DOES A CYTOPLASMIC FACTOR STIMULATE THE TRANSFER OF PHOSPHATIDYLSERINE FROM LIPOSOMES TO MITOCHONDRIA?

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1. Introduction

Mitochondria are the only site in the cell where decarboxylation of phosphatidylserine to phosphatidylethanolamine occurs [1,2]. This process is located in the inner mitochondrial membrane [2]. On the other hand, in view of the available experimental data, the only pathway known for phosphatidylserine formation in mammalian tissues is the base-exchange reaction proceeding in the endoplasmic reticulum [3]. A transfer of [^{14}C]phosphatidylserine from microsomes and liposomes to mitochondria, promoted by a soluble factor from rat liver homogenate, was postulated by Butler and Thompson [4] who also showed that the radio-activity accumulated in mitochondria was present mainly in phosphatidylethanolamine. These authors therefore concluded that phosphatidylserine was transported by a transferring factor to mitochondria where it was subsequently decarboxylated. However, previous studies from this laboratory [5] showed that phospholipids of microsomes could be exchanged mainly and primarily with those of the outer mitochondrial membrane and that the transfer to the inner membrane was very slow.

The present study was undertaken in order further to elucidate the problem of transfer and decarboxylation of phosphatidylserine in animal cells. The system contained liposomes labelled in phosphatidylserine as the phospholipid donor and either intact mitochondria or mitoplasts (inner membrane-matrix particles) as the acceptor. It was found that the cytoplasmic fraction of rat liver homogenate promoted the accumulation of labelled phosphatidylethanolamine but not that of phosphatidylserine in the acceptor particles. These results are discussed in terms of the folowing alternatives: (1) stimulation of phosphatidylserine decarboxylation, and (2) a limited ability of the cytoplasmic fraction to promote the transfer of phosphatidylserine.

2. Materials and methods

Liver mitochondria and microsomes from male Wistar rats were isolated by conventional procedures [6] and mitoplasts (inner membrane-matrix particles) according to Schnaitman and Greenawalt [7]. The cytoplasmic fraction was obtained according to Butler and Thompson [4] as follows. The 105 000 $\times g$ supernatant of rat liver homogenate was adjusted to pH 5.1, the precipitate discarded and the soluble fraction re-adjusted to pH 7.2.

Labelled phosphatidylserine was prepared essentially as described by Bjerve [3]. Liver microsomes, about 150 mg protein, were incubated in 60 mM imidadazole–HCl buffer (pH 7.2) containing 3 mM $CaCl_2$ and 50 μCi L-[U-^{14}C]serine (specific activity 105 mCi/mmol) in final volume of 10 ml. After 30 min at 37°C, microsomes were precipitated with trichloroacetic acid (final concentration 10%) and the precipitate washed two times with water containing unlabelled serine. Lipids were extracted using a mixture of methanol and chloroform (1 : 2, v/v) [8]. Liposomes were prepared by suspending total microsomal lipids in 250 mM sucrose – 2 mM Tris–HCl – 1 mM EDTA (pH 7.4) and sonicating under nitrogen during 20 min. Analysis of the resulting liposomes revealed about 96% radioactivity in phosphatidylserine.

The transfer of phospholipids was examined by incubating mitochondria or mitoplasts (about 10 mg protein/ml) with liposomes (0.5–1.0 μmol total lipid phosphorus/ml, corresponding to 100 000–150 000 counts/min [^{14}C]phosphatidylserine/ml) in 250 mM sucrose – 2 mM Tris–HCl – 1 mM EDTA (pH 7.4) with or without the cytoplasmic fraction (about 3 mg protein/ml) under constant gentle shaking at 37°C. The incubation was terminated by a rapid centrifugation of 0.3 ml aliquots of the mixture through a layer of 0.2 ml of 20% sucrose in a microcentrifuge (Beckman, Model 152). The supernatants were sucked off and the pellets extracted overnight with methanol and chloroform (1 : 2, v/v). Extracted phospholipids were separated by thin layer chromatography on silica gel G. The plates were developed in chloroform/methanol/27% ammonia/water (60 : 25 : 2.5 : 2.5, v/v/v/v) and the spots of phosphatidylserine and phosphatidylethanolamine were counted for radioactivity.

Protein was determined by the Biuret method [9] and phospholipid phosphorus according to Bartlett [10].

3. Results

As shown in fig.1 A, the cytoplasmic fraction of rat liver substanstially stimulated a transfer of the label from liposomes labelled with ^{14}C in phosphatidylserine to mitochondria and mitoplasts. The analysis of the acceptor particles revealed, however, that the label was mainly recovered in phosphatidylethanolamine (fig.1 B). It was therefore evident that the accumulation of phosphatidylethanolamine and not that of phosphatidylserine was potentiated by the cytoplasmic fraction. This was especially clear in case of mitoplasts where only negligible amounts of phosphatidylserine accumulated. Increasing the amount of the cytoplasmic fraction produced a proportional increase in the accumulation of labelled phosphatidylethanolamine in mitoplasts (fig.2). Contrary to this, the accumulation of labelled phosphatidylserine was not increased.

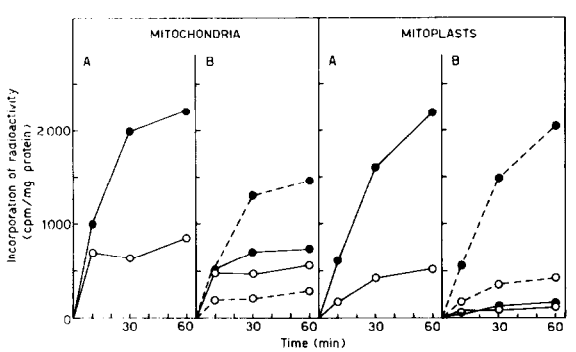

Fig.1. Transfer of [^{14}C]phosphatidylserine from liposomes to mitochondria and mitoplasts (inner membrane-matrix particles). The ordinate indicates radioactivity found in total (A) and individual (B) phospholipids per mg of the acceptor particle protein minus radioactivity transferred in zero time samples. Open symbols, without the cytoplasmic fraction; full symbols, with the cytoplasmic fraction. In B: solid lines, phosphatidylserine; dashed lines, phosphatidylethanolamine.

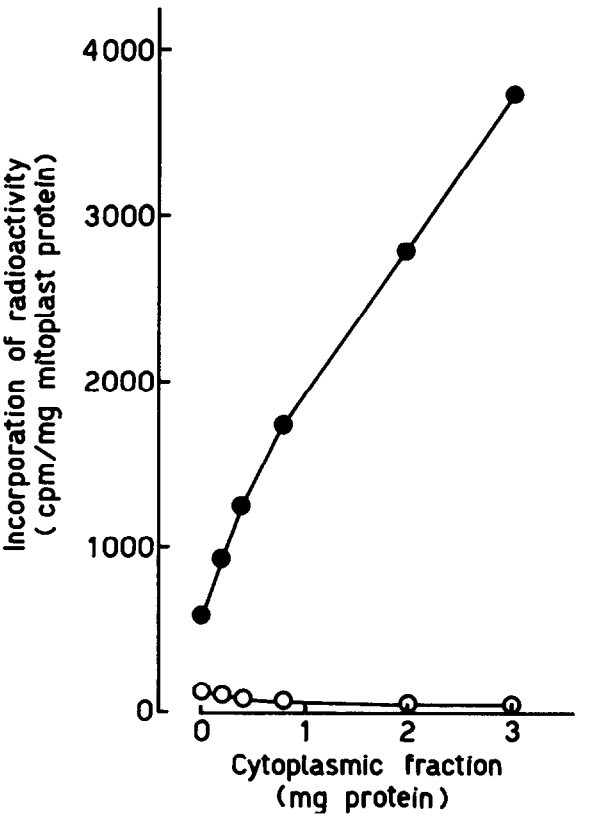

Fig.2. Effect of increasing amounts of the cytoplasmic fraction on the transfer of [^{14}C]phosphatidylserine from liposomes to mitoplasts and the formation of phosphatidylethanolamine. Incubation time 60 min. Values for zero time samples were subtracted. Open symbols, phosphatidylserine; full symbols, phosphatidylethanolamine.

Table 1
Transfer of [^{14}C]phosphatidylserine from liposomes to fresh and heat-treated mitochondria

Acceptor particles	Cytoplasmic fraction	Radioactivity transferred (counts/min/mg particle protein)			Stimulation factor[a]		
		Total	In phosphatidylserine	In phosphatidylethanolamine	Total	For phosphatidylserine	For phosphatidylethanolamine
Fresh mitochondria	None	700	350	340	1.0	1.0	1.0
	Untreated	1400	395	1000	2.0	1.1	2.9
	Heat-treated[b]	980	350	610	1.4	1.0	1.8
Heat-treated mitochondria[c]	None	1410	More than 90%		1.0		
	Untreated	1770	in phosphatidyl-		1.3		
	Heat-treated[b]	1685	serine		1.2		

[a] The stimulation factor is expressed as the ratio of the radioactivity transferred in the presence of the cytoplasmic fraction to that transferred in its absence under otherwise identical conditions.
[b] Heated at 100°C for 5 min.
[c] Heated at 100°C for 5 min and re-homogenized.

To eliminate a possible effect of the decarboxylation of phosphatidylserine to phosphatidylethanolamine, which occurs in the inner mitochondrial membrane [2], heat-treated mitochondria were used as acceptor particles (table 1). Such denatured mitochondria appeared to be a better acceptor for liposomal phosphatidylserine, since twice as much of the total radioactivity was transferred upon them than upon an equal amount of untreated mitochondria. Nevertheless, the cytoplasmic fraction had a negligible stimulatory effect. In contrast to this and in agreement with experiments shown in figs 1 and 2, the cytoplasmic fraction stimulated radioactivity accumulation in untreated mitochondria (table 1), but again this was due to the formation of phosphatidylethanolamine rather than to the accumulation of phosphatidylserine.

Table 1 also shows that heating the cytoplasmic fraction at 100°C greatly diminished its ability to promote the accumulation of phosphatidylethanolamine in mitochondria. Contrary to this, dialysing the cytoplasmic fraction against a sucrose–Tris–EDTA solution had no effect (not shown). The effect of the cytoplasmic fraction on phosphatidylethanolamine accumulation could not be simulated by pyridoxal 5-phosphate, a co-factor of phosphatidylserine decarboxylase [11].

4. Discussion

The present results may cast some doubt as to the existence of an exchange factor for phosphatidylserine in liver cytoplasm, as postulated by Butler and Thompson [4]. It is clearly evident that liver cytoplasmic fraction stimulates a transfer of labelled phosphatidylserine to mitochondria only under conditions when this phospholipid can be immediately decarboxylated to phosphatidylethanolamine and that only the accumulation of phosphatidylethanolamine is increased by the cytoplasmic fraction. Thus, the stimulation of the transfer is the highest in case of mitoplasts where the inner membrane, which is the site of the decarboxylase [2], is directly exposed. It is lower in 'intact' mitochondria and is none in heat-treated mitochondria where the enzyme is inactivated.

The latter observation is in contrast to that reported by Butler and Thompson [4]. However, these authors compared the transfer of phospholipids to heated mitochondria in the presence of the cytoplasmic fraction with that to untreated mitochondria without the cytoplasmic fraction (table 1 of [4]) and gave no control for heated mitochondria without the cytoplasmic fraction. Since heated particles are a better acceptor for phosphatidylserine, even without any cytoplasmic fraction, as shown in table 1 of the

present paper, the conclusion of Butler and Thompson should be taken with caution.

Two explanations may be proposed for the observations made in this study:

(1) There is no real exchange factor specific for phosphatidylserine in the cytoplasmic fraction of rat liver, and the exchange of this phospholipid between membranes occurs spontaneously. The cytoplasmic fraction, however, contains a factor potentiating the activity of phosphatidylserine decarboxylase of the inner mitochondrial membrane. Therefore, phosphatidylserine transferred spontaneously to the inner mitochondrial membrane can be faster decarboxylated in the presence than in the absence of the cytoplasmic fraction, thus enabling new portions of this phospholipid to be accepted. Since the factor in question is non-dialysable, its effect on whole mitochondria must be due to the presence of a certain percentage of particles with disrupted outer membrane [12,13].

(2) The cytoplasmic fraction does contain a phosphatidylserine-specific exchange factor whose activity can be, however, observed only with the inner mitochondrial membrane as acceptor. It may be speculated that the saturation of the outer mitochondrial membrane by [^{14}C]phosphatidylserine, under experimental conditions used, occurs so rapidly that no effect of the exchange factor can be noticed. In contrast, the transport of phosphatidylserine to the inner membrane may proceed for a longer time because of a continuous decarboxylation of this phospholipid to produce phosphatidylethanolamine. It may also be possible that the exchange factor facilitates a transfer of phosphatidylserine specifically to those sites in the inner mitochondrial membrane where the decarboxylase is located.

With the experimental data available so far it is difficult to decide between these two possibilities and therefore further elucidation of this problem is required.

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