CORRELATION BETWEEN LOSSES OF MITOCHONDRIAL ATPase ACTIVITY AND CARDIOLIPIN DEGRADATION

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SUMMARY.- Peroxidation changes induced by ascorbate or cysteine on phospholipids of isolated rat liver inner mitochondrial membranes are accompanied by losses in some enzyme activities. The rate of degradation for each phospholipid is different and can be also modified by the addition of antioxidant BHT. A close correlation between the alteration of cardiolipin and ATPase activity has been found, indicating the existence of specific association between this enzyme and cardiolipin, independently of other phospholipids.

Reports from several laboratories have shown that ATPase activity in different mitochondrial preparations can be stimulated in the presence of added phospholipids (1, 2). Removal of phospholipids by cholate extraction of submitochondrial particles from rat liver resulted in a marked decrease of ATPase activity; this activity was partially recovered by the addition of different phospholipids; although acidic phospnolipids proved to be more active in the ATPase stimulation (3), so far no definite specificity has been shown (1, 3, 4).

In previous work from this laboratory it has been shown that mitochondrial membranes incubated in the presence of ascorbate or cysteine undergo profound changes in their phospholipid constituents through a peroxidation process (5). These phospholipid alterations are accompanied by a loss of some enzyme activities, among them that of ATPase (6). The possibility that the degradation rate of the individual phospholipids could be

different prompted us to follow both the levels of each lipid and enzyme activities, in an attempt to determine whether some specific correlations between phospholipids and enzymes exist. In this study the use of antioxidants, such as 2,6-tert-butyl-4-methylphenol (BHT), which can protect against peroxidation reactions, has proved very useful, since the degradation rate of certain phospholipids can be modified.

The data here presented strongly suggest that mitochondrial ATPase from rat liver is specifically associated with cardiolipins.

MATERIALS AND METHODS

Liver mitochondria were isolated from male Wistar rats weighing approximately 200 g following the method of Hogeboom (7). Inner mitochondrial membranes were prepared according to the method of Parsons et al.(8) with the modifications already described (9). Incubation of inner mitochondrial membranes were carried out in a medium containing 1 mM ascorbate or 8×10^{-4} M cysteine, 0.02 M Tris-HCl buffer, pH 7.4, and 0.25 M sucrose, at 30ºC. Controls without ascorbate or cysteine were incubated simultaneously. Inner membranes resuspended in 0.25 M sucrose were added to the incubation medium to give an approximate concentration of 0.8 mg of protein per ml. The optical density (O.D.) of this suspension read at 520 nm in a 1 cm cuvette was approximately 1.100. Lysis of the membranes was followed at this wavelength.

Proteins were determined by the method of Lowry et al.(10). Lipids were extracted from the sediment obtained after precipitating the proteins with enough conc. $HClO_4$ to give a final concentration of 0.3 N, as previously described (11). Phospholipids were separated by thin layer chromatography as described by

Neskovic et al. (12). Lipid phosphorus was determined as described by Bartlett (13). ATPase activity was determined by the method of Pullman et al. (14).

RESULTS

Incubations with cysteine.- Aliquots were taken at different incubation times and phospholipids extracted and separated.

The values corresponding to phosphatidylcholine, phosphatidylethanolamine and cardiolipin at different degrees of membrane disaggregation indicated by the changes of O. D. are plotted in figure 1. O. D. changes took place after 50 minutes. It can be seen that while there was a gradual loss of extractable phosphatidylcholine and phosphatidylethanolamine, cardiolipin remained unchanged until the membrane disaggregation was large enough to give a decrease of approximately 0.400 optical units, and even then these changes were very limited. In the incubated controls the changes in O. D. as well as the levels of extractable phospholipids were negligible.

ATPase activity was also determined in aliquots taken at different times during the incubation. The results are shown in figure 3. Changes in the initial values took place only after the O. D. decreased in approximately 0.400 units.

Incubations with ascorbate.- Figure 2 shows the values of the different phospholipids, determined in aliquots taken at different times corresponding to different degrees of membrane disaggregation. Phosphatidylcholine and phosphatidylethanolamine gradually decreased after the lag of approximately 7 minutes preceding the O. D. changes; cardiolipin was also affected showing a rather sharp decrease. In the controls both O. D. and the levels of extractable phospholipids remained constant troughout the incubation time.

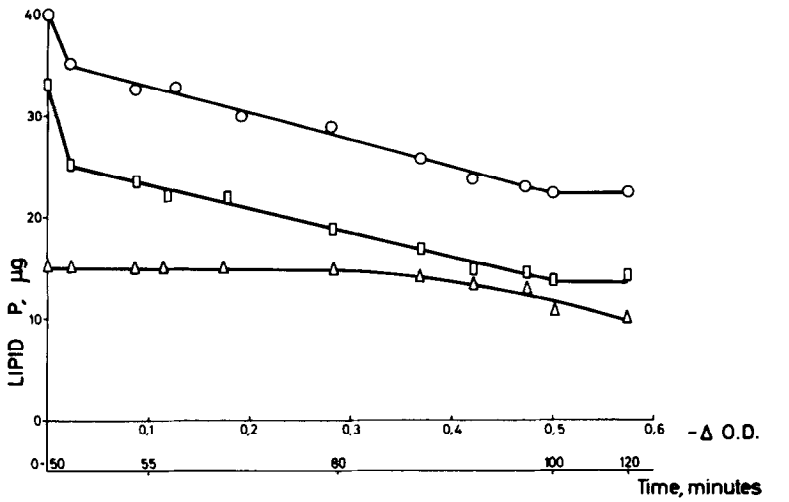

Figure 1.- Optical density (O. D.) and phospholipid changes during incubation of isolated rat liver inner mitochondrial membranes in the presence of cysteine. Incubation conditions are given in the text. Number of experiments, 5. The values of lipid P corresponding to each phospholipid are referred to 20 mg of membrane protein. (○) Phosphatidylcholine; (□) Phosphatidylethanolamine; (Δ) Cardiolipin.

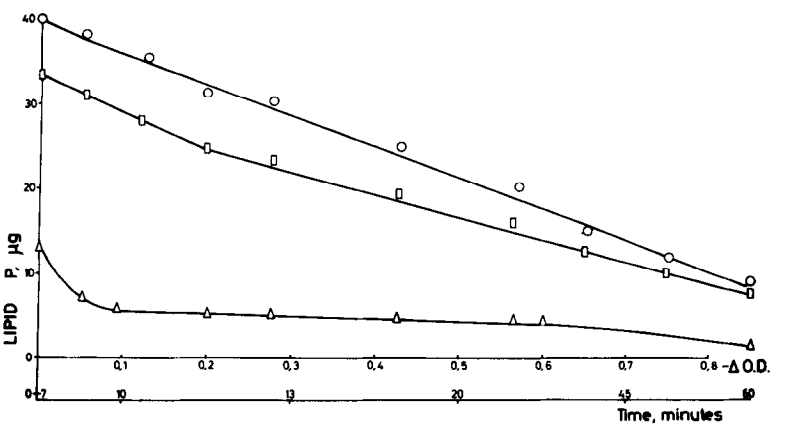

Figure 2.- Optical density (O. D.) and phospholipid changes during incubation of isolated rat liver inner mitochondrial membranes in the presence of ascorbate. Incubation conditions are given in the text. Number of experiments, 5. The values of lipid P corresponding to each phospholipid are referred to 20 mg of membrane protein. (○) Phosphatidylcholine; (□) Phosphatidylethanolamine; (Δ) Cardiolipin.

The values of ATPase activity at different intervals during the incubation are plotted in figure 3. A decrease in activity started to take place immediately after the lag preceding the O. D. changes.

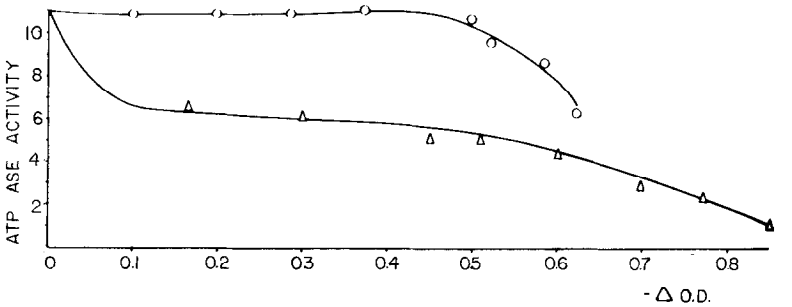

Figure 3.- Changes in ATPase activity along the incubation of isolated rat liver inner mitochondrial membranes in the presence of either cysteine or ascorbate. ATPase activity is expressed as μmoles of ATP hydrolyzed/mg protein in 10 minutes. (○) ATPase activity in the presence of 8×10^{-4} M cysteine; (Δ) ATPase activity in the presence of 1 mM ascorbate. Number of experiments, 5.

Incubations with ascorbate plus BHT.- In another series of experiments with 1 mM ascorbate, BHT was added to the incubation medium after 5 or 7 minutes to give a final concentration of 5×10^{-5} M. Table I shows the O. D. changes and the percent decrease in phosphatidylcholine, phosphatidylethanolamine, cardiolipin and ATPase activity in the presence of ascorbate, and when BHT was also present in the medium. A close relationship between the alteration of cardiolipin and the loss of ATPase activity can be readily observed.

DISCUSSION

The loss of ATPase activity in inner mitochondrial membranes incubated with ascorbate or cysteine along the incubation is always parallel to the alterations of cardiolipin, whereas no relationship has been observed with the degradation of other lipids. When cardiolipin was totally or partially protected with the antioxidant BHT, independently of other phospholipids, ATPase activity was also preserved in the same extent. This fact strongly suggests a specific association of ATPase with cardiolipins.

TABLE I

CORRELATION BETWEEN DECREASE IN ATPase ACTIVITY AND PHOSPHOLIPID DEGRADATION

Additions	-Δ O.D.	% Decrease PC	PE	CL	ATPase activity
None	0	0	0	0	0
Ascorbate	0.870	80	80	90	90
Ascorbate+BHT (0 minutes)	0.000	0	0	0	0
Ascorbate+BHT (5 minutes)	0.220	25	26	0	0
Ascorbate+BHT (7 minutes)	0.480	53	48	10	11

Decrement in optical density (-Δ O.D.), percent decrease of phosphatidylcholine (PC), phosphatidylethanolamine (PE), cardiolipins (CL) and ATPase activity in the presence of ascorbate and ascorbate + BHT. Incubation was carried out for 60 minutes at 30ºC. BHT was added as indicated, at the beginning of the incubation or after 5 or 7 minutes. Final concentration of BHT was 5×10^{-5} M. Number of experiments, 3.

These findings would be in agreement with the mosaic model of membrane structure as proposed by Singer et al. (15); according to this model proteins would be "floating in a sea of lipid"; however, it should be added that some or perhaps all membrane proteins would be surrounded by specific phospholipids, allowing the complexes so formed to move more freely within the lipid.

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