Cardiolipin is essential for higher proton translocation activity of reconstituted $F_0$

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 $F_0$ membrane domain of $F_0F_1$-ATPase complex had been purified from porcine heart mitochondria. SDS-PAGE with silver staining indicated that the purity of $F_0$ was about 85% and the sample contained no subunits of $F_1$-ATPase. The purified $F_0$ 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 $F_0$ was investigated. The results demonstrated that CL, PA and PI could promote the proton translocation of $F_0$ with the order of CL $>$ PA $>$ PI, while PS inhibited it. Meanwhile ADM (adriamycin) severely impaired the proton translocation activity of $F_0$ vesicles containing CL, which suggested that CL's stimulation of the activity of reconstituted $F_0$ might correlate with its non-bilayer propensity. After $F_0$ was incorporated into the liposomes containing PE (phosphatidylethanolamine), DOPE (dioleoylphosphatidylethanolamine) as well as DEPE (dielaidoylphosphatidylethanolamine), it was found that the proton translocation activity of $F_0$ 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 $F_0$ at cysteine indicated that CL could induce $F_0$ to a suitable conformation resulting in higher proton translocation activity.

Keywords: CL, propensity of non-bilayer structure formation, reconstituted $F_0$, 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\(\|\) phase-forming promoters or bilayer stabilizer into the bilayer lipids, indicating the importance of H\(\|\) phase formation for higher activity of these reconstituted enzymes.

The F\(_o\) membrane domain of F\(_{1}\)F\(_o\)-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\(\|\) 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 KPi, 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\(^\circ\)C. 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\(^\circ\)C. The emulsion was centrifuged at 11000 \(g\) for 10 min at 20\(^\circ\)C. The top aqueous layer was collected and centrifuged at 105000 \(g\) for 30 min at 20\(^\circ\)C. The supernatant was collected and saturated (NH\(_4\))\(_2\)SO\(_4\) 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\(^\circ\)C. The supernatant was collected again and saturated (NH\(_4\))\(_2\)SO\(_4\) was added to 52.5% saturation. The suspension was incubated for 15 min on ice, and centrifuged at