PHOSPHOLIPID ACTIVATION OF LACTOBACILLUS PLANTARUM UNDECAPRENYL PYROPHOSPHATE SYNTHETASE[1]

by

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SUMMARY: The ability of a number of phospholipids to stimulate Lactobacillus plantarum undecaprenyl pyrophosphate synthetase was investigated. The detergent Triton X-100, which is added to stabilize the enzyme during purification and is required for in vitro activity, was removed with the non-ionic resin XAD-2. The effects of cardiolipin, phosphatidyl ethanolamine, phosphatidyl choline, and phosphatidyl glycerol on the activity of XAD-2 treated undecaprenyl pyrophosphate synthetase were determined. Of the phospholipids studied only cardiolipin stimulated in vitro enzymic activity as effectively as Triton X-100.

INTRODUCTION

Undecaprenyl monophosphate serves as a carbohydrate carrier in the biosynthesis of some bacterial cell-wall polysaccharides. An undecaprenyl phosphokinase which catalyzes the ATP dependent formation of undecaprenyl monophosphate from undecaprenol has been isolated from Staphylococcus aureus membranes (1). This enzyme is insoluble in water but soluble in a number of organic solvents and requires phospholipid cofactor for in vitro activity (1,2). We have recently reported the isolation and partial purification of an undecaprenyl pyrophosphate synthetase from Lactobacillus plantarum (3). This enzyme is isolated from the 100,000 x g supernatant and requires the non-ionic detergent Triton X-100 for stability during purification procedures and for in vitro activity.

In light of the well documented ability of phospholipids to stimulate undecaprenyl phosphokinase (2, 4, 5, 6), the ability of a number of phospholipids

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to stimulate the activity of undecaprenyl pyrophosphate synthetase was investigated.

MATERIALS AND METHODS

L. plantarum undecaprenyl pyrophosphate synthetase, farnesyl pyrophosphate and [1-^{14}C] -Δ^3-isopentenyl pyrophosphate were prepared as previously described (3).

Amberlite XAD-2 beads were obtained from Mallinckrodt Chemical Works. Triton X-100, bovine heart cardiolipin and phosphatidyl choline dimyristoyl were obtained from Sigma Chemical Co. Bacterial phosphatidyl glycerol was obtained from Supelco Inc. Bacterial phosphatidyl ethanolamine, and egg lecithin were obtained from Analabs Inc.

Enzyme Assay

Enzyme was incubated for 30 min at 37^{o} in 0.5 ml total volume containing 0.1 M Tris pH 7.5, 0.2 mM $MgCl_2$, 50 μM farnesyl pyrophosphate, 64 μM [1-^{14}C]-Δ^3-isopentenyl pyrophosphate (32,000 dpm) and either 0.5% Triton X-100 or the indicated amount of phospholipid. The appropriate volume of an ethanolic solution of phospholipid was added to a reaction vessel and evaporated to dryness with a stream of nitrogen. The assay solution was then added and thoroughly mixed with the phospholipid on a Vortex Jr. Mixer. Each reaction vessel was then immersed in a Heat Systems-Ultrasonics Inc. sonic bath and sonicated. The assay solution was mixed a second time on a Vortex Jr. Mixer and the enzyme was added.

The product was isolated for analysis after acid hydrolysis as previously described (3).

Preparation of Detergent Free Enzyme

XAD-2 beads were prepared for use according to the procedure of Holloway (7). Moist XAD-2 beads 51 gr/gr Triton X-100 were added to a slowly stirred solution of the enzyme at 0-4^{o}. The solution was stirred for 30 min and filtered to remove the XAD-2 beads. The detergent concentration was determined from the absorbance at 275 nm (7).

Protein Determination

An aliquot of the protein solution was brought to 0.4 ml final volume with deionized water, then 10 mM $ZnSO_4$ (0.05 ml), 10mM NaOH (0.05 ml), and absolute ethanol (2 ml) were added. The resulting solution was cooled to -20° for 20 min and centrifugated at 17,300 x g for 30 min. The supernatant was carefully removed with a transfer pipette and discarded. The pellet was resuspended in absolute ethanol (3 ml) and recentrifugated at 17,300 x g for 30 min. The resulting pellet was washed with a second portion of absolute ethanol (3 ml) and then heated at 100° to evaporate any residual ethanol. The two ethanol washes remove Triton X-100 which interferes with the protein determination. The amount of protein was then determined by the procedure of Lowry et al. (8)

RESULTS AND DISCUSSION

Previous results from this laboratory described a requirement of L. plantarum undecaprenyl pyrophosphate synthetase for Triton X-100. In the absence of Triton X-100 the enzyme is unstable to chromatography and inactive

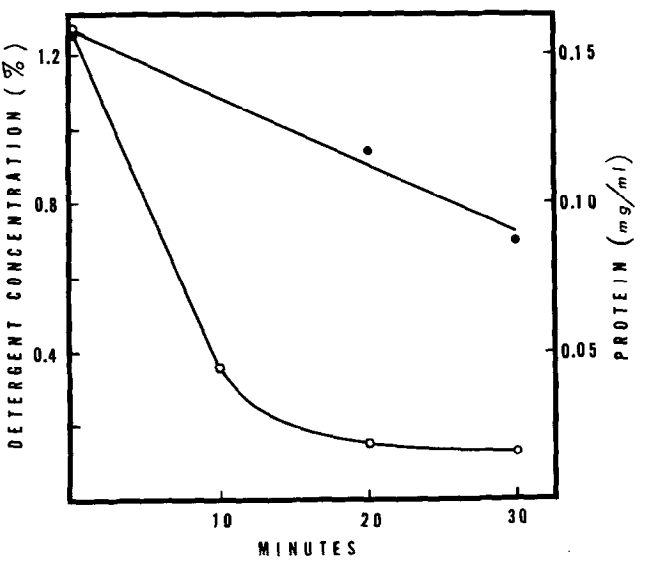

Figure 1. Time Dependent Removal of Detergent From Enzyme Using XAD-2

Enzyme was treated with XAD-2 beads at 0-4° as described in the Methods Section. Aliquots were taken at the indicated times and assayed for detergent (○) and protein (●) concentration.

(3). Further studies on the detergent or lipid requirements for enzymic activity necessitated a method for removal of detergent from the partially purified enzyme in a manner which would permit subsequent stimulation of the enzyme to essentially full activity. The use of the non-ionic resin XAD-2, previously described by Holloway (7) for the removal of Triton X-100 from bovine serum albumin, has been applied in the removal of Triton X-100 from a solution of undecaprenyl pyrophosphate synthetase. The time-dependent removal of detergent from an enzyme solution is described in Fig. 1. As can be seen at levels of XAD-2 of 51 grams per 1 gram of Triton X-100 most of the detergent is removed after 20 min. Routinely, there is an approximate 33% decrease in the protein concentration of the enzyme solution with a corresponding irreversible loss of enzyme activity. The enzyme activity is restored to approximately 66% of its original value by adding back Triton X-100 to a final concentration of 0.5% in the assay solution. This is in contrast to the previous method of

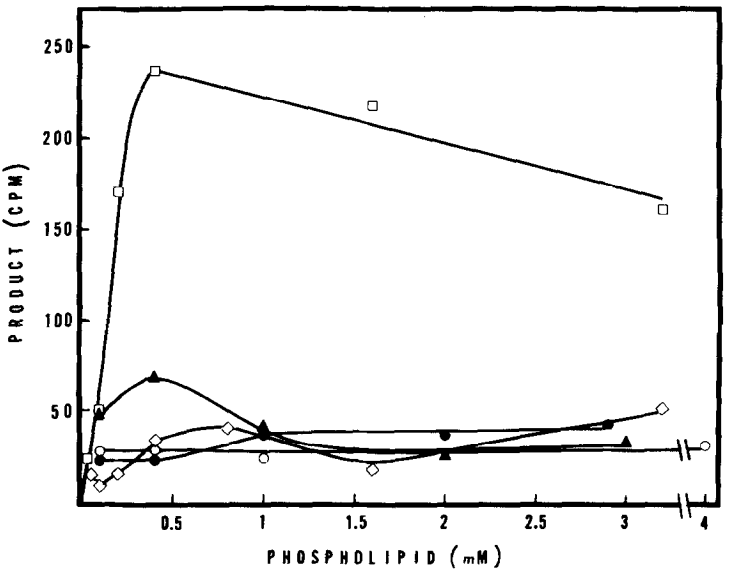

Figure 2. Phospholipid Dependence of Enzymic Activity

The enzyme assays were carried out under the standard reaction conditions for each phospholipid tested: cardiolipin (□), phosphatidyl choline dimyristoyl (●), lecithin (○), phosphatidyl ethanolamine (▲), and phosphatidyl glycerol (◇). When 0.5% Triton X-100 was used instead of phospholipid the reaction yielded 256 cpm in the product. The blank was 27 cpm.

detergent removal, ethanol extraction, which gave only 31% of the original activity after adding back detergent (3).

A variety of phospholipids were tested for their ability to stimulate the enzyme which had been depleted of Triton X-100. The results of these studies are described in Fig. 2.

It is of considerable interest that of all the phospholipids studied only cardiolipin stimulates enzymic activity in the concentration range studied. Neither phosphatidyl glycerol nor the nitrogen containing phospholipids, phosphatidyl choline or phosphatidyl ethanolamine demonstrate the ability to substitute effectively for Triton X-100 or cardiolipin. It is noteworthy that, whereas the nitrogen containing phospholipids tested have not been demonstrated in this bacterium, cardiolipin is a common phospholipid of bacterial membranes (9). Cardiolipin also stimulates undecaprenyl phosphokinase activity (2), although it is not found associated with highly purified active enzyme (4).

The effectiveness of Triton X-100 and cardiolipin as stimulants of this soluble enzyme strongly implicates the natural site of undecaprenyl pyrophosphate synthesis at the membrane surface where the water-soluble pyrophosphate substrates are readily available. The enzymic product may then enter the lipid phase of the membrane for subsequent utilization in its well described role of carbohydrate carrier for the synthesis of cell wall components.

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