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IONIC CHARGE ON PHOSPHOLIPIDS AND THEIR INTERACTION WITH THE MITOCHONDRIAL ADENOSINE TRIPHOSPHATASE

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Summary

The activity of the lipid-depleted, oligomycin-sensitive mitochondrial ATPase has been measured in the presence of liposomes prepared from mixtures of phosphatidylglycerol and phosphatidylglycerol lysine. Enzyme activity increased linearly with an increase in the negative charge of liposomes prepared from the phosphatidylglycerol-phosphatidylglycerol lysine mixtures. The electrophoretic mobility and activating capacity of liposomes of several other phospholipids were determined. A linear relationship between electrophoretic mobility of the liposomes and oligomycin-sensitive activity was again apparent. These observations demonstrate that the activity of the ATPase is directly proportional to the ionic charge on phospholipid activators if the acyl chain composition of the phosphoglycerides is relatively constant.

The oligomycin-sensitive ATPase of the mitochondrion [1], functioning either as an ATPase [2] or in energy-linked reactions such as ATP-P_i exchange [3], requires phospholipid for activity. Previous reports have demonstrated that acidic phospholipids maintain the activity of oligomycin-sensitive ATPase preparations at higher levels than do neutral phospholipids [4,5]. In this study we have measured the influence of a positively charged phospholipid, phosphatidylglycerol lysine, on the activity of the ATPase. We have also established quantitatively the relationship between ionic charge on liposomes and their ability to activate the oligomycin-sensitive ATPase.

The oligomycin-sensitive ATPase was prepared as earlier described [5]. The preparation obtained is contaminated with some inner membrane proteins, but is very low in respiratory components and in its phospholipid content. This preparation was selected for the kinetic measurements carried out in this study because of its low phospholipid content, its low activity in the absence of added phospholipids, and its excellent oligomycin sensitivity [5]. The

phosphatidylglycerol and phosphatidylglycerol lysine were prepared from cultures of *Staphylococcus aureus* (ATCC 15564) by a slight modification of the procedure described by Houtsmuller and van Deenen [6]. The phosphatidylglycerol was obtained in pure form by fractionating the lipid extract on a silicic acid column with a continuous gradient of methanol (20—50%) in chloroform. The phosphatidylglycerol lysine was further purified by thin-layer chromatography on silica gel G, using a chloroform/methanol/acetic acid (70 : 30 : 3, v/v) solvent system. Analyses of hydrolysis (0.05 M sodium borate, pH 10.0, 37°C, 12 h) products of phosphatidylglycerol lysine revealed only phosphatidylglycerol and lysine. The hydrolysis products were identified by thin-layer chromatography upon comparison with authentic phosphatidylglycerol and lysine standards.

Liposomes were prepared for microelectrophoresis measurements, and for reconstitution with the ATPase, by suspending 2—3 mg of phospholipid in 1—1.1 ml of 0.045 M Tris sulfate, pH 7.4, and then sonicating in an Ultrasonics Model 13 sonifier for 5—15 s at 22°C. The above buffer was also used in the ATPase assays. From 0.1 to 0.2 μmol of the lipid was utilized for microelectrophoresis measurements, with the remainder being used in the ATPase assays. The electrophoretic mobilities of liposomes were measured as described by Bangham et al. [7] on a Rank Mark I microelectrophoresis apparatus (Rank Brothers, Cambridge, England).

Reconstitution of the ATPase with phospholipids, and the ATPase assays, were carried out as described earlier [5]. Lipid phosphorus determinations were performed by the procedure of Chalvardjian and Rudnicki [8] and protein determinations by the Lowry method [9]. The phospholipids used in the second experiment (Fig. 2) were obtained from Serdary Research Laboratories.

Studies on the interaction of the ATPase with phospholipids [4,5,10] indicate that the stimulatory activity of the phosphoglycerides depends on the number of fatty acids per molecule, the degree of unsaturation in the fatty acids, and the ionic properties of the phospholipids. In this investigation of ionic interactions between the ATPase and phospholipids we have attempted to keep the acyl chains as constant as possible while varying the net charge on the phospholipid molecule itself from positive to negative values. We therefore isolated both phosphatidylglycerol (negatively charged) and phosphatidylglycerol lysine (positively charged) from *Staphylococcus aureus* for the present studies. The acyl chain composition of the two lipids was identical, with anteiso C_{15} and anteiso C_{17} being the predominant fatty acids.

Initial experiments demonstrated that while the ATPase exhibited relatively high oligomycin sensitive activity in the presence of liposomes prepared from phosphatidylglycerol, no oligomycin-sensitive activity was observed in the presence of phosphatidylglycerol lysine alone. To determine more quantitatively the relationship between ionic charge on the phospholipid liposomes and the activity of the lipid stimulated ATPase we prepared liposomes containing varying proportions of phosphatidylglycerol and phosphatidylglycerol lysine and measured their ability to activate the enzyme complex. The stimulatory activities of the liposomes were then compared with their electrophoretic mobilities as measured by microelectrophoresis. Fig. 1 illustrates a

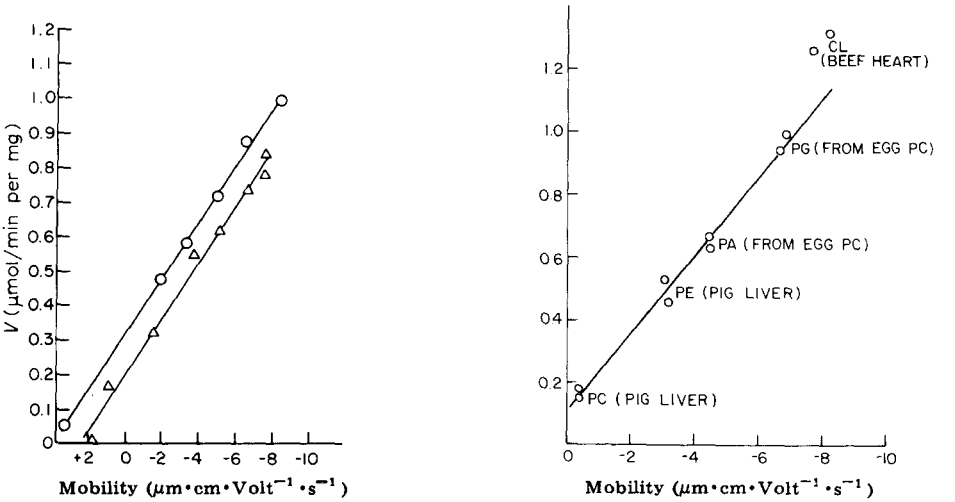

Fig. 1. ATPase activity versus electrophoretic mobility of liposomes prepared from phosphatidylglycerol-phosphatidylglycerol lysine mixtures. In the ATPase assay mixtures ATP and Mg^{2+} were in excess (2.5 mM). The phospholipids added were varied from 25—400 nmol, and V was determined by extrapolating reciprocal plots to infinite concentration of lipid activator. The results of two experiments performed at different times (○—○, △—△) are shown.

Fig. 2. ATPase activity versus electrophoretic mobility of liposomes prepared from various phospholipids. Reconstitution of the ATPase with liposomes, the ATPase assay, and the method for determining V are described in the text and in the legend of Fig. 1. The duplicate points are from two separate experiments carried out with liposomes prepared immediately before use. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PA, phosphatidic acid; PG, phosphatidylglycerol; CL, cardiolipin.

linear relationship between enzyme activity and negative charge on the liposome. In this figure the results of two experiments performed at different times are illustrated. The variation in V was due to the loss of enzyme activity which occurs with time of storage of the ATPase preparation.

A similar relationship is noted when liposomes of different phospholipids are compared for their stimulatory activity and electrophoretic mobility (Fig. 2). The linear correlation is obvious with all the phospholipids except cardiolipin. In experiments where 2.5 mM ATP, 2.5 mM $MgSO_4$, and 2.5 mM phosphoenolpyruvate were included in the buffer used for electrophoretic mobility measurements the linear correlation (correlation coefficient = 0.98 with cardiolipin omitted) was retained, but the slope of the line increased 15%. Because of the difficulties in matching the fatty acid compositions of the phospholipids used in this latter experiment, it is possible that the acyl chains in cardiolipin enhanced the stimulatory activity of this phospholipid. Moreover, the increased number of acyl chains per molecule may affect the interaction between cardiolipin and the ATPase; we have observed that the characteristics of activation of the enzyme with monoacyl phospholipids are different than with diacyl phosphoglycerides [5].

In neither experiment was there any indication that the increase in activity was associated with an increased affinity for phospholipid. The concentration of phospholipid which gave rise to one-half V, determined from double reciprocal plots, was somewhat variable from one phospholipid mixture or phospholipid type to the next, and did not correlate with either V or electro-

phoretic mobility of liposomes. With the exception of phosphatidylglycerol lysine alone, all phospholipids and phospholipid mixtures employed in this study elicited an ATPase activity that could be inhibited by oligomycin. The phosphatidylglycerol-phosphatidylglycerol lysine mixtures did, however, elicit a low level oligomycin-insensitive activity from the ATPase. Whereas this insensitive activity remained low and rather constant, the oligomycin-sensitive activity increased dramatically as the proportion of phosphatidylglycerol was increased in the liposomes.

The observations in this study demonstrate that the ionic charge of the phospholipids is of primary importance in eliciting an oligomycin-sensitive activity in the ATPase. Liposomes composed entirely of positively charged phospholipids do not activate the ATPase. Moreover, the activity of the enzyme is directly proportional to the electrophoretic mobility of the phospholipid liposomes, as was demonstrated in both Figs. 1 and 2. The chemical composition of the head-group portion of the phospholipid appears, therefore, to be important in influencing the activity of the ATPase since it dictates the ionic properties of the phospholipid. These results also suggest that phospholipids are not simply a matrix in which the ATPase resides, but also directly influence the functioning of the enzyme complex by modulating its catalytic properties. If one assumes that the ATPase in the intact mitochondrion has the potential for maximal catalytic activity, then the enzyme probably has a microenvironment comprised of negatively charged phospholipid. Cardiolipin would provide this negatively charged ionic environment within the inner membrane.

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