Cardiolipin is essential for higher proton translocation activity of reconstituted F_o

YANG Hui (杨 辉), HUANG Youguo (黄有国), ZHANG Xujia (张旭家) & YANG Fuyu (杨福愉)

National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China

Correspondence should be addressed to Yang Fuyu (email: yangfy@sun5.ibp.ac.cn)

Received July 25, 2000

Abstract The F_o membrane domain of F_oF_1-ATPase complex had been purified from porcine heart mitochondria. SDS-PAGE with silver staining indicated that the purity of F_o was about 85% and the sample contained no subunits of F_1-ATPase. The purified F_o was reconstituted into liposomes with different phospholipid composition, and the effect of CL (cardiolipin), PA (phosphatidic acid), PI (phosphatidylinositol) and PS (phosphatidylserine) on the H^+ translocation activity of F_o was investigated. The results demonstrated that CL, PA and PI could promote the proton translocation of F_o with the order of CL>PA≫PI, while PS inhibited it. Meanwhile ADM (adriamycin) severely impaired the proton translocation activity of F_o vesicles containing CL, which suggested that CL's stimulation of the activity of reconstituted F_o might correlate with its non-bilayer propensity. After F_o was incorporated into the liposomes containing PE (phosphatidylethanolamine), DOPE (dioleoylphosphatidylethanolamine) as well as DEPE (dielaidoylphosphatidylethanolamine), it was found that the proton translocation activity of F_o vesicles increased with the increasing content of PE or DOPE, which has high propensity of forming non-bilayer structure, but was independent of DEPE. The dynamic quenching of the intrinsic fluorescence of tryptophan by HB (hypocrellin B) as well as fluorescent spectrum of acrylodan labeling F_o at cysteine indicated that CL could induce F_o to a suitable conformation resulting in higher proton translocation activity.

Keywords: CL, propensity of non-bilayer structure formation, reconstituted F_o, proton translocation activity, conformation.

The inner membrane of porcine heart mitochondria mainly contains PC (27%), PE (38%), PI (3.4%), CL and PA (~25%)[1]. Among them, PE, CL and PA have strong propensity of forming non-lamellar phase, and are able to form non-bilayer structure under certain circumstance[2]. The non-bilayer structure, or hexagonal II phase is inverted micelle in which the hydrophobic fatty acid chains of phospholipids interact with solvent and the hydrophilic head groups of phospholipids aggregate together. The propensity of H_{II} phase formation exhibits the tendency of lipids such as PE, CL, PA to adopt H_{II} phase under certain circumstance, albeit the lipids are not in real H_{II} phase. This propensity is characterized by the phase transition temperature at which lipids turn to H_{II} phase[3,4].

The effect of the propensity of H_{II} phase formation on the activity of mitochondrial

ubiquinol-cytochrome reductase and H^+-ATPase was reviewed[3,4]. It was found that PE, DOPE or PA under lower pH could enhance their activities. Their activities could also be either enhanced or inhibited by incorporation of H_{II} phase-forming promoters or bilayer stabilizer into the bilayer lipids, indicating the importance of H_{II} phase formation for higher activity of these reconstituted enzymes.

The F_o membrane domain of F_1F_o-ATPase, which is responsible for the proton translocation, has been studied extensively. However, the knowledge of F_o activity as a function of phospholipids is very limited. How does the propensity of H_{II} phase formation affect the activity and conformation of F_o? In this paper, the purified F_o was reconstituted successfully into liposomes to produce the functional proton translocation vesicles, and the effect of phospholipids on its activity and conformation was studied. Our results demonstrate that CL is essential for higher proton translocation activity of F_o.

1 Materials and methods

1.1 Materials

Fresh porcine heart was bought from a slaughterhouse. ADM, CHAPS {3-[(3-cholamidopropyl) dimethylammonio]-propanesulfonate}, PS, oligomycin, valinomycin and CCCP (carbonylcyanide m-chlorophenylhydrazone) were all purchased from Sigma. CL was from Fluka. PC (phosphatidylcholine), PE, PI, PA, DOPE and DEPE were from Avanti Polar Lipids. ACMA (9-amino-6-chloro-2-methoxyacridine), acrylodan were from Molecular Probes. DTT (DL-Dithiothreitol) was from ICN. PMSF (phenylmethylsulfonyl fluoride) was from Promega. HB was extracted and purified in our lab[5]. Other reagents were of analytical grade.

1.2 Methods

1.2.1 Preparation of submitochondria. Lutter's procedure[6] was used. The submitochondrial stocking solution was PA buffer (0.15 mol/L KP_i, pH 7.9, 1 mmol/L ATP, 25 mmol/L EDTA, 0.5 mmol/L DTT, 5% ethylene glycol, 0.001% PMSF).

1.2.2 Purification of F_1-ATPase. According to Beechey's method[7] with some modifications, submitochondrial suspension was centrifuged at 105000 *g* for 45 min at 4℃. The pellet was suspended in 10 mmol/L Tris-SO_4, pH 7.5, containing 0.25 mol/L sucrose, 1 mmol/L EDTA, 0.5 mmol/L DTT and 0.001% PMSF (protein concentration: 5 mg/mL). Chloroform (0.5 *v*) was added and the suspension was vigorously mixed for 30 s at 20℃. The emulsion was centrifuged at 11000 *g* for 10 min at 20℃. The top aqueous layer was collected and centrifuged at 105000 *g* for 30 min at 20℃. The supernatant was collected and saturated $(NH_4)_2SO_4$ solution was added to 37.5% saturation. The suspension was incubated for 15 min on ice, and then centrifuged at 15000 *g* for 15 min at 4℃. The supernatant was collected again and saturated $(NH_4)_2SO_4$ was added to 52.5% saturation. The suspension was incubated for 15 min on ice, and centrifuged at

15000 *g* for 15 min at 4℃. The resulting pellet was suspended in 52.5% saturation of $(NH_4)_2SO_4$ and stored at 4℃.

1.2.3 Isolation and purification of F_1F_o-ATPase. McEnery's method[8] was used with some improvements. Submitochondrial suspension was centrifuged at 105000 *g* for 45 min at 4℃. The pellet was suspended in TA buffer (50 mmol/L Tricine, pH 7.9, 1 mmol/L ATP, 25 mmol/L EDTA, 5% ethylene glycol, 0.5 mmol/L DTT, 0.001% PMSF) at a protein concentration of 8 mg/mL. Freshly prepared 10% CHAPS in TA buffer was added to a final concentration of 1.2%. The suspension was gently stirred for 30 min on ice and centrifuged at 105000 *g* for 1 h at 4℃. The supernatant was collected. Then 6.5 mL supernatant was layered on 32 mL 25% sucrose in TA buffer containing 0.2% CHAPS, and centrifuged at 25000 r/min for 10 h at 2℃ in a Beckman SW28Ti rotor. The top gold-yellow layer and 3 mL solution at the bottom were discarded, as they contained very low ATPase activity. The rest was collected and saturated $(NH_4)_2SO_4$ was added to 50% saturation. The suspension was incubated for 15 min on ice, then centrifuged at 105000 *g* for 15 min. The pellet was suspended in 50 mL TA buffer and concentrated to 5 mL by ultrafiltration to remove the excess CHAPS precipitated with protein. The solution was stored at −80℃ before use.

1.2.4 Extraction and purification of F_o from membrane vesicles. Solid NaBr and sucrose were added to 30 mL submitochondrial suspension (protein concentration: 10 mg/mL) with stir to a final concentration of 4 mol/L NaBr and 0.25 mol/L sucrose respectively. The mixture was incubated for 30 min on ice, then centrifuged at 105000 *g* for 20 min. The floating pellet was suspended in PA buffer (protein concentration: 10 mg/mL) and the procedure mentioned above was repeated. Then the pellet was washed twice in PA buffer. Finally, the pellet was suspended in TA buffer at a protein concentration of 5 mg/mL. 5 mL freshly prepared 10% CHAPS was added to vesicles' suspension. The final concentration of protein and CHAPS were 4 mg/mL and 1.2% respectively; the total volume was 40 mL. The suspension was gently stirred for 30 min on ice. Then the samples were centrifuged at 105000 *g* for 1 h at 4℃. The supernatant was collected. Every 6.5 mL supernatant was layered on 32 mL 20% sucrose in TA buffer containing 0.2% CHAPS and centrifuged at 27500 r/min for 10 h in the Beckman SW28Ti rotor at 2℃. Then the top 11 mL of yellow solution containing few F_o was discarded and the rest solution was collected. The sample was concentrated by ultrafiltration on a PM-30 membrane (Amicon) and stored at −80℃ until use.

1.2.5 Reconstitution of F_o into liposomes and examination of passive proton translocation activity. F_o was reconstituted into liposomes according to McEnery's method[9] with some improvements. Appropriate amount of different kinds of phospholipid in organic solvent was mixed and dried under nitrogen, Tricine-KOH buffer (10 mmol/L Tricine, pH 7.5, 0.5 mmol/L

DTT, 1 mmol/L $MgCl_2$) and freshly prepared 10% CHAPS in the same buffer was added; final concentration of phospholipid and CHAPS was 30 mg/mL and 1.2% separately. The suspension was sonicated by an ultrasonic homogenizer (CPX 600, Cole Parmer) for 10 min on ice until it became clear. F_o was added to this suspension at a suitable protein: lipid weight ratio and the mixture was incubated for 30 min on ice. Then at least 20 times the mixture's volume of TK buffer (10 mmol/L Tricine, pH 7.5, 0.2 mol/L KCl, 0.5 mmol/L DTT, 1 mmol/L $MgCl_2$) was added. The emulsion was centrifuged at 105000 *g* for 30 min. The pellet was suspended in TN buffer (10 mmol/L Tricine, pH 7.5, 0.2 mol/L NaCl, 0.5 mmol/L DTT, 1 mmol/L $MgCl_2$).

20 μg F_o in vesicles loaded with KCl was added to 2 mL TN buffer, 5 μL 1 mmol/L ACMA was added and the mixture was incubated for 5 min at 20℃. Then 5 μL 20 μg/mL valinomycin was added to elicit K^+ efflux leading to proton translocation through F_o into vesicles. Proton influx into proteoliposomes was monitored by quenching of ACMA, a highly permeant fluorescent probe (excitation wavelength: 410 nm, emission wavelength: 490 nm), with fluorescence intensity change being quantitatively proportional to the change of pH difference across membrane. Finally, 1 μL 1 mmol/L CCCP was added. K^+-loaded liposomes were used as control.

1.2.6 HB quenching[5] of tryptophan intrinsic fluorescence in F_o. 20 μg F_o vesicles was added to 2 mL TN buffer, and incubated for 5 min at 20℃; the fluorescence intensity of sample (excitation wavelength: 295 nm, emission wavelength: 335 nm) was measured. Then 1 μL 2 mmol/L HB was added and incubated for 2 min. The fluorescence intensity was measured again. This procedure was repeated until 5 μL HB was added.

1.2.7 The fluorescence intensity of acrylodan[10] labeled to F_o in proteoliposomes. F_o was incubated with acrylodan (acrylodan : F_o > 5 : 1, molar ratio) for 12 h at 4℃, then reconstituted into liposomes by CHAPS dilution method; at the same time, the unlabelled acrylodan was removed from the vesicles. 20 μg F_o in vesicles was added to 2 mL TN buffer and incubated for 5 min at 20℃. To get the fluorescence spectrum, excitation wavelength was fixed at 370 nm; emission wavelength was scanned from 400 nm to 550 nm.

1.2.8 Protein determination and SDS-PAGE. Membrane protein was estimated by the Simpson's method[11] in the presence of 0.5% SDS. BSA was used as a standard.

SDS-PAGE was carried out on Bio-Rad electrophoresis system with 4% stacking gel and 18% separating gel. The samples were precipitated by equal volume of organic solvent (chloroform : methanol = 1 : 3, volume ratio) to concentrate and delipidate the protein. The floating pellet was suspended in sample buffer and boiled for 2 min before loading.

2 Results

2.1 Purification and subunit composition of F_o from porcine heart mitochondria

The bands in fig. 1 show that the purified F_o does not contain F_1 subunits (α, β, γ, δ, and ε),

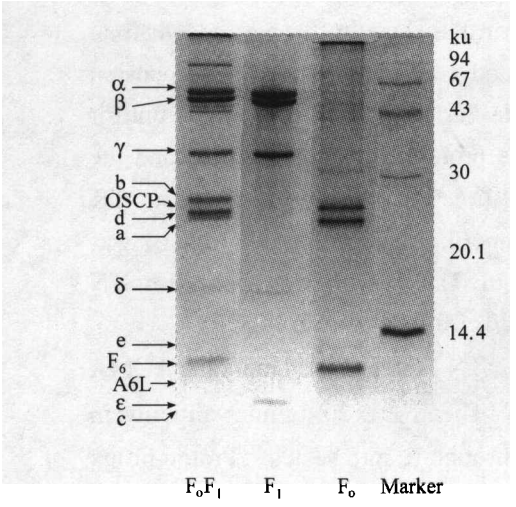

Fig. 1. SDS-PAGE profiles of F_oF_1-ATPase, F_1-ATPase and F_o from porcine heart mitochondria.

but has some high molecular weight impurities. Compared with components of F_o from bovine heart mitochondria[12], at least 8 subunits could be assigned in porcine heart mitochondria F_o. They are subunits b, OSCP, d, a, e, F_6, A6L and c respectively, and their molecular weight are all below 30 ku. The purity is about 85% judged by a Bio-Rad Imaging Densitometer Model GS-670. The purified F_o has no ATP hydrolysis activity, but is able to transport proton after it was reconstituted into liposomes, and this ability could be inhibited by oligomycin. Its assembly with F_1-ATPase could produce a functional F_oF_1-ATPase which could hydrolyze ATP (1.52 μmolPi/min/mg) and is sensitive to oligomycin (56.2% inhibition). Thus, the functional F_o was obtained.

2.2 Reconstitution of the purified F_o into liposomes

In order to study proton translocation activity of F_o as a function of phospholipid composition, F_o was reconstituted into defined-component liposomes. Considering the major phospholipid compounds such as PC (27%), PE (38%), CL (25%, including minor PA) and minor PI (3.4%) in the inner mitochondrial membrane[1], we reconstituted F_o into liposomes containing PC, PE and CL. Fig. 2 shows a typical ΔpH-dependent quenching of ACMA fluorescence in a time course.

Valinomycin elicits proton influx resulting in the fluorescence quenching of ACMA, which indicates that the reconstituted F_o has proton translocation activity. The fluorescent intensity becomes stable finally, indicating that the proteoliposomes have no obvious leaking.

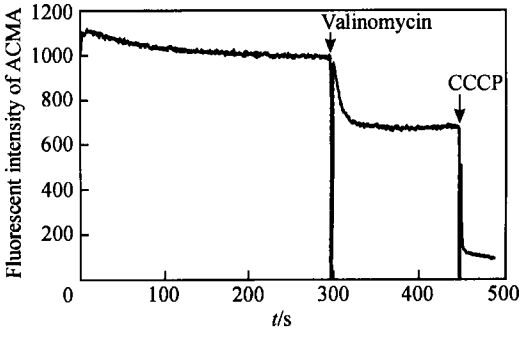

Fig. 2. Proton translocation of F_o vesicles containing 30% CL (PC : PE : CL = 23 : 47 : 30). 20 μg F_o in vesicles loaded with KCl was added to 2 mL buffer containing 10 mmol/L Tricine, pH 7.5, 0.2 mol/L NaCl, 0.5 mmol/L DTT, 1 mmol/L $MgCl_2$, and then 5 μL 1 mmol/L ACMA was added. The mixture was incubated for 5 min at 20℃, and 5 μL valinomycin (20 μg/mL) was added. Proton influx into proteoliposomes was monitored by fluorescent quenching of ACMA.

2.3 Effect of acidic phospholipid on the proton translocation activity of reconstituted F_o

The inner membrane of mitochondria contains a large amount of acidic phospholipids as CL and PA (25% total). Their effect on the proton translocation activity of the reconstituted F_o is not well understood yet. After successfully reconstituting F_o into liposomes, the effect of CL or PA on the activity of F_o was examined. The results are illustrated in fig. 3.

As shown in fig. 3, the activity of F_o enhances largely as the content of CL or PA increases. F_o vesicles with 20% CL or 15% PA give maximal activity, but the activity goes down when more

CL and PA is incorporated into liposomes. As the inner membrane of mitochondria contains 3.4% PI, we also examine the effect of PI on the activity of F_o. The obvious increment of proton translocation activity is observed beyond 20% PI and the activity reaches maximum at 40% PI, however, the effect of PI is not so efficient as CL and PA. PS is another acidic phospholipid with little content in inner membrane of mitochondria. PS totally inhibits the activity of F_o when it is incorporated into liposomes. The results demonstrate that acidic phospholipids enhance the proton translocation activity of reconstituted F_o with the order of CL>PA>>PI, while PS totally inhibits the activity.

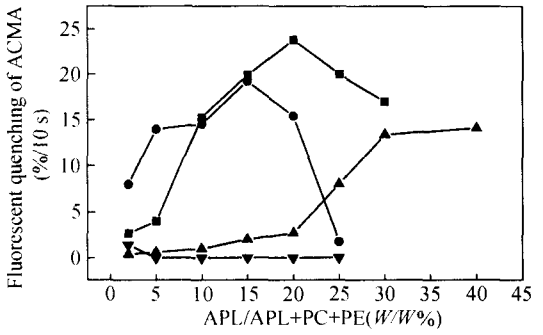

Fig. 3 Effect of CL, PA, PI and PS on the proton translocation activity of reconstituted F_o. Experimental conditions was as for fig. 2. Time scan spectrum of ACMA quenching after addition of valinomycin (fig 2) was fitted by exponential decay. Proton translocation activity of F_o vesicles was expressed as percentage of fluorescent quenching exactly 10 s after addition of valinomycin. APL stands for one of the four kinds of acidic phospholipid. ■, CL; ●, PA; ▲, PI; ▼, PS.

2.4 Effect of ADM on the proton translocation activity of F_o

ADM has a positive charge, which could react with negatively charged head group of acidic phospholipids[13]. ADM can specifically react with CL and significantly inhibit its formation of non-lamellar structure[14].

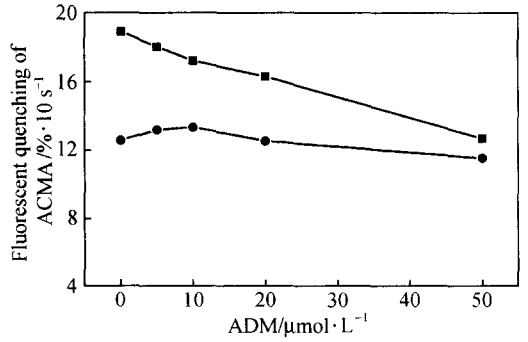

Fig. 4. Effect of ADM on the proton translocation activity of F_o in proteoliposomes containing 15% CL or 30% PI. ADM was added to the mixture just before addition of ACMA. Other experimental conditions were the same as for fig. 2. ■, PC : PE : CL = 28 : 57 : 15; ●, PC : PE : PI = 23 : 47 : 30.

As shown in fig. 4, the proton translocation activity of F_o proteoliposomes containing 15% CL decreases evidently with increasing concentration of ADM, while the activity of F_o vesicles with 30% PI is independent of ADM.

2.5 Effect of PE, DOPE and DEPE on the proton translocation activity of the F_o proteoliposomes

DOPE, DEPE have an unsaturated double bond with *cis*- or *trans*-configuration respectively, so DOPE (10℃) has much lower phase transition temperature of bilayer (L_α) to hexagonal II (H_{II}) phase as compared to DEPE (65℃). Therefore, DOPE has strong propensity of H_{II} phase formation at room temperature, while DEPE has not. To examine the effect of the propensity of H_{II} phase formation on the proton translocation activity of F_o further, we studied the effect of PE, DOPE and DEPE on the activity of reconstituted F_o. The results are shown in fig. 5.

The proton translocation activity of reconstituted F_o increases as the content of PE increases

in the proteoliposomes containing PE, PC and 10%CL (fig. 5(a)). The result indicates that PE can enhance the proton transport of F_o vesicles.

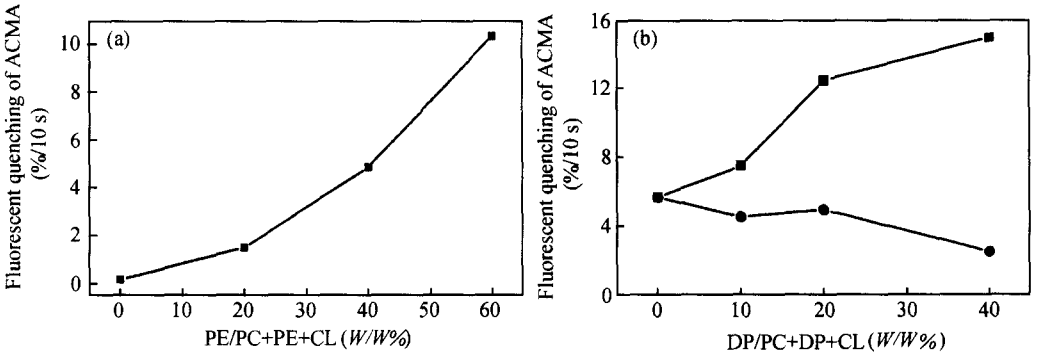

Fig. 5. Effect of PE and DOPE, DEPE on the proton translocation activity of reconstituted F_o. (a) Effect of PE on the proton translocation ability of reconstituted F_o containing 10% CL; (b) effect of DOPE and DEPE on the proton translocation activity of F_o in vesicles containing 20% CL. DP stands for DOPE or DEPE. ■, DOPE; ●, DEPE.

When DOPE or DEPE is substituted for PE, it is observed that DOPE considerably promotes the proton translocation of reconstituted F_o, and the activity of F_o vesicles with 40% DOPE is 2.6 times as much as control (fig. 5(b)). On the contrary, DEPE has no obvious effect within the same concentration range.

2.6 Effect of CL and PI on the conformation of F_o in proteoliposomes

It is well known that the activity of membrane proteins is tightly related to their conformation. The different effect of CL and PI on the proton translocation activity of F_o proteoliposomes must relate to the corresponding different conformations. So the effect of CL and PI on the conformation of F_o in proteoliposomes was studied.

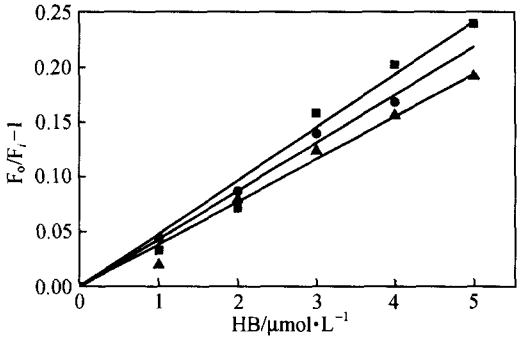

Fig. 6. Stern-Volmer plot for HB quenching of tryptophan intrinsic fluorescence of F_o in vesicles with different content of phospholipids. The experimental procedure refers to sec. 1.2.6. ■, PC : PE : CL = 33 : 65 : 2; ▲, PC : PE : CL = 27 : 53 : 20; ●, PC : PE : PI = 23 : 47 : 30.

2.6.1 Quenching of intrinsic fluorescence of F_o in vesicles by HB. HB acts as a very efficient collision quencher of fluorescence of tryptophan residues embedded in the hydrophobic domain of membrane proteins. The quenching efficiency reflects the conformation of membrane protein[5]. We have studied the dynamic quenching of fluorescence of tryptophan residues of F_o in proteoliposomes with different phospholipid component. The results are shown in fig. 6.

As shown above, HB quenching efficiency of tryptophan fluorescence of F_o in vesicles with 20% CL is lower than that of F_o in vesicles with 30% PI, suggesting different conformation of F_o in various proteoliposomes. Meanwhile, 2% CL incorporated into F_o proteoliposomes leads to more efficient fluorescent quenching by HB than 20% CL, providing further evidence for the

inference that CL can regulate the conformation of F_o in vesicles.

2.6.2 Fluorescent measurement of acrylodan labeled F_o in proteoliposomes. The F_o membrane portion of F_oF_1-ATPase from bovine heart mitochondria has at least six cysteine residues, each in a, b, c, d, f, and OSCP subunit[15]. Acrylodan can specifically label cysteine residue of proteins and emit fluorescence. Its fluorescent intensity decreases and its emission maxima shift toward blue when the associated cysteine residue is in more hydrophobic environment.

Table 1 suggests that the cysteine residues of F_o in proteoliposomes containing 30%PI are in more hydrophilic environment than that of F_o in vesicles with 20%CL, and the conformation of the former is relatively loose as the fluorescent intensity of acrylodan labeled F_o in proteoliposomes containing 30% PI is higher as well as the fluorescence spectrum is red shifted. Accordingly, F_o in proteoliposomes containing 2% CL adopts much looser conformation than F_o in vesicles with 20% CL as well.

Table 1 Investigation of the conformation of F_o in vesicles by fluorescence measurement

Phospholipid composition	PC : PE : CL = 33 : 65 : 2	PC : PE : CL = 27 : 53 : 20	PC : PE : PI = 23 : 47 : 30
Fluorescence intensity	3145±7.2	1221±4.1	2089±6.4
Emission maxima/nm	492.4±0.2	485.8±0.1	490.6±0.1

3 Discussion

3.1 Purification of F_o from porcine heart mitochondria

The study on the structure and function of F_o-membrane domain of F_oF_1-ATPase has been of great interest since the three-dimensional structure of F_1-ATPase from bovine heart mitochondria was resolved in 1994. However, only F_o from bovine heart mitochondria was purified to high homogeneity[12]. Because the hydrophobic F_o embedded into membrane consists of many subunits, some of its subunits might be easily lost during extensive purification and this might result in inactivation of F_o. We developed a modified purification procedure to obtain high quality F_o from porcine heart mitochondria. F_1-ATPase was removed from submitochondria by NaBr, CHAPS was used to extract F_o from membrane, and the highly purified F_o was obtained after sucrose gradient centrifugation. The purified F_o contains no F_1-ATPase subunits, but has OSCP.

3.2 Effect of CL on the proton translocation activity of F_o proteoliposomes

CL is an important phospholipid in inner membrane of porcine heart mitochondria, and could regulate the activity of cytochrome c oxidase, complex I, complex II as well as F_oF_1-ATPase which participate in oxidative phosphorylation of mitochondria[1]. The effect of CL on the activity of F_oF_1-ATPase complex has been extensively investigated, but there is little information about the effect of CL on the activity of F_o in defined-component liposomes. So the effect of CL on the reconstituted F_o was studied here.

3.2.1 CL is essential for higher proton translocation activity of F_o proteoliposomes. It was demonstrated that higher proton translocation activity of F_o-incorporated proteoliposomes could

only appear when a certain amount of CL was present in proteoliposomes containing PC and PE. The activity of F_o increases with increasing content of CL and reaches maximum at 20% CL (fig. 3). Meanwhile, CL enhances the activity of F_o more strongly than PA and PI. Interestingly, PS, another acidic phospholipid not existing in inner membrane of mitochondria, completely inhibits the activity of F_o. The results described above demonstrate that CL is definitely essential for higher proton translocation activity of F_o proteoliposomes.

3.2.2 The effect of CL on the activity of F_o is related with the propensity of H_{II} phase formation. The fact that various acidic phospholipids have diverse effect on the activity of F_o proteoliposomes suggests that their effect cannot be attributed to their negatively charged head groups. It is further demonstrated by the inhibition of ADM on the proton translocation activity of F_o proteoliposomes containing CL, but no effect of ADM on that of F_o vesicles containing PI.

Both CL and PA have the propensity of H_{II} phase formation[2]. This could be the reason for their similar promotion effect on the activity of F_o vesicles. The inhibition of ADM on the activity of F_o (fig. 4) and the promotion of PE, DOPE which have the propensity of H_{II} phase formation on the activity of F_o proteoliposomes (fig. 5) strongly suggest the above inference.

3.2.3 CL is favorable for maintaining a suitable conformation of reconstituted F_o. The activity of protein is based upon its suitable conformation. Investigation on the quenching of intrinsic fluorescence of tryptophan by HB and the fluorescent spectra of acrylodan labeling F_o at cysteine implied that F_o in the proteoliposomes containing 20% CL might be more compact than F_o in vesicles with 30% PI. This might be ascribed to the strong propensity of H_{II} phase formation of CL.

Summing up, because of the difficulty in obtaining highly purified F_o as well as its reconstitution, there is very little information about the effect of phospholipids on the activity of F_o so far. In this work, the fact that CL enhances the activity of F_o suggests that the strong propensity of H_{II} phase formation of CL might result in the compact conformation of F_o, subsequently its higher activity. This conclusion is consistent with our previous reports that the propensity of H_{II} phase formation could enhance the activity of F_oF_1-ATPase and cytochrome c reductase[3,4]. The results here further demonstrate that CL is essential for the activity of enzymes related with energy transduction in mitochondria, and provide evidence for the conclusion that the activity of F_0 is closely related with its associated phospholipids.

Acknowledgements This work was supported by the National Natural Science Foundation of China (Grant No. 39730130) and the Chinese Academy of Sciences.

References

1. Daum, G., Lipids of mitochondria, Biochim. Biophys. Acta, 1985, 822: 1.
2. Cullis, P. R., De Kruijff, B., Lipid polymorphism and the functional roles of lipids in biological membranes, Biochim. Biophys. Acta, 1979, 559: 399.
3. Li, L., Zheng, L. X., Yang, F. Y., Effect of propensity of hexagonal II phase formation on the activity of mitochondrial

ubiquinol-cytochrome c reductase and H^+-ATPase, Chem. Phys. Lipids, 1995, 76: 135.

4. Yang, F. Y., Huang, F., Effect of non-bilayer lipids on the activity of membrane enzymes, Chem. Phys. Lipids, 1996, 81: 197.
5. Yue Jiachang, Li Shengguang, Zhu Xiongwei, Conformational change of F_o moiety during uni-site ATP hydrolysis of H^+-ATPase, Acta of Biophysica Sinica (in Chinese), 1996, 12: 585.
6. Lutter, R., Abrahams, J. P., Van Raaij, M. J. et al., Crystallization of F_1-ATPase from bovine heart mitochondria, J. Mol. Biol., 1993, 229: 787.
7. Beechey, R. B., Hubbard, S. A., Linnett, P. E. et al., A simple and rapid method for the preparation of adenosine triphosphatase from submitochondrial particles, Biochem. J., 1975, 148: 533.
8. McEnery, M. W., Buhle, E. L. Jr., Aebi, U. et al., Proton ATPase of rat liver mitochondria, Preparation and visualization of a functional complex using the novel zwitterionic detergent 3-[(3-cholamidopropyl) dimethylammonio]-1-propane-sulfonate, J. Biol. Chem., 1984, 259: 4642.
9. McEnery, M. W., Hullihen, J., Pedersen, P. L., F_o "Proton channel" of rat liver mitochondria, J. Biol. Chem., 1989, 264: 12029.
10. Prendergast, F. G., Meyer, M., Carlson, G. L. et al., Synthesis, spectral properties, and use of 6-acryloyl-2-dimethylaminonaphthalene (Acrylodan), A thiol-selective, polarity-sensitive fluorescent probe, J. Biol. Chem., 1983, 258: 7541.
11. Simpson, I. A., Sonne, O., A simple, rapid, and sensitive method for measuring protein concentration in subcellular membrane fractions prepared by sucrose density ultracentrifugation, Anal. Biochem., 1982, 119: 424.
12. Collinson, I. R., Runswick, M. J., Buchanan, S. K. et al., F_o Membrane domain of ATP synthase from bovine heart mitochondria: Purification, subunit composition and reconstitution with F_1-ATPase, Biochemistry, 1994, 33: 7971.
13. Goormaghtigh, E., Chatelain, P., Caspers, J. et al., Evidence of a specific complex between adriamycin and negatively-charged phospholipids, Biochim. Biophys. Acta, 1980, 597: 1.
14. Goormaghtigh, E., Vandenbranden, M., Ruysschaert, J. M. et al., Adriamycin inhibits the formation of non-bilayer lipid structures in cardiolipin-containing model membranes, Biochim. Biophys. Acta, 1982, 685: 137.
15. Walker, J. E., Lutter, R., Dupuis, A. et al., Identification of the subunits of F_1F_o-ATPase from bovine heart mitochondria, Biochemistry, 1991, 30: 5369.