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# ABSORPTION KINETICS AND ELECTRIC SIGNALS MEASURED ON *LEPTOSPHAERIA* RHODOPSIN

K. Magyari*, V. Simon, Y. Fan[a], L. S. Brown[a], G. Váró[b]

Babes-Bolyai University, Faculty of Physics and Institute for Interdisciplinary Experimental Research, Cluj-Napoca, Romania
[a]University of Guelph, Department of Physics, Guelph, ON, Canada
[b]Institute of Biophysics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary

*Leptosphaeria* rhodopsin (LR) is a light-driven proton pumping retinal protein found in *Leptosphaeria maculans*, a eukaryotic organism. LR pumps protons in a manner similar to bacteriorhodopsin (BR), a light-driven proton pump of halobacteria. The protein structure around the retinal chromophore in LR and its structural change upon retinal photoisomerization resemble those in BR. The photocycle intermediates of LR resemble the K, L, M, N and O states of BR. The electric signals provided information about the charge motion during the photocycle. There is one basic difference between the electric signals of BR and LR: the positive part typical for BR could not be detected in the electrical signal of LR.

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## 1. Introduction

Rhodopsins are currently known to belong to two distinct protein families, which show no significant sequence similarity and may have different origins. These are visual rhodopsins, found in eyes throughout the animal kingdom (type II rhodopsin), which serve as photosensory pigments, and microbial rhodopsins, found in extreme haloarchaea, bacteria and eukaryotic microorganisms (type I rhodopsins). Type I rhodopsins were first discovered in halobacteria, so this protein family is called the family of rhodopsins of the halobacterial type [1]. They do share identical topologies characterized by seven transmembrane α helices that form a pocket in which retinal is covalently linked, as a protonated Schiff base, to a lysine in the seventh transmembrane helix (helix G). They have two primary function, photosensory transduction, where optical signals are transformed into conformational changes and communicated to a transducer protein mediating phototaxis or other responses to light, and light-driven ion transport as exemplified by light-driven pumping of protons or chloride ion [1]. The retinal chromophore experiences all-*trans* to 13-*cis* photoisomerization followed by a thermal relaxation called the photocycle. During the photocycle the ion is transported across the membrane.

*Leptosphaeria* rhodopsin (LR) is a light-driven proton pumping retinal protein from eukaryotes which belongs to the type I rhodopsin family and is found in fungi. Amino acid sequence comparison reveals a 25.7% homology of LR with the proton transporter BR [2]. The protein structure around the retinal chromophore in LR is similar to that in BR, and low-temperature FTIR study revealed that the structural changes upon retinal photoisomerization in LR resemble those in BR [2]. Rapid-scan FTIR difference spectroscopy and time-resolved laser spectroscopy confirmed the BR-like character of the LR photocycle. The photocycle intermediates of LR resemble the K, L, M, N and O states of BR [3]. In the proton-transporting photocycle of the all-*trans*, 15-*anti* isomeric form of BR,

*Corresponding author: kmagyari@phys.ubbcluj.ro

after photoisomerization of the retinal to 13-*cis*, 15-*anti* (in the L, M and N states), the protein oscillates between extracellular and cytoplasmic configurations. In the L intermediate, the Schiff-base of the retinal deprotonates, by transferring a proton to the acceptor Asp-85 and the proton is released from the release group on the surface of the membrane to the external medium, giving rise to M. $M_1$ is in the extracellular, and $M_2$ is in the cytoplasmic configuration of the protein. In $M_2$, a proton is transferred from the proton donor Asp-96 to the Schiff base, producing the N intermediate, followed by the uptake of another proton from the surrounding medium. When the retinal isomerizes back to the all-*trans* form, the red-shifted intermediate O appears. In the final step, going from O to BR, the protein relaxes back to the initial BR state [4, 5].

The LR photocycle exhibited a strong $D_2O$-dependence, most notably in the rise and decay of the M- and O-like intermediates, implying that there are proton-transfer steps in the photocycle [3].

The ion translocation through the membrane is accompanied by an electric signal, which reflects charge rearrangements perpendicular to the membrane surface. The isolated biological membrane fragments usually emerge as closed vesicles. In some special cases the fragments do not close, remaining as thin sheets of 4-5 nm thickness. An example is the purple membrane from *Halobacterium salinarum* where supporting lipids are combined with a single type of protein, BR. The non-closing membrane fragments of biological origin have permanent electric dipole moment $\mu$ perpendicular to the membrane sheet. The fragments also have polarizability $\alpha$ capable of exhibiting induced electric dipole moment [6].

The electric signals measured for different BR-containing oriented purple membrane systems demonstrated a remarkably good correlation with the photocycle [7, 8]. In principle, the electric dipole moment of each intermediate will be the function of the charge configuration in the protein, which depends on the positions of the amino acid side chains and the transported ion.

To understand the details of the photocycle, and therefore the process of ion translocation through cell membranes, we measured optical absorption kinetics and electric signal in LR. Based on similarity of LR and BR, we compared the obtained LR signals with those of BR.

## 2. Materials and methods

The *L. maculans ops* gene was modified and cloned into the pHIL-Sl vector similarly to the procedure previously used for *Neurospora* rhodopsin (NR) [9]. The protein was expressed in the methylotrophic yeast *P. pastoris*, strain GS115, following the optimized procedure for NR [10].

The absorption kinetic measurements were carried out on BR and LR polymerized in acrylamide gel, following the procedure described elsewhere [11]. The thoroughly washed gels were soaked overnight in 100mM NaCl, 50mM MES (2-[N-morpholino]ethanesulfonic acid) buffer at pH 6.

During the experiment the sample was in a 4x10 mm cuvette in a temperature controlled sample holder. The photocycle was initiated by a laser flash from a frequency-doubled Nd-YAG laser (Surelite I-10, Continuum, Santa Clara, CA) of 1.52 $mJ/cm^2$ energy density at 532 nm. The measuring light was provided by a 55W halogen lamp with heat filter. The absorption kinetic measurements were performed at three wavelengths (420, 560, and 620 nm) selected by a monochromator placed between the sample and the photomultiplier. In this configuration, the change of the measuring wavelength did not affect the light intensity going through the sample. Signals were recorded with a transient recorder card, having 16 MB memory and 50 ns time resolution (NI-DAQ PCI-5102, National Instruments, Austin, TX). Each measurement was the average of 100 individual signals. Data were recorded, after laser excitation of the sample, on a linear time scale, and converted to a logarithmic time scale. The linear-to-logarithmic conversion was accomplished by averaging the linear time points between two logarithmic time points, which improved the signal-to-noise ratio at later time points.

For electric signals measurements, oriented gel samples were prepared. They were oriented by an electric field and immobilized in acrylamide gel as described elsewhere for purple membranes [12]. The gels were equilibrated with a bathing solution containing 100mM NaCl and buffers, 25mM MES and 25mM TRIS, the pH was set to 6, 7, or 8, and the temperature of the sample was $20^0$C.

The electrogenicity of the BR intermediates was defined earlier [13] as the change in the dipole magnitude of the intermediate relative to the ground state.

The electric current signal in the measuring circuit by the change of the electrogenity is

$$i(t) = B\sum_j E_j \frac{dC_j}{dt}$$

were B is a constant determined by the electric circuit and $C_j$ is the concentration of the *j*th intermediate. If an intermediate is missing or its concentration is constant throughout the whole photocycle, it has no electric signal. The charge of the protein depends on the external condition (pH, ionic strength, etc), and the electrogenicity of the intermediates could also depend on these conditions. The electric signals arise only from the dipole magnitude changes in the direction perpendicular to the membrane. The sign of the electrogenicity is considered positive in the case of BR when the change in dipole momentum is equivalent to a shift of a positive charge in the proton transporting direction of the membrane [14].

## 3. Results and discussion

Taking as a starting-point the similarity between LR and BR, we performed absorption kinetic and electrical signal measurements on both proteins and compared the resulting signals.

Absorption kinetic measurements were performed on membranes containing LR at three wavelengths at pH 6 with 100mM NaCl, buffered by 50mM MES. Every wavelength was chosen to characterize mainly one of the intermediates. The signal measured at 420 nm is characteristic for the M intermediate, containing deprotonated Schiff-base, after a proton is transferred to the acceptor. The signals measured at 560 nm and 620 nm are characteristic of the N (plus the ground state) and O intermediates, respectively. The absorption kinetic signals measured at 560 and 620 nm were similar to those measured by Waschuk et. al. [3]. In case of the signal measured at 420 nm there is a difference in the magnitude of the two signals; this could be due to the differences in the measurement techniques (fig. 1), as well as due to the high turbidity/low transmission of our sample at this wavelength.

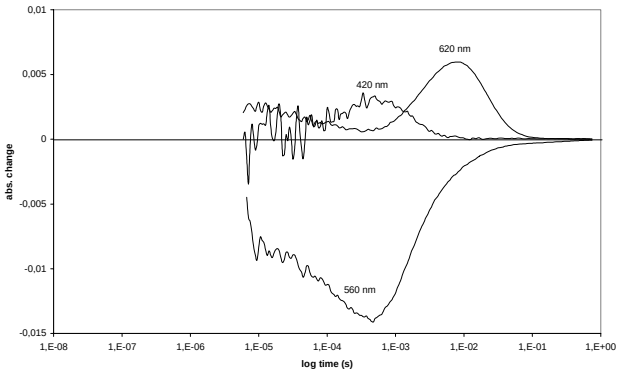


*Fig. 1. Time-dependent light-induced absorption difference signals measured at three wavelengths on Leptosphaeria rhodopsin. Measuring conditions were 100 mM NaCl, 50 mM MES, pH 5, and 20 $^0$C.*

The electric measurements do not give exact information about the proton release or uptake step but show that the transfer of protons from one side of the membrane to the other occurs in well-determined steps. The electrogenicity of the photocylce intermediate is defined as the change of the dipole moment in the direction perpendicular to the membrane [14]. The electrogenicity is the sum of all charge displacements, originating both from the transported ion and amino acid side chain motion inside the protein during the lifetime of that intermediate. This type of electric signal measurement is insensitive to charge motion in the electrolyte outside the membrane, because of shielding by the existing free charges.

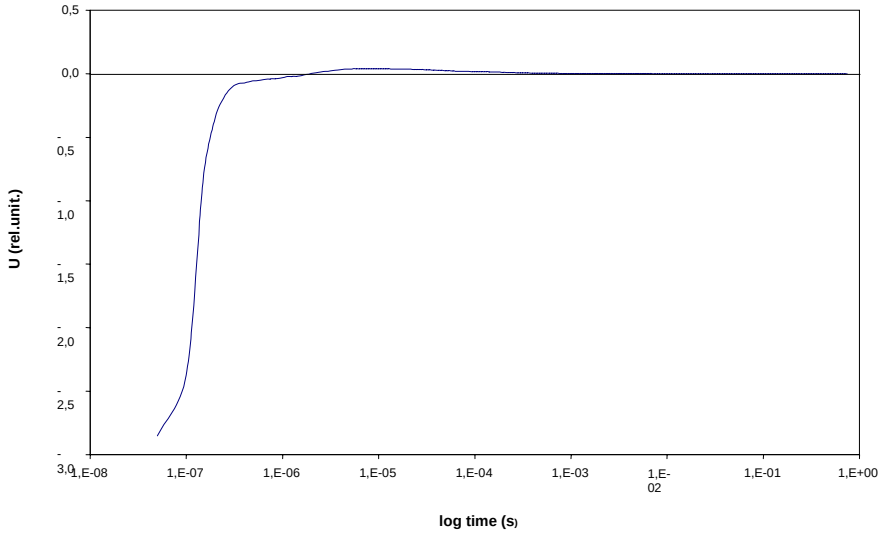


*Fig. 2. The electric signal measured on bacteriorhodopsin oriented sample. Measuring conditions were 100 mM NaCl, 50 mM MES, pH 6, and $20^0$C.*

With oriented samples encased in gels, and the method described before [13, 14], charge motion can be determined inside the protein. The external electric field, applied during the polymerization of the gel sample, produced orientation of the membranes due to their charge asymmetry.

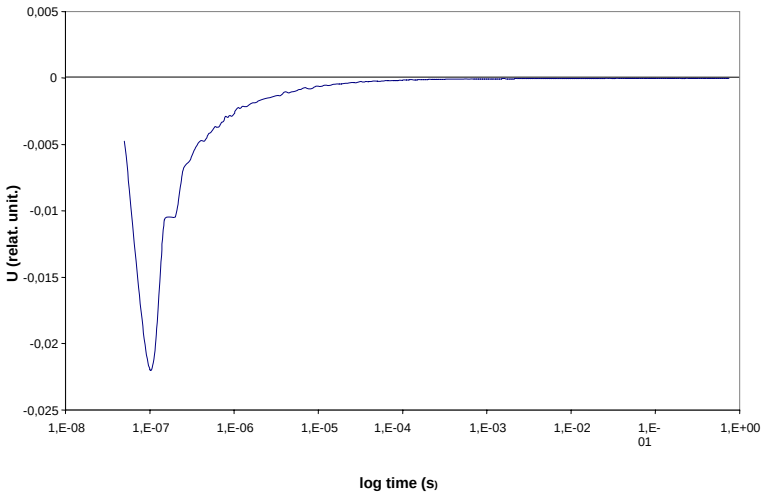


*Fig. 3. The electric signal measured on Leptosphaeria rhodopsin oriented sample. Measuring conditions were 100 mM NaCl, 50 mM MES, pH 6, and $20^0$C.*

The electric signal measurements were performed at pH 6 with 100mM NaCl and buffers, 25mM MES and 25mM TRIS. The result on the electric signals of BR were very similar to those published earlier (Fig. 2). In BR, the electrogenicity of the K intermediate was negative, for the L also negative and about two times larger, resulting in a fast negative signal, which crosses the zero line, becoming positive in several microseconds. The positive current corresponds to the rise of the M intermediate [14]. We compared this to the electrical signal of LR measured at pH 6 (fig. 3). There is one basic difference between the two signals: the positive part typical for BR doesn't appear in the electrical signal of LR. In order to exclude errors in the measurements, we measured the electrical

signal of LR at pH 5, 6, 7, and 8 as well. The electrical signals at the different pH values were of the same type. One of the possible reasons for the observed behavior may lie in the different sequence of proton transfers in LR, where the proton release does not happen on the submillisecond scale, but delayed until the end of the photocycle [3].

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## Reference

[1] J. L. Spudich, C. S. Yang, K. H. Jung, E.N. Spudich, Annu Rev Cell Dev Biol **16**, 365 (2000).
[2] M. Sumii, Y. Furutani, S. A. Waschuk, L. S. Brown, H. Kandori, Biochemistry **44** 15159 (2005).
[3] S. Waschuk, A. G. Bezerra, Jr., L. Shi, L. S. Brown, PNAS **102**, 6879 (2005).
[4] D. Oesterhelt, J. Tittor, E. Bamberg, J. Bioenerg. Biomembr. **24,** 181 (1992).
[5] J. K. Lanyi, Int. Rev. Cytol. **187**, 161 (1999)
[6] L. Keszthelyi, Colloids and Surfaces **209,** 173 (2002)
[7] L. Keszthelyi, P. Ormos, FEBS Lett. **109,** 189 (1980) -193.
[8] E. Bamberg, A. Fahr, Ann NY Acad. Sci. **358**, 324 (1980) -327
[9] J. A. Bieszke, E. N. Spudich, K. L. Scott, K. A. Borkovich, J. L. Spudich, Biochemistry **38** 14138 (1999).
[10] Y. Furutani, A. G. Bezerra Jr., S. Waschuk, M. Sumii, L. S. Brown, H. Kandori, Biochemistry **43,** 9636 (2004)
[11] P. C. Mowery, R. H. Lozier, Q. Chae, Y. W. Tseng, M. Taylor, W. Stoeckenius, Biochemistry **18,** 4100 (1979).
[12] A. Dér, P. Hargittai, J. Simon, J. Biochem. Biophys. Meth. **10** (5-6), 295 (1985).
[13] C. Gergely, C. Ganea, G. I. Groma, G. Váró, Biophys J. **65**, 2478 (1993).
[14] K. Ludmann, C. Gergely, A. Dér, G. Váró, Biophys. J. **75**, 3120 (1998).