NONIONIC INTERACTION BETWEEN PROTEINS AND LIPIDS IN THE MITOCHONDRIAL MEMBRANES

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Received January 7, 1969

SUMMARY. The interaction between lipid-depleted mitochondria and phospholipids is not inhibited by high ionic strength of the medium, and therefore is not electrostatic but is conceivably hydrophobic in nature. The proteins responsible for the interaction, both in high and low ionic strength media, are located in the basepieces of the membrane. These findings are not compatible with the unit membrane model of a lipid bilayer sandwiched between two extended protein layers.

The nature of the interactions which link together proteins and lipids in membranes is of primary importance for the understanding of membrane structure. The "unit membrane" theory (1) demands that protein and lipid be linked together mainly by electrostatic interactions (1-3). On the other hand, criticisms of the unit membrane model have been published recently (4,5). The importance of hydrophobic interactions in natural systems has been stressed (6), also, a structural protein has been isolated from mitochondria (7) that interacts hydrophobically with micellar phospholipids (8). Recent studies on mitochondrial structural protein (9,10) have demonstrated that this protein is contained in readily detachable sectors (4) of the mitochondrial membranes, which do not contain phospholipids. The interaction of this protein with phospholipids could then be of little physiological significance. The question is therefore pertinent: Are the hydrophobic interactions those responsible for the lipid-protein bonds found physiologically in membranes? In this

TABLE I

EFFECT OF 1 M NaCl ON THE BINDING OF PHOSPHOLIPIDS TO LIPID-DEPLETED MITOCHONDRIA

Experiment	Mitochondria added (mg protein)	Phospholipids added (µg lipid P)	NaCl in medium (1 M)	Phosphorus bound (µg P per mg protein)
1	10.2	-	-	4.8
	10.2	-	+	4.2
	10.2	1410	-	17.4
	10.2	1410	+	19.4
2	4.7	1400	-	22.1
	4.7	1400	+	18.4
3	5.0	855	-	14.3
	5.0	855	+	13.9

Incubation was for 30 min at 30°. The amount of P in intact mitochondria was usually 18-20 µg per mg protein.

communication we present evidence that polar bonds are not significantly involved in the binding of phospholipids to lipid-depleted mitochondria.

Beef heart mitochondria were prepared by a small scale procedure (11); they were depleted of their lipids according to Fleischer and Fleischer (12). Acetone plus NH_3 were used as the extracting medium. Soybean phospholipid micelles (Asolectin) were prepared by sonication. Binding to the lipid-depleted mitochondria was assayed as described by Fleischer and Fleischer (12). Lipid P was determined according to Marinetti (13) and protein was measured with a biuret method (14).

The acetone-NH_3 treatment extracted most of the phosphorus from mitochondria (Table I); when such mitochondria were incubated at 30° with phospholipid micelles, the phosphorus content was restored. High ionic strengths inhibit polar interactions; therefore we have tested the nature of the bonds established between the lipid-extracted mitochondria and phospholipids by increasing the ionic strength of the medium. We observed no alteration of the binding when NaCl was

TABLE II

EFFECT OF TEMPERATURE ON THE BINDING OF PHOSPHOLIPIDS TO LIPID-DEPLETED MITOCHONDRIA

Temperature	NaCl in medium (1 M)	Phosphorus bound (°) (µg P per mg protein)
0°	-	4.98
	+	6.78
31°	-	11.82
	+	12.70
45°	-	14.31
	+	15.74

In each tube 7.6 mg of mitochondrial protein were incubated for 30 min. with 885 µg of lipid P.
(°) After subtraction of residual P.

present at a concentration of 1 M in the medium. By comparison we mention the fact that the electrostatic interaction between micellar phospholipids and cytochrome c is inhibited already at 0.1 M ionic strength (15). Table II shows that the interaction not only was temperature-dependent but that the total extent of the interaction also increased with temperature. At 0° there was more binding in the presence of NaCl than in its absence in all experiments. The kinetics of the interaction has been studied only at 0° (Figure 1) because at higher temperature the binding was too fast to follow. To avoid complications due to the possible solubilization of the detachable sectors during the binding studies, and to test whether the binding of the phospholipids (either in the presence or absence of salt) was directly to the membrane (and not to the detachable sectors thereof) we prepared mitochondrial membranes from which all detachable sectors were extracted with 10 mM HCl (10). Extraction with acetone and ammonia depleted these membranes, as well as intact mitochondria, of their phospholipids. Addition of Asolectin micelles at low or high ionic strengths brought about a replenishment of the component phospholipids (Table III). The bonding in

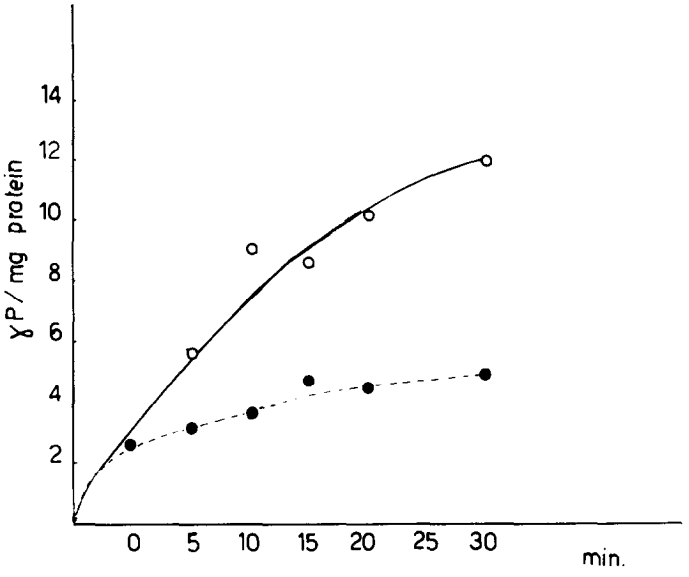

Fig. 1. Time course of phospholipid binding to lipid-depleted mitochondria; 5.9 mg of mitochondrial protein were incubated at 0° with 1000 µg of lipid P; the open circles represent the data obtained in the presence of added salt; the closed circles represent the findings without added salt. The results plotted are expressed as µg of lipid P bound per mg of protein (after subtraction of residual P).

TABLE III

BINDING OF PHOSPHOLIPIDS TO MITOCHONDRIA FREED FROM DETACHABLE SECTORS AND EXTRACTED WITH ACETONE PLUS NH_3

Experiment	Mitochondria added (mg protein)	Phospholipids added (µg lipid P)	NaCl in medium (1 M)	Phosphorus bound (µg P per mg protein)
1	4.74	-	-	4.4
	4.74	765	-	21.6
	4.74	765	+	25.7
2	4.25	675	-	18.1
	4.25	675	+	24.5

The original phosphorus content of mitochondria freed from detachable sectors was µg 33.5 per mg protein in Exp. 1 and µg 35.5 per mg protein in Exp. 2. Incubation was carried out at 30° for 30 min.

high NaCl was therefore not due to hydrophobic interactions of depolymerized headpieces (vz. of their structural protein subunits) with phospholipids; rather, it was actual binding to membrane proteins located in the basepieces. That this was the case is also demonstrated by the restoration of succinoxidase activity in lipid-depleted mitochondria treated with phospholipids both in absence and in presence of salt. Although direct addition of NaCl in the assay medium destroyed all succinoxidase activity, incubation of phospholipid-reconstructed mitochondria (but not of depleted mitochondria) with the addition of Coenzyme Q only (16), restored identical activity, both when the previous incubation with phospholipids was made either without or with salt (Table IV).

These experiments demonstrate, in our opinion, that electrostatic

TABLE IV

RESTORATION OF SUCCINOXIDASE ACTIVITY BY PHOSPHOLIPIDS IN LIPID-DEPLETED MITOCHONDRIA

Particles	Addition to incubation medium[d]	Specific activity mμatoms O_2/min/mg
LDM[a]	-	2
LDM	Asolectin	14
LDM	Asolectin, Q_{10}	73
LDM	Asolectin, Q_{10} in 1 M NaCl	0
LDM+PL (-NaCl)[b]	Q_2	50
LDM+PL (-NaCl)[b]	Q_{10}+ Asolectin	52
LDM+PL (+NaCl)[c]	Q_2	55
LDM+PL (+NaCl)[c]	Q_{10}+ Asolectin	45

(a) Lipid-depleted mitochondria.
(b) Lipid-depleted mitochondria incubated for 30 min. at 30°C with Asolectin (250 μg lipid P per mg protein), then washed.
(c) Lipid-depleted mitochondria incubated with Asolectin as above but in 1 M NaCl, then washed.
(d) Asolectin and Coenzymes Q, when added, were 30 μg of lipid P and 0.1 μmoles, respectively, in each Warburg flask. For other experimental details see ref. 16.

interactions have to be excluded as the main type of interaction between lipid and protein in the assembly of the mitochondrial membranes. The characteristics of the interaction also suggest its possible hydrophobic character. This finding is not compatible with the unit membrane model, at least in its original most simple formulation; and membrane models must account for the physiological predominance of nonionic interactions between lipid and proteins.

Acknowledgement. We wish to thank Dr. D.E. Green for his interest and for critical reading of the manuscript.

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