X-Ray Structure of Bacteriorhodopsin at 2.5 Å Resolution

Ferdinand Hucho*

Scientific researchers often approach broad topics by working with models. Brain power and funding are applied in amounts that outsiders sometimes find absurd, but which are justified by leaps in fundamental understanding. In the end the "ultimate truth", chapters in textbooks, can be written.

Biochemists work this way, too. They investigate various molecules to clarify fundamental principles of life, which then flow, almost unnoticed, into the vast pool of basic knowledge. Hemoglobin, for example, is one of these model molecules. It allowed us to understand how molecular activity (such as oxygen intake and output) is regulated, how a protein molecule is put together, and how hereditary diseases are brought about by point mutations. The *lac* operon became a model for gene regulation, the acetylcholine receptor a model for molecules processing signals, and glycogen phosphorylase a model for key regulatory enzymes in metabolism.

Now bacteriorhodopsin (BR), another model molecule, grabs our attention once again (Figure 1). After twenty years of attempts, researchers have finally been able to crystallize this molecule and elucidate its X-ray structure.[1] Is bacteriorhodopsin, a "light-driven proton pump" essential for the survival of a purple bacterium that lives in warm saline solution, merely an exotic molecule crucial only for that one somewhat uninteresting organism? The pioneer researchers in this field, Oesterhelt, Stoeckenius,[2] and others, would disagree. BR demonstrates a fundamental concept:[3] How can light energy be directly (and in a much simpler process than that used by plants) transformed into chemical energy?[4] How can membranes be used for energy conservation? How does "active transport" work? In other words, how are ions or molecules transported against a concentration gradient? (Biochemists call this active process "pumping".)

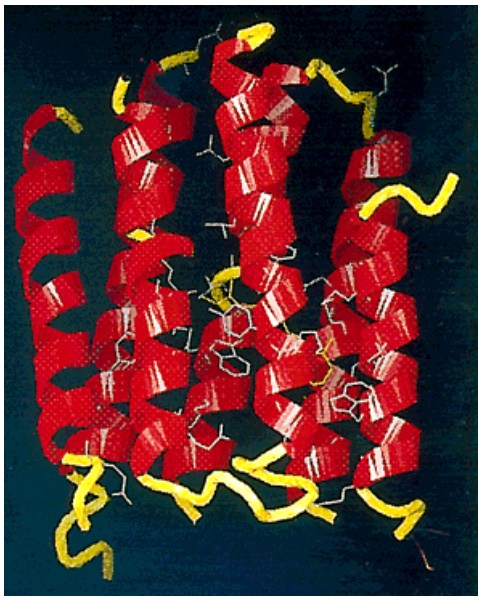

Figure 1. Ribbon model of the bacteriorhodopsin monomer. α Helices are red, loops are yellow, and the side chains crucial for proton transfer are white. The bundle made up of the seven transmembrane helices is perpendicular to the plane of the cell membrane. (The figure was kindly provided by Eva Pebay-Peyroula, IBS Grenoble, and Jurg Rosenbusch, Biozentrum Basel.)

[*] Prof. Dr. F. Hucho
Institut für Biochemie der Freien Universität
Thielallee 63, D-14195 Berlin (Germany)
Fax: (+49) 30-838-3753
E-mail: hucho@chemie.fu-berlin.de

[**] Financial support from the Deutsche Forschungsgemeinschaft (SFB 312) and the Fonds der Chemischen Industrie is gratefully acknowledged.

BR is found in two-dimensional crystalline arrays in the membrane of the halophile archaebacteria *Halobacterium salinarium*. It spans the lipid membrane that separates the inside of the bacterium (containing low salt concentration) from its oxygen-deficient, inhospitable surroundings. BR consists of a single polypeptide chain folded into seven transmembrane helices.

The chromophore retinal, which is bound as a Schiff base to a lysine residue (for the experts: Lys216), is embedded between the seven helices (Figure 2). In the resting state retinal exists in its all-*trans* conformation, and after absorbing a photon, it isomerizes to the 13-*cis* isomer. The movement triggered by this isomerization is carried over to the protein, so that the carboxylate group of aspartate residue 85 takes up the proton from the Schiff base and then passes it on via a network of water molecules to glutamate 204 and, finally, to the extracellular surface of the bacterium. Then the chromophore reisomerizes to all-*trans*-retinal after taking up a proton from the inside of the cell and passing it on via aspartate 96 to the Schiff base. This reestablishes the inactive state and completes the cycle. The process can be observed spectroscopically and can be described by the intermediate states J, K, L, M, N, and O (see Figure 2).

This (and much more) was known before the elucidation of the recently published X-ray structure.[1] Over the past 25 years data accumulated from high-resolution electron microscopy,[5] time-resolved FTIR spectroscopy,[6] neutron scattering,[7] and classic biochemical methods (spectroscopy and molecular biology) has provided a detailed picture of the proton's path from the inside to the outside, which can be described almost Ångström by Ångström and millisecond by millisecond. Three basic questions pop up when one reads the recent *Science* article: 1) Was the old model of the light-driven proton pump wrong, and do we need to correct it based on the crystal structure at hand? 2) What is the principally new aspect of the recently published X-ray analysis that justifies the sensation of publishing in a "high-impact journal"? 3) Is this the end of BR research; do we know everything now, and can we write the definitive textbook chapter?

  1433-7851/98/03711-1518 $ 17.50+.50/0

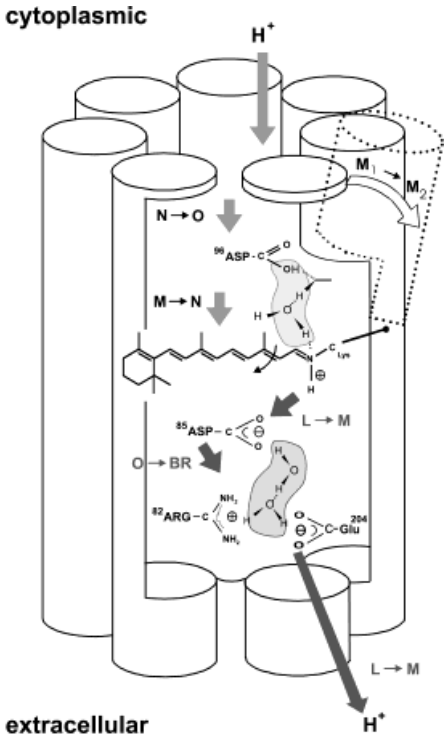

Figure 2. The proton pump model of bacteriorhodopsin. After the isomerization of retinal, the proton of the Schiff base is passed on to Asp 85 in the L→M transition. Simultaneously, an additional proton is released to the outside from the hydrogen bonding network. The Schiff base is reprotonated by Asp 96 in the M→N reaction. Internal water molecules between the Schiff base and Asp 96 play an important role here. Furthermore, the movement of a helix in the $M_1 \rightarrow M_2$ step is important for the pump's directionality. In the N→O reaction Asp 96 is reprotonated and retinal reisomerizes to the all-*trans* form. Finally, Asp 85 is deprotonated in the O→BR reaction, bringing the system back to the initial state. (The figure was kindly provided by Klaus Gerwert, Bochum.)

In answer to the first question: Nothing in the model was completely wrong. Luckily, we can state that the results obtained by "classical" methods were correct and that our interpretations and models still stand. Minor corrections are required on the loops that connect the seven transmembrane helices. There are, however, bigger problems in the proton canal: the distance between aspartate 85 and the Schiff base is too large for the proton to manage in one step.

In answer to the second question: If the mere confirmation of "classical" biochemical and biophysical methods is not sufficient, then we would like to point to the quantum leap achieved with these analyses. For the first time microcrystals with dimensions of 20–40 μm (and a mere 5 μm thick) were used. This was possible with a new beam focus in Grenoble's electron synchrotron.[1] The second breakthrough: a membrane protein was crystallized in a totally new way by using a special lipid phase obtained by mixing lipids with water.[8] Researchers hope that this method will solve the fundamental problem of crystallizing integral membrane proteins.

In answer to the last question: Please be patient—we are near the end of the BR chapter, not because we know everything now, but because we finally have information and tools that allow us to really understand the proton pump process. As mentioned above, there are problems with the distances that the "pumped" proton has to overcome. Here we need X-ray structures with higher resolutions (≤ 2 Å) which will let us more accurately localize additional water molecules that can serve as proton carriers[9] (hopefully they won't be too mobile for the X-ray!). Other missing pieces in the puzzle are X-ray structures of artificial mutants, which would be able to prove the significance of individual protein side chains, in particular, for the water structure. And then the final phase of BR research should follow. A satisfactory description of the pumping process includes not only the proton's exact path, but more importantly, the energetics of the process: How are the intermediate states formed? How are the barriers of activation energy separating the various states overcome? We still need the molecular dynamics, the calculation of the mobility, and the energetics of the structures involved. X-ray analysis provides a snapshot, but the movie is missing. The secret of biomolecules' special activity lies in their dynamics, and with BR we may, for the first time ever, see the connection between dynamics and activity.

German version: *Angew. Chem.* **1998**, *110*, 1600–1601

Keywords: bacteriorhodopsin · membrane proteins · protein structures · proton transfer · structure elucidation

[1] E. Pebay-Peyroula, G. Rummel, J. P. Rosenbusch, E. M. Landau, *Science* **1997**, *277*, 1676–1681.
[2] D. Oesterhelt, W. Stoeckenius, *Proc. Natl. Acad. Sci. USA* **1973**, *70*, 2853–2857.
[3] U. Haupt, J. Tittor, E. Bamberg, D. Oesterhelt, *Biochemistry* **1997**, *36*, 2–7.
[4] D. Oesterhelt, J. Tittor, E. Bamberg, *J. Bioenerg. Biomembr* **1992**, *24*, 181–191.
[5] N. Grigorieff, T. A. Ceska, K. H. Downing, J. M. Baldwin, R. Henderson, *J. Mol. Biol.* **1996**, *259*, 393–421.
[6] K. Gerwert, *Biospektrum* **1997**, *2*, 34–39.
[7] N. A. Dencher, D. Dresselhaus, G. Zaccai, G. Büldt, *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 7876–7879.
[8] E. M. Landau, J. P. Rosenbusch, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 14532–14535.
[9] G. Papadopoulos, N. A. Dencher, G. Zaccai, G. Büldt, *J. Mol. Biol.* **1990,** *214*, 15.

  1433-7851/98/03711-1519 $ 17.50+.50/0