

**Photoelectric responses of bacteriorhodopsin  
intermediates**

Ph. D. Thesis

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## I. PRELIMINARIES AND GOALS

My work deals with the retinal protein bacteriorhodopsin (BR), which resides in the cell membrane of *Halobacterium salinarum* (earlier *Halobacterium halobium*) and pumps protons when irradiated with light.

The protein molecules form a two dimensional crystalline array, with trimers at each lattice points. The regions of the plasma membrane that are composed solely of BR and lipids are called purple membrane (PM), in which the BR molecules are uniformly oriented with the carboxyl terminus on the cytoplasmic side. The PMs are easy to isolate from the cell. The BR in these retains full functionality for many months and longer, even at room temperature. In suspension, PMs (diameter ca. 500nm) remain as flat disks. BR has seven transmembrane helices oriented perpendicular to the plane of the membrane. A retinal molecule is bound to the G-helix via a protonated Schiff-base interacting with Lys-216. The retinal is situated parallel to the membrane plane in the center of the space enclosed by the helices. Upon absorption of a photon BR undergoes a cyclic series of reactions, in which the protein successively changes its conformation (the photo cycle). These quasistable conformations are called intermediates and designated as K, L, M, N and O. Transitions between intermediates are thermally activated. The intermediates not only differ from one another in conformation but also in their optical absorption spectrum, which enabled the investigation of the photocycle, following its initiation by a flash of light. During the photocycle one proton is moved across the membrane (vectorial transport). The resulting charge movements in an external electrical circuit can induce a measurable displacement current. In order to be able to measure a macroscopic

displacement current a large number of identically oriented BR molecules have to be simultaneously excited. The first requirement can be satisfied by exciting bR with a powerful laser pulse at an appropriate wavelength. The second requirement can be achieved by noting that each membrane fragment has a large permanent electric dipole moment perpendicular to the membrane, hence the initially randomly orientated fragments in suspension can be aligned by applying a low (10-20V/cm) static electric field. The orientation can be done in a polymerizable polyacrylamide gel. Gel samples technically simplify the electrical measurements.

The displacement current induced by a short light pulse serves to elucidate the kinetics of the proton transport.

The flash induced displacement current has two important properties:

- 1) The kinetics of the displacement current are closely correlated with those of the photocycle.
- 2) The time integral is not zero, implying vectorial charge transport.

From the examination of the electric response information about photocycle kinetics and the proton pumping behavior can be obtained.

It is known from the literature that the intermediates are light sensitive. The series of reactions following light excitation of the intermediates no longer correspond to the thermally activated succession, but follows a different path.

Displacement current measurements established that proton pumping ceases if the K and M intermediates are photoexcited.

The aim of my work was to determine 1) the photoreactions of the L, N and the O intermediates and the accompanying intramolecular charge movements with the help of

photoinduced displacement current measurements, 2) how the proton pumping behavior depends on the photoexcitation of these intermediates.

## II. METHODS

I developed a two-flash technique for investigating the photoreactions of the intermediates and the consequently modified photocycles. In these experiments I illuminated a gel sample containing oriented BR molecules with two successive laser flashes. The first flash induced the photocycle while the second one excited the desired transiently accumulating intermediate by suitable selection of wavelength and delay time. Such experiments elucidated both the studied intermediate photoreaction and the modified photocycle. Because I used oriented samples, the accompanying intramolecular charge motions could be measured via the displacement currents induced in an external electrical circuit. I recorded such currents. The interpretation of these data is found in part III..

In my experiments I used such samples in which the photocycle intermediates under examinations accumulated under proper measuring conditions. In wild type BR-containing samples, at room temperature and neutral pH only the L intermediate accumulates following flash excitation. Under such condition the rates of formation and decay of the N and O intermediates occur at comparable rates, hence there is no accumulation. Nevertheless by choosing other temperatures or pHs, or via point mutations, the decay of these intermediates can be hindered and the desired accumulation achieved.

For studying the N intermediate I used two gel samples: 1.) high pH (9.5), wild type BR; 2.) neutral pH, T46V point mutation.

For studying the O intermediate I also used two gel samples: 1.) low pH (4.5), wild type BR; 2.) neutral pH, L93A point mutation.

I determined the quantity of photocycle intermediate by absorption kinetic measurement.

### III. RESULTS

- 1.) From the analysis of the displacement currents I established that photoexcitation of the L intermediate causes proton pumping to cease, and the induced electrical movements in the protein relax within microseconds.
  
- 2.) From the analysis of the absorption kinetics together with the electrical measurements I established that: a.) the N intermediate has two substates in wild type BR; b.) only one of these substates gives rise to a measurable displacement current following photoexcitation; c.) photoexcitation of N does not change the protonpumping behavior of BR; d.) certain intramolecular charge movements accompany the photoreaction of the N intermediate, but there is no resultant charge translocation [1].
  
- 3.) I showed that the T46V point mutant pumps protons following flash excitation. The results with this mutant confirmed the above findings concerning the N intermediate [1].
  
- 4.) Using wild type BR, with both flash and continuous excitation of the ground state and the O intermediate, I established that the light-driven proton pumping activity is similar to, but greater than, that following excitation of the ground state alone [2].

5.) The following conclusions can be made on the bases of the optical and electrical measurements of L93A point mutant: a.) In contradiction to the literature, I found that during the entire photocycle the optical and accompanying electrical events are kinetically correlated with each other. b.) There are two substates of the O intermediate (OI and OII). c.) From the optical measurements of proton release and uptake, using pH sensitive pyranine dye, during the photocycle with the mutant, I established that OI and OII substates occur consecutively (i.e. in series). d.) Using two-flash excitation I demonstrated that only the OI photoreaction is accompanied by a measurable displacement current. e.) Using flash and continuous illumination, I established that excitation of the O intermediate increases proton pumping [3, 4, 5].



#### IV. PUBLICATIONS

1. R. Tóth-Boconádi, A. Szabó-Nagy, S.G. Taneva and L. Keszthelyi  
*Photoelectric response of the N intermediate of bacteriorhodopsin and its mutant T46V*  
FEBS Letters **459** (1999) 5-8
2. R. Tóth-Boconádi, S.G. Taneva and L. Keszthelyi  
*Photoexcitation of the O intermediate of bacteriorhodopsin and its mutant E204Q*  
Journal of Biological Physics and Chemistry **1** (2001) 58-63
3. R. Tóth-Boconádi, L. Keszthelyi and W. Stoeckenius  
*Late Events in the Photocycle of Bacteriorhodopsin Mutant L93A*  
Biophysical Journal **84** (2003) 3848-3856
4. R. Tóth-Boconádi, L. Keszthelyi and W. Stoeckenius  
*Photoexcitation of the O-intermediate in Bacteriorhodopsin Mutant L93A*  
Biophysical Journal **84** (2003) 3857-3863
5. R. Tóth-Boconádi and L. Keszthelyi  
*Photocurrent from illuminated O-intermediate in bacteriorhodopsin mutant L93A:  
Further evidences.*  
Journal of Biological Physics and Chemistry (2004 accepted)

