Biochimica et Biophysica Acta, 600 (1980) 79—90

BBA 78798

PHOSPHOLIPID-DEPENDENT ASSEMBLY OF MITOCHONDRIAL ATPase COMPLEX

A. PITOTTI, F. DABBENI-SALA and A. BRUNI

Institute of Pharmacology, University of Padova, Largo E. Meneghetti 2 and CNR Unit for the Study of the Physiology of Mitochondria, Via Loredan 16, Padova (Italy)

(Received December 5th, 1979)

Key words: Mitochondrial ATPase; Oxidative phosphorylation; Complex assembly; Phospholipid

Summary

1. Phosphatidylcholines of different acyl-chain composition and a preparation of ATPase complex depleted of phospholipids have been employed in order to evaluate the contribution of lipid bilayer to the assembly of this multisubunit component of mitochondrial membrane.

2. At the minimal requirement for bilayer assembly (dinonanoylphosphatidylcholine, mixtures of lysophosphatidylcholine and phosphatidylcholine), fragments with oligomycin-insensitive ATPase activity are reconstituted. Conformational changes with dislocation of ATPase complex subunits may explain these results.

3. At increased strength of acyl-chain interaction (dilauroylphosphatidylcholine and higher homologues), the damage to the ATPase complex is prevented but this is not sufficient to achieve functional restoration. Bilayers with a tendency to coalesce and fuse aggregate in large amounts with the complex and yield low ATPase reactivation. Bilayers of high stability yield complexes with physiological content of phospholipids and efficient ATPase activity. Transition between these two possibilities is found at sixteen carbon acyl-chains. Only at this chain length does the cholate dialysis procedure of reconstitution become feasible.

4. It is concluded that a minimum of 16 carbon atoms in each chain are required to organize a bilayer structurable to maintain the ATPase complex conformation and to sustain the transmembrane position of the whole assembly.

Abbreviation: Mops, 2-(*N*-morpholino)ethanesulfonic acid.

Introduction

As proton translocator, the mitochondrial ATPase complex spans the membrane and is subjected to regulation by the membrane phospholipids (reviewed in Ref. 1). Previous studies [2—6] have shown that the ATPase complex is responsive to both charged and isoelectric phospholipids but a different pattern of reactivation can be described in the two cases. Negatively charged phospholipids maximally activate the ATPase complex at low lipid to protein content (0.1—0.3 μmol · mg^{-1}). Since this amount of phospholipid is not sufficient to assemble a lipid bilayer around the complex [7] the activation probably results from the formation of a 'boundary' lipid layer sufficient to prevent protein aggregation and to exert a regulatory effect. Consistently, this activation has been shown to be independent of both phospholipid acyl-chain length and composition [5]. Moreover, negative phospholipids resolve the association between the F_1-ATPase and the ATPase inhibitor subunit [3,4]. At variance with the effect of charged phospholipids, activation by isoelectric phospholipids is sensitive to both acyl-chain length and composition and is produced at higher lipid to protein content (0.7—1.5 μmol · mg^{-1}). In this case the activation is thought to result from the possibility to form around the membrane segment of the complex the fluid bilayer structure required for protein mobility and conformational changes. The difference between negatively charged and isoelectric phospholipids is in agreement with a parallel study on the specificity of phospholipids required for energy transfer reactions [8]. Studies on the ATPase complex from sarcoplasmic reticulum [9,10] and on the β-hydroxybutyric dehydrogenase apoprotein [11,12] have demonstrated that the stability of the lipid-protein complex depends upon the composition of phospholipid bilayer. This phospholipid-dependent stabilization might be of particular importance in the case of the mitochondrial ATPase complex in view of the multiplicity of its constituent subunits and of the ease of their resolution under relatively mild conditions. Previous experiments [5] have shown that reconstitution of phospholipid deficient ATPase complex preparations with short-chain phosphatidylcholines elicits insensitivity to oligomycin a known inhibitor of transmembrane proton translocation. The same effect has been also found in ATPase complex added with long-chain fatty acids [13]. This effect might reflect a dislocation of the catalytic moiety of the complex from the subunits forming the proton channel. The influence of the membrane lipids on the stability of the ATPase complex is examined in detail in this paper by the use of synthetic phosphatidylcholines that yield bilayers of definite properties.

Materials and Methods

The synthetic 1,2-diacyl-*sn*-glycerol-3-phosphatidylcholines used in this study were made according to published procedures [14]. Egg lysophosphatidylcholine was from Sigma. Thin-layer chromatographic controls established the purity of phospholipids. The liposomes of phospholipids were dispersed in 0.25 M sucrose, 25 mM Tris-Mops (pH 7.4) by sonication at temperature above the gel to liquid-crystalline phase transition. Mixtures of phospholipids were made in chloroform/methanol (2 : 1, v/v). The liposomes were then prepared

after evaporation of the solvent under a stream of nitrogen. The phospholipid-deficient ATPase complex was prepared from bovine heart submitochondrial particles by extraction with cholate [3,13]. In eight preparations, the oligomycin-sensitive ATPase activity of the original submitochondrial particles (2.7 ± 0.3 μmol $\cdot$ mg^{-1} $\cdot$ min^{-1}) was reduced 0.19 ± 0.04 by the procedure. The phospholipid content dropped from 1.03 ± 0.13 μmol/mg to 0.14 ± 0.01. To obtain this extensive phospholipid depletion some loss of F_1-ATPase in the prolonged incubation with cholate could not be avoided. However, the residual ATPase activity was increased 20-fold by the addition of endogenous phospholipids under optimal conditions. The electrophoretic pattern of the preparation can be seen in Fig. 1A. Its major components were the α and β subunits of F_1-ATPase and peptides of approx. 30 000 molecular weight, suggested [15,17] to include the adenine nucleotide transporter and the uncoupler binding protein. These components are found in the most purified preparations of the ATPase complex from bovine heart [15—17].

Reactivation of the ATPase complex

Reactivation of phospholipid-deficient ATPase complex was tested according to three procedures. (a) An aliquot of the ATPase complex preparations (25—30 μg) was added to 0.2 ml of 0.25 M sucrose, 25 mM Tris-Mops (pH 7.2) containing the phospholipids and warmed to 37°C. After 10 min at 37°C the ATPase reaction was started by appropriate addition of a stock solution containing the other components of the incubation medium. (b) 1 mg of the ATPase complex was added to 0.6 ml of 25 mM Tris-Mops (pH 7.2) containing the phospholipids and incubated 10 min at 20°C. The mixture was transferred on top of a 20% sucrose solution and centrifuged 30 min at 20°C at 30 000 rev./min in a SW-50 Beckman rotor. The sediment was collected and tested for its ATPase activity, protein and phospholipid content. Occasionally, this fraction was washed by repeating several times the centrifugation against the sucrose solution. (c) (Ref. 18) 0.5 mg of the ATPase complex was added to 0.3 ml of a solution containing 10 mM Tris-Mops (pH 7.4), 2 μmol of phospholipids, 0.17% cholate, 1 mM dithiothreitol. This mixture was dialyzed 15 h at 4°C against 170 vols. of 10 mM Tris-Mops (pH 7.4), 0.1 mM dithiothreitol with a change at 4 h. After dialysis the sample was transferred on top of a 20% sucrose solution and centrifuged as described above. The sediment was collected and tested.

The ATPase activity was assayed in a medium containing 167 mM sucrose, 7 mM Tris-Mops, 600 μg bovine serum albumin, 2.7 mM phospho*enol*pyruvate (monopotassium salt), 20 μg pyruvate kinase, 4 mM ATP, 4 mM $MgCl_2$ 30—40 μg of ATPase complex (pH a 37°C, 7.2). Final volume, 0.6 ml. Incubation time, 10 min. The temperature of incubation was 37°C , and therefore above the transition temperature of all phospholipids tested except the dipalmitoylphosphatidylcholine. Inorganic phosphate, phospholipid phosphorus and protein were determined as outlined earlier [5]. Polyacrylamide gel electrophoresis under dissociating conditions was performed as described [19]. An estimate of apparent molecular weight was based on a titration curve obtained with a purified preparation of F_1-ATPase, using the subunit molecular weight given in Ref. 20.

Results

Phospholipid-dependent resolution

When the phospholipid-deficient ATPase complex preparation was incubated with egg phosphatidylcholine and centrifuged against a 20% sucrose solution, the mixture separated in two fractions: a layer floating on top of the sucrose solution and a sediment. The former contained the unbound phospholipids and a low amount of protein whereas the latter contained most of the protein with a phospholipid content close to that of the original submitochondrial particles (Table I). All the ATPase activity was recovered in the sediment. When the bilayer organization was loosened by inclusion of 50% lysophosphatidylcholine [21], a greater amount of phospholipids was bound to the sediment and more protein remained on top of the sucrose solution. The ATPase activity of the sediment became very variable and largely oligomycin-insensitive. Similar results were obtained with dinonanoylphosphatidylcholine. Although this phospholipid yields liquid crystals in water [22] and forms stable monolayers at the air/water interface [23], its surface tension measurements reveal an appreciable monomer activity in aqueous media (critical micellar concentration, 27 μM, Ref. 22). With these two phospholipid preparations, both protein extraction and the loss of oligomycin sensitivity were dependent upon the phospholipid concentration, each decreasing at lower phospholipid to protein content.

Polyacrylamide gel electrophoresis in the presence of sodium dodecylsulfate of floating and sedimented fractions, showed that the phospholipids induced a different distribution of the 30 000 molecular weight component present in the preparation. With dinonanoylphosphatidylcholine (Fig. 1B,C) this component was concentrated in the sediment, whereas with the monoacyldiacylphosphatidylcholine mixture it was found in the floating layer. As shown in Fig. 2 this last finding was further documented in a preparation sequentially

TABLE I

RESOLUTION OF THE ATPase COMPLEX BY LAMELLAR PHOSPHATIDYLCHOLINES

1 mg of lipid-deficient ATPase complex was incubated 10 min at 20°C in 0.6 ml of 25 mM Tris-Mops (pH 7.2) containing 4 μmol of the indicated phospholipid. The mixture was transferred on top of 4.5 ml 20% sucrose and centrifuged 30 min at 30 000 rev./min in a swinging rotor at 20°C. The ATPase activity of sediment and the distribution of protein in the sediment and in the layer remained on top of the sucrose solution were determined. Conditions for ATPase activity: 167 mM sucrose, 7 mM Tris-Mops, 600 μg bovine serum albumin, 2.7 mM phospho*enol*pyruvate (monopotassium salt), 20 μg pyruvate kinase, 4 mM ATP, 4 mM $MgCl_2$, 30—40 μg ATPase complex (pH 7.2). Final volume 0.6 ml. Incubation 10 min at 37°C. The reaction was stopped with 0.2 ml cold 50% trichloroacetic acid. Oligomycin 40 μg/mg protein.

	Egg phosphatidylcholine	Egg phosphatidylcholine/lysophosphatidylcholine (1:1, mol/mol)	Dinonanoylphosphatidylcholine
Percent of protein in the sediment	78	35	31
Percent of protein in the floating layer	11	49	53
Phospholipid content of sediment (μmol/mg protein)	0.82	3.6	4.5
ATPase activity of sediment ($\mu mol \cdot mg^{-1} \cdot min^{-1}$)	3.0	0.4—2.7	0.7—1.2
Percent inhibition by oligomycin	81	3—34	6—29

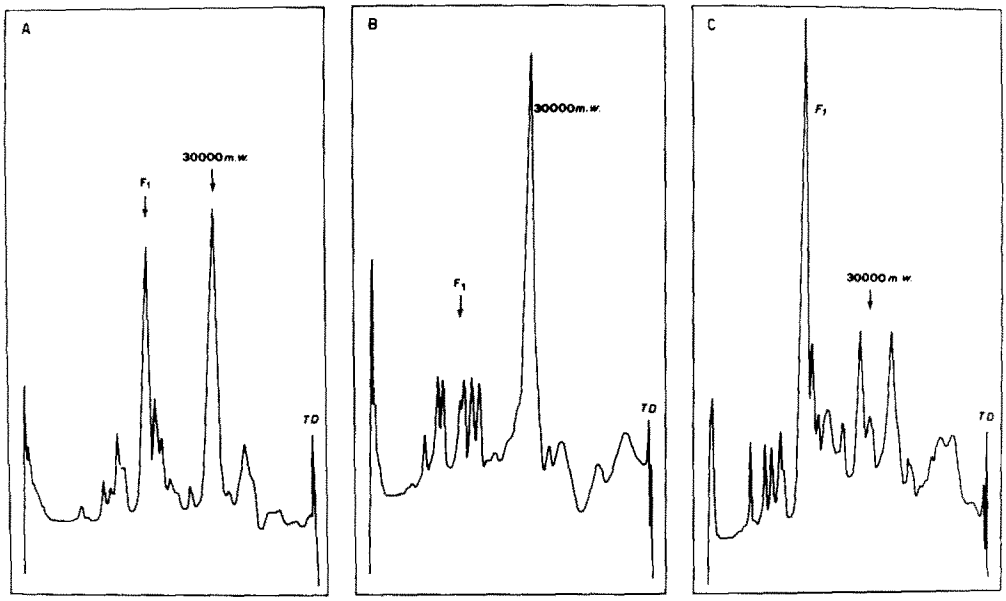

Fig. 1. Resolution induced by dinonanoylphosphatidylcholine. 2 mg of lipid-deficient ATPase complex were incubated with 8 μmol dinonanoylphosphatidylcholine in the conditions described in Table I. The sediment and the floating fraction were examined by gel electrophoresis. Gel loading, 22 μg. TD, tracking dye. A, densitometric trace of ATPase complex before the incubation with the phospholipid; B, sedimented fraction; C, floating fraction.

treated [24] with Sephadex, urea and cholate to eliminate any interference by the γ subunit of F_1-ATPase. This subunit has a molecular weight of 33 000 and may not be well separated from the 30 000 molecular weight component. As

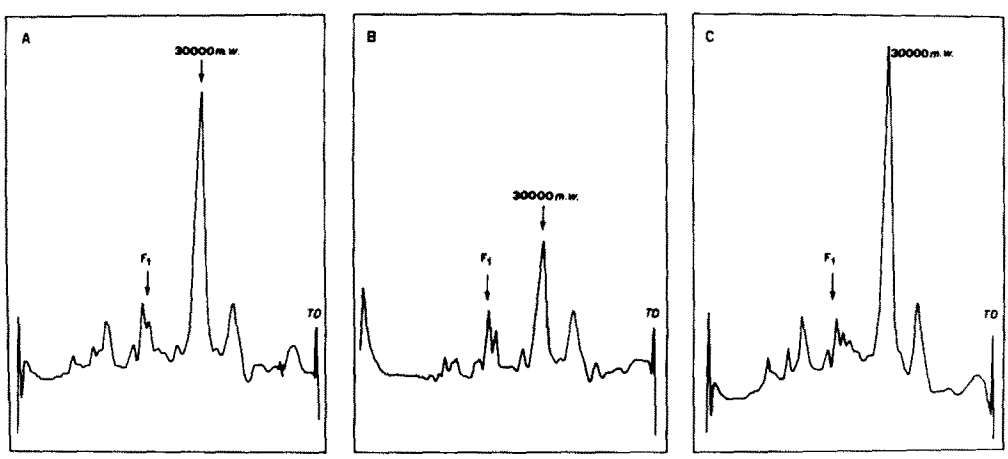

Fig. 2. Resolution in Sephadex-urea-cholate particles. Submitochondrial particles were extracted with cholate after sequential treatment with sephadex and urea [24]. 1 mg of the resulting preparation was incubated with 4 μmol egg phosphatidylcholine/lysophosphatidylcholine (1 : 1, mol/mol) in the conditions described in Table I. The sediment and the floating fraction were examined by gel electrophoresis. Gel loading, 22 μg. TD, tracking dye. A, densitometric trace before the incubation with phospholipids; B, sedimented fraction; C, floating fraction.

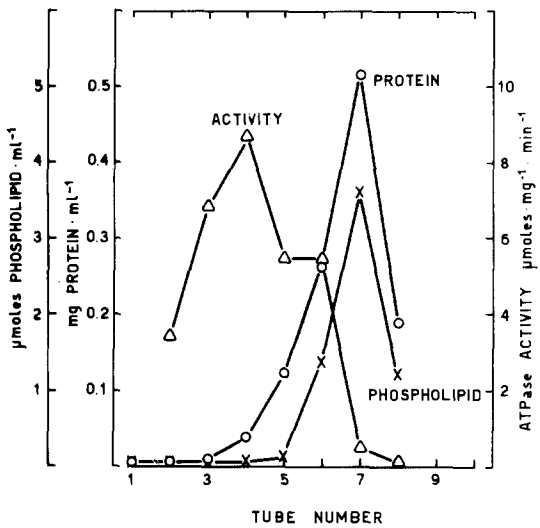

Fig. 3. Isolation of fragments with oligomycin-insensitive ATPase activity. 2.5 mg lipid-deficient ATPase complex were incubated with 10 μmol lysophosphatidylcholine/egg phosphatidylcholine (1 : 1, mol/mol), 10 min at 20°C in 1 ml of 25 mM Tris-Mops (pH 7.2). The mixture was transferred on top of a 11 ml gradient of 5, 10, 15, 20, 50% sucrose and centrifuged 4 h at 27 000 rev./min at 4°C in a SW-27 Beckman, rotor. 1 ml fractions were collected above a particulate material found between 20 and 50% sucrose. Fraction 8 corresponded to the top of the gradient. The oligomycin sensitivity of fractions showing ATPase activity was 0—15%.

reported previously, a component of apparent 30 000 molecular weight was separated from the ATPase complex during purification procedures that employed a deoxycholate/lysophosphatidylcholine mixture [15] or Triton X-100 [16]. In the former procedure the oligomycin sensitivity was retained, in the latter it was lost. In Fig. 3 it is seen that after the incubation of the ATPase complex with the lysophosphatidylcholine/phosphatidylcholine mixture, fractions with oligomycin-insensitive ATPase activity could be recovered by centrifugation against a discontinuous sucrose gradient. The ATPase activity of these fractions was cold-stable and remained oligomycin-insensitive upon the addition of the oligomycin sensitivity conferring protein [25]. Gel electrophoresis showed a multisubunit composition with a low content of 30 000 molecular weight peptide. This component was retained near the top of the sucrose gradient (fraction 7).

Since the above results pointed at a correlation between the resolution of the ATPase complex and a loose association of lipid molecules in the bilayer, attempts were made to prevent resolution by increasing the packing of lipid molecules. This has been possible by the employment of cholesterol. This compound is known to produce a condensing effect in phosphatidylcholine dispersions, provided that the acyl-chain length exceeds 10 carbon atoms [26]. The same effect in lysophosphatidylcholine dispersions makes possible the assembly of a lamellar structure [27,28]. Indeed in Table II it is shown that the presence of cholesterol preserved the oligomycin sensitivity in a complex treated with the lysophosphatidylcholine/phosphatidylcholine mixture.

TABLE II

CHOLESTEROL-INDUCED OLIGOMYCIN SENSITIVITY

25 μg lipid-deficient ATPase complex were incubated with 0.1 μmol of the indicated phospholipid in 0.2 ml of 0.25 M sucrose, 10 mM Tris-Mops (pH 7.2). After 10 min at 37°C, 0.4 ml of a stock solution containing or not oligomycin (40 μg/mg protein) were added to initiate the ATPase reaction. The final composition of the incubation mixture was described in Table I. The egg phosphatidylcholine/lysophosphatidylcholine mixture was 1:1 (mol/mol). That of phosphatidylcholine, lysophosphatidylcholine and cholesterol was 1:1:1 (mol/mol/mol).

Phospholipid	ATPase activity (μmol · mg^{-1} · min^{-1})		
	Without oligomycin	With oligomycin	% inhibition
None	0.39	0.21	54
Phosphatidylcholine	3.44	0.99	71
Phosphatidylcholine, lysophosphatidylcholine	4.47	2.75	38
Phosphatidylcholine, lysophosphatidylcholine, cholesterol	2.40	0.51	79

Phospholipid dependent assembly

When the ATPase complex was incubated with phosphatidylcholines containing 12—14 carbon atoms in each chain, the resulting lipid-protein complex did not separate after centrifugation against a 20% sucrose solution. The high oligomycin sensitivity confirmed that splitting of ATPase complex did not occur. In agreement with earlier results [5], in Table III it is shown that these phospholipids produced only low restoration of ATPase activity. To reach significant activation, a phosphatidylcholine with 16 carbon atoms in each chain was required (dipalmitoleylphosphatidylcholine). At this chain length this unsaturated phosphatidylcholine was more active than the saturated

TABLE III

ACTIVATION OF THE ATPase COMPLEX BY PHOSPHATIDYLCHOLINES

1 mg lipid-deficient ATPase complex was incubated with 4 μmol of the indicated phosphatidylcholines in the conditions described in Table I. After centrifugation 80% of the added protein and all the ATPase activity were recovered in the sediment. ATPase activity was 70—80% oligomycin-sensitive. A phospholipid sediment was not detected in the absence of protein. T_t denotes the temperature of the gel-to-liquid crystalline phase transition taken from Refs. 47 and 48.

Phosphatidylcholine	T_t (°C)	Phospholipid content of sediment (μmol/mg protein)	ATPase activity of sediment (μmol · mg^{-1} · min^{-1})
(a) None	—	0.1	0.32
(b) Saturated			
dilauroyl	0	2.0	0.47
dimyristoyl *	23	3.0	0.71
dipalmitoyl	41	1.9	0.71
(c) Unsaturated			
dipalmitoleyl	—	1.1	3.20
dielaidoyl	13	0.8	2.70
egg phosphatidylcholine	—15/—7	0.8	3.00

* High value of phospholipid binding and low ATPase stimulation were found also when the procedure of reconstitution was performed at 30°C.

homologue, dipalmitoylphosphatidylcholine. This phospholipid was however a separate case since it was the only one that could not be tested above the gel-to-fluid state transition temperature (41°C), because of ATPase complex thermal instability. As shown previously [5], the all-*trans* configuration of phospholipid acyl-chains is unsuitable to support the ATPase activity. Dielaidoylphosphatidylcholine (chains of 18 carbon atoms containing a *trans* double bond) elicited ATPase activity. In spite of greater chain length, the transition temperature of this phospholipid is similar to those of dilauroyl and dimyristoylphosphatidylcholine. This precluded explanation of the low effectiveness of these phosphatidylcholines with their relatively high values of melting temperature.

It is manifest from the data of Table III that the phospholipids producing a poor stimulation were largely bound to the membrane fragments. Appropriate controls established that the activation was not improved when the amount of added phospholipids was decreased in order to have a physiological value of binding. The protein-bound phospholipids could not be displaced by four consecutive washings against the concentrated sucrose solution. The inclusion of cholesterol (1 : 1, mol/mol) into these phospholipid bilayers in order to prevent the fusion among the vesicles [29], reduced by 60—70% the binding of lipid to protein. The same effect was obtained by the dilution of the phospholipids in a large volume before their incubation with the ATPase complex. These observations suggested that vesicles aggregation occurred and was responsible for the high lipid content of these complexes. Aggregation and coalescence among vesicles is rapid in dilauroylphosphatidylcholine dispersions

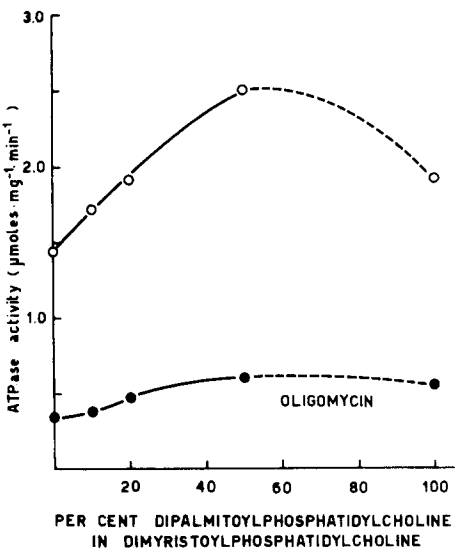

Fig. 4. Mixtures of dimyristoyl and dipalmitoylphosphatidylcholine. Liposomes of individual or mixed phosphatidylcholines were prepared and sonicated at 37°C. 0.25 mg ATPase complex were incubated with 1 μmol phospholipids in 0.25 M sucrose, 10 mM Tris-Mops (pH 7.2) at 37°C. After 10 min the mixing of protein and phospholipids was completed by a brief (40 s) sonication at 37°C. The ATPase activity was then tested in the medium described in Table I.

TABLE IV

RECONSTITUTION BY CHOLATE-DIALYSIS PROCEDURE

0.5 mg ATPase complex were mixed with 0.3 ml of 1 mM dithiothreitol, 0.17% cholate, 2 μmol phosphatidylcholine, 10 mM Tris-Mops (pH 7.4). The mixture was dialyzed 15 h at 4°C against 50 ml of 0.1 mM dithiothreitol, 10 mM Tris-Mops (pH 7.4) with a change at 4 h. After dialysis the samples were transferred on top of 20% sucrose solution and centrifuged 30 min at 30 000 rev./min in a swinging rotor. The sediments were tested for phospholipid content and ATPase activity. To reveal any latent ATPase activity, bovine brain phosphatidylserine (1 μmol/mg protein) was added to the incubation medium where indicated.

	Dilauroyl-phosphatidyl-choline	Dimyristoyl-phosphatidyl-choline	Dipalmitoyl-phosphatidyl-choline	Egg phosphatidyl-choline
Phospholipid content (μmol/mg protein)	3.7	3.4	1.5	1.7
ATPase activity ($\mu mol \cdot mg^{-1} \cdot min^{-1}$)	0.24	0.39	0.77	3.30
Per cent inhibition by oligomycin	63	50	73	88
Further activation by phosphatidylserine ($\mu mol \cdot mg^{-1} \cdot min^{-1}$)	+0.46	+0.80	+2.4	+0.1

[30]. In this and other studies [31,32] the low stability of sonicated dimyristoylphosphatidylcholine vesicles has been also evident and explains their failure to trap ions [33]. Evidence that the lack of ATPase stimulation by dilauroyl and dimyristoylphosphatidylcholines was due to insufficient acyl-chain length is presented in Fig. 4. These experiments were based on the observation that dimyristoyl and dipalmitoylphosphatidylcholines form ideal mixtures with transition temperatures dependent upon the relative proportion of the components [34,35]. On improving the bilayer structure of dimyristoylphosphatidylcholine by the inclusion of increasing amounts of dipalmitoylphosphatidylcholine, the activation gradually increased. The improvement was detected up to 50% of each component when the fluid state of lipid mixture was still ensured by the temperature of incubation. These experiments showed also that unsaturation was not an absolute requirement for the activation of the ATPase complex. Cholate dialysis experiments [18] served to elucidate further the contribution of lipid-chain length to the activation of the ATPase complex. This procedure permitted a complete penetration of protein into the lipid structure by the cholate-induced dispersion of both components. As shown in Table IV, dilauroyl and dimyristoylphosphatidylcholines were bound in large amounts but failed to yield activated complexes. When in these samples a state of latent ATPase activity was monitored by the addition of phosphatidylserine [36] only a slight activation was detected. This suggested that either inactivation of the F_1-ATPase occurred or the complex was trapped inside the aggregated vesicles. In contrast, dipalmitoylphosphatidylcholine, although prevented from yielding fully activated complexes due to the high melting temperature, did maintain the possibility of subsequent phosphatidylserine induced activation. Active preparations required a phospholipid of both long acyl-chain and low melting temperature. Unsaturated egg phosphatidylcholine served this purpose.

Discussion

Distinct patterns of ATPase complex reconstitution correlate with definite changes of phospholipid bilayer. At low bilayer stability, fragments with oligomycin-insensitive ATPase activity are obtained. At increased bilayer stability, the reconstitution of ATPase complex has been dependent on the acyl-chain length. Phosphatidylcholines with less than 16 carbon atoms in each chain yield complexes with low ATPase activity and high phospholipid content. At longer acyl-chains, complexes with optimal phospholipid content and oligomycin-sensitive activity are obtained.

Loss of oligomycin sensitivity is produced by dinonanoylphosphatidylcholine, the shortest chain phosphatidylcholine that forms a lamellar organization, or by a combination of lysophosphatidylcholine and egg phosphatidylcholine despite the long acyl-chain of each component. This shows that the damage to the ATPase complex does not primarily depend on chain length. Rather, a favourable balance between the phospholipid hydrophilic and lipophilic regions is involved. The induced loss of oligomycin sensitivity may be the consequence of ATPase complex conformational change with dislocation of catalytic from the proton channel forming subunits. However the ability of these phospholipids to extract a peptide of apparent 30 000 molecular weight present in the complex, indicates that resolution of ATPase complex may even occur. Investigations are in progress to establish whether this effect is useful for further complex purification. The interaction of this component with egg phosphatidylcholine has been reported [37]. The phospholipid-induced resolution can be compared to the solubilizing effect of non-ionic surfactants on membranes [38,39]. The presence of short chains in dinonanoylphosphatidylcholine is sufficient to yield a free monomer activity (27 μM, Ref. 22) compatible with the affinity of membrane protein for soluble amphiphiles. Similarly, when the soluble lysophosphatidylcholine is added to phosphatidylcholine, the monomer concentration is expected to increase. The presence of adequate monomer concentration is believed to be a necessary condition to achieve membrane solubilization by detergents [38]. In contrast, calculation of diacylphosphatidylcholine critical micellar concentration [31,40,41] indicates that membrane proteins interact only with stable lipid aggregates when added to dispersions of phosphatidylcholines with 12—14 carbon atoms in each chain. In accord, resolution of ATPase complex is not detected with phospholipids of this acyl-chain length. These conclusions are consistent with the cholesterol effect which prevents the ATPase complex resolution by increasing the packing of phosphatidylcholine molecules.

The increased interaction among phosphatidylcholine molecules with two chains of 12—14 carbon atoms is sufficient to avoid the loss of oligomycin sensitivity. However, only low ATPase activity is restored. The large amount of these phospholipids accumulating in reconstituted complexes documents the phospholipid aggregation and shows that the bilayer formed by these phosphatidylcholines is still not adequate to serve as stable support for the multisubunit complex. To this end the optimal bilayer structure is reached at 16 carbon atoms. Increased bilayer stability and optimal thickness of hydrocarbon region may concur to yield this result. The effectiveness of long-chain phos-

phatidylcholines to sustain protein incorporation is documented by recent findings on the phosphatidylcholine-serum apolipoprotein (Apo C-III) interaction [42,43]. With dimyristoylphosphatidylcholine the binding of protein is followed by the breakdown of phospholipid lamellae whereas the bilayer structure is retained with dipalmitoylphosphatidylcholine. Moreover, studies on the influence of phosphatidylcholines on β-hydroxybutyric dehydrogenase apoprotein show that this lipid dependent enzyme is poorly activated at acyl-chains of 12—14 carbon atoms whereas the activation increases at 16 carbon atoms [11,12]. The limited activity of phosphatidylcholines with 12—14 carbon atoms has been also observed in the ATPase complex from sarcoplasmic reticulum [9,10]. Since the ATPase complex is a transmembrane proton translocator, the optimal activation by long-chain phospholipids may also originate from the appropriate fitting of proton channel into the lipid hydrophobic region. In this respect the 5 Å difference [44] between the bilayer thickness of dipalmitoyl and dimyristoylphosphatidylcholines may be relevant. Recent papers [45,46] document the role of optimal bilayer thickness in the formation of conducting channels by the peptide gramicidin A and the polyene antibiotic amphotericin B, when included in liposomes.

Considering the requirement for long chain phosphatidylcholines in order to activate the ATPase complex, the apparent role of phospholipid unsaturation observed earlier [5] is explained by the possibility of unsaturated phospholipids to combine the long acyl-chain with fluidity at physiological temperature. In this respect it is of interest to note that the ATPase complex shows no preference for the *cis* or *trans* oriented double bonds.

Acknowledgement

The synthetic phospholipids used in this study were generously provided by Professor J. de Gier and Dr. P.W.M. van Dijck of the Biochemistry Department, University of Utrecht. Their stimulating discussions and advice are most gratefully acknowledged. This work has been supported by the Italian Consiglio Nazionale delle Ricerche (C.N.R.).

References

1 Kagawa, Y. (1978) Biochim. Biophys. Acta 505, 45—93
2 Pitotti, A., Contessa, A.R., Dabbeni-Sala, F. and Bruni, A. (1972) Biochim. Biophys. Acta 274, 528—535
3 Dabbeni-Sala, F., Furlan, R., Pitotti, A. and Bruni, A. (1974) Biochim. Biophys. Acta 347, 77—86
4 Bruni, A. and Bigon, E. (1974) Biochim. Biophys. Acta 357, 333—343
5 Bruni, A., van Dijck, P.W.M. and de Gier, J. (1975) Biochim. Biophys. Acta 406, 315—328
6 Bruni, A., Pitotti, A. and Dabbeni-Sala, F. (1978) Bull. Mol. Biol. Med. 3, (Suppl. 2), 150s—159s
7 Vanderkooj, G. (1974) Biochim. Biophys. Acta 344, 307—345
8 Kagawa, Y., Kandrach, A. and Racker, E. (1973) J. Biol. Chem. 248, 676—684
9 Warren, G.B., Toon, P.A., Birdsall, N.J.M., Lee, A.G. and Metcalfe, J.C. (1974) FEBS Lett. 41, 122—124
10 Warren, G.B., Toon, P.A., Birdsall, N.J.M., Lee, A.G. and Metcalfe, J.C. (1974) Biochemistry 13, 5501—5507
11 Bock, H.G. and Fleischer, S. (1975) J. Biol. Chem. 250, 5774—5781
12 Grover, A.K., Slotboom, A.J., de Haas, G.H. and Hammes, G.G. (1975) J. Biol. Chem. 250, 31—38
13 Kagawa, Y. and Racker, E. (1966) J. Biol. Chem. 241, 2467—2474
14 Van Deenen, L.L.M. and de Haas, G.H. (1964) Adv. Lipid Res. 2, 168—229

15 Serrano, R., Kanner, B.J. and Racker, E. (1976) J. Biol. Chem. 251, 2453—2461
16 Berden, J.A. and Voorn-Brouver, M.M. (1978) Biochim. Biophys. Acta 501, 424—439
17 Stiggal, D.L., Galante, Y.M. and Hatefi, Y. (1978) J. Biol. Chem. 253, 956—964
18 Kagawa, Y. and Racker, E. (1971) J. Biol. Chem. 246, 5477—5487
19 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406—4412
20 Knowles, A.F. and Penefsky, H.S. (1972) J. Biol. Chem. 247, 6624—6630
21 Mandersloot, J.G., Reman, F.C., van Deenen, L.L.M. and de Gier, J. (1975) Biochim. Biophys. Acta 382, 22—26
22 Tausk, R.J.M., Karmiggelt, J., Oudshoorn, C. and Overbeek, J.T.C. (1974) Biophys. Chem. 1, 175—183
23 Joos, P. and Demel, R.A. (1969) Biochim. Biophys. Acta 183, 447—457
24 Racker, E. and Horstman, L.L. (1967) J. Biol. Chem. 242, 2547—2551
25 MacLennan, D.H. and Tzagoloff, A. (1968) Biochemistry 7, 1603—1610
26 Demel, R.A. and de Kruijff, B. (1976) Biochim. Biophys. Acta 457, 109—132
27 Rand, R.P., Pangborn, W.A., Purdon, A.D. and Tinker, D.O. (1975) Can. J. Biochem. 53, 189—195
28 Kitagawa, T., Inoue, K. and Nojima, S. (1976) J. Biochem. 79, 1123—1133
29 Breisblatt, W. and Ohki, S. (1976) J. Membrane Biol. 29, 127—146
30 Hauser, H. and Barrat, M.D. (1973) Biochem. Biophys. Res. Commun. 53, 399—405
31 Martin, F.J. and MacDonald, R.C. (1976) Biochemistry 15, 321—327
32 Lawaczeck, R., Blackman, R., Kaionosho, M. (1977) Biochim. Biophys. Acta 468, 411—422
33 Nicholls, P. and Miller, N. (1974) Biochim. Biophys. Acta 356, 184—198
34 Chapman, D., Urbina, J. and Keough, K.M. (1974) J. Biol. Chem. 249, 2512—2521
35 Van Dijck, P.W.M., Kaper, A.J., Oonk, H.A.J. and de Gier, J. (1977) Biochim. Biophys. Acta 470, 58—69
36 Bruni, A., Pitotti, A., Contessa, A.R. and Palatini, P. (1971) Biochem. Biophys. Res. Commun. 44, 268—274
37 Montecucco, C., Bisson, R., Pitotti, A., Dabbeni-Sala, F. and Gudweniger, H. (1979) Biochem. Soc. Trans. 7, 954—955
38 Helenius, A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29—79
39 Egan, R.W., Jones, M.A. and Lehninger, A.L. (1976) J. Biol. Chem. 251, 4442—4447
40 Smith, R. and Tanford, C. (1972) J. Mol. Biol. 67, 75—83
41 Blake, R., Hager, L.P. and Gennis, R.B. (1978) J. Biol. Chem. 253, 1963—1971
42 Träuble, H., Middelhoff, G. and Brown, V.W. (1974) FEBS Lett. 49, 269—275
43 Novosad, Z., Knapp, R.D., Gotto, A., Pownall, H.J. and Morrisett, J.D. (1976) Biochemistry 15, 3176—3183
44 Seelig, A. and Seelig, J. (1974) Biochemistry 13, 4839—4845
45 Boehler, B.A., de Gier, J. and van Deenen, L.L.M. (1978) Biochim. Biophys. Acta 512, 480—488
46 Van Hoogevest, P. and De Kruijff, B. (1978) Biochim. Biophys. Acta 511, 397—407
47 Phillips, M.C. and Chapman, D. (1968) Biochim. Biophys. Acta 163, 301—313
48 Op den Kamp, J.A.F., Kauerz, M.T.H. and van Deenen, L.L.M. (1975) Biochim. Biophys. Acta 406, 169—177