Bacteriorhodopsin: Crystallography Under Extreme Conditions

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Bacteriorhodopsin (BR) is a light-driven proton-translocating machine, which converts the energy of photons into an electrochemical potential across the cell membrane. The pioneering studies of Henderson and others using electron microscopy and image reconstruction provided the first insight into the structural organization of a membrane protein at a resolution of 7 Å. During the past 20 years, major advances in electron crystallography have allowed significant improvements of the resolution, which has reached 3.5 Å,[1] and more recently 3.0 Å.[2] The results revealed that the structure of bacteriorhodopsin consists of seven membrane-spanning alpha-helices. The pigment retinal, bound to lysine 216, is buried in the interior of the protein. The mechanism of action of BR has been studied extensively by investigations of the photocycle, using several spectroscopic and structural methods, as well as dynamical modeling. It could thus be concluded that the absorption of a photon causes the release of a proton into the medium, and that the isomerization of the retinal from the all-trans to the 13-cis configuration is followed by a series of intermediate states that include the reprotonation of the Schiff-base by a proton from the cytoplasmic compartment. A re-isomerization to the ground state completes the photocycle. In order to understand the mechanistic details of this process, atomic resolution is required. In particular, it was shown that water molecules inside the putative proton channel are essential for the proton translocation. A novel concept that exploits bicontinuous lipidic cubic phases for the crystallization of membrane proteins was recently developed.[3] Lipidic cubic phases provide a three-dimensional bilayer matrix that facilitates growth of bacteriorhodopsin microcrystals. The BR crystals are thin hexagonal plates [20 (50) x 20 (50) x 5 micron^3]. Despite their small dimensions, they diffract isotropically to 2.0 Å in a highly focused monochromatic x-rays (beamline ID13, ESRF). The structure was solved to a resolution of 2.4 Å by molecular replacement using the electron microscopy structure as a starting model.[4] Some water molecules could be positioned in the proton pathway. This structure reveals the atomic positions of BR, the retinal in the ground state of the photocycle. Nevertheless, the consequence of the retinal isomerization during the photocycle on the BR structure and the water molecules bound, as well as its role in the proton translocation, are still open questions.

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