Membrane Proteins in Thin Films: a DSC Study

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Molecular functions and structural changes of membrane proteins in an aqueous environment can be elucidated by reaction-induced FTIR difference spectroscopy in the spectral range between 2000 and 950 cm^{-1}, for example upon photolysis of caged compounds (1,2). The achieved detection of IR band changes even due to single amino acid residues is, however, only possible in the presence of very high protein concentrations implying that a low water content must be present. For this purpose, techniques have been developed where thin protein films with a thickness around 5 µm and protein concentrations up to 1 mM are investigated. In general, the films are formed by controlled dehydration of membrane protein suspensions at reduced pressure and low temperature. For the retention of enzymatic activity of Na,K-ATPase, for example, a cosolvent such as glycerol is required. For the interpretation of the results obtained by FTIR spectroscopy, it is important to know whether essential properties of the proteins such as hydration are changed upon the formation of such thin films. Therefore, a differential scanning calorimetry (DSC) study has been carried out with purified Na,K-ATPase and Ca-ATPase in suspension, as pellet obtained by high-speed ultracentrifugation and in thin films. As relevant thermoanalytical properties, the endothermic denaturation transitions of the proteins as well as the heat of water melting in the sample have been determined.

For Na,K-ATPase in the presence of a cosolvent (20% glycerol), a single, comparatively narrow endothermic and irreversible denaturation transition around 70°C and a denaturation enthalpy of about 1.7 MJ/mol is found in concentrated suspension and in the state of the pellet. In the case of the thin films suitable for IR spectroscopy, a characteristic change is observed in a reproducible manner. The enthalpy change of the remaining transition around 70°C is reduced but an additional transition at about 77°C is observed. Based on control experiments, the new high-temperature transition is attributed to a partially dehydrated state of a region of the protein. In addition, a reversible, comparatively broad endothermic transition around 20°C is found under conditions of high protein concentrations (pellet or film), which is tentatively assigned to a transition of the lipid environment of this integral membrane protein. Similar results are found for Ca-ATPase films in the presence and absence absence of glycerol. In the absence of glycerol, the deoxycholate treated enzyme in suspension exhibits a narrow endothermic main transition at 52°C with a denaturation enthalpy around 0.9 MJ/mol. For the film, two equally large endothermic transitions are found at 59 and as high as 87°C. The latter marked increase in transition temperature can be considered as characteristic of an even more pronounced partial protein dehydration than observed for Na,K-ATPase. These results show clearly that DSC can easily be applied in a sensitive manner to control and characterize the hydration properties of very concentrated protein samples.

1. A. Barth, W. Kreutz, M. Mäntele, FEBS Lett. **277** (1990), 147-150.
2. M. Stolz et al., Biopolymers **82** (2006) 368-372.