Three different aspects will be discussed.

1. The interaction of the lipid with the protein and the role of stabilization
2. The potential participation of the lipid in proton translocation
3. The interaction with the cofactor and the influence on the redox potential.

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16P1

Synthesis of cardiolipin analogs and characterization of their effects on induction of the peroxidase activity of cytochrome c

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Cardiolipin (CL), a negatively charged phospholipid bearing four fatty acyl chains, is a major phospholipid found in mammalian mitochondria (up to ~20%) with a multitude of biological functions. For instance, CL is responsible for regulation of the activity of several proteins involved in ATP biosynthesis, though the precise molecular mechanism of its regulation remains to be elucidated. Activation of the peroxidase activity of cytochrome c (cyt c) by CL and hydrogen peroxide in mitochondria has been suggested to be a key event in early apoptosis stage. To explore molecular mechanisms of the formation of cyt c–CL complex and the induction of peroxidase activity of cyt c, biochemical studies using structurally diverse CL analogs are needed.

We have developed a concise procedure for the synthesis of CL using phosphoramidite chemistry [1], which produces diverse CL analogs bearing any acyl chain compositions. This approach also allows for the production of CL containing a biophysical probe (nitroxide spin-label, fluorescent label, etc.) in one of four acyl chains. With numerous CLs in hand, we examined the structural factors of CL required for the formation and the induction of peroxidase activity of cyt c–CL complex in liposomal system. The activation efficiencies of CLs are well correlated with the binding affinities to cyt c. Our results revealed that at low CL content, the saturated acyl chain of CL is favorable for the activation of peroxidase activity of CL-bound cyt c and the proposed critical role of double bond in the acyl chains is not a general feature of the cyt c–CL interaction [2]. Moreover, using CL analogs in which a central glycerol head moiety was modified, we revealed that the natural structure of the polar head moiety is not critical for the formation of active cyt c–CL complex.

References


16P2

Does lipid saturation change affect the aggregation of proteoliposomes in a semi-lamellar system?

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Chloroplast membranes – unique assemblies of lipid, protein and pigment molecules – accommodate all light-harvesting and energy-transducing functions. In higher plants thylakoid membranes are differentiated into grana and stroma regions, also known as stacked and non-stacked regions, respectively. Various agents, including stress agents, change the proportion of stacked regions. The mechanism of membrane aggregation is not yet well-known. A good model of origination of the stacked regions is modulation by concentration of magnesium ions.

An attempt was made to characterize aggregation of artificial systems of liposomes and to compare it with the native thylakoid membranes. Liposomes containing chloroplast membranes’ lipids, such as MGDG, DGDG, and incorporated LHClII (isolated from spinach thylakoids) were used as a semi-lamellar system for a multi-method study by infrared spectroscopy — FTIR, absorption and fluorescence, confocal laser scanning microscopy (CLSM) and atomic force microscopy (AFM) imaging.

Spectroscopic data showed the type of protein–protein and lipid–protein interactions during the stacking as well as possible orientation of the trimeric form of LHClII complexes within the liposome membranes. The topographic images obtained by means of AFM microscopy as well as 3D CLSM images of aggregated liposomes revealed structures very similar to the grana membranes of higher plants.

Examination of the type of interactions observed in an artificial, less complicated system, makes mechanisms of specific thylakoid membrane in vivo organization foreseeable.

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16P3

Unraveling the architecture of the A1 complex of the Nanoarchaeum equitans A1A0 ATP synthase

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ATP synthases are complex molecular motors found in all living organisms. While the mitochondrial and the vacuolar ATP synthases have been widely studied and understood both structurally and functionally, there remain some extremophile archaeal A1A0 ATP synthases that are still uncharacterized. Nanoarchaeum equitans is a nano-sized hyperthermophilic parasitic organism with several ancestral traits found in its proteins and structural RNA. Genomic annotation indicates that some typical archaeal A1A0 ATPase subunits are missing, suggesting that the N. equitans ATP synthase is either compromised or constructed partially of subunits donated by its host [1]. We noted that the annotated B subunit of the A1 complex which is supposed to regulate the ATP synthesis/hydrolysis is significantly shortened compared to its own A subunit and compared to the B subunits of other archaeal ATP synthases [2]. These putative N. equitans ATPase subunit proteins were expressed in Escherichia coli in order to determine whether they could reconstitute a complex, and to elucidate the organization of A and B subunits. The minimal stalk formed by the D subunit was also expressed to determine whether it was also capable of forming a complex with the A and B subunits. An in-vitro reconstitution of purified A and B subunits has been