enzyme.

In conclusion, the present paper describes conditions for the solubilization of rat liver mitochondrial phosphatidylserine decarboxylase and some properties of the solubilized enzyme. Further purification steps are required to learn on its structure and properties.
REFERENCES


