INTRODUCTION OF TWO DIFFERENT MEMBRANE PROTEINS WITH
MODEL MEMBRANES INVESTIGATED BY FT-IR SPECTROSCOPY

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Introduction

There are several ways of how membrane proteins are attached to a lipid bilayer. We investigated two proteins with respect to their interactions with model membranes.

1. mitochondrial creatine kinase (Mi-CK) is known to bind effectively to the surface of negatively charged bilayers like cardiolipin (CL). Mitochondrial CK is important for energy metabolism in cells of high and fluctuating energy requirements.

2. Alkaline phosphatase (AP) belongs to a wide group of enzymes which catalyze the non-specific hydrolysis of phosphate monoesters in an alkaline environment. AP is attached to the outer leaflet of the plasma membrane by the lipid moiety of a glycosyl-phosphatidylinositol (GPI) anchor. The hydrophobic hydration chains of this moiety are responsible for the attachment of the enzyme to the lipid bilayer.

Materials and Methods

In case of Mi-CK as model membrane, a supported lipid bilayer was prepared using the Langmuir Blodgett (LB) Vesicle-Method. With the help of a film balance, the first layer consisting of dipalmitoyl phosphatidic acid (DPPA, Fig. 2) was transferred to the surface of a clean germanium plate. The second layer consisting of negatively charged cardiolipin (CL) was produced by spontaneous adsorption to the hydrophobic DPPA film from a vesicular solution (Fig. 3, path 1: IV). In case of Mi-CK immobilized on a supported lipid bilayer, the first layer was transferred at 30 mN/m from an aqueous subphase (10 M CaCl2) to a germanium ATR plate. Spectra were measured against air. Surface concentration was calculated with the thin film equation: surface concentration = 3.80 10 mole cm-2. Surface concentrations were estimated with the thin film approximation: angle of light incidence 6, 45°; number of active internal reflections N, 3.

AP tends to precipitate in aqueous buffer solutions and needs some detergent for solubilization. Therefore, the transfer of AP from solution to the lipid bilayer is more critical than that of Mi-CK. At first, AP solubilized with [I]-octylglucoside ([I]-OG) was directly adsorbed to DPPA, then a palmitoyl oleyl phosphatidylcholine (POPC) vesicle solution was circulated through the ATR cell via a syringe pump (Figs. 2, IV, VI). Afterwards, the activity of the immobilized AP was measured by pumping substrate solution (I-nitrophenylphosphate, pNPP) through a flow-through cuvette. Enzymatic activity was determined from the rate of p-nitrophenol production by a spectrophotometer. FT-IR spectroscopy was done at 420 nm. A generalized scheme for carrying out in situ activity measurements is shown in Fig. 1.

Results and Discussion

Fig. 3: Schematic description of two pathways for immobilizing enzymes on lipid model membranes attached to an IRE-plate. Path I: immobilization of Mi-CK. (I) Transfer of the inner IRE-attached DPPA-bilayer. We investigated two proteins with respect to their interaction with model membranes. Mitochondrial creatine kinase (Mi-CK) is known to bind effectively to the surface of negatively charged bilayers like cardiolipin (CL). Mi-CK is important for energy metabolism in cells of high and fluctuating energy requirements.

Polarized IR ATR absorbance spectra of POPC-AP and POPC-Mi-CK immobilized on a supported lipid bilayer are shown in Fig. 4 and 5. A 0.55 mg/ml enzyme-solution in phosphate buffer (pH 7.0) was slowly pumped over the DPPA/CL bilayer at 25°C. The process could be monitored in situ because replacement in situ could be achieved by VIS spectroscopy at 420 nm. A generalized scheme for carrying out in situ activity measurements is shown in Fig. 1.

In contrast to Mi-CK adsorbing to the negatively charged bilayer, AP exhibits a much slower adsorption process (saturation of the adsorption to DPPA after 7.5 h from a 50 µg/ml 20 mM Tris buffer solution). AP will remain solubilized and will not adsorb to the DPPA layer; if it is too large, AP will aggregate from the beginning.

Fig. 4: Polarized IR ATR absorbance spectra of CL and immobilized Mi-CK. Top: Cardiolipin from E. Coli (CL) assembled from a vesicle solution (0.06 filament/ml CL) on a DPPA-layer. This bilayer was in contact with 20 ml phosphate buffer solution (pH 7.0, T 18°C). Buffer in the phosphate buffer; dichroic ratio R, 1.16; surface concentration = 1.68 10 mole cm-2; Bottom: Polarized IR ATR absorbance spectra of Mi-CK immobilized on a DPPA/CL-bilayer in 10 mM D O phosphate buffer solution pH 7.0, T 25°C; reference DPPA/CL/bilayer in D O buffer; dichroic ratio R, 1.85; surface concentration = 1.68 10 mole cm-2. Both surface concentrations were estimated with the thin film approximation: angle of light incidence 6, 45°; number of active internal reflections N, 3.

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References

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Fig. 5: Polarized IR ATR spectra of AP. Top: Single channel spectra of AP immobilized on DPPA; 20 mM D O Tris buffer pH 7.0, T 25°C. Bottom: Corresponding polarized I R ATR absorbance spectra; reference, DPPA in D O Tris buffer; dichroic ratio R, 1.62; surface concentration = 1.84 10 mole cm-2; angle of light incidence 6, 45°; number of active internal reflections N, 3.

Regardless whether the interactions between enzymes and lipid membranes are of electrostatic or hydrophobic nature, our results show that it is possible to build up well defined and stable protein-lipid assemblies. The simultaneous application of FT-IR ATR measurements with methods for determining the native enzymatic activity will give new insight into mechanisms of interactions between different types of membrane proteins and their lipid matrices.

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