EXPERIMENTAL REPORT

LS-1616, ID14-EH2, 05/06/00 to 10/06/00

Applicability of lipidic cubic phases to the crystallization of various membrane proteins.

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The aim of this experiment was to test the applicability of lipidic cubic phases, developed in our laboratory for bacteriorhodopsin [1-4], as a general matrix for producing well-ordered crystals of membrane proteins. The routine availability of such crystals is still the most critical obstacle towards their structural elucidation by X-ray crystallography. So far, we were able to show that 5 membrane proteins can be crystallized, and diffract to various resolutions. These systems are: Two photosynthetic reaction centers from *Rhodopseudomonas viridis* (RCvir) and *Rhodobacter sphaeroides* (RCsph), the light harvesting complex 2 from *Rhodopseudomonas acidophila* (LH2) and the chloride pump halorhodopsin (hR) [5]. Despite marked differences in molecular dimensions, subunit composition and membrane origin, one single lipid, monoolein, is sufficient to form a crystallization matrix for all aforementioned systems.

Recently, we obtained crystals of the archeabacterial sensory rhodopsin II (SRII) from lipidic cubic phases. This light sensor triggers photorepellent responses via the tightly-bound transducer protein HtrII that in turn induces a phosphorylation cascade regulating the flagellar motors. The crystals diffracted to 3.5 Å resolution on ID14-EH2, and further improvement in crystal quality will be instrumental in elucidating the high resoluton structure and understanding the mode of action of this important membrane protein.

The project on the elucidation of the L intermediate in the photocycle of bacteriorhodopsin (LS 1617) was grouped under this project. Bacteriorhodopsin passes through a series of structural intermediates with well-defined lifetimes and spectral properties during its photocycle. We have previously we trapped at low temperature an early intermediate of the photocycle within wild type bR crystals grown in a lipidic cubic

phase and determined its X-ray structure [6]. We showed that directly after photoexcitation, a key water molecule is dislocated enabling the primary proton acceptor, Asp85, to move. Movement of main chain Lys216 locally disrupts the hydrogen bonding network of helix G, facilitating structural changes in later stages of the photocycle. In the current experiment we solved the 2.1 Å resolution structure of the next (L) state in bR's photocycle [7], deciphering the structural changes immediately preceding the primary proton transfer event, which occurs in the $L_{550} \rightarrow M_{412}$ transition. The structural rearrangements of the K state [6] are observed to propagate from the protein's core towards the extracellular surface, disrupting a network of hydrogen bonded water molecules which, in the ground state, stabilises helix C. Concomitantly, a bend of this helix enables the negatively charged primary proton acceptor, Asp85, to further approach the positively charged primary proton donor, the Schiff base.

[1] Landau, E.M. and Rosenbusch, J.P. *Proc. Natl. Acad. Sci. USA* **93**, 14532-14535 (1996)

[2] Pebay-Peyroula, E. et al. *Science* **277**, 1676-1681 (1997)

[3] Rummel, G. et al. J. *Struct. Biol.* **121**, 82-91 (1998)

[4] Belrhali, H. et al. *Structure.* **7**, 909-917 (1999)

[5] Chiu, M.L. et al. *Acta Cryst. D,* **56**, 781-784 (2000)

[6] Edman, K. et al. *Nature* **401**, 822-826 (1999)

[7] Royant, A. et al. *Nature* **406**, 645-648 (2000)