

Structural Organisation of Membrane Bound H-ras Protein

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The anchoring of the H-ras-protein into a phospholipid model membrane has been investigated by neutron reflectometry. In oriented phospholipid multilayer stacks the protein is embedded within the lipid bilayers increasing the repeat spacing from 54.8 Å to 57.1 Å. The presence of the protein influences the hydration of system with increased hydration at lower temperatures.

Ras proteins are mediators in the signal cascade from receptor tyrosine kinases to the cell nucleus and must be associated with the plasma membrane to function; non lipid-modified ras is cytosolic and thus inactive. Therefore most Ras-proteins are attached to one or more lipid anchors, which are often essential for the signal transmitting function of these structures. In recent FT-IR, NMR and neutron diffraction experiments clearly the anchoring of the lipidated N-ras peptide fragment in the hydrophilic region of the membrane has been shown and the distribution of the lipid anchor and peptide fragment parts in the hydrophobic membrane region [1]. Since the C-terminus of the ras protein is unstructured, a mobile peptide conformation appears to be relevant for the structure of the full-length membrane bound ras protein. The detailed penetration depth of such a structure into the membrane and the protein orientation towards the membrane surface is unclear.

To investigate the structural organisation of the complete membrane bound ras protein we started to investigate fully hydrated multilamellar vesicles oriented on solid supports of complete ras proteins with modified lipidated C-terminus embedded in a phospholipid matrix (DMPC) (molar ratio protein:lipid 1:150). The protein lipid anchor at the C-terminus was a deuterated palmitoyl chain (d33). As shown in Fig. 1 the obtained oriented multilamellar lipid-protein stacks show a number of lamellar diffraction peaks. Hydrated in 100% relative humidity in H₂O or D₂O water vapour the diffraction peaks reveal the membrane in lamellar fluid phase with a thickness of $d = 57.1$ Å. For comparison the repeat spacing in samples of pure DMPC revealed a value of 54.8 Å. The small increase in bilayer thickness indicates a flat, pancake like structure of ras protein embedded within the lipid multilayer.

Calculated neutron scattering length density profiles perpendicular to the membrane surface are shown in Fig. 2. Despite the low resolution of the calculated density profiles with only up to three lamellar diffraction peaks the increase in scattering length density on exchange of H₂O with D₂O in the hydrophilic region of the bilayer system indicates a large hydration of the system in the presence of the ras protein. Measurements at different temperatures between 10 and 50 °C of the system also did show increased (10 °C) and decreased (50 °C) lamellar repeat spacings (data not shown) related to a strong temperature dependent hydration of the protein lipid mixture.

To reveal a more detailed structure of the ras protein lipid system measurements with additional contrast variations will be needed (e.g. deut. DMPC, H₂O/D₂O mixtures) and

improvement of the lamellar ordering to amend the current resolution of the neutron scattering length density profiles obtained.

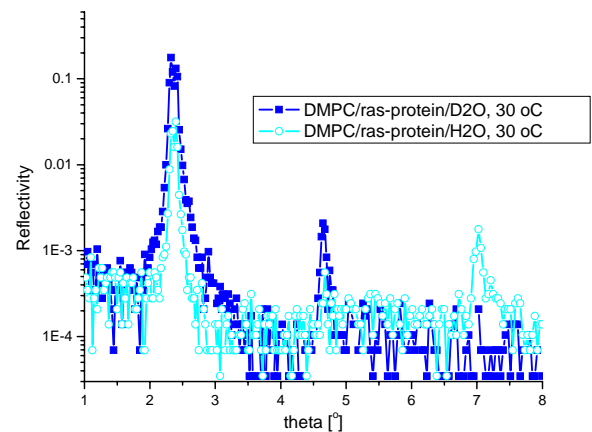


Figure 1: Neutron reflectivity patterns of DMPC/ras protein (150:1 molar ratio) at 100 % relative humidity in H₂O and D₂O.

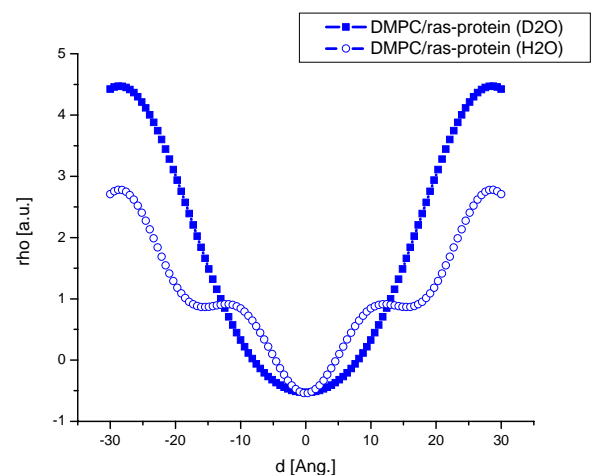


Figure 2: Neutron scattering length density profiles of DMPC/ras protein system calculated from data shown in Fig. 1.

[1] D. Huster et al., JACS **125**, 4070 (2003)

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