

FORMATION OF PROTEIN-CONTAINING SUPPORTED LIPID BILAYERS ON SiO₂.

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INTRODUCTION: One way to study transmembrane proteins is to mimic the membrane structure by incorporating the proteins into lipid membranes deposited on solid supports. The focus of this project is to combine spontaneous formation of lipid membranes on solid supports, known to occur on SiO₂ [1], with incorporation of transmembrane proteins. Understanding and mastering of this process has important implications in the field of biosensors, bioenergetics, biomaterials and for a basic understanding of membrane processes.

In this work we use small unilamellar proteoliposomes to form a lipid bilayer with incorporated transmembrane proteins on a hydrophilic SiO₂-surface. The vesicle and protein adsorption is studied with the QCM-D technique where the frequency shift (mass adsorbed on the surface including the mass of water coupled to the molecules bound to the surface) and the energy dissipation shift (reflecting the viscoelastic properties of the overlayer) are measured simultaneously [2]. Proton translocating nicotinamide nucleotide transhydrogenase (TH) is used as a model protein. The presence of TH in the supported lipid bilayers was detected by trypsin-cleavage of water soluble subunits of the protein.

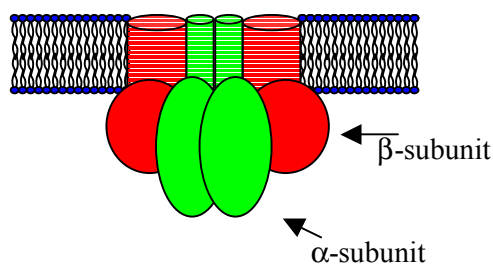


Fig. 1. TH in the lipid membrane. The enzyme consists of two subunits. In the cell membrane it is a dimer.

METHODS: The purified TH comes from *E-coli*, it is the wild-type enzyme and it is purified according to Mueller et al [3]. The liposomes consists of 50% bovine heart PE, 45% egg-PC and 5% bovine heart PS and has been prepared by sonication, followed by ultra-centrifugation [4]. The lipids are purchased from Sigma-Aldrich.

The TH is reconstituted by mixing pre-formed liposomes, Triton X-100 and protein. The Triton X-100 is removed by bio-beads SM-2, Bio-Rad. The 1 inch diameter, piezoelectric quartz sensor crystals, with gold electrodes, used in the QCM-D experiments are purchased from Maxtec. 3 nm Titanium followed by 100 nm SiO₂ are evaporated onto the sensor electrodes to constitute the bilayer forming SiO₂ surface [ref]. The sensors are cleaned with detergent and UV-Ozon-treatment. The buffer used through the entire experiment is 100 mM NaCl, 10 mM Tris-HCl, pH 8.0. The bilayer is incubated with trypsin for 30 minutes with and without the presence of the substrate for the β -subunit of TH, which is NADPH [5]. When trypsin is added to the bilayer without NADPH only the α -subunit is cleaved off. When NADPH is present both soluble subunits are cleaved off.

RESULTS: The supported lipid bilayers containing protein are spontaneously formed onto the silicon wafers when exposing them to the proteoliposomes. When the protein concentration is too high, the desired total transformation of the proteoliposomes to bilayer doesn't occur. Instead there are some intact proteo-liposomes remaining on the surface, indicated by the final higher frequency and dissipation shift compared to the results of the fusion of the liposomes in fig 2.

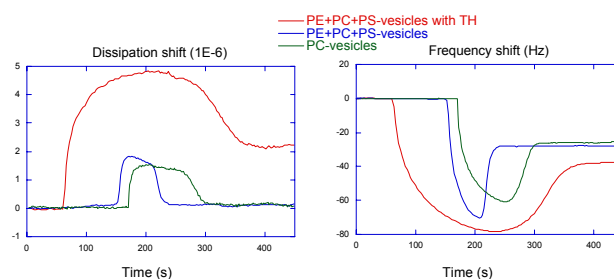


Fig 2. QCM-D results. Comparison of frequency and dissipation shifts between liposomes containing different kinds of lipids and proteoliposomes. The maximum and minimum of the curves show the collapse of the liposomes and proteoliposomes where the mass and energy dissipation of bound water inside the liposomes are lost.

When the protease Trypsin is added to the formed bilayer, containing incorporated proteins, the soluble subunits are cleaved off resulting in complete transformation of the vesicles to a bilayer. See fig 3 where the loss of mass and energy dissipation is shown when the subunits are cleaved off.

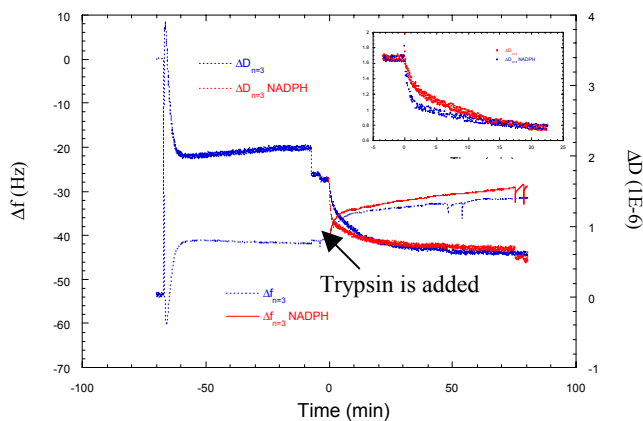


Fig 3. Trypsin cleaving of subunit α and β of TH in supported membrane

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