WAVELENGTH DEPENDENCE OF THE ELECTRIC SIGNALS GENERATED IN DRIED BR SAMPLES

M. DIMA, Z. BÁLINT**, CONSTANȚA GANEA*, G. VÁRÓ***

*Department of Biophysics, "Carol Davila" University of Medicine and Pharmaceutics, 8, Eroii Sanitari Blvd, 050474 Bucharest, Romania
**Institute of Biophysics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary, H-6726

Abstract. The electrical signals from Bacteriorhodopsin-containing air dried purple membranes (PM) were recorded at various wavelengths by using a 250 W halogen lamp and narrow band interference filters to excite the probe. The amplitude of the signals was plotted as a function of the wavelength and then compared to the absorption spectrum of dried PMs. The action spectrum thus obtained shows a good correlation with the absorption spectrum in the long wavelengths region but not in the short wavelengths one in contrast to the results obtained in AFM measurements where the correlation was very good at all wavelengths. This puzzling result was tentatively explained taking into account the fact that the samples were excited with continuous light having as a consequence the simultaneous presence of several intermediates of the photocycle, each with a different electrogenicity. Further experiments, employing as exciting laser pulses of various wavelengths, are required in order to elucidate this problem.

Key words: bacteriorhodopsin, electric signals, absorption spectrum, photocycle intermediates.

INTRODUCTION

The light-driven retinal protein bacteriorhodopsin (BR) is a very well studied proton pump but, nevertheless, several details concerning the structural changes induced by the photon absorption-initiated reaction cycle are still to be elucidated. BR is the main component of the purple membranes (PMs), part of the cell membrane of *Halobacterium salinarum* [12]. The structures of BR and its intermediates are known with high resolution. Retinal is bound to lysine 216 through a protonated Schiff-base [15], which plays a key role in the proton transporting process [3]. Its orientation inside the protein is well defined [9, 11]. A detailed description of the photocycle can be found in review papers [7, 8, 10]. After absorbing the light quanta, a charge separation along the retinal occurs in the femtosecond time domain [5], followed by an all-*trans* to 13-*cis* isomerization in

Received: October 2007.

several picoseconds, reaching the K state. In the K $\rightarrow$ L transition, a local rearrangement around the retinal occurs in less than 10 μs. In the L $\rightarrow M_1$ transition, about 100 μs after the excitation, the Schiff-base of the retinal deprotonates by transferring its proton to the acceptor Asp 85, and a proton is released from the release group on the surface of the membrane close to the external medium [2, 14]. A conformational change in the BR occurs during the $M_1 \rightarrow M_2 \rightarrow N$ transitions, when the protein switches from the extracellular to the cytoplasmic conformation in about 100 μs. The accessibility of the Schiff-base changes from the extracellular to the cytoplasmic side [3]. The Schiff-base is reprotonated by the proton donor Asp 96 on the cytoplasmic side, reflected by the appearance of intermediate N. In the N $\rightarrow$ O $\rightarrow$ BR transition, the retinal reisomerizes to its original all-*trans* form, a proton is taken up from the cytoplasmic side, and the proton from the acceptor is transferred to the proton release group in the millisecond time domain [8, 13]. At low pH, below the p*K*a of the proton acceptor group, the photocycle does not translocate protons across the membrane, and the M intermediate is missing from the photocycle [17]. When the PMs are allowed to air-dry, the BR still preserves its photocycle up to the M intermediate, and, once the sample is rehydrated, the protein regains its original proton-pumping function [6, 4, 18]. Using the permanent electric dipole and the net negative charge of the PM, highly oriented membrane samples were prepared electrophoretically on a conductive surface [6, 16].

In a recent paper [1] we have reported the study of the conformational changes occurring inside the membrane during the photochemical reaction cycle of the BR by measuring the deflection change of a conductive AFM cantilever, on which PMs were electrophoretically deposited. In order to get new insight into the mechanism of conformational changes of the protein during its photocycle, we have proposed ourselves to study the electrogenic activity of BR in the same conditions as those in which the AFM measurements were done, namely we have recorded the electrical signals elicited by light at various wavelengths on air dried oriented PM samples. We have found that in the long wavelength region the action spectrum of the electrical signals thus obtained showed a good correlation with that obtained from AFM measurements for the mechanical changes, while at the short wavelength part the spectrum presented significant differences.

MATERIALS AND METHODS

PMs were isolated from *Halobacterium salinarum* strain S9 according to a standard procedure [12]. The preparation was further washed by centrifugation in freshly prepared tridistilled water to remove any traces of salt from the suspension.

The oriented samples were prepared on conductive indium-tin-oxide (ITO) covered glass slides, connected as the anode in the electric circuit. The cathode was a platinum disc. The suspension was in contact with both electrodes, which were at a distance of 1–3 mm. An electric field of 20–40 V/m oriented the purple membranes by their permanent electric dipole moment and deposited them on the anode by electrophoresis due to their negative charge. The excess water was carefully removed and the samples let air dry. The photoelectric measurements were performed on a setup described earlier [16]. The sample was placed inside a humidity and temperature controlled sample holder. During the photoelectric measurements, a platinum plate as a second electrode was placed on the surface of the sample. The PMs were excited by continuous illumination. The light source was a 240 W (12 V / 20 A) incandescent light bulb powered by a home-made stabilized power supply. A series of 9 narrow band interference filters was used, corresponding to 409, 465, 528, 545, 577, 595, 610, 650 and 698 nm wavelengths. An infrared filter (a water tank) was also used to prevent the heating of the filters. The light was focalized on the BR sample using lenses and mirrors. The electric signal of the BR sample was passed through a home-made amplifier/filter and then fed to a digital oscilloscope connected to a computer. To compensate for the change in light intensity due to different absorption intensities of the filters, the transmission spectrum of the filters was measured using a Unicam absorption spectrophotometer. The emission spectrum of the unfiltered light source was also measured with an emission spectrophotometer, to compensate for the nonlinear characteristic of the incandescent light bulb.

RESULTS AND DISCUSSIONS

The oriented dried samples were illuminated with light of various wavelengths by using narrow band interference filters in the 409–698 nm range. Prior to PM light excitation, the light intensity for each filter was measured with an Ophire power-meter in order to normalize the signals to the same light intensity. Then, the electrical signals were recorded and the final signal for each wavelength was the average of 10 individual signals. The resulting averaged signals (Fig. 1) were corrected taking into account the intensity measurements. The wavelength dependence of the signal amplitude, normalized to the incident light intensity (Fig. 2), represents the action spectrum of the sample for the measured signals.

It can be noticed that in the wavelengths range 580–685 nm the action spectrum follows the shape of the BR absorption spectrum (solid line). The presented absorption spectrum was measured on a light-adapted PM dried sample.

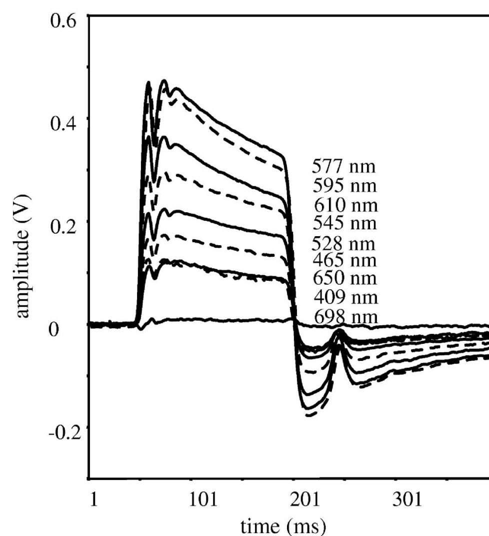

Fig. 1. Normalized electric signals from dried PM samples at various wavelengths. The pH of the sample was 7, and the temperature 22 ºC.

On the contrary, in the 403–580 nm range, the spectrum is shifted both with regard to the absorption spectrum and to the action spectrum obtained in AFM experiments (dashed line, Fig. 2).

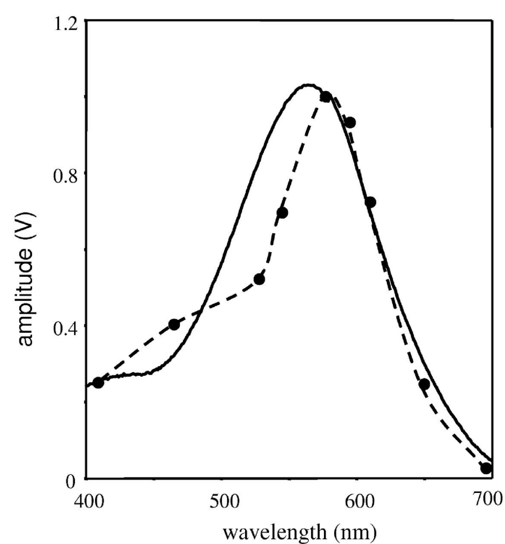

Fig. 2. The action spectrum for the electrical signals of dried PMs (dashed line) as compared to the absorption spectrum of PMs in the same conditions of pH and temperature as in Fig. 1.

This result is puzzling, as it is considered that the photocycle of BR should be the same measured at each wavelength. A tentative explanation would take into account the fact that the sample was illuminated with continuous light and not with light pulses. This means that during the measurements the protein has undergone many turnovers and the intermediates were also light excited. This means that during the experiment in the sample is present a mixture of intermediates each one with a different electrogenicity. In order to solve this problem, additional experiments should be carried out in which the light source should be replaced with a short pulse laser light of various wavelengths.

CONCLUSIONS

Electrical signals recorded from illuminated dried oriented samples containing purple membranes revealed that in the wavelengths range from 580– 685 nm the action spectrum follows the shape of the BR absorption spectrum. However, in the 403–580 nm range, the spectrum is shifted both with regard to the absorption spectrum and to the action spectrum obtained in AFM experiments. These results were interpreted as due either to the fact that the illumination was with continuous light or to the presence of a mixture of intermediates at the same time. In order to give a more precise interpretation of the above results, more experiments, in which laser pulses should be used instead of continuous light for sample excitation, are still needed.

Acknowledgements. The National Science Research Fund of Hungary (OTKA T048706) supported this work. M.D. and C.G. performed this research in the frame of the Romanian–Hungarian Bilateral Cooperation, 2006-2007, under number RO-4/05 by TÉT Hungary and ANCS Romania.

REFERENCES

1. BÁLINT, Z., G.A. VÉGH, A. POPESCU, M. DIMA, C. GANEA, G. VÁRÓ, Direct observation of the protein motion during the photochemical reaction cycle of the bacteriorhodopsin, *Langmuir*, **23**, 7225–7228.
2. BROWN, L.S., J. SASAKI, H., KANDORI, A. MAEDA, R. NEEDLEMAN, J.K. LANYI, Estimated acid dissociation constants of the Schiff base, Asp-85, and Arg-82 during the bacteriorhodopsin photocycle, *J. Biol. Chem.*, 1995, **270**, 27122–27126.
3. BROWN, L.S., A.K. DIOUMAEV, R. NEEDLEMAN, J.K. LANYI, Local-access model for proton transfer in bacteriorhodopsin, *Biochemistry* , 1998, **37,** 3982–3993.
4. GANEA, C., C. GERGELY, K. LUDMANN, G. VÁRÓ, The role of water in the extracellular half channel of bacteriorhodopsin, *Biophys. J.*, 1997, **73**, 2718–2725.
5. GROMA, G.I., A. COLONNA, J.C. LAMBRY, J.W. PETRICH, G. VÁRÓ, M. JOFFRE, M.H. VOS, J.L. MARTIN, Resonant optical rectification in bacteriorhodopsin, *Proc. Natl. Acad. Sci. U.S.A.*, 2004, **101**, 7971–7975.

6. KESZTHELYI, L., P. ORMOS, G. VÁRÓ, Fast components of the electric response signal of bacteriorhodopsin protein, *Acta Phys. Acad. Sci. Hung*., **1982**, **53**, 143–157.
7. KREBS, M.P., H.G. KHORANA, Mechanism of light-dependent proton translocation by bacteriorhodopsin G., *J. Bacteriol.,* 1993, **175**, 1555–1560.
8. LANYI, J.K., Bacteriorhodopsin, *Int. Rev. Cytol*., 1999, **187**, 161–202.
9. LANYI, J.K., B. SCHOBERT, Mechanism of proton transport in bacteriorhodopsin, *J. Mol. Biol.*, 2003, **328**, 439–450.
10. LANYI, J.K., Bacteriorhodopsin, *Annu. Rev. Physiol.*, 2004, **66**, 665–688.
11. LUECKE, H., B., SCHOBERT, H.T. RICHTER, J.P. CARTAILLER, J.K. LANYI, Structure of bacteriorhodopsin at 1.55 Å resolution, *J. Mol. Biol.*, 1999, **291**, 899–911.
12. OESTERHELT, D., W. STOECKENIUS, Isolation of the cell membrane of *Halobacterium halobium*, *Methods. Enzymol.,* 1974, **31**, 667–678
13. OESTERHELT, D., J. TITTOR, E. BAMBERG, A unifying concept for ion translocation by retinal proteins, *J. Bioenerg. Biomembr.*, 1992, **24**, 181–191.
14. SPASSOV, V.Z., H. LUECKE, K., GERWERT, D. BASHFORD, pK(a) calculations suggest storage of an excess proton in a hydrogen-bonded water network in bacteriorhodopsin, *J. Mol. Biol.*, 2001, **312**, 203–219.
15. STOECKENIUS, W., R.H. LOZIER, R.A. BOGOMOLNI, Bacteriorhodopsin and the purple membrane of Halobacteria, *Biochim. Biophys. Acta*, 1979, **505,** 215–278.
16. VÁRÓ, G., Dried oriented purple membrane samples, *Acta Biol. Acad. Sci. Hung.*, 1981, **32**, 301–310.
17. VÁRÓ, G., J.K. LANYI, Photoreactions of bacteriorhodopsin at acid pH, *Biophys. J.*, 1989, **56**, 1143–1151.
18. VÁRÓ, G., J.K. LANYI, Distortions in the photocycle of bacteriorhodopsin at moderate dehydration, *Biophys. J.* 1991, **59**, 313–322.