THE EFFECTS OF PHOSPHOLIPIDS ON THE ACTIVITY OF ORNITHINE DECARBOXYLASE FROM RAT LIVER

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1. Introduction

Ornithine decarboxylase (EC 4.1.1.17) is believed to catalyze the rate-limiting step in the biosynthesis of the polyamines [1–5] which is known to play a key role in the control of cell growth, proliferation and differentiation [6–9] and therefore the regulatory mechanism of its activity is of particular interest. Inhibitor proteins were demonstrated in rat hepatoma cells [10,11], in other mammalian cells and also in *Escherichia coli* [9]. In [12] non-dialyzable activator in *E. coli* was shown, suggesting the involvement of a post-translational regulatory mechanism in the regulation of the activity of ornithine decarboxylase. A naturally occurring polyanion, heparin, as well as synthetic polyanions such as poly(L-glutamic acid) and dextran sulfate, inhibited ornithine decarboxylase activity presumably as a result of an interaction between the enzyme and a polyanionic compound [13]. In [14], serum albumin, as well as synthetic non-ionic detergents such as Tween 80 and Triton X-100, stimulated the enzyme activity of a highly purified enzyme preparation presumably as a result of the conversion of a less active form to an active form of the enzyme. Here, modulatory effects of phospholipids on the activity of highly purified ornithine decarboxylase from rat liver in vitro are presented: a stimulatory effect by phosphatidylcholine and phosphatidylethanolamine and an inhibitory effect by phosphatidylserine and phosphatidylinositol.

2. Materials and methods

D,L-[1-^{14}C]Ornithine (40–60 mCi/mmol) was purchased from Radiochemical Centre (Amersham). L-α-Phosphatidylcholine (from bovine liver, type III-L), L-α-phosphatidylethanolamine (from egg yolk, type III), and L-α-phosphatidylinositol (from soybean, grade III) were obtained from Sigma. L-α-Phosphatidylserine (from bovine brain) was from Calbiochem. If shipped in a organic solvent solution the organic solvents were removed by a stream of argon and the phospholipids were suspended and then sonicated in water for 1–2 min under an argon atmosphere. Ornithine decarboxylase was purified ~10 000-fold from liver cytosol of rats which had been treated with thioacetamide by acid treatment, ammonium sulfate fractionation, acetone fractionation and chromatography on DE-52, Sephacryl S-200, hydroxylapatite and phenyl–Sepharose CL-4B as in [14]. The activity of ornithine decarboxylase was determined by measurement of the rate of $^{14}CO_2$ evolution from L-[1-^{14}C]ornithine as in [13]. One unit of the enzyme was defined as the amount of enzyme which catalyzed the release of 1 nmol CO_2 from L-ornithine/min at 37°C.

3. Results

The addition of four naturally occurring phospholipids to the assay mixture of a highly purified ornithine decarboxylase (a preparation from the phenyl–Sepharose step) resulted in marked changes in enzyme activity (table 1). Anionic phospholipids such as phosphatidylinositol and phosphatidylserine inhibited the activity of the enzyme while phospholipids without a net negative charge, phosphatidylcholine and phosphatidylethanolamine, stimulated enzyme activity. These four phospholipids were ineffective in modulating the activity of a partially purified ornithine

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Table 1
Effects of phospholipids in the activity of ornithine decarboxylase

	Enzyme from the DE-52 step [nmol $^{14}CO_2$ evolved (% control)]	Enzyme from the phenyl–Sepharose step
None	3.02 (100)	1.70 (100)
Phosphatidylcholine	3.09 (102)	3.70 (218)
Phosphatidylethanolamine	3.20 (106)	3.82 (226)
Phosphatidylinositol	2.79 (92)	0.10 (6)
Phosphatidylserine	2.98 (98)	0.72 (42)

The activities of two enzyme preparations, one from the DE-52 step and the other from the phenyl–Sepharose step of the purification procedures, were estimated under standard conditions except that phospholipids, 100 μg/ml, were added. The specific activities of the enzyme preparations from the DE-52 step and from the phenyl–Sepharose step were ~250 and 3 units/mg protein, respectively

decarboxylase (a preparation from the DE-52 step). Effect of various concentrations of phosphatidylcholine and phosphatidylinositol on the modulation of the activity of a highly purified enzyme is shown in fig.1. Phosphatidylcholine at a final concentration as low as 5 μg/ml caused an almost maximal increase in enzyme activity. In contrast, the concentration of phosphatidylinositol required for a maximal inhibition was ~100 μg/ml. The time course of modulation of the enzyme activity by phosphatidylcholine and phosphatidylinositol is shown in fig.2. Under standard assay conditions, the activation of the enzyme by phosphatidylcholine added to a final concentration of 20 μg/ml occurred very rapidly but a slight time-dependent increase in the activation was observed over the entire 60 min incubation period. Incubation of the enzyme with 20 μg/ml phosphatidylinositol resulted in a progressive inactivation of the enzyme, leading to almost complete loss of the enzyme activity after a 60 min period of incubation. Thus, both the activation and the inactivation of the enzyme by phospholipids appeared to be time-dependent reactions.

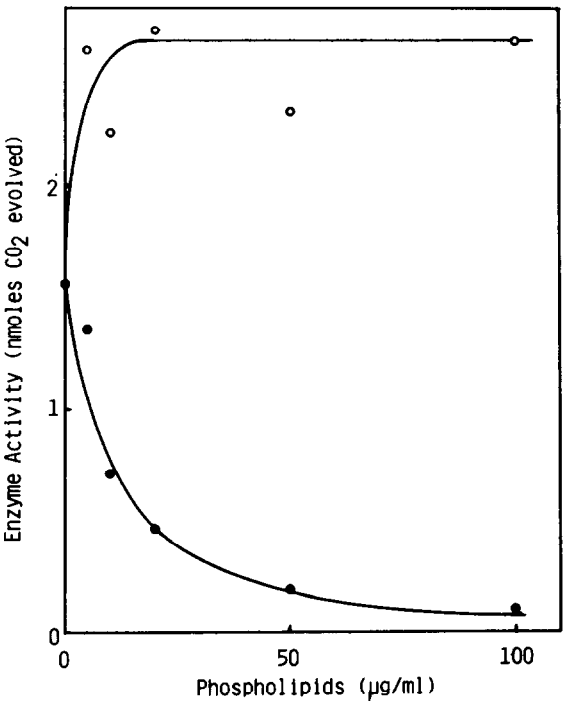

Fig.1. Effect of varying the concentrations of phospholipids on the activity of highly purified ornithine decarboxylase from rat livers. The activity of the enzyme preparation from the phenyl–Sepharose step of the purification procedures was measured under standard conditions except that phosphatidylcholine (○) or phosphatidylinositol (●) was added as indicated.

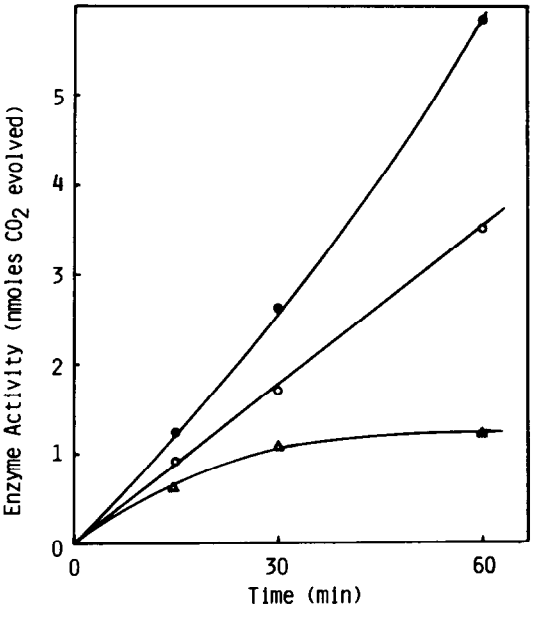

Fig.2. Time course of the activity of highly purified ornithine decarboxylase in the presence of phospholipids. The activity of the enzyme preparation from the phenyl–Sepharose step of the purification procedures was measured in the presence of phosphatidylcholine (●) or phosphatidylinositol (△) added to a final concentration of 20 μg/ml and in their absence (○). The incubation period was varied as indicated.

4. Discussion

Several lines of evidence suggested that ornithine decarboxylase served an important regulatory function in the control of cell division and growth, and a wide variety of stimuli, including partial hepatectomy, various hormones and carcinogenic compounds, resulted in a dramatic rise in enzyme activity, and furthermore the enzyme activity decreased with an extremely short apparent half-life in vivo, indicating an important role of ornithine decarboxylase in biological functions [7]. Although the regulation of ornithine decarboxylase by de novo synthesis and degradation of the enzyme was suggested by inhibitor experiments [7], the findings [9–12] of inhibitor proteins and a non-dialyzable activator of ornithine decarboxylase suggested the regulation of the enzyme by an activation–inhibition mechanism as well as by a de novo synthesis–degradation mechanism. We have shown [13,14] that some general cellular components were also effective in stimulating or inhibiting the enzyme activity: stimulatory effects of serum albumin as well as synthetic non-ionic detergents such as Tween 80 and Triton X-100 and inhibitory effects of heparin as well as synthetic polyanions such as poly-(L-glutamic acid) and dextran sulfate. The effects of phospholipids on the activity of ornithine decarboxylase, as described here, are of particular interest, because anionic phospholipids such as phosphatidylinositol and phosphatidylserine caused a marked inactivation of the enzyme, while non-ionic phospholipids such as phosphatidylcholine and phosphatidylserine caused a marked stimulation of the enzyme activity. These phospholipids were ineffective in modulating the enzyme activity of a more crude preparation, possibly due to the existence of some materials in this enzyme preparation which can bind to added phospholipids.

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