ELSEVIER

Biophysical Chemistry 56 (1995) 159–163

Biophysical Chemistry

Introduction of a method for three-dimensional mapping of the charge motion in bacteriorhodopsin

András Dér *, Pál Ormos

Institute of Biophysics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Temesvári krt. 62, P.O.B. 521, H-6701 Szeged, Hungary

Abstract

Electric signals associated with the photocycle of bacteriorhodopsin carry valuable information about the proton transport process. Photocurrents measured by different experimental methods are interpreted in terms of intramolecular charge displacements [1,2]. Permanent electrical asymmetry of the sample is considered to be a prerequisite for the detection of electric signals. The various photoelectric measuring techniques can be distinguished by the way of achievement of this asymmetry [3]. A common feature of the available methods, however, is that the samples are cylindrically symmetric. Consequently, intramembraneous charge displacements can normally be monitored only along the axis of the membrane normal. We developed a novel method that allows also the detection of the in-plane components of the charge displacements. Samples containing oriented purple membrane fragments were used in the experiments [4], and the rotational symmetry was transiently broken via anisotropic excitation of the bR molecules by linearly polarized light. Kinetics of the normal and in-plane components were measured and interpreted as a result of spatial charge displacements associated with the proton transport process in bacteriorhodopsin.

Keywords: Purple membrane; Orientation; Photoselection; Charge displacements

1. Introduction

Bacteriorhodopsin (bR) is one of the best-characterized ion pumps known in biological membranes. It pumps protons from the intracellular side of the cell membrane to the extracellular space [5]. Among others, mainly spectroscopic techniques (i.e. visible and UV absorption kinetics, FTIR, CD and resonance Raman spectroscopy) were used to investigate its photoreaction cycle accompanying the proton transport process. Photoelectric signals also carry valuable information about the photocycle independent of those supplied by other techniques (for a review see [3]). We have developed a method for the kinetic measurement of photoelectric signals of membrane-bound ion pumps within a wide time range (from submicroseconds to seconds) [1,4] that allows simultaneous measurement of absorption kinetic signals, as well. As a result of a complex evaluation procedure of electric and absorption kinetic signals it is possible to determine the size of intramolecular charge displacements accompanying the photocycle of bR [1,6] in the direction of the overall pumping process, i.e. parallel with the normal membrane. Because of an inherent rotational

* Corresponding author.

SSDI 0301-4622(95)00028-3

symmetry of both the available measuring techniques and samples, the in-plane components of the charge displacements have not been detected yet. Here we introduce a novel method allowing the measurement of bR photoelectric signals in all three space dimensions. For the orientation of membrane fragments a combination of electric and magnetic fields was applied, while excitation with polarized light (photoselection) was used to break the rotational symmetry of the system. The first results prove the existence of the in-plane components.

2. Materials and methods

Purple membranes prepared by the usual procedures [5] were oriented by the combination of electric and magnetic fields. First an electric field of 20 V/cm was applied to the membrane suspension containing polyacrylamide gel-forming materials (see e.g. [4]), and the sample was subsequently introduced into a magnetic field of 14T, where the alignment of the membranes is close to perfect [7]. After a duration of 20 s the electric field was switched off in order to minimize the consequences of electrolysis and electrophoresis, while the magnetic field was kept on until the polymerization was completed. The average polymerization time was 4 min. Samples were prepared only by magnetic field ordering. Note that in this case the membrane fragments are only aligned, i.e. the membranes are parallel but their sidedness (pumping direction) is random.

Cubes of 1 cm length were cut out of the gels of $OD_{575} = 2$, and were put into a cubic measuring cell with 3 pairs of platinized platinum electrodes on the walls of the cell. The distance between the electrodes was 2 cm. Special care was taken to shield electrodes from the exciting light in order to avoid the usual light-scattering artifacts.

Samples were excited by polarized light flashes of 100 μJ energy. The plane of the polarization made an angle of 45° with the z-axis (Fig. 1). Signals were amplified by voltage amplifiers (home-made) and collected in a transient recorder (LeCroy). The time resolution of the measuring system was 2 μs. Exponential fitting was carried out by the routines of the program package MATLAB for Windows 3.0.

3. Results and discussion

3.1. Principles of the new method

An intrinsic rotational symmetry of the measuring systems available for the detection of photoelectric currents of bacteriorhodopsin is the reason why only the normal component of the electric signal has been measured so far. In our novel method, this rotational symmetry was transiently broken by an appropriate excitation with polarized light. Fig. 1a shows the geometry of the optical properties of a purple membrane oriented by its permanent dipole moment along the z direction. The Descartian component vectors of the electric signal are denoted by i_x, i_y and i_z, respectively. Let us consider the contribution of the bR molecules having chromophores in the 'left' half space ($-\infty < x < \infty$; $-\infty < y < 0$; $-\infty < z < \infty$) to the i_x and i_y components to be positive. Then, because of symmetry reasons, the contribution of molecules in the 'right' half space ($-\infty < x < \infty$; $0 < y < \infty$; $-\infty < z < \infty$) to the same components is negative. In order to maximize the measurable in-plane components, the difference between the excitation probabilities in the left and right half spaces (D) should be maximized too. Using the notations of Fig. 1a, D can be written as:

$$D \sim \int_{\Omega_l} |<\underset{\sim}{e},\underset{\sim}{\mu}_T>|^2 \mathrm{d}\Omega$$

$$- \int_{\Omega_r} |<\underset{\sim}{e},\underset{\sim}{\mu}_T>|^2 \mathrm{d}\Omega = \frac{2}{\pi} \cdot \sin^2\beta \cdot \sin^2\vartheta$$

where ϑ is the half-angle of the retinal cone, and β is the angle which the plane of polarization makes with the z-axis. Ω_l is related to the left, while Ω_r is to the right half-space. D is maximal for $\beta = 45° \pm k \cdot 180°$, where k is a natural number.

As for the case of facing membranes (alignment with magnetic field only), it is demonstrated in Fig. 1b that the i_y and i_z components are compensated, while the i_x components are not.

The arguments presented above are valid for the case of perfect orientation and alignment of membrane fragments. Note, that a non-complete orientation results in a contribution of i_z to the signal measured in the y direction.

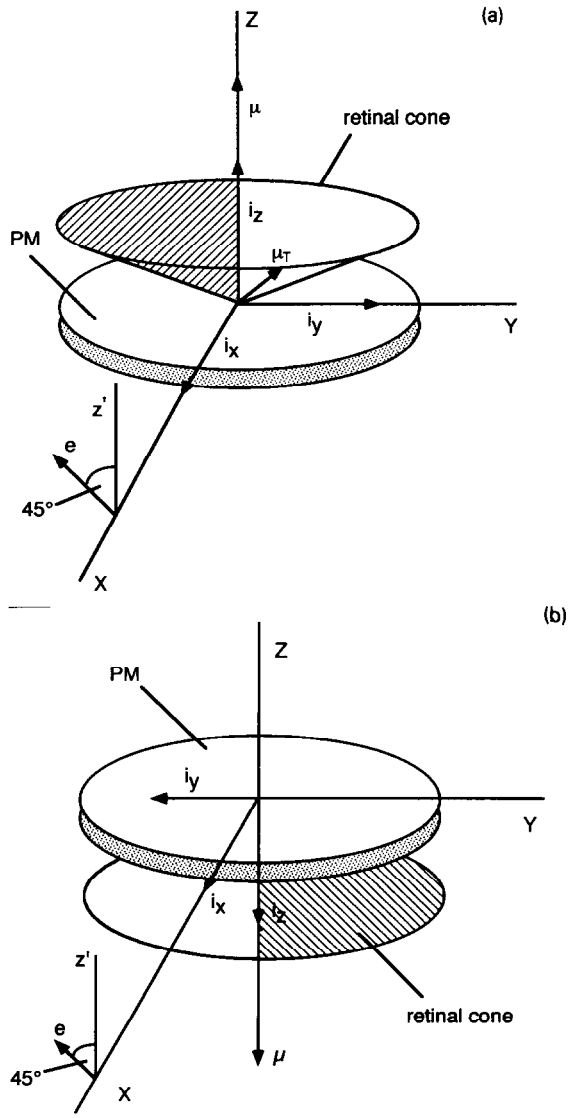

Fig. 1. Geometrical representation of the optical properties of purple membranes oriented in two opposite directions (a and b, respectively). The photocurrent components are denoted by i_x, i_y and i_z; μ is the permanent $-$, and μ_T is the transition dipole moment of the membrane and the retinal, respectively. The electric field vector of the exciting light is denoted by e.

3.2. Electric signals

In order to prove the existence of an in-plane component of the electric signal of bR, the first measurements were performed on samples containing purple membranes aligned in a magnetic field. Because of symmetry reasons, in this case no contribution from the y and z component should occur in any direction, while the x components from facing membranes are not compensated by each other, as it is demonstrated in Fig. 1b. In fact, the measurements revealed the existence of an i_x component, which changed sign upon changing the polarization plane of the exciting flash from 45° to −45°. The fast

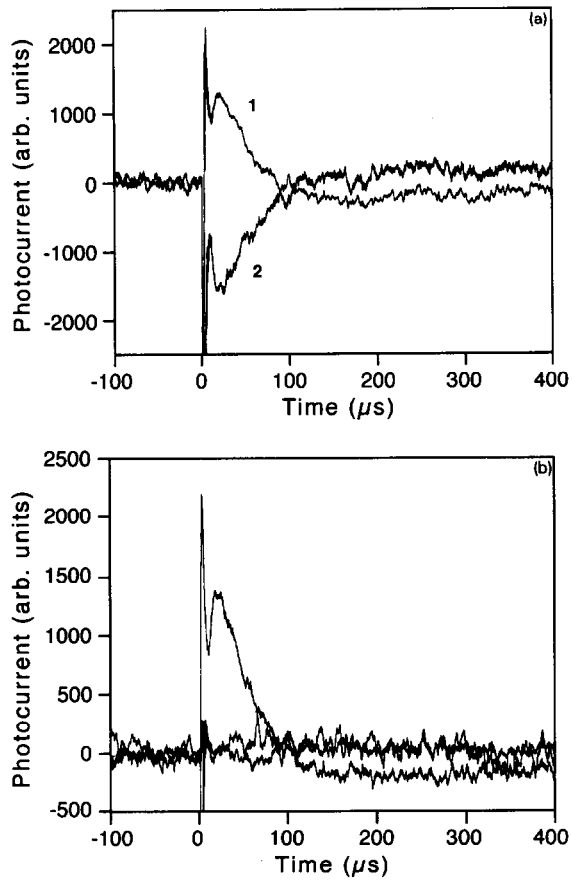

Fig. 2. (a) Electric signals of bacteriorhodopsin in the x direction (i_x) measured with two different polarizations of the exciting light ($\beta = 45°$ (1) and $-45°$ (2)) on a sample containing pm aligned in the magnetic field. The gels were washed in distilled water at pH 6.5. (b) The averaged subtraction of the signals in Fig. 2a together with the traces measured in the y and z direction under the same conditions.

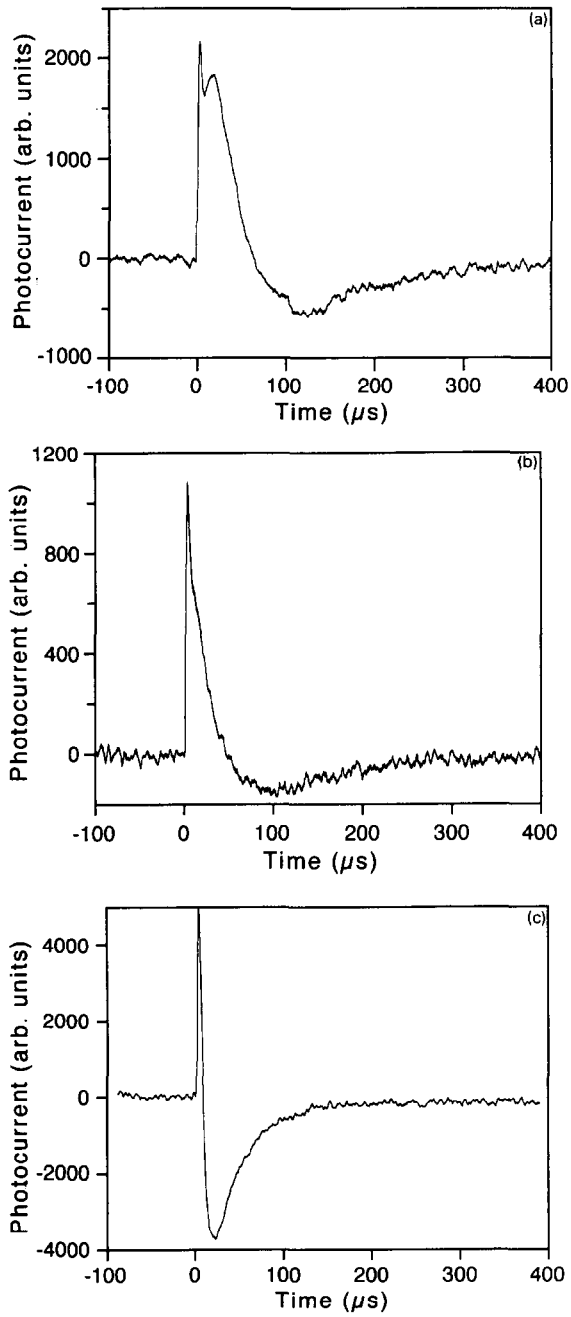

Fig. 3. Electric signals of bR in all the three Descartian space dimensions: i_x, i_y and i_z corresponding to a, b and c, respectively, on a sample containing pm oriented by a combination of electric and magnetic fields. Measuring conditions were the same as for the traces of Fig. 2.

electromagnetic artifact is expected to be diminished by the subtraction of signals (1) and (2) in Fig. 2a. The result is shown in Fig. 2b, together with the traces measured in the y and z directions. The observations are in a complete accord with the symmetry properties of the measuring system. Note, that this is the first demonstration of photocurrents measured in a *non-oriented* (but *aligned*) membrane system.

The y component of the electric signal, however, can only be measured in a sample containing *oriented* membrane fragments. For this purpose we used a gel with purple membrane fragments oriented by the combination of an electric and a magnetic field. First, the membrane fragments were oriented by an electric field, subsequently, a magnetic field was switched on in order to improve the optical anisotropy of the sample. This procedure resulted in a nearly perfect alignment of the membranes with a preferential orientation in one direction. (For details, see Materials and Methods). As it is shown in Fig. 1a, using an excitation by polarized light, both in-plane components should be detected in this case.

The results are depicted in Figs. 3a–c. The life times of the exponential components from a global fit of all curves to 4 exponentials are informative ($2\,\mu s$ [1], $4\,\mu s$, 40 μs and 200 μs), however, a complex evaluation procedure using the results of simultaneous absorption kinetic measurements would be necessary to obtain the charge displacement vectors. Some normalization problems may also occur for the following reason. The y and z components are dependent on the net orientation, while the x component is only alignment-dependent. Thus, in case of a non-perfect orientation (in the sense that a non-negligible amount of purple membranes face each other) the size of the x component is overestimated as compared to the other two components. This problem would be solved by using a higher magnetic field during the sample preparation in order to get a more perfect orientation, by which also the possible contribution of i_z to i_y could be avoided, resulting in 'clean' current components. With these improve-

[1] Limited by the measuring system.

ments, we expect the new method to supply us with results having important implications in bR research, and, possibly, in the case of other biological systems, too.

Acknowledgements

The authors are indebted to Prof. Lajos Keszthelyi and Prof. Walther Stoeckenius for valuable discussions and help in the access to the magnetic facilities. Thanks are due to Prof. Maarten Heyn and Dr. Harald Otto in the Free University, Berlin, and to Prof. György Mihály and Dr. Richárd Gál in the Technical University, Budapest for allowing us to use their 14 T magnets, as well as to Mr. Miklós Szücs for the preparation of the excellent sample holder. During his stay in Berlin, A.D. was supported by a NATO travel grant (CRG 930419), while Hungarian research grants (OTKA T-914 and OTKA T-5130) were used to finance the experimental work. The technical assistance of Mrs. Gabriele Schubert and Mrs. Helga Volk in Max-Planck-Institut für Biophysik, Frankfurt is gratefully acknowledged.

References

[1] L. Keszthelyi and P. Ormos, FEBS Lett., 109 (1980) 189.
[2] A. Fahr, P. Läuger and E. Bamberg, J. Membrane Biol., 60 (1981) 51.
[3] H.-W. Trissl, Photochem. Photobiol., 51 (1990) 793.
[4] A. Dér, P. Hargittai and J. Simon, J. Biochem. Biophys. Methods, 10 (1985) 295.
[5] D. Oesterhelt and W. Stoeckenius, Methods Enzymol., 31 (1974) 667.
[6] A. Dér, R. Toth-Boconádi and L. Keszthelyi, Colloque INSERM, 221 (1992) 197.
[7] H. Otto and M.P. Heyn, FEBS Lett., 293 (1991) 111.