THE EFFECTS OF THE LYOTROPIC ANION NITRITE ON THE TRANSIENT AND STATIONARY PHOTOCURRENTS OF BACTERIORHODOPSIN

A. IFTIME, IOANA PLAJER, CONSTANŢA GANEA

Department of Biophysics, "Carol Davila" University of Medicine and Pharmaceutics, 8, Eroii Sanitari Blvd, 050474 Bucharest, Romania

Abstract. The effects of the lyotropic anion nitrite on the transient and stationary photocurrents of bacteriorhodopsin (bR) adsorbed to planar lipid bilayers were followed up at different nitrite concentrations. On raising the nitrite concentrations, both transient and stationary photocurrents increased and, at the same time, the kinetics of the bR photocycle was modified, showing an acceleration at nitrite concentrations below 100 mM and a slowing down above this value. The effects of nitrite were explained taking into account the slightly chaotropic character of this anion and the possible interaction both with the dipole potential of the membrane and/or with the charged groups of the protein.

Key words: bacteriorhodopsin, BLM, nitrite, photocurrents.

INTRODUCTION

Bacteriorhodopsin (bR), one of the retinal proteins present in the plasma membrane of *Halobacteria*, functions as a light-driven proton pump [7, 12, 13], converting the light energy into an electrochemical proton potential across the membrane [15]. A structural model for bR was proposed on the basis of electron crystallographic studies and recently a 0.25 nm resolution structure was published [11, 17], allowing a better correlation between its structure and function. Bacteriorhodopsin consists of a single polypeptide chain, folded into seven transmembrane α-helices, forming a channel that is, in the middle, divided into two parts by the chromophore retinal [10, 11, 17]. The retinal is covalently bound to the protein via a protonated Schiff base, formed with the ε-amino group of Lys 216. The positive electric charge of the Schiff base is equilibrated by a complex counterion which consists of two negatively charged carboxyl groups of Asp 85 and Asp 212, a positively charged Arg 82 and several water molecules [11, 17]. The absorption of a photon leads to the isomerization of the chromophore from all-*trans* to 13-*cis* and initiates, thus, a photochemical cycle having as a result the

Received May 2005.

release of one proton to the extracellular surface of the membrane and the uptake of another one from the cytoplasmic side. A number of biochemical and biophysical investigations, carried out on point-mutated bRs (for a review see [13]) revealed the central role for proton translocation of two aspartic acid residues, Asp 85, located near the Schiff base in the extracellular "half-channel" and Asp 96, in the cytoplasmic "half-channel". After the isomerization of retinal and the subsequent drop of the pK_a of the Schiff base, this one deprotonates and its proton is accepted by Asp 85. In the second half of the photocycle, Asp 96 serves as the proton donor to the Schiff base. 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 returning the protein to its original state [13, 16].

In the last years the interest for the effects of anions belonging to Hofmeister (or lyotropic) series at the level of cell membranes grew steadily up [1, 8, 9]. The effects of some anions seem to increase in the following order: $SO_4^{2-} < F^- < Cl^- < Br^- < NO_3^- < I^- < SCN^- < ClO_4^-$ [4]. The salts belonging to this series can have stabilizing or destabilizing effects on cell membrane structures [1]. The Hofmeister anions are frequently encountered in various food products and drugs. For this reason, the study of their effects in membrane structures could contribute not only to elucidating of the mechanisms underlying the function of these structures but also to the development of some applicative domains such as food industry and pharmacology. We have proposed ourselves to study how the lyotropic anion nitrite, slightly chaotropic, can modify the electrical characteristics of lipid bilayers and of lipid bilayers on which bacteriorhodopsin containing purple membranes were adsorbed. Previous studies on the effects of some of the lyotropic anions on bR photocycle [6, 14] indicated a slowing down of the photocycle in the presence of chaotropic ions (SCN^-) while the kosmotropic anions had no effect on photocycle kinetics. The authors have explained these effects by modifications in the bR flexibility.

MATERIALS AND METHODS

The photocurrents generated in the bR containing membranes were measured using the BLM technique [8]. The black lipid films, having an area of 10^{-2} cm^2, are formed in a Teflon cuvette, consisting of two compartments, each with a volume of 1.5 ml. The compartments were filled with an appropriate electrolyte solution (1.3 ml for each compartment). The film forming solution contained 1.5% (wt/vol) diphytanolphosphatidylcholine (Avanti Chemicals, Birmingham, AL) and 0.025% (wt/vol) octadecylamine (Riedel-de-Haen, Hannover, Germany) in n-decane to obtain a positively charged membrane surface [18]. The membrane fragments were suspended in the appropriate buffer solution (OD = 5) and sonicated for 1 min. in a

sonication bath. Then, aliquots of 20 μl were added under stirring to the rear compartment of the Teflon cell containing the same buffer. The membrane was illuminated with a mercury lamp (100 W) and the actinic light passed through appropriate filters, including a heat protection filter. The intensity of the continuous light source was up to 2 W/cm^2 at the membrane surface. For "yellow" light, a cutoff filter, $\lambda > 515$ nm (Schott, Mainz) was used. The suspensions on both sides of the black membrane were connected to an external measuring circuit via Ag/AgCl electrodes, separated by salt bridges from the Teflon cell. The current was measured with a current amplifier (Stanford Research System - SR570). The buffer solution used for the experiment consisted of 100 mM NaCl and 20 mM Hepes, pH 6.8 plus $NaNO_2$ at various concentrations. The nitrite concentration was adjusted by using a stock solution of 4 M $NaNO_2$ and titrating in the cuvette. To obtain the stationary currents we added the blue-UV light insensitive protonophore 1799 (2,6-dihydroxy)-1,1,1,7,7,7-hexafluoro-2,6-bis(trifluoromethyl)heptane-4-one (Dr.P.Heydtler, DuPont Nemours), which permeabilized the lipid membrane for protons. For further details see [8]. Throughout this paper, the outwardly directed currents, that is in the direction of the normal proton pumping in wild type bR, were taken as negative.

RESULTS AND DISCUSSIONS

In order to follow up the effects of nitrite on lipid bilayers, the capacitance and the conductance of the lipid membranes were measured at sodium nitrite concentrations ranging from 4–1040 mM by using the BLM method.

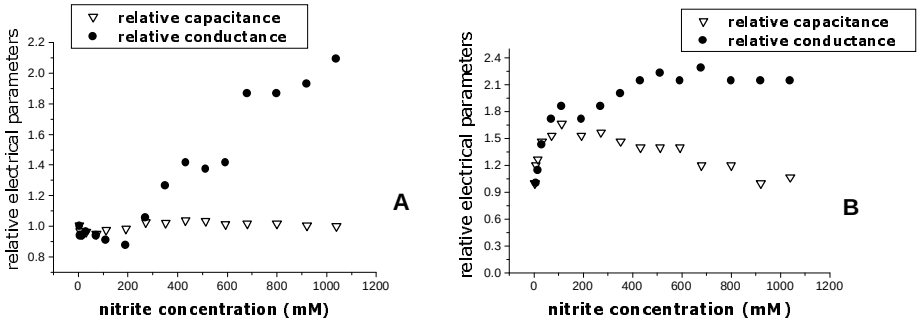

Fig. 1. The effects of nitrite on the capacitance and conductance of lipid bilayers (A) and of lipid bilayers containing bacteriorhodopsin (B). The buffer solution contained 100 mM NaCl, 20 mM Hepes at pH 6.8.

It can be noticed (Fig. 1A) that raising the nitrite concentration the capacitance of the bilayer remains unaffected while its conductance increases, by a factor more than 2 at 1040 mM sodium nitrite. A possible interpretation, based also on previous findings [2, 3], would refer to the dipol potential of the lipid membrane. The chaotropic anions have the tendency to enter the hydrophobic core of the membrane leading thus to the increase of its conductance. They can thus screen the positive end of the intrinsic dipol potential [2, 3].

The same measurements were performed on bacteriorhodopsin containing lipid bilayers (Fig. 1B). In this case not only the conductance is modified, but the capacitance as well, depending on the concentration of the anion. At small concentrations, less than 100 mM, the capacitance increases by a factor bigger than two for 100 mM sodium nitrite and when the concentration is raised further, the capacitance decreases until it reaches again its initial value. The conductance increases steadily with increasing nitrite concentration until it reaches a plateau at about 400 mM. The differences in the effects produced at the level of lipid bilayers as compared to bilayers containing purple membranes should arise from the specific effects on bacteriorhodopsin. On one hand, the slightly chaotropic nitrate could affect the purple membrane adsorption on the lipid bilayer and on the other hand it can modify the dielectric constant of the composed membrane (lipid membrane plus purple membranes). This effect should reflect itself in the capacitance variation as compared to the constant value found in simple bilayers. At the same time, the conductance can increase as the nitrite enters not only in the lipid membrane, but at the same time in the proton channel of bacteriorhodopsin. The plateau obtained after 400 mM nitrite concentration could indicate saturation in nitrite binding to the protein.

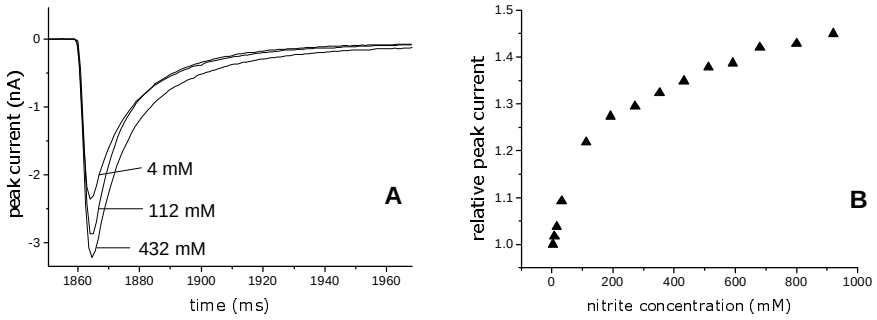

Fig. 2. Transient photocurrents of bacteriorhodopsin at three nitrite concentrations (A) and their concentration dependence (B). Conditions like in Fig. 1.

In order to see if nitrite has specific effects on bR photocycle, transient (Fig. 2) and stationary (Fig. 3) photocurrents were recorded for various nitrite concentrations. The transients were fitted to a bi-exponential function with the view to get some information about the effects of nitrite on the photocycle kinetics. As the time

resolution of the method is too poor for extracting quantitative information about the kinetics of different steps of the photocycle, the calculated parameters, i.e. the two time constants calculated from the fit, can give us only a qualitative information about the time course of the transient currents.

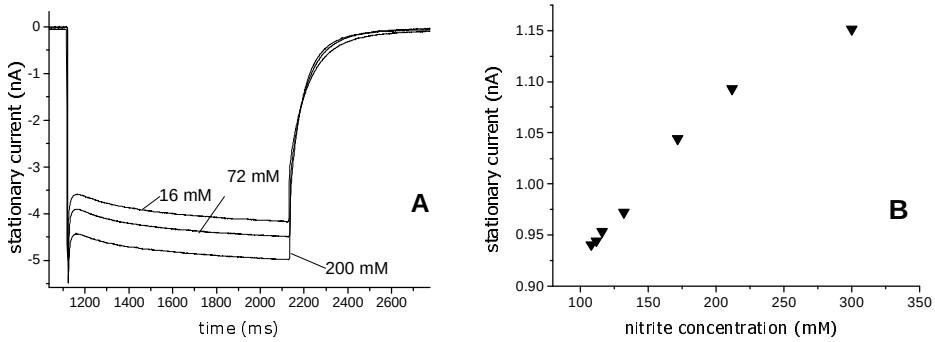

Fig. 3. Stationary photocurrents of bacteriorhodopsin at three nitrite concentrations (A) and their concentration dependence (B). Conditions like in Fig. 1

Only the time constant for the first component of the fit was plotted as a function of nitrite concentration, as the values for the second one were in the range of 100 ms, therefore too high to reflect changes in the bR photocycle. It can be easily seen that both transient and stationary photocurrents increase as the nitrite concentration increases (Fig. 3). At the same time, the kinetics is differently modified according to the concentration range on which the measurements were performed (Fig. 4). Thus, for nitrite concentrations up to 100 mM, the kinetics is accelerated and when the concentration is further raised it is slowed down.

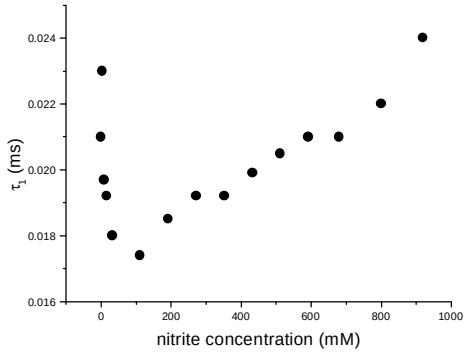

Fig. 4. The evolution of the time constant of the first component of the bi-exponential fit to the transient currents as the nitrite concentration increases.

A tentative explanation for the decrease of the time constant in the case of small concentrations would refer to a possible acceleration of the proton release at the extracellular side of the membrane due to the negative charge, which accumulates at the extracellular end of the proton channel. As the concentration of the anion increases, the nitrite can penetrate the proton channel and screen the positively charged residues that line the proton path and/or influence the interactions of the proton with the residues important for the reactions leading to proton translocation.

CONCLUSIONS

We carried out electrical measurements on planar lipid bilayers, without and with bacteriorodopsin incorporation, with the view to study the effects of the lyotropic anion nitrite. It was found that both the capacitance and conductance of the lipid bilayers on which bacteriorhodopsin containing purple membranes were adsorbed were affected when sodium nitrite at various concentrations was added to the bathing solution. Moreover, the transient and stationary photocurrents elicited by a light pulse in BR increased steadily as the nitrite concentration increased. In contrast, the kinetic behavior of the transient currents differed in the small concentrations range (up to 100 mM) from that for concentrations exceeding 100 mM. The kinetics was accelerated at small concentrations and slowed down at large concentrations. All the effects were tentatively explained by the capacity of nitrite ions to create a negative charge in the vicinity of the opening of the proton channel to the extracellular side of the membrane, due to the slightly chaotropic properties of the nitrite anion.

Acknowledgements. The authors thank Professor Bamberg for allowing them to perform part of the measurements in the laboratories of MPI for Biophysics, Frankfurt/Main, Germany. The research was partially funded from the research grant No. 827, CNCSIS.

REFERENCES

1. CACACE, M.G., E.M. LANDAU, J.J. RAMSDEN, The Hofmeister series: salt and solvent effects on interfacial phenomena, *Q. Rev. Biophys.*, 1997, **30**, 241–278.
2. CLARKE, R.J., Effect of lipid structure on the dipole potential of phosphatidylcholine bilayers, *Biochim. Biophys. Acta*, 1997, **1327**, 269–278.
3. CLARKE, R.J., C. LUEPFERT, Influence of Anions and Cations on the Dipole Potential of Phosphatidylcholine Vesicles: A Basis for the Hofmeister Effect, *Biophys. J.***,** 1999, **76**, 2614–2624.
4. COHN, E.J., J.T. EDSALL, Amino Acids and Peptides, In: *J.T. Proteins*, Reinhold Publishing Corp., N.Y., 1943, pp. 370–381.
5. DANCSHÁZY, Z., B. KARVALY, Incorporation of bacteriorhodopsin into a bilayer lipid membrane; a photoelectric-spectroscopic study, *FEBS. Lett.*, 1976, **72**, 136–138.

6. DÉR, A., J.J. RAMSDEN, Evidence for Loosening of a Protein mechanism, *Naturwiss*., 1998, **85**, 353–355.
7. EBREY, T.G., Light energy transduction in bacteriorhodopsin, In: *Thermodynamics of Membrane Receptors and Channels*, M. Jackson ed., CRC Press, New York 1993, pp. 353–387.
8. FAHR, A., P. LÄUGER, E. BAMBERG, Photocurrent kinetics of purple-membrane sheets bound to planar bilayer membranes, *J. Membr. Biol*., 1981, **60** , 51–62.
9. GANEA, C., A. BABES, C. LUEPFERT, E. GRELL, K. FENDLER, R.J.CLARKE, Hofmeister Effects on the Kinetics of Partial Reactions of the Na^+,K^+-ATPase, *Biophysical J*,. 1999, **77**, 267–281.
10. HENDERSON, R., J.M. BALDWIN, T.A. CESKA, F. ZEMLIN, E. BECKMANN, K.H. DOWNNING, Model for the structure of bacteriorhodopsin based on high resolution electron cryo microscopy, *J. Mol. Biol*., 1990, **213**, 899–929.
11. KIMURA, Y., D.G. VASSYLYEV, A. MIYAZAWA, A. KIDERA, M. MATSUSHIMA, K. MITSUOKA, K. MURATA, T. HIRAI, Y. FUJIYOSHI, Surface of bacteriorhodopsin revealed by high-resolution electron crystallography, *Nature*, 1997, **389**, 206–211.
12. LANYI, J.K., G. VÁRÓ, The photocycle of bacteriorhodopsin, *Isr. J. Chem.*, 1995, **35**, 365–385.
13. LANYI, J. K., Bacteriorhodopsin, *Int. Rev. Cytol*., 1999, **187**, 161–202.
14. NEAGU, A., M. NEAGU, A. DÉR, Fluctuations and the Hofmeister, *Biophysical J*., 2001, **81**, 1285–1294.
15. OESTERHELT, D., W. STOECKENIUS, Functions of a new photoreceptor membrane, *Proc. Natl. Acad. Sci. U. S. A* ., 1973, **70**, 2853–2857.
16. OESTERHELT, D., J. TITTOR, E. BAMBERG, A unifying concept for ion translocation by retinal proteins, *J. Bioenerg. Biomembr*., 1992, **24**, 181–191.
17. PEBAY-PEYROULA, E., G. RUMMEL, J.P. ROSENBUSCH, E.M. LANDAU, X-ray structure of bacteriorhodopsin at 2.5 Angstroms from microcrystals grown in lipidic phase, *Science*, 1997, **277**, 1676–1681.
18. VOGEL, R., G.B. FAN, M. SHEVES, F. SIEBERT, Salt dependence of the formation and stability of the signaling state in G protein-coupled receptors: Evidence for the involvement of the Hofmeister effect, *Biochemistry*, 2001, **40** , 483–490.