ESRF

Experiment title:
High resolution structures of membrane protein crystals grown in lipidic cubic phases

Experiment number:
LS-435

Beamline:
D2-D2AM

Date of Experiment:
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3

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Report:

In contradistinction to soluble proteins, only very few high resolution structures of membrane proteins, with rather unique properties, have been reported to date: Reaction centers [1,2] porins[3,4,5] a light harvesting complex[6] and two cytochrome c

[1]Deisenhofer, J., Epp, O., **Miki,** K., Huber, **R. and Michel, H. (1985)** *Nature* **318,** 618-624.
[2]Roth, M., Lewit-Bentley, A., Michel, H., Deisenhofer, J., Huber, R. and Oesterhelt, D.(1989) Nature 340, 659-662.
[3]Cowan, SW., Schirmer, T., Rummel, G., Steiert, M., Ghosh, R., Pauptit, R. A., Jansonius, J.N. and Rosenbusch, J.P. (1992) ***Nature 358,727-733.***
Schirmer, T., Keller, T.A., Wang, Y.-F. and Rosenbusch, J.P. (1995) Science 267,512-514.
[4]Weiss, M. S., Abele, U., Weckesser, J., Welte, W., Schiltz, E. and Schulz, G.E. (199 1) Science 254, 1627-1630,
Kreusch, A., Neubüser, E., Schiltz, E., Weckesser, J. and Schulz, G.E. (1994) ***Science*** 3,58-63.
[5]Pebay-Peyroula, E., Garavito, R.M., Rosenbusch, J. P., Zulauf, M. and Timmins, p.A. (1995) Structure 3, 1051-1059.
[6]McDermott, G., prince, S,M., Freer, A, A., Hawthornwaite-Lawless, A. M., Papiz, M. Z., Cogdell, R.J. and Isaacs, N.W. (1995) ***Nature 374,517-521.***

oxidases[7,8]. Other crystallization attempts with plasma membrane proteins have failed, despite intensive efforts, e.g. with lactose permease. A critical step involved in conventional membrane protein crystallization is that their purification requires solubilization out of their natural habitat, the lipid bilayer, using mild detergents. This causes release of lateral pressure of the membrane, which may lead to confirmational perturbations and consequently to heterodispersity. In order to overcome these problems, we have initiated a novel approach which uses lipidic cubic phases, which are rigid, isotropic and transparent membrane-mimetic materials, as matrices for the crystallization of membrane proteins. These materials exhibit a bicontinuous structure of curved bilayer interpenetrated by a network of aqueous channels, which we envisage as a structured yet flexible matrix that affects water activity. Initially, we tested this approach with simple systems such as ionic salts, amino acids and lysozyme. The latter was added to the aqueous solution prior to the formation of the cubic phase and yielded large rhombic crystals that diffract X-rays to a resolution of 2.2 Å. These crystals were nucleated inside the transparent gel matrix, and inspection revealed that they grow freely in three dimensions, indicating crystal nucleation in the cubic phase ("seeding") to take place, and that lateral diffusion ("feeding") facilitated crystal growth without perturbing the lipidic matrix or being perturbed by it.

We have now successfully crystallized bacteriorhodopsin (BR) from a bicontinuous monoolein/water system to yield hexagonal crystals with sizes ranging between 25 and 100 mm. Microspectrophotometry has shown that the crystals were proteinaceous and that no bleaching had occurred. An earlier diffraction experiment at the synchrotrons beam in Grenoble has yielded a resolution of 6Å. In a recent set of diffraction experiments at the ESRF (experiment LS-435, conducted on Feb 15, 1996), we were able to diffract a 75 mm BR crystal to a resolution of 4.5Å.

[7] 1wata, S., Ostermeier, C., Ludwig, B. and Michel, H. (1995) ***Nature 376,660-669.***

[8] Tsukihara, T., Aoyama, H., Yamashita, E., Tomizaki, T., Yamaguchi, H., Shinzaa-Itoh, K., Nakashima, R., Yaono, R. and Yoshikawa, S. (1995) Science 269, 1069-1074.