EFFECTS OF PHOSPHOLIPIDS ON SOLUBLE PHOSPHATIDYLSERINE SYNTHETASE OF *ESCHERICHIA COLI*

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1. Introduction

Phosphatidylserine synthetase occurs in a soluble form and participates in the biosynthesis of phosphatidylethanolamine by its association with the membrane of *Escherichia coli* [1,2]. However, the other enzymes responsible for the biosynthesis of phospholipids are firmly bound to the membrane [3–5]. It was thought of interest to see how the soluble enzyme functions in the membrane. The present paper deals with the effects of major phospholipid classes of *E. coli* membrane on the kinetic parameters of the soluble enzyme.

2. Materials and methods

Phosphatidylethanolamine *(E. coli),* phosphatidylglycerol (Egg lecithin), phosphatidylserine (Bovine brain) and cardiolipin (Beef heart) were the products of Serdary Research lab.

Soluble phosphatidylserine synthetase was purified from *E. coli* B according to the method described previously [1].

The reaction mixture contained 33 mM potassium phosphate buffer, pH 7.2, 15 mM $MgCl_2$, 0.11 mM L-[U-^{14}C]serine (11 455 cpm/nmole), 1.5 mM CDP-diolein and an appropriate amount of soluble phosphatidylserine synthetase in a volume of 0.6 ml. Prior to initiation of the reaction by the addition of CDP-diolein, the mixture was incubated for 1 min at 37°C with or without a phospholipid at a concentration of 0.1%. The reaction was allowed to proceed for 10 min at 37°C and terminated by the addition of chloroform–methanol (2:1, v/v) and 200 μg phosphatidylserine. After being washed twice with 30 ml 2M KCl, pH 2.0, and distilled water, chloroform layer was dried under nitrogen and separated by thin-layer chromatography with the solvent of chloroform–methanol–acetic acid (65:25:8, by vol). The area of phosphatidylserine was scraped and transferred to a scintillation vial. Radioactivity was measured in the toluene scintillation solution on a Packard liquid-scintillation counter.

Phospholipids suspended in 83 mM potassium phosphate buffer, pH 7.2, at a concentration of 0.25% were sonicated for 3 min with a sonicator, Kaijo Denki T-A-4201 (20KHz), by cooling in an ice bath.

3. Results and discussion

Association of soluble phosphatidylserine synthetase with the membrane [1] may raise a possibility that membrane phospholipids interact with the soluble enzyme. Effects of phosphatidylethanolamine, phosphatidylglycerol and cardiolipin, the major classes of the phospholipids of *E. coli,* on the soluble enzyme were examined. As shown in fig. 1, cardiolipin activated the enzyme and lowered K_m for L-serine from 85 μM to 29 μM. Phosphatidylethanolamine slightly activated the enzyme and decreased K_m from 85 μM to 25 μM. However, in the presence of phosphatidylglycerol, the enzyme was strongly inhibited, and K_m for L-serine was 50 μM. Phosphatidylserine exhibited no effect. An intracellular L-serine concentration has been described to be 80–90 μM in *E. coli* [6]. K_m for L-serine of the enzyme was 85 μM and decreased to 25–27 μM in the presence of

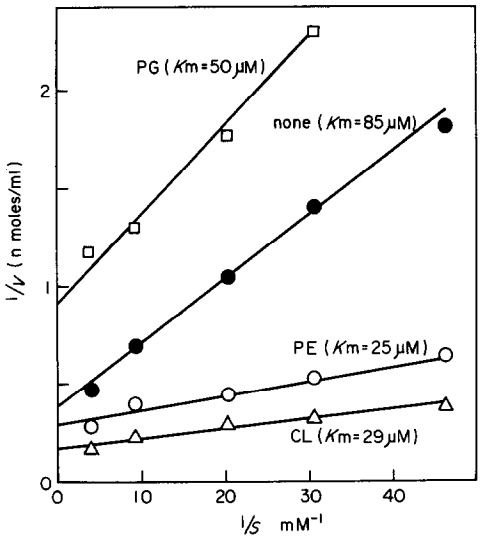

Fig. 1. K_m for L-serine with or without phospholipids. PE, phosphatidylethanolamine; PG, phosphatidylglycerol; CL, cardiolipin.

Table 1
Desensitization of phospholipids effects by heat treatment

Heat treatment	Addition	Phosphatidyl serine formed, nmoles/ml	Activity, %
0 min	None	2.08	100
	Cardiolipin	5.67	273
	Phosphatidyl-ethanolamine	3.08	148
	Phosphatidyl-glycerol	0.59	28
5 min	None	1.62	78
	Cardiolipin	2.04	98
	Phosphatidyl-ethanolamine	1.98	95
	Phosphatidyl-glycerol	0.37	18

The mixture containing 50 mM potassium phosphate buffer, pH 7.2, 91 mM $MgCl_2$, 0.5 mM dithiothreitol and soluble phosphatidylserine synthetase (9.1 units) in a volume of 1 ml was incubated for 5 min at 40°C. The activity obtained in the absence of phospholipids without heat treatment was regarded as 100%.

phosphatidylethanolamine and cardiolipin. Such changes in K_m values are assumed to bear a close relationship to the pool size of L-serine.

It was examined whether the effects of phospholipids were caused by their binding to the soluble enzyme. The enzyme was completely desensitized by treatment for 5 min at 40°C against the effects of cardiolipin and phosphatidylethanolamine without loss of the activity (table 1). Phosphatidyglycerol may bind to a site on the enzyme different from those for cardiolipin and phosphatidylethanolamine, since heat treatment failed to change the phosphatidylglycerol effect. Effects of cardiolipin and phosphatidylglycerol may raise the possibility that phosphatidylserine synthetase, and finally the phosphatidylethanolamine biosynthesis, is controlled by phospholipid species of the alternate biosynthetic pathway in the membrane.

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