Structure Dynamics of Energized Biological Membranes – Time-Resolved Dynamic Light Scattering TR-DLS and Time-Resolved Neutron Scattering TR-SANS

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Biological membranes of energy metabolism get their function by energization, i.e. generation of an electro- chemical proton potential difference across a membrane. This couples the energy of respiration, photosynthesis or ion transport to membrane proteins. Those processes can be studied with liposomes as model membranes. Liposomes (small unilamellar vesicles SUV) with reconstituted ATP-synthase from Micrococcus luteus [1,2] were prepared from DMPC-D54, protein-free SUV from protonated lecithins. The energized membrane state was estimated by time resolved dynamic light scattering TR-DLS and time resolved neutron small angle scattering TR-SANS of liposomes after a large pH-jump (delta-pH > 1) at the D22-beamline at ILL in frames of logarithmic time resolution. The pH-jump was achieved by two techniques:
i) by rapid acid addition using a stopped flow device and
ii) by flash photolysis of novel caged acids (caged proton), namely caged SulfoBenzoate cSB.
With pure liposomes, the effect of a large electrochemical proton potential difference across the membranes sufficient for ATP-synthesis, which is called "membrane energization", on the membrane structure was investigated. Size changes of the liposomes after a large pH-jump was investigated by time resolved neutron scattering and dynamic light scattering, the proton flow was tracked using entrapped indicator dyes with respect to proton binding to the membrane surface. As a novel result we observed a change in the lipid bilayer structure upon membrane energization (delta-pH > 0.5). The thickness of the hydrophobic core shrinked by 1 A while no swelling was observed. Spectroscopic experiments with pH-indicator entrapped liposomes showed an increase of the proton permeability by an order, which is consistent with a transition of transient hydrogen bond chain (tHBC) pores of type-C to -A.
The experiments were extended to ATP-synthase-liposomes. The proteoliposomes were prepared form preformed empty liposomes (SUV) by detergent-assisted reconstitution of the membrane protein. The structure of the proteoliposomes was estimated by neutron scattering, freeze fracture electron microscopy and dynamic light scattering. For neutron scattering the lipid entity was matched by solvent-contrast variation [2]. The liposomes from DMPC-D54 were matched by 85% D2O-buffer, while the lipid contributed 98% of the mass. Subtraction of the scattering of matched protein-free reference liposomes, the contribution of the protein in situ was obtained. It was compared to the neutron scattering of ATP-synthase in detergent solution (TDOC).

[1] H. Freisleben; K.Zwicker; T. Nawroth et al. (1995) Chem.Phys.Lip 78, 137
[2] T. Nawroth; K. Dose; H. Conrad (1989) Physica 156 B, 489-492