THE EFFECTS OF SOME LYOTROPIC ANIONS ON TRANSIENT AND STATIONARY PHOTOCURRENTS OF BACTERIORHODOPSIN

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Abstract. The effects of the lyotropic anions acetate, chloride and nitrate on the transient and stationary photocurrents of bacteriorhodopsin (bR) adsorbed to planar lipid bilayers were followed up at different anion concentrations. A different behavior was found in the case of kosmotropic (acetate) and chaotropic (nitrate) anions, both from the point of view of the transient and stationary currents dependency on anion concentration and from that of the photocycle kinetics. The effects of these anions were explained taking into account the possible interaction both with the dipole potential of the membrane and/or with the charged groups of the protein on the one side and the influence on the transport properties of the protein on the other side.

Key words: bacteriorhodopsin, BLM, lyotropic anions, photocurrents.

INTRODUCTION

Bacteriorhodopsin (bR), a retinal protein present in the plasma membrane of *Halobacterium salinarum,* functions as a light-driven proton pump [8, 14, 15], converting the light energy into an electrochemical proton potential across the membrane [17]. Several structural models for bR were proposed in the past, on the basis of electron crystallographic studies and recently a 0.25 nm resolution structure was published [13, 19]. Bacteriorhodopsin spans the plasma membrane in seven transmembrane α-helices, forming a channel divided in the middle into two parts by the chromophore retinal [11, 13, 19] which is covalently bound to the protein via a protonated Schiff base. 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 [13, 19]. The first event after the absorption of a photon is the isomerization of the chromophore retinal from all-*trans* to 13-*cis* and this initiates a

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photochemical cycle having as a result the release of one proton to the extracellular surface of the membrane and the uptake of another one from the cytoplasmic side. Biochemical and biophysical investigations, carried out on point-mutated bRs (for a review see [15]), 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 [15, 18].

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, 9, 10]. 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 study of the effects of Hofmeister anions in membrane structures could contribute, not only to elucidating the mechanisms underlying the function of these structures, but also to the development of some applicative domains such as food industry and pharmacology. In a previous study [12] we have found that the slightly chaotropic anion nitrite modifies the electrical and kinetic parameters of purple membranes adsorbed to BLM (**B**lack **L**ipid **M**embranes). We have proposed ourselves to study also another lyotropic anions like acetate (kosmotropic), chloride (neutral) and nitrate (chaotropic) in order to find if their different character modifies differently the electrical characteristics 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 [7, 16] indicated an acceleration of the photocycle in the presence of chaotropic ions (SCN^-) while it was found that kosmotropic anions had no effect on photocycle kinetics. The authors have explained these effects by modifications in the bR flexibility. In our case, both kosmotropic and chaotropic anions influenced the electrical response of the protein.

MATERIALS AND METHODS

The photocurrents generated in the bR containing membranes were measured using the BLM technique [9]. 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 [20]. 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 Na acetate, NaCl or $NaNO_3$ at various concentrations. The anion concentration was adjusted by using a stock solution of 4 M 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 [9]. 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

Transient (Fig. 1) and stationary (Fig. 2) photocurrents elicited by a light pulse were recorded from bR containing purple membranes (PM) adsorbed to a lipid bilayer at various anion concentrations ranging from 4 to 800 mM. The measurements for a given anion were performed on the same system PM/BLM.

It can be easily seen that both transient and stationary photocurrents increase as the acetate concentration increases (Fig. 3A, C). On the contrary, the currents in the presence of the chaotropic anion nitrate decrease as the nitrate concentration increases. The neutral chloride induces a different behavior of bR. At small chloride concentrations, up to 100 mM, the amplitude of the transient current decreases and afterwards it remains constant. When calculating the integral of the current, that is, the corresponding electrical charge transported, one can notice that it increases as the chloride concentration increases (Fig. 3 B). This can be partially explained by the change in the photocycle kinetics as the chloride concentration increases.

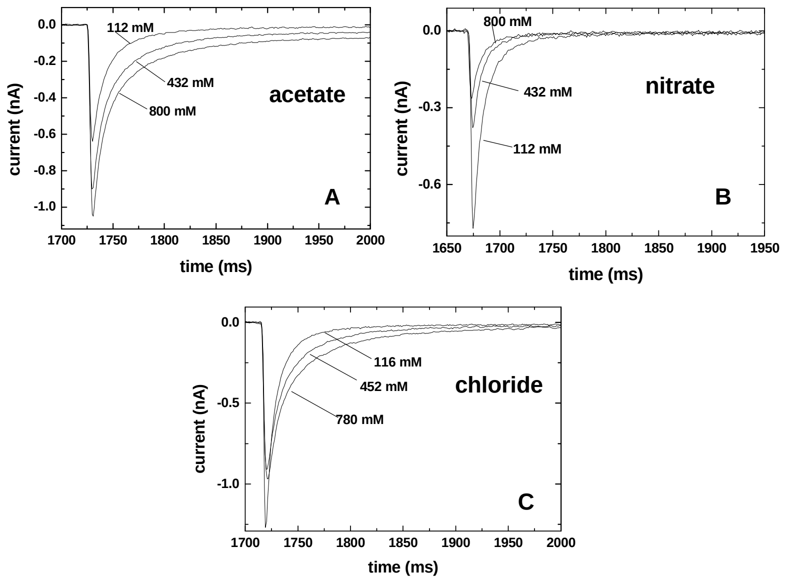

Fig. 1. Transient photocurrents of bacteriorhodopsin at three anion concentrations in the case of: acetate (A), nitrate (B) and chloride (C). The buffer solution contained 100 mM NaCl, 20 mM Hepes at pH 6.8.

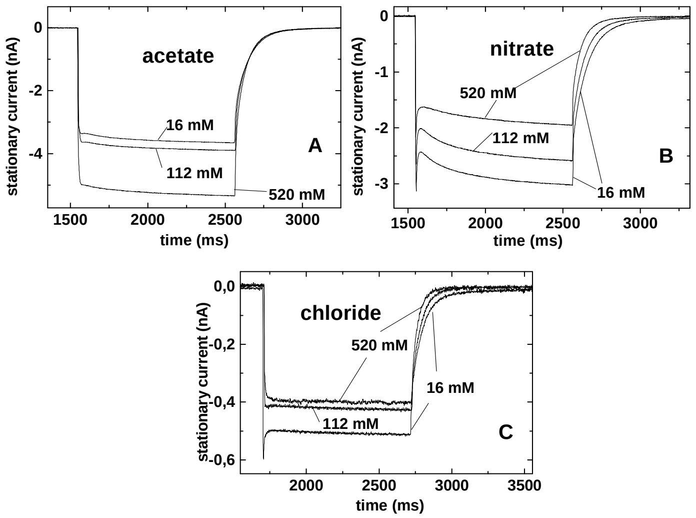

Fig. 2. Stationary photocurrents of bacteriorhodopsin at three anion concentrations in the case of: acetate (A), nitrate (B) and chloride (C). Conditions like in Fig. 1.

With the view to get some information about the effects of the anions on the photocycle kinetics the transients were fitted to a bi-exponential function.

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 qualitative information about the time course of the transient currents. Only the time constant for the first component of the fit was plotted as a function of anion 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.

Fig. 4 shows the evolution of time constant corresponding to the first exponential as the anion concentration increases. From the figure it can be seen that while the time constant is increasing significantly as chloride concentration increases, the time constant for the other two anions has only a slight increase. At the same time, at all concentrations the time constant for the chaotropic nitrate is smaller than that for kosmotropic acetate. This suggests an acceleration of the photocycle by the chaotropic anions, as already shown for other chaotropic anions (thiocyanate) [7]. On the contrary, the photocycle is slower for acetate and chloride.

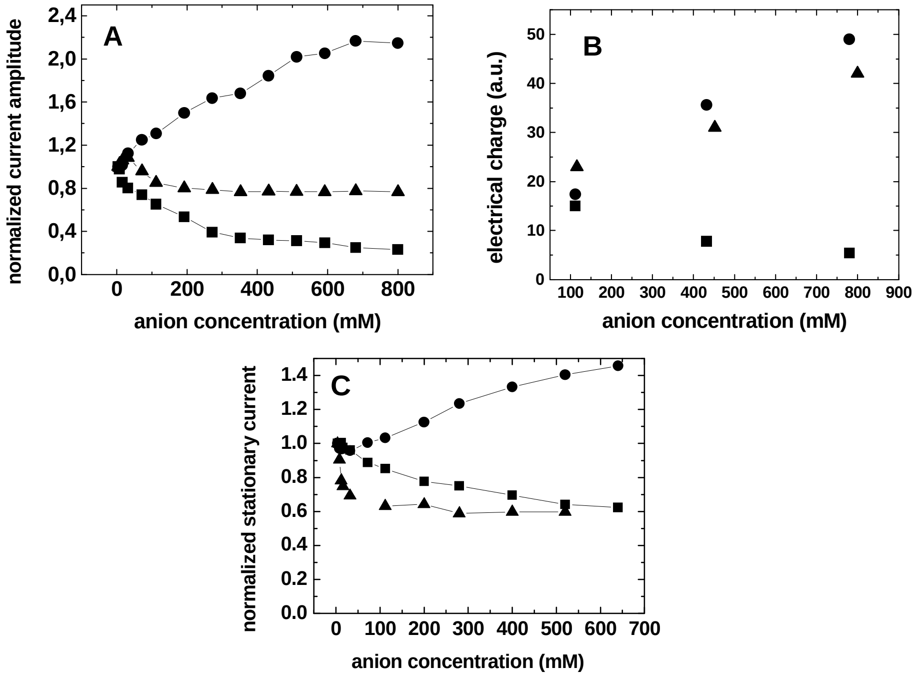

Fig. 3. Anion concentration dependence of the transient (A) and stationary (C) photocurrents of bacteriorhodopsin at three anion concentrations in the case of: acetate – solid circles, nitrate – solid squares, chloride – solid triangles and the electrical charge transported (B). Conditions like in Fig. 1.

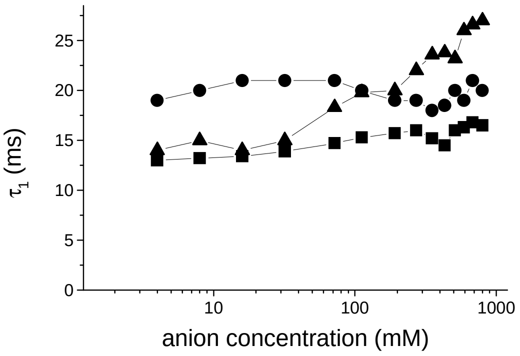

Fig. 4. The evolution of the time constant τ_1 of the first component of the bi-exponential fit to the transient currents, as the anion concentration increases (acetate – solid circles, nitrate – solid squares, chloride – solid triangles). The solid line is drawn to guide the reader's eye.

A possible interpretation of the effects induced by the chaotropic anion nitrate in the evolution of the transient and stationary photocurrents of bR, that is based also on previous findings [2, 3], would refer to the dipole potential of the lipid membrane. The dipole potential is believed to have a positive value in the interior of the membrane. The chaotropic anions have the tendency to enter the hydrophobic core of the membrane leading thus to the screening of the positive end of the intrinsic dipole potential [2, 3] and to a decrease of its value. As in the process of proton transport bR undergoes conformational changes, some conformations might be stabilized by the dipole potential of the membrane. Consequently, the changes induced by chaotropic anions in the dipole potential of the membrane might affect the stability of such conformations and, therefore, influence the photocycle kinetics. On the other hand, such a hypothesis is in good agreement with a previous interpretation [7] of the effect of the chaotropic anion thiocyanate on bR photocycle. The authors proposed that chaotropes could induce a slight loosening of the protein mechanism, increasing its flexibility. On the other hand, the kosmotropic anions, like acetate in our case, can accumulate at the membrane surface and contribute thus to a change in the electric field at the surface of the membrane. It is known that kosmotropes tend to organize the water structure and bR is very sensitive to the water content and water structure around purple membranes and inside the proton channel. Thus, it is to be expected that the structure of the water would become more rigid in the presence of such kosmotropes and this could lead to a slowing down of some steps in the photocycle. It is interesting to note that at concentrations up to 200 mM the time constants for the three anions decrease according to their position in the Hofmeister series, i.e. $\tau_{1\text{acetate}} > \tau_{1\text{chloride}} > \tau_{1\text{nitrate}}$. At higher concentration, however, in the case of chloride the time constant increases much more than for acetate as the anion concentration increases. It should be mentioned that chloride has a special role in the functioning of bR. Thus, it has been previously shown [6] that at very low pH chloride can be transported by bR. Therefore, the special behavior of chloride could be correlated to the fact that chloride can penetrate the proton channel and accumulate in the vicinity of the Schiff base creating a negative counterion, or even binding to it, and possibly slowing down the release of the proton to its acceptor D85.

CONCLUSIONS

We carried out electrical measurements on wild-type bacteriorhodopsin containing purple membranes adsorbed to planar lipid bilayers with the view to study the effects of the lyotropic anions nitrate (chaotropic) and acetate (kosmotropic) as compared to chloride effects. It was found that the transient and stationary photocurrents elicited by a light pulse in BR increased steadily as the acetate concentration increased and decreased as the chloride or nitrate concentration increased. At the same time, the kinetic behavior of the transient currents differed from one type of anion to the other. Thus, in the small concentrations range (up to 200 mM) the time constants decreased according to the position of the anion in the Hofmeister series. At higher concentrations the time constant of the transients currents in the presence of chloride increased much more than for two other anions, their evolution suggesting the binding of chloride, possibly in the vicinity of the Schiff base. At all concentrations the photocycle in the presence of nitrate was accelerated as compared to that in the presence of acetate. The effects were tentatively explained by the specific capacity of the anions to act on the electrostatic environment of the lipid membrane and of the protein and on the water structure at membrane surface and/or in the proton channel.

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