ANNA DYGAS, JÓZEF ZBOROWSKI and LECH WOJTCZAK

DECARBOXYLATION OF PHOSPHATIDYLSERINE BY RAT LIVER MITOCHONDRIA

Department of Cellular Biochemistry, Nencki Institute of Experimental Biology,
ul. Pasteura 3; 02-093 Warszawa, Poland

1. The decarboxylation of phosphatidylserine was studied using particles obtained
by sonication of rat liver mitochondria as the source of the enzyme, and liposomes
prepared from total microsomal phospholipids labelled in phosphatidylserine. The
reaction was followed by measuring formation of either CO₂ or phosphatidylethanol-
amine.

2. The reaction was inhibited when isotonic sucrose was substituted by equiosmotic
solutions of electrolytes (acetate or phosphate).

3. Optimum pH for the reaction was 5.0 - 5.2.

4. At pH 7.4 the reaction was stimulated by the cytoplasmic fraction from rat
liver, most likely due to the action of phospholipid transfer protein(s).

5. The reaction was stimulated by Mg²⁺ and Mn²⁺. Maximum stimulation
occurred at 2 - 3 mM-concentration of the divalent cation.

Phosphatidylserine decarboxylase (EC 4.1.1.65) has been found in mammalian
liver (Borkenhagen et al., 1961; Taki & Matsumoto, 1973; Suda & Matsuda, 1974),
bacteria (Kanfer & Kennedy, 1964; Patterson & Lennarz, 1971) and Protozoa
(Dennis & Kennedy, 1970). According to the available data, decarboxylation of
phosphatidylserine is the only pathway for phosphatidylethanolamine formation
in bacteria. In contrast, in rat liver the CDP-ethanolamine-dependent pathway
is also operating.

In rat liver, phosphatidylserine decarboxylase was found to be located in mito-
chondria (Dennis & Kennedy, 1972). There is, however, a discrepancy concerning
the intramitochondrial localization of the enzyme. Taki & Matsumoto (1973) sug-
gested that the outer membrane is the locus of the decarboxylase in mouse liver mito-
chondria, whereas Van Golde et al. (1974) provided evidence for its presence in the
inner membrane of rat liver mitochondria.

[153]
In mammalian tissues, phosphatidylserine is formed mainly by the base-exchange reaction which proceeds extramitochondrially (Hübscher, 1962; Porcellati et al., 1971; Bjerke, 1973). Thus, to be decarboxylated, phosphatidylserine must be transported into the mitochondria. The participation of a soluble cytoplasmic protein in this transport has been first proposed by Butler & Thompson (1975).

Most of the studies on the properties of phosphatidylserine decarboxylase and conditions in which the reaction proceeds have been done so far on bacterial and protozoan material. The present investigation provides a more detailed information on the properties of this enzyme in rat liver mitochondria.

MATERIALS AND METHODS

Albino rats of the Wistar strain, about six months old, fed a standard laboratory diet, were used.

Liver mitochondria and microsomes were isolated in 250 mm-sucrose - 2 mm-Tris/HCl (pH 7.4) or 225 mm-mannitol - 75 mm-sucrose - 3 mm-Tris/HCl (pH 7.4) by the conventional procedure. The cytoplasmic fraction, i.e. the post-microsomal supernatant, was used directly or was made free of lipoproteins by sedimenting them at pH 5.1 (Wirtz & Zilversmit, 1969).

Microsomes labelled in phosphatidylserine were obtained by incubation with 50 μCi L-[U-14C]serine (105 mCi/mmol) or DL-[1-14C]serine (10.7 mCi/mmol) and 3 mm-CaCl₂ in 60 mm-imidazole buffer (pH 7.4) according to Bjerke (1973). The incubation was terminated by addition of ice-cold EDTA to the final concentration of 10 mm, and the mixture was allowed to stand for 15 min. Lipids were extracted by the method of Bligh & Dyer (1959).

Submitochondrial particles were obtained by sonication of mitochondria at 0°C for 4 min using MSE sonicator (16 Kcycles/sec) working at maximum output. The particles were collected by centrifugation at 160 000 gₛ for 60 min. This preparation contained fragments of the outer and inner mitochondrial membranes. Usually, the particles were used immediately after preparation. In some experiments, they were stored for a few days at -20°C which resulted in a loss of 10 - 20% of the activity.

Liposomes were prepared from total microsomal lipids by sonication at 30°C for 2 min.

Decarboxylation of phosphatidylserine was followed by measuring either the production of CO₂ or the formation of phosphatidylethanolamine from [14C]phosphatidylserine. In the first method, used in all experiments shown except that of Fig. 1, the assay was carried out at 37°C in a Warburg vessel. The main compartment contained submitochondrial particles (3 mg protein) and liposomes (1.25 μmol of phospholipid phosphorus and 40 000 counts per min of [14C]phosphatidylserine labelled in the carboxyl carbon of serine) in the total volume of 1.5 ml. The center compartment contained a strip of filter paper soaked with 250 μl of Hyamine 10-X hydroxide in methanol (10%, w/v). The side arm was filled with 0.5 ml of
3 m-HClO₄ that was added to stop the reaction. After the reaction was stopped, shaking was continued for 30 min in order to bind the liberated CO₂. The content of the center compartment was then quantitatively transferred into a vial containing the scintillation cocktail and counted for radioactivity. In the second method (Fig. 1 only) liposomes contained [¹⁴C]phosphatidylserine labelled uniformly in the serine moiety. The incubation conditions were the same as those for the first method. The incubation was terminated by the addition of 10% trichloroacetic acid and the lipids were extracted by the mixture of chloroform-methanol (2:1, v/v). Before chromatography, the solvents were evaporated and the lipids re-extracted with chloroform-petroleum ether (1:1, v/v).

Separation of phosphatidylserine and phosphatidylethanolamine was performed by thin-layer chromatography according to Wagner et al. (1961) or Rouser et al. (1970). Spots were visualized by iodine vapour and, after evaporation of iodine, scraped off for the determination of phospholipid phosphorus (Rouser et al., 1970) and radioactivity.

Radioactivity was measured by liquid scintillation. The counting was corrected for efficiency on the basis of the channel ratio.

Protein was estimated by the biuret method (Gornall et al., 1949) after solubilization of the material with deoxycholate. Bovine serum albumin was used as a standard.

The experimental results presented are average of duplicate incubations. The values of the non-enzymatic decarboxylation were usually low and were subtracted.

RESULTS

The decarboxylation of phosphatidylserine was strongly dependent on pH and proceeded at maximum rate at pH 5.2 in acetate and phosphate buffers (Fig. 1). This optimum was somewhat shifted in citrate and phthalate buffers.

The effect of incubation time is shown in Fig. 2. The experiment was performed in 250 mM-sucrose buffered with either 20 mM-acetate (pH 5.2) or 20 mM-Tris/HCl (pH 7.4). Similarly as in Fig. 1, the rate of decarboxylation at pH 5.2 was much higher than at pH 7.4. At pH 7.4 the decarboxylation was strongly enhanced by the cytoplasmic fraction. In both sucrose-acetate and sucrose-Tris/HCl the reaction was almost linear for 30 min and gradually decreased thereafter. In the absence of the cytoplasmic fraction the reaction was very slow and proceeded for at least 2 h. The stimulatory effect of the cytoplasmic fraction at pH 7.4 was the same, irrespective whether the cytoplasmic fraction was or was not made free of lipoproteins (see Materials and Methods). In contrast, no stimulation by the cytoplasmic fraction was found when the reaction was carried out at pH 5.2 in the sucrose-acetate medium, and even some inhibition was occasionally observed.

The dependence of phosphatidylserine decarboxylation on mitochondrial protein content is shown in Fig. 3.
Fig. 1. Effect of buffer composition and pH on phosphatidylserine decarboxylation. The concentration of the buffers was 100 mM and the incubation time 60 min. The curves represent three separate experiments: (1) in acetate and phosphate buffers (2.9 mg protein); (2) in citrate and phthalate buffers (3.3 mg protein); and (3) in imidazole-HCl buffer (3.5 mg protein). Sonicated mitochondria instead of submitochondrial particles were used. Buffers: O, Na-acetate; ●, Na,K-phosphate; ▼, Na-citrate; □, Na-phthalate; ▽, imidazole-HCl.

The reaction was also strongly dependent on the ionic strength of the medium. In Fig. 4 two separate experiments performed at two pH values using acetate and phosphate buffers are presented. In both experiments the tonicity of the medium was kept constant by adding sucrose. It is evident that the rate of the phosphatidylserine decarboxylation was much higher at low ionic strength and rapidly decreased with increasing concentration of the buffer. Additional data are also given in Table 1 in which the decarboxylation in sucrose and KCl at two pH values is compared. It can be seen that phosphatidylserine decarboxylation at both pH values studied proceeded more effectively in sucrose than in KCl solutions.

Phosphatidylserine decarboxylation was stimulated by divalent cations, manganese and magnesium. The dependence of the reaction on varying Mn$^{2+}$ concentration measured at pH 5.2 in sucrose - acetate is presented in Fig. 5. The optimum of the cation concentration was found to be 2 - 3 mM. The same optimum was obtained for Mg$^{2+}$ (data not shown). The stimulation produced by Mn$^{2+}$ and Mg$^{2+}$ was of the same range. At pH 7.4 in sucrose - Tris/HCl, the effect of Mn$^{2+}$ was much less pronounced than at pH 5.2 in sucrose - acetate. In the presence of the cytoplasmic fraction the addition of 2.5 mM-Mn$^{2+}$ was without effect or even slightly inhibitory.
Fig. 2. The dependence of phosphatidylserine decarboxylation on the time of incubation. The incubation media contained 250 mM sucrose and the following additions: Δ, 20 mM-Na-acetate (pH 5.2); ○, 20 mM-Tris/HCl (pH 7.4); •, 20 mM-Tris/HCl (pH 7.4) and the post-microsomal supernatant, 14 mg protein.

Fig. 3. The dependence of phosphatidylserine decarboxylation on protein content of submitochondrial particles. The medium contained 250 mM sucrose and the following additions: Δ, 20 mM-Na-acetate (pH 5.2); ○, 20 mM-Tris/HCl (pH 7.4); •, 20 mM-Tris/HCl (pH 7.4) and the post-microsomal supernatant, 10 mg protein. The incubation time was 15 min at pH 5.2 (Δ) and 60 min at pH 7.4 (○, •).
The decarboxylation of phosphatidylserine was strongly inhibited by the non-ionic detergent Triton X-100. In experiments in which submitochondrial particles were briefly preincubated with liposomes and then the detergent was added, 50% inhibition was obtained at 1.7 mM concentration of Triton.

Table 1

Decarboxylation of phosphatidylserine in KCl and sucrose media

Liposomes were incubated with submitochondrial particles for 15 min at pH 5.2 and for 60 min at pH 7.4 in 250 mM-sucrose or 100 mM-KCl buffered with either 20 mM-Na/acetate (pH 5.2) or 20 mM-Tris/HCl (pH 7.4).

<table>
<thead>
<tr>
<th>Medium</th>
<th>Phosphatidylserine decarboxylated (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 5.2</td>
</tr>
<tr>
<td>Sucrose</td>
<td>11.9</td>
</tr>
<tr>
<td>KCl</td>
<td>3.9</td>
</tr>
</tbody>
</table>
Fig. 5. Effect of MnCl₂ concentration on phosphatidylserine decarboxylation. The medium contained 250 mm-sucrose and 20 mm-Na-acetate (pH 5.2). Incubation time was 15 min.

DISCUSSION

The rate of phosphatidylserine decarboxylation may depend on two factors: (1) the catalytic activity of the enzyme and (2) the accessibility of the substrate to the enzyme. Since both submicrosomal particles, as the source of the enzyme, and liposomes made of total microsomal phospholipids have a net negative surface charge, the second factor may play a substantial role. Stimulation of the decarboxylation by divalent cations Mg²⁺ and Mn²⁺, as observed here, may be explained by facilitating the accessibility of liposomal phosphatidylserine to the enzyme. A similar stimulation has been observed for phosphatidylserine decarboxylase from Bacillus megaterium (Patterson & Lennarz, 1971). However, with the decarboxylase from Tetrahymena pyriformis, Warner & Dennis (1975) found no effect of Mg²⁺ and, with the enzyme of mouse liver, Taki & Matsumoto (1973) observed a stimulation by chelating agents. The discrepancy between our result and that of the latter authors is not clear.

Another discrepancy concerns the pH dependence of phosphatidylserine decarboxylation. Taki & Matsumoto (1973) found the pH optimum of 7.4 - 7.6, whereas Suda & Matsuda (1974) working with the same material, i.e. mouse liver mitochondria, used the medium of pH 5.8. A rather sharp pH optimum of 5.0 - 5.2 was found in the present study, whereas the reaction carried out by the membranes of Bacillus megaterium (Patterson & Lennarz, 1971), Escherichia coli (Dowhan et al., 1974) and Tetrahymena pyriformis (Worner & Dennis, 1975) showed broad pH optima.
in the range of 5 - 8. Low pH may not only affect the catalytic properties of the enzyme but also, by lowering the negative charge of the particles and liposomes, may facilitate the interaction between the decarboxylase and its substrate.

By demonstrating the inhibition by Triton X-100, the present investigation confirms the inhibitory effect of detergents on phosphatidylserine decarboxylase of mammalian mitochondria, first described by Taki & Matsumoto (1973) and Suda & Matsuda (1974) for deoxycholate and mouse liver mitochondria. Contrary to this, the enzyme from *Tetrahymena pyriformis* (Dennis & Kennedy, 1970; Warner & Dennis, 1975) and *Escherichia coli* (Dowhan et al., 1974) was activated by Triton X-100.

The accessibility of phosphatidylserine to its decarboxylase can be facilitated by phospholipid-transfer proteins which have been found in rat liver cytoplasm (Bloj & Zilversmit, 1977; Zilversmit & Hughes, 1977; Barańska & Grabarek, 1979). A similar stimulatory action of the cytoplasmic fraction as that shown in Fig. 2 was also found in the case of intact mitochondria and mitoplasts (Barańska & Wojtczak, 1976).

In conclusion, the present investigation shows that certain properties of phosphatidylserine decarboxylase of rat liver mitochondria differ from those described for this enzyme in bacteria and *Tetrahymena*.

REFERENCES


DEKARBOKSYLACJA FOSFATYDYLOSERINY PRZEZ MITOCHONDRIA WĄTROBY SZCZURA

Streszczenie


2. Wykazano, że dekarboksylacja fosfatydyloseryny zależy od siły jonowej środowiska i jest hamowana przy zastąpieniu izotonicznej scharozy ekwiwalarnymi stężeniami elektrolitów (octanu lub fosforanu).

3. Wyznaczono, że optimum pH reakcji przypadła na pH 5.0 - 5.2.

4. W pH 7.4, w środowisku o niskiej sile jonowej, reakcja stymulowana jest przez frakcję cytoplazmatyczną wątrobę szczura. Stymulacja ta prawdopodobnie spowodowana jest przy obecne we frakcji cytoplazmatycznej białka przynoszące fosfolipidy.

5. W nieobecności frakcji cytoplazmatycznej, w środowisku o niskiej sile jonowej, dekarboksylacja fosfatydyloseryny stymulowana jest przez jony Mg²⁺ i Mn²⁺. Maksimum stymulacji stwierdzono przy 2 - 3 mm stężeniu każdego z tych jonów.

Received 5 November, 1979.