In situ Preparation and Electric Field Excitation of an Oriented Film of Omp32 monitored by FTIR ATR Spectroscopy

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Introduction
Gram-negative bacteria are characterized by an outer membrane which serves as an effective protective against numerous toxins. The uptake of nutrients and ions is enabled by the outer membrane proteins related to porins. The porin protein often show a selectivity to either cations or anions. The Omp32 from Eschei.

Results
In situ Preparation of an oriented, monomolecular Omp32/lipid bilayer. Dialysis: In situ monitoring of dialysis shows the progress of the adsorption process onto the Ge IRE (Fig. 3). The time dependent behaviour of the components as shown in Fig. 4 were evaluated from time resolved difference spectra, taking the spectrum at the end of dialysis as reference. The observation of a saturation in the course of Omp32 adsorption strongly suggests that a monolayer is formed.

Calculation of surface concentrations: Quantification of porin, DMPC and DM ODC in adsorbed films has been performed using the spectra measured after 17.19 h of H2 exchange. Surface concentrations were calculated from measurements with parallel and perpendicular polarized incident infrared light, using the f band for Omp32, and the symmetric stretching vibration of the methylene groups (CH2) and (CO2) for DMPC and DM ODC, respectively. Omp32 bilayers resulted in a mean area per Omp32 trimer of 67±13 Å2. This finding is in good agreement with electron microscopic studies of 200 crystals from Omp32 which showed a trimer area of 87±16 Å2. In addition, Omp32 exists in 2D crystal forms. The one with the smaller unit cell size (f2f2) exhibits a lateral arrangement leading to an area per trimer of 67±16 Å2 which fits exactly our mean area. This result approves the assumption of a monomolecular film adsorption. Two to six DMPC molecules per Omp32 monomer were found. 

Orientation: By means of dynamic infrared spectroscopy a strong orientation of the films found. The film is oriented preferentially perpendicular to the surface of the Ge IRE. A more detailed analysis of the results obtained by dialysis is found in (8).

Electric field excitation
Phase resolved absorbance spectra: The spectra (Fig. 6) of the modulated Omp32 monolayer showed D0 components which were much stronger as found in a corresponding experiment without applied electric field. This indicates a electric field depending orientation of D0 in the water filled pore of Omp32. Small absorbance bands at 1640 cm-1, 1631 cm-1, 1620 cm-1 and a broad band around 1560 cm-1 were reproducibly detected in the modulated absorbance of Omp32. Further experiments have to be done, to decide whether this bands are induced by the electric field or by the existence of amino acid residues (probably of arginine, lysine and acidic amino acids, e.g. by varying the pH of the buffer). 

Materials and Methods
Fig. 1: Three dimensional structure of Omp32 monomer (7). As type III secretion channel the Omp32 monomer forms a channel. A monomer is build up of 16 amphipathic antiparallel strands connected by seven short periplasmic and eight external loops resulting in a molecular area of 34.7 kDa. Each monomer of Omp32 forms a complex with a 5.8 kDa porin. Porin and peptide together form a funnel like homodimer where the peptides are located close to the trimer axis of the Omp32. One of the external loops (L3) folds back into the center of the channel and determines together with a second loop (PP) a particularly narrow constriction zone. A cluster of these charged arginine residues in this constriction in combination with further arginine residues at the external and periplasmic pore entrances create a large positive electric potential within the channel. This is suggested to represent the selectivity filter of the porin.

Fig. 2: Arrangement of the ATR dialysis cell for in situ FTIR measurements of monolayer polymer assembly. Dialysis membrane

Detergent solubilized protein and lipids in buffer

ATR Internal reflection element (IRE)

Fig. 3: Adsorption of Omp32 and deuterated DMPC to the ATR IRE of the dialysis cell. The spectra (pp-pp) show the fast decrease of the intensity of the acid band and the slower vanishing of OPOE bands. The appearance of increasing protein bands indicates unambiguously adsorption of Omp32 to the IRE. The development of the shape of the amide I is characteristic of a protein rich in anti-parallel platted sheet structure. After 17.8 h no further increase of the protein bands could be observed after removal of the electric field. There are weak, but unambiguously detectable bands resulting from the stretching vibrations (CD2) favouring the conclusion that Omp32 protein adsorption is parallalised by the adsorption of small amounts of phospholipids.

Fig. 4: Kinetics of the dialysis. Normalised integrated absorbance of Omp32 (a), NaCl (b) and deuterated DMPC (c) and absorbance of the protein at 1524 cm-1. The decrease of NaCl, and the detergent can be fitted by a single exponential function resulting in a time constant for the NaCl, antraxtion at 35°C of 1.0±0.4 h, whereas the corresponding time constant for the detergent is found to be 4.3±0.05 h. Omp32 porin adsorption shows a different characteristic. Within the first hour of dialysis the amide I band decreases. After 1.2 h of Omp32 detection starts after about 1.2 h when the Omp32 concentration is reduced to 0.07 rag/ml. At the end of the dialysis stage 0.07 rag/ml is achieved after about 4 hours and almost finished after 5 hours. The critical imide concentration (concm) of Omp32 of 0.25 % is reached after 3 h of dialysis.

References
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