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Hydration in Purple Membrane as a Function of Relative Humidity

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LETTERS TO THE EDITOR

Hydration in Purple Membrane as a Function of Relative Humidity

Neutron diffraction experiments on the purple membrane of *Halobacterium halobium* as a function of H_2O–D_2O exchange in a wide relative humidity range, are described.

Increasing relative humidity leads primarily to hydration of the lipid area in the membrane. The exchanged H density is higher in the centre of the protein than at the protein–lipid interface, in support of the hypothesis that the molecule has a hydrophilic interior. However, there is no aqueous pocket in the protein.

Continuing the study of hydration in the purple membrane of *Halobacterium halobium* by neutron scattering using H_2O–D_2O exchange (Zaccai & Gilmore, 1979) we have collected and analyzed neutron diffraction data as a function of relative humidity. The main conclusion of the previous study, i.e. that at 100% RH†, hydration is predominantly in the lipid areas of the membrane with very few water molecules associated with the protein, bacteriorhodopsin, is confirmed and extended.

Apart from aspects of sample preparation and data handling which have been improved (Engelman & Zaccai, 1980) the materials and methods of the present work are identical to those described by Zaccai & Gilmore (1979). All data were collected on the same sample and different RH and isotopic conditions were obtained by changing a saturated salt bath in a sample chamber without disturbing the sample itself. The following conditions were examined: "dry" (over silica gel), 47% RH (KCNS), 66% RH ($NaNO_2$), 85% RH (KCl) in both H_2O and D_2O. The "dry" D_2O data were obtained by putting silica gel in the sample container after it had equilibrated over KCl in D_2O. The temperature was maintained at 20 ± 1 deg.C. A structure factor table is available from the authors.

Difference Fourier maps were calculated in the standard manner (Blundell & Johnson, 1976), i.e. using components $|F_{D_2O}| - |F_{H_2O}|$ with the phases of F_{H_2O}; these were derived from electron microscopy (Henderson & Unwin, 1975) as was the ratio of reflections contributing to the observed intensity in a powder peak. This approach is justified by Zaccai & Gilmore (1979). The difference Fourier maps show the distribution of exchanged hydrogen density in the unit cell. They appear to be made up of the same distinct protein and lipid areas at each RH condition. Varying the relative humidity allowed us to follow the stepwise increases in exchanged hydrogen density in the lipid region from a mean density below that of the protein (in the dry state, Fig. 1) to one above it (the map at 100% RH is shown by Zaccai & Gilmore, 1979). The mean hydrogen–deuterium exchange levels in the protein and lipid were very close to each other at 47% RH. At that humidity, fluctuations in

† Abbreviation used: RH, relative humidity.

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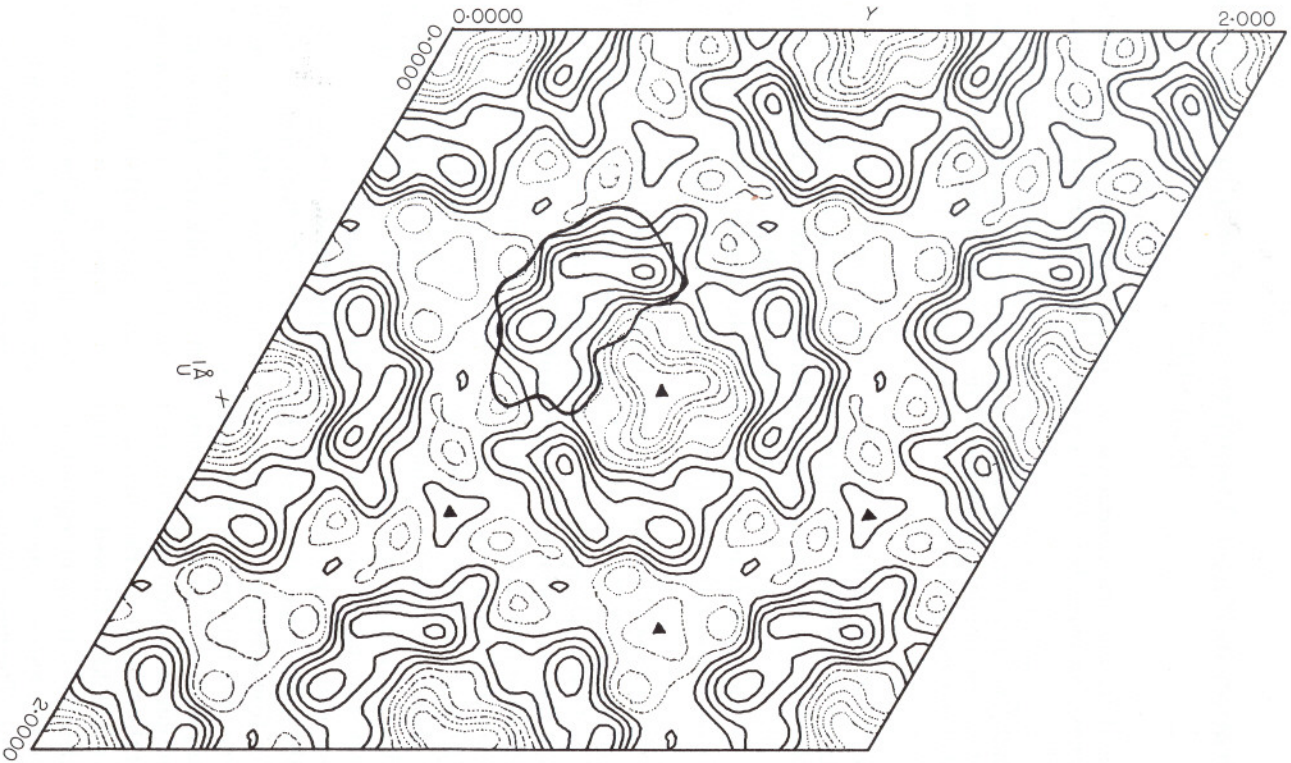

FIG. 1. Difference Fourier map between D_2O and H_2O at 0% RH. Contouring is the same as the difference map shown by Zaccai & Gilmore (1979). An approximate outline of the protein is shown.

the exchange density within the protein molecule appeared most clearly. There were no peaks, however, that were significantly higher than noise, confirming that there is no substantial aqueous channel in the protein.

The D_2O–H_2O dry difference Fourier is presented in Figure 1, with the protein area showing more exchange. It is consistent with the removal of water from the lipid. If most of the exchange observed in the protein were due to labile hydrogen on the molecule itself or to strongly bound water, it should not be affected by the drier atmosphere. The density of exchanged hydrogen is higher in the centre of the protein molecule than on its boundaries. This independently supports a previous deduction that bacteriorhodopsin is an "inside-out" protein with the hydrophilic sides of the α-helices directed towards each other (Engelman & Zaccai, 1980).

Additional support for the hypothesis that drying the membrane removed water from the lipid component comes from the Fourier map of the dry condition. Although superimposing the wet and dry maps shows the protein contours to coincide, the dry in-plane lattice dimension is 61·2 Å compared with 62·3 Å for 85% RH, corresponding to a difference in area of the unit cell of 120 $Å^2$. Also, since the structure factors for the dry and wet membranes are significantly different, the dried membrane projection is not a scaled down version of its wet counterpart. In other words, protein and lipid areas are not reduced in the same ratio. Most, if not all of the difference in area can be attributed to an expansion of the lipid area as headgroups absorb water. In this aspect the behaviour of purple membrane lipids appears to be similar to that of pure lipid bilayers (Tardieu *et al.*, 1973).

In conclusion: the protein structure in purple membrane is maintained (to 7 Å resolution) in the entire relative humidity range from a silica gel atmosphere to 100% RH. Increasing relative humidity leads primarily to an increase of the lipid area coupled to increased exchanged H density in that area, due presumably to increased hydration around the lipid headgroups. The exchanged H maps are made up of distinct protein and lipid areas. Below about 47% RH, the mean exchange on the protein is higher than on the lipid. This exchange density, being higher in the centre of the protein than at the protein–lipid interface, supports the notion that bacteriorhodopsin has a hydrophilic interior and a hydrophobic exterior.

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