THE EFFECT OF ENVIRONMENT ON PHOTOCYCLE OF PROTEORHODOPSIN

Abstract of the Ph.D. Thesis

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Introduction

All life depends on light from the sun (with possible excepts of a few organisms near deep-sea vents). Taking in consideration how important is light to life, there are many types of interactions between light and living organisms: visualization and spatial orientation, temporal orientation, conversion of light into chemical energy.

Living cells preserve their functions by building up and maintaining ion gradients across their cell and organelle membranes. The ions are driven across these membranes by active pumps that use chemical or light energy. Proton pumping is based on Mitchell's theory of chemiosmotic coupling, whereby light or electrochemical energy is first converted to a proton-motive potential by chain of integral membrane proton pumps. The energy thus stored within this potential is harvested by ATP synthase.

Halobacteria belong to the Archaea type organisms. They are widespread in the nature and populate habitats with salt (NaCl) concentrations exceeding four molars. A special class of retinal protein occurs in their cell membrane each with different function:

- A light-driven proton pump, the retinal protein bacteriorhodopsin, transports proton out of the cell thereby generating proton-motive force, which then derives ATP synthesis, flagella rotation or secondary transport processes.
- A second light-driven ion pump with similar structure, halorhodopsin, transports chloride ion into the cell against the membrane potential to maintain the osmotic equilibrium during cell growth.

- Light signals are converted by two photoreceptors, sensory rhodopsin I and II, which transmit signal to the flagellar motor of the cell.

These retinal proteins are intrinsic membrane proteins, with an approximate molecular weight of 26 kDa. They share a common seven transmembrane helix topology. The seven helices are arranged in two arcs, an inner one comprising helices B, C and D and an outer one with helices E, F, G and A. A transmembrane pore is formed mainly between helices B, C, F and G. The retinal is bound to a lysine in helix G as a protonated Schiff-base, which interrupts the pore and separates the extracellular half channel from the cytoplasmic half channel.

The recently identified light-activated proton pump, proteorhodopsin belongs to the retinal protein family type I rhodopsins. It was discovered in the genome of an uncultivated marine bacterium of the 'SAR86' phylogenetic group, which is present in the oceanic surface waters.

The proteorhodopsin gene encoded a polypeptide of 249 amino acids, with a molecular weight of 27 kDa. Hydropathy plots indicated seven transmembrane domains, which aligned well with the corresponding helices of the archaeal rhodopsins. The amino acid residues that form a retinal binding pocket in archaeal rhodopsins are also highly conserved in proteorhodopsin. The retinal is bound to a lysine residue (Lys-231) in helix G through the protonated Schiff-base. Analysis of structural model of proteorhodopsin indicates that the majority of active side residues are well conserved between proteorhodopsin and bacteriorhodopsin. The essential steps of light-driven proton pumping by proteorhodopsin likely follow the same path as in bacteriorhodopsin.

The early studies had shown that the photoisomerization of all-*trans* retinal to 13-*cis* initiates a photocycle, and a proton is transported across the cell membrane from the cytoplasm to the extracellular space. This photocycle is influenced by pH. The pK_a of the proton acceptor Asp-97 was determined by

spectral titration to be between 7.1 and 7.68. It was shown that at pH above this pK_a the protein is a proton pump, below the photocycle is a non-transporting one.

The aim of this work is to study the kinetic properties of the proteorhodopsin photocycle, and therefore the process of ion translocation through cell membranes, at high and low pH. In addition there were performed measurements to study the effect of humidity on the photocycle of proteorhodopsin. We used the following techniques: spectroscopic titration, time-resolved spectroscopy in the visible, absorption kinetic and electric signal measurements.

Materials and methods

Wild type proteorhodopsin was expressed in *Escherichia coli* (strain UT5600), as described before. J.K. Lanyi kindly supplied the proteorhodopsin membranes.

To study the photocycle of proteorhodopsin by transient spectroscopy and absorption kinetic measurements, acrylamide gel samples were prepared. The electric signal measurements were carried out on oriented gel samples. During the sample preparation no salt was used, to avoid the aggregation of the membranes. To study the high and low pH photocycle, prior the measurements, the gel samples were soaked overnight in the bathing solution containing CAPSO and MES buffers respectively.

To study the role of water in the photoactive reaction cycle and the proton transport of proteorhodopsin, dried samples were prepared from suspension of proteorhodopsin, by drying it on a glass surface. A so-called “high pH” and “low pH” samples were prepared by soaking the sample in a high pH and low pH solution, respectively and let them dry again.

During the measurements the sample was kept in a temperature and humidity controlled holder. In the case of dried samples to equilibrate at a given relative humidity the sample was kept overnight in the sample holder, over various salt solutions.

Time-resolved spectroscopy, with a gated optical multichannel analyzer, provided difference spectra at various time points during the photocycle, as described elsewhere. From the measured difference spectra the absolute spectra of intermediates were calculated.

Absorption changes were recorded at several characteristic wavelengths after laser excitation, at six different temperatures between 5 and 30°C on both high and low pH samples. The data collected with a sampling rate of 50 ns were converted to logarithmic time scale, by averaging on logarithmically equidistant time intervals. The fit of the absorption kinetic traces was done with the RATE program. The thermodynamic parameters of the transitions were determined from the temperature dependencies of the rate constants with the EYRING program.

The model was accepted or rejected on the bases of the goodness of the fit and the predicted behavior of the kinetic parameters to the change of the external conditions, such as temperature or humidity.

From the electric signal measurements the electrogenicity of the intermediates was calculated. This characterizes the charge motions inside the protein. The fit of the electric signal was carried out in MATHLAB.

Results and discussion

The main results of the measurements are:

High pH photocycle of proteorhodopsin {Váró, 2003 2358 /id}:

- I determined the electrogenicity of each intermediate based on the electric signal measurements, which I carried out on oriented gel sample. The small negative value of the first intermediates characterizes the charge shifts at around the retinal, while the positive and two-order-of-magnitude larger electrogenicities correspond to the proton translocation through the membrane.
- I showed the similarities between the charge motions in proteorhodopsin and bacteriorhodopsin by comparing the calculated electrogenicities.

Low pH photocycle of proteorhodopsin {Lakatos, 2003 2390 /id}:

- I identified with time-resolved spectroscopy and absorption kinetic measurements three spectrally distinct intermediates of the photocycle, K, L and N, and another silent one, noted PR'.
- I showed that the 410 nm absorption signal representing the deprotonated Schiff-base containing M-like intermediate, characteristic for proton pumping activity, does not accumulate in the low pH photcycle.
- I determined the photocycle model:

$$K \Leftrightarrow L \Leftrightarrow N \Leftrightarrow PR' \Rightarrow PR.$$

- I proved that all reactions between the intermediates are close to equilibrium, except the last transition from PR' to PR, when the protein returns to its initial, unexcited state in a quasiunidirectional reaction.
- I showed by electric signal measurements that although there are detectable charge motions inside the protein, their net translocation is zero, indicating that contrary to the earlier reported results, at low pH no charged particle is transported across the membrane.

The role of water in proteorhodopsin photocycle {Lakatos, 2004 2467 /id}:

- I carried out spectroscopic and absorption kinetic measurements to study the role of water in the photocycle of proteorhodopsin at high and low pH on dried samples. I determined the minimum number of intermediates of both photocycles.
- I determined the photocycle model at low pH sample at 0.5 relative humidity having a K like red shifted intermediate, decaying through an energized PR' intermediate to the ground state protein.
- I showed that at high pH photocycle model, at 0.5 relative humidity, beside of the red shifted intermediate an M like intermediate could be identified, having a deprotonated Schiff-base. This blue shifted intermediate decays through PR' intermediate, which is spectrally identical to the unphotolysed ground state.
- I calculated from temperature-dependent absorption kinetic measurements the energetics of both photocycles. The humidity effects measured on proteorhodopsin were very similar to that measured earlier on bacteriorhodopsin.

PUBLICATION LIST

Papers related to the thesis

1. G. Váró, L.S. Brown, M. Lakatos and J.K. Lanyi (2003)
   Characterization of the photochemical reaction cycle of proteorhodopsin.
   Biophysical Journal 84. 1202-1207
2. M. Lakatos, J.K. Lanyi, J. Szakács and G. Váró (2003)
   The photochemical reaction cycle of proteorhodopsin at low pH.
   Biophysical Journal 84. 3252-3256
3. M. Lakatos and G. Váró (2004)
   The influence of water on the photochemical reaction cycle of proteorhodopsin at low and high pH.
   Photochem. Photobiol. B. 73. 177-182

Other papers

4. G.I. Groma, L. Kelemen, Á. Kulcsár, M. Lakatos and G. Váró (2001)
   Photocycle of dried acid purple form of bacteriorhodopsin.
   Biophysical Journal 81. 3432-3441
5. M. Lakatos, G.I. Groma, C. Ganea, J.K. Lanyi and G. Váró (2002)
   Characterization of the azide-dependent bacteriorhodopsin-like photocycle of salinarum halorhodopsin.
   Biophysical Journal 82. 1687-1695
6. Z. Bálint, M. Lakatos, C. Ganea, J.K. Lanyi and G. Váró (2004)
   The nitrate transporting photochemical reaction cycle of the pharaonis halorhodopsin.
   Biophysical Journal 86. 1655-1663