FTIR spectroscopy of bacteriorhodopsin microcrystals at Beamline 1.4

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Bacteriorhodopsin (bR) is the sole protein component of the purple membrane of *Halobacterium salinarium* [1]. The function of bR *in vivo* is to convert solar energy into a pH gradient across the cell membrane which the organism uses to drive ATP synthesis [2]. Bacteriorhodopsin undergoes a light-induced cycle of physicochemical changes for for every proton it pumps out of the cell. The photocycle of bR has been well-characterized by both visible and IR spectroscopy. The major intermediates are identified as the K, L, M, N and O intermediates, and each has a distinct visible color and a distinct IR spectrum [3]. The Schiff base that connects the side chain of Lys 216 to the retinal molecule buried within the core of the apoprotein [4] is deprotonated upon formation of the M intermediate, and reprotonated when the M intermediate decays. Since access to the Schiff base switches from the extracellular side of the membrane to the cytoplasmic side between these two proton transfer events, the M intermediate is of particular interest.

High resolution x-ray diffraction experiments on microcrystals of bR have recently become possible, through the discovery by Landau and Rosenbusch that the solubilized protein can be crystallized from the bicontinuous lipid-water gel that is formed by mono-olein [5]. Structural studies on intermediate states of the photocycle thus become a high priority, allowing the visualization of the structural changes that are responsible for converting light energy into a proton-motive force.

Previously we collected high resolution x-ray diffraction data from crystals of wild type bR trapped in both the L and M states by illumination at low temperature, using Fourier Transform IR (FTIR) spectroscopy to confirm the identity of the photointermediate[6]. This year we turned our attention this year to the F219L mutant of bR, which forms N, the intermediate following M, more readily than does the wild type protein[7]. We have succeeded in characterizing two photointermediates of the bacteriorhodopsin mutant F219L by means of FTIR spectroscopy. At 213K an early phase of the M photostate is trapped, whereas at 173K the IR signature strongly suggests that the L photostate is trapped. Further studies of this mutant are planned, with an eye towards trapping the N intermediate.

ACKNOWLEDGMENTS

We thank Dr. Michael Martin for his continued assistance at Beamline 1.4.3.

REFERENCES

1. Oesterhelt, D. & Stoeckenius, W. Rhodopsin-like protein from the purple membrane of Halobacterium halobium. *Nature New Biology* **233**, 149-152 (1971).
2. Racker, E. & Stoeckenius, W. Reconstitution of purple membrane vesicles catalyzing light-driven proton uptake and adenosine triphosphate formation. *Journal of Biological Chemistry* **249**, 662-663 (1974).
3. Lozier, R. H., Bogomolni, R. A. & Stoeckenius, W. Bacteriorhodopsin: a light-driven proton pump in *Halobacterium halobium*. *Biophysical Journal* **15**, 955-962 (1975).
4. Henderson, R. et al. Model for the structure of bacteriorhodopsin based on high-resolution electron cyro-microscopy. *Journal of Molecular Biology* **213**, 899-929 (1990).
5. Landau, E. M. & Rosenbusch, J. P. Lipidic cubic phases: A novel concept for the crystallization of membrane proteins. *Proceedings of the National Academy of Sciences USA* **93**, 14532-14535 (1996).
6. Facciotti, M. T. et al. Structure of an early intermediate in the M-state phase of the bacteriorhodopsin photocycle. *Biophysical Journal* **81**, 3442-3455 (2001).
7. Vonck, J. A three-dimensional difference map of the N intermediate in the bacteriorhodopsin photocycle: Part of the F helix tilts in the M to N transition. *Biochemistry* **35**, 5870-5878 (1996).

This work was supported NIH grant GM51487.

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