DISPOSITION OF PHOSPHATIDYLGLYCEROL IN METABOLIZING CELLS OF *ACHOLEPLASMA LAIDLAWII*

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1. Introduction

In microorganisms, transport of metabolites, energy production and energy conservation are closely interrelated and for the greater part confined to the cytoplasmic membrane [1]. The lipid dependency of some well-defined steps in these processes has been studied extensively. Lipid dependency is shown in an indirect way by measuring the activation energy of β-galactoside-transport in the *Escherichia coli* membrane. Within the temperature range of the lipid phase transition the activation energy of the process is increased. Both phenomena can be correlated as shown [2]. Purification of the proteins II-A and II-B of the phosphoenolpyruvate–sugar phosphotransferase system from *E. coli* membranes, followed by reconstitution experiments show that phosphatidylglycerol is involved in the activity of these proteins [3]. Finally, specific removal of phosphatidylglycerol from the *Acholeplasma laidlawii* membrane inactivates the Mg^{2+}-dependent ATPase. From all the lipids present in this organism, phosphatidylglycerol is the only one which can be used to reconstitute this enzymatic activity [4]. It is obvious that a lipidic environment is prerequisite for such membrane-found proteins to become activated.

Alternatively, one can envisage the possibility that when proteins in the membrane are active, they influence their immediate environment and thus the surrounding lipids. Such an effect on the lipids can involve a differential localization, lateral mobility, fluidity or even turnover. An indication that this type of activated protein–lipid interaction does occur, comes from [5]. It was found that ATP-depletion of rat erythrocytes results in an increased accessibility of membrane phospholipids towards exogenous phospholipase C. Additional evidence to support the above hypothesis is described here. We have studied the fate of a single lipid species, phosphatidylglycerol, as a consequence of energization of the membrane of *A. laidlawii*.

It is shown that the availability of phosphatidylglycerol for hydrolysis by exogenous phospholipase A_2, depends on the state of membrane energization. In the presence of glucose hydrolysis proceeds at a lower rate and is limited, whereas in the non-energized state hydrolysis goes to completion quite rapidly. Nigericin abolishes this energization effect, but valinomycin in the presence of FCCP does not.

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2. Experimental

Acholeplasma laidlawii B was grown of a defatted tryptose medium supplemented with oleic acid to final concn 1.12 mM [6]. Lipids were radioactively labeled by adding 1 μCi [1-^{14}C]oleic acid/100 ml medium. When the culture had reached A_{640} 0.10–0.25, cells were harvested by centrifugation at 8000 × *g*. Cells were washed once at 5°C in a solution containing 250 mM NaCl and 10 mM $MgCl_2$. The cell pellet was resuspended to a concentration of 0.5 mg cell protein/ml in a buffer containing: 50 mM Tris, 130 mM NaCl, 2 mM $MgCl_2$, 5 mM $CaCl_2$, 1.2 mM

Na_2HPO_4 and 1 mM KCl adjusted with HCl to pH 7.4 at 37°C. When desired 20 mM (final concn.) glucose or 3-*O*-methylglucoside (Sigma) was added to the cell suspension. Ethanolic solutions of FCCP (*p*-trifluoromethoxy-carbonyl-cyanide phenylhydrazone, generously supplied by Dr P. G. Heyter, Dupont Co., DE) and valinomycin (Calbiochem, A grade, Los Angeles, CA) were added to the incubation mixtures at concn 5.10^{-6} M and 10^{-7} M, respectively (final concn ethanol in the mixture never exceeded 0.5%). Nigericin (Eli Lilly, Indianapolis, FN) was used at a concentration of 2 μg/ml.

Each incubation was carried out at 37°C with exactly the same amount of phospholipase A_2 from pig pancreas (Boehringer, Mannheim). The final concentration of this enzyme was 5 μg/ml (4 units). At different time intervals samples were taken from the incubation mixture and the phospholipase action was stopped by addition of an excess EDTA to complex the Ca^{2+} ions. Lipids were extracted as in [7] and analysed by thin-layer chromatography and radioactivity counting [8]. The K^+ content of the cells was determined by flame spectrophotometry. The capability of cells to metabolize was tested before each experiment was started. This was done by measuring the capacity of the cells for acidification of the medium upon addition of 20 mM glucose. At the same time a yellow–white color change of the cell suspension was observed.

3. Results and discussion

Phosphatidylglycerol is present in three pools in the membrane of *A. laidlawii* [8]. Half of the phosphatidylglycerol can be found in the outer layer of the cytoplasmic membrane, 20% is probably in the inner layer and 30% in close contact with proteins. These pools can be recognized by phospholipase A_2 incubations but only at low incubation temperatures. Upon temperature increase redistribution of the phospholipid can occur and at 37°C all the phosphatidylglycerol seems to be present in one pool only. That is shown also in fig.1. Phospholipase A_2 from porcine pancreas does hydrolyze phosphatidylglycerol in intact cells of *A. laidlawii* quite rapidly at 37°C and eventually the reaction goes to completion. By contrast, when the membrane is energized by the addition of glucose, hydrolysis of phosphatidylglycerol proceeds at a lower rate. Furthermore, it is evident that only a limited amount of phosphatidylglycerol is accessible. From 30–50% of this lipid can be hydrolyzed and addition of an excess of phospholipase A_2 does not increase this amount of accessible phosphatidylglycerol.

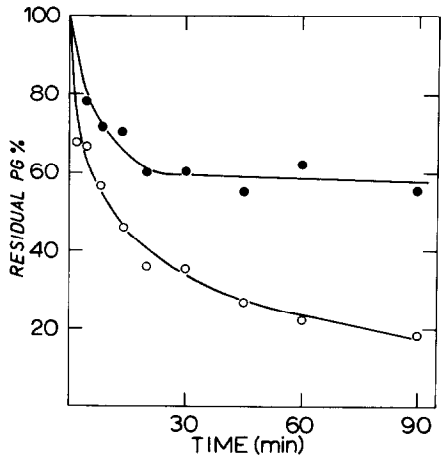

Fig.1. Inhibition of phosphatidylglycerol hydrolysis in energized cells of *A. laidlawii*. Cells of *A. laidlawii* were incubated at 37°C with pancreatic phospholipase A_2 as described in section 2. At different time intervals cells were analyzed for the residual phosphatidylglycerol (PG) content. Experiments were carried out in the absence (○) and presence (●) of glucose. Addition of 3-*O*-methylglucose resulted in an identical hydrolysis patterns as obtained with the control cells (○).

Evidence for the uptake and metabolism of glucose is provided by pH and K^+ ion-leak measurements. When cells were allowed to metabolize in a non-buffered solution, a gradual decrease in the pH of the medium from pH 7.5–6.5 was observed. However, under the conditions applied (incubation medium buffered at pH 7.5) the pH of the medium decreased by about 0.2 units within 1 h incubation time in the presence of glucose. Furthermore, K^+ is actively retained and even taken up by the cells in the presence of glucose whereas without glucose, K^+ is readily lost. Thus, uptake and breakdown of glucose alters the membrane in such a way that phosphatidylglycerol is not fully accessible from the outside in contrast to the situation at rest.

The uptake and metabolism of glucose involves at

least 3 membrane associated processes in which lipid might play a role:

(i) The transport of glucose
(ii) The formation of ATP during glucose breakdown
(iii) The energy conservation by means of a potential and pH gradient over the membrane.

A functional role of phosphatidylglycerol in the transport process is unlikely. The glucose transport system can be utilized by 3-*O*-methyl-D-glucose, a non-metabolized glucose analogue [9]. When this compound is added to the cells, phosphatidylglycerol is fully accessible (fig.1). The energized state of the membrane which is required for transport of carbohydrates may be due to the hydrolysis of ATP generated by glycolysis [9]. The involvement of phosphatidylglycerol in energy production is an attractive hypothesis because the Mg^{2+}-dependent ATPase of this organism was shown to depend on the presence of phosphatidylglycerol in the membrane [4]. Evidence to substantiate this idea is indirect because the ATPase activity is located at the cytoplasmic side of the membrane and therefore difficult to affect from the outside [10]. The use of uncouplers, however, might provide some insight in the coupling of lipid accessibility and ATPase activity. The accessibility of phosphatidylglycerol in energized cells is considerably increased upon addition of nigericin (fig.2). Addition of uncoupler before the addition of phospholipase A_2 gives an increased rate of hydrolysis exceeding even the phosphatidylglycerol hydrolysis in the absence of glucose. This observation suggests that freshly isolated cells still contain some residual metabolic activity. The combined addition of valinomycin + FCCP, however, hardly affects the rate and extent of phosphatidylglycerol hydrolysis (fig.2).

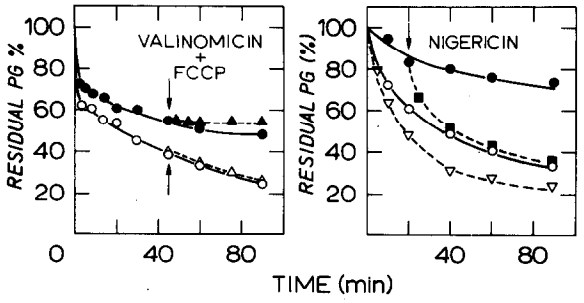

Fig.2. Effect of uncouplers and ionophores on phosphatidylglycerol hydrolysis in energized cells of *A. laidlawii*. Cells were incubated without (open symbols) and in the presence (closed symbols) of glucose. Left: Valinomycin + FCCP were added to control cells (△) and energized cells (▲) at the time indicated in the figure. Right: Nigericin was added at zero time to the control cells (▽) and at the time indicated in the figure to energized cells (■).

It is obvious that the data obtained with these uncouplers and ionophores are open for several explanations especially because the precise effects of these compounds on this particular membrane have not been investigated. Nevertheless, it can be concluded that during energy production and/or conservation the disposition of phosphatidylglycerol in the membrane greatly alters. If this is caused by changes in overall fluidity of membrane lipids, binding to membrane proteins, reduction in flip–flop rates or other membrane characteristics is open for further investigations.

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