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Solubility of mitochondrial membrane proteins in acidic organic solvents

The DANIELLI–DAVSON[1] model for biological membranes does not provide a suitable explanation for the role played by non-enzymatic protein. There is considerable evidence[2–4] that there are largely hydrophobic, rather than electrostatic, interactions between the proteins and phospholipids of membranes. One might then expect non-enzymatic membrane protein to show some affinity for non-polar solvents. Thus, myelin membranes will dissolve in chloroform–methanol[5] and 2-chloroethanol–water (9:1, v/v) will dissolve plasma membranes of Ehrlich's ascites carcinoma[6]. In addition, GREEN *et al.*[7] noted that the core protein fraction from beef heart mitochondria dissolved in acidic organic solvents. It is, in fact, possible to dissolve all the protein from rat liver mitochondrial membranes in acidic organic solvents if the phospholipids of the membrane are removed carefully. The necessity of acid in an organic solvent for dissolving membrane proteins would seem to indicate that the most important protein–protein interactions in a hydrophobic environment are ionic bonds.

Rat liver mitochondria were obtained by the method of SCHNEIDER[8], and membranes from the mitochondria were prepared by osmotic disruption in distilled water, followed by two washes with distilled water of the pellet obtained by centrifugation at 30000 × *g* for 30 min. Two procedures were used to remove phospholipids. One utilised chloroform–methanol. 1 vol. mitochondrial membrane suspension in distilled water was rapidly dispersed into 50 vol. ice-cold chloroform–methanol (2:1, v/v). The bulk of the protein was collected by centrifugation at 1000 × *g* for 10 min and washed with 20 vol. ice-cold chloroform–methanol, giving the low speed pellet (Table I). Centrifugation of the supernatant and the washing at 30000 × *g* for 30 min yielded more protein (high speed pellet), while 5 % of the total protein still remained in the supernatant fraction. The low speed pellet could be dissolved completely by suspending the protein in a small volume of chloroform–methanol followed by the stepwise addition of hydrochloric acid in chloroform–methanol until a clear solution was obtained (approx. 1 μmole H^+ per mg of protein) (Table II). The high speed pellet only partially dissolved under these conditions. Methanol could similarly be

TABLE I

EXTRACTION PROCEDURES FOR MEMBRANES

Protein estimated by the biuret procedure[9]; phospholipid by a modified method of Fiske and SubbaRow[10].

		% *Total protein*	% *Total phospholipid*
1	Acetone		
	Pellet	97	15.8
	Supernatant	0	84.0
2	Chloroform–methanol		
	Pellet (low speed)	85	6.2
	Pellet (high speed)	15	2.2
	Supernatant	5	92.0

used to dissolve the low speed pellet. The alternative procedure required that 9 vol. acetone at room temperature were mixed with 1 vol. mitochondrial membrane suspension followed by low speed centrifugation at room temperature in a bench centrifuge. The pellet dissolved readily in methanol when hydrochloric acid was added stepwise until a clear solution was obtained (approx. 1 μmole H^+ per mg protein). The pellet dissolved in water if it was suspended, after extraction, rapidly in a small volume of water containing acid or alkali at a concentration of 1 μmole H or OH^- per mg protein.

TABLE II

TITRATION OF EXTRACTED PROTEIN WITH ACID

1.5 mg extracted protein (by chloroform–methanol) suspended in 2 ml methanol; 0.1 ml methanol containing increasing concentration of HCl added, suspension centrifuged at 1000 × *g* for 10 min and protein estimated in the supernatant liquid.

H^+ (μmoles)	0	0.5	1.0	1.5	2.0	3.0	10.0
Soluble protein (mg)	0	0.5	1.15	1.30	1.47	1.55	1.55

TABLE III

OPTICAL ROTATORY DISPERSION OF THE SOLUBLE MEMBRANE PROTEINS

Measurements made on a Bendix polarimeter in a 10-mm cell at protein concentrations of 1–2 mg/ml at 22°. (a) Phospholipid extracted with acetone, pellet dissolved in water containing HCl at 1 μmole H^+ per mg protein, and the solution dialysed against distilled water overnight. (b) Phospholipid extracted with chloroform–methanol; the low speed pellet dissolved in methanol containing HCl at 1 μmole H^+ per mg protein. (c) As (b) but with chloroform–methanol in place of methanol.

Solvent	a_0	b_0	% *Helix*
(a) Water	−157	− 27.5	4.4
(b) Methanol	+ 21	−330	52
(c) Chloroform–methanol	+120	−336	53

A solution of mitochondrial membrane protein in an organic solvent remained optically clear indefinitely if kept at 0–4°, though in water the solution normally became more opaque after 1 day, but never with obvious precipitation. Neutralisation of the acid in the non-polar solution resulted in a precipitate which, however, readily dissolved on addition of more acid.

Optical rotary dispersion (ORD) studies on the mitochondrial membrane proteins in organic solvents and water showed that they possessed more α-helix in chloroform–methanol than in water (Table III). ORD measurements were made in the visible region (600–300 mμ) and were treated according to the procedure of MOFFITT AND YANG[11] to give a_0, b_0, and percentage helicity. λ_0 was assumed to be 212 mμ, and b_0 as −630 for 100% helix. Cell membranes showed a similar amount of helicity in 2-chloroethanol–water, but as an aqueous suspension, the ORD showed significant differences[3,4]. The differences, however, may be due to the influence of the phospholipids[12] or the presence of structures other than the α-helix-coil structures[4], instead of simply an increase of helicity due to a transfer from an aqueous to a non-polar solvent.

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Comments on recent efforts to estimate the molecular weight of the $(Na^+ + K^+)$-ATPase

A recent paper from the laboratory of M. Nakao reports on efforts to approximate the molecular weight of the $(Na^+ + K^+)$-ATPase (ATP phosphohydrolase, EC 3.6.1.3) using an enzyme fraction solubilized from pig brain microsomes[1]. No attempt was made to place this estimate in perspective with prior work. There are a number of reports in the literature wherein techniques similar to those used by MIZUNO *et al.*[1] and NAKAO *et al.*[9] were applied to the problem of estimating the molecular weight of the $(Na^+ + K^+)$-ATPase[2–6]. Since the results from various groups differ, it may be useful to summarize the current situation, and to suggest some basis for evaluating available data.

Because the $(Na^+ + K^+)$-ATPase has not yet been isolated in a pure form, attempts to estimate the molecular weight have had to rely on impure enzyme fractions from red cell membranes and brain or kidney microsomes. One approach suited to such conditions is the target theory analysis of radiation inactivation data[7,8]. Usually, samples are irradiated in air or *in vacuo* and one reports the D_{37} (dose to reduce initial enzyme activity to 1/e = 37 %) which is related to the molecular weight by the equation: mol. wt. = constant/D_{37}. Either kind of data can be used to estimate the enzyme molecular weight, but different constants must be used since the D_{37}^{air}