

Bacteriorhodopsin as a possible chloride pump

András Dér, Rudolf Tóth-Boconádi and Lajos Keszthelyi

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

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Purple membranes oriented and immobilized in gel show charge transfer at pH 0.55 if the pH is set by HCl. Current appears as laser flash driven transient and also as continuous current by quasi-continuous illumination. If the pH value 0.55 is set by H₂SO₄ continuous current is not observed. The results suggest that bacteriorhodopsin may pump chloride ions at low pH.

Bacteriorhodopsin; Membrane, oriented purple; Photocurrent; Chloride pump

1. INTRODUCTION

The retinal protein bacteriorhodopsin (BR) from *Halobacterium halobium* (Hh) is the simplest proton pump known in biological systems [1]. Comparative studies between BR and other retinal proteins are the subject of current interest in bioenergetics [1–4]. Bacteriorhodopsin and the Cl[−]-transporter protein of Hh, halorhodopsin (HR), show a number of structural similarities [3,5]. Many of these might be expected from the functional analogy of the two proteins, but there are common features in BR and HR which play an unelucidated role in the operation of these ion pumps. For example, BR contains two arginine residues in similar positions as HR [3,5]. These are probably the two anion binding sites involved in Cl[−] pumping of HR [3,5], yet BR is not known to transport anions. In this paper we report on photoelectric experiments carried out on the low-pH forms of BR, in order to clarify whether this unique chromoprotein can be excluded as a possible chloride pump or not.

2. MATERIALS AND METHODS

Purple membrane fragments prepared by the standard procedure [6] from *Halobacterium halobium* were oriented by a static electric field, and incorporated into a polyacrylamide gel matrix [7]. Gel slabs were washed in ion-free water, then incubated in H₂SO₄ and HCl solutions (pH 0.55), overnight.

Samples were excited by laser pulses of an excimer laser-pumped Rhodamine 6G dye laser (wavelength: 590 nm, 1 mJ pulse energy). Photoelectric signals were amplified by a fast, home-made current amplifier, and recorded by a computer-controlled transient recorder (Thurlby DSA-524) at room temperature (24°C).

Correspondence address: A. Dér, Institute of Biophysics, Biological Research Center of the Hungarian Academy of Sciences, H-6701 Szeged, P.O.B. 521, Hungary

Quasi-stationary photoelectric signals were detected by the same method, with quasi-permanent illumination. Samples were excited by a series of 16 laser pulses (repetition frequency: 50 Hz). Signals were filtered by a low-pass filter (cut-off frequency: 30 Hz).

Absorption spectra were taken by a Shimadzu UV-160 spectrophotometer.

3. RESULTS AND DISCUSSION

Why bacteriorhodopsin does not pump Cl[−] ions under normal conditions was discussed in a recent review [3]. Among others, either a lowered pK for arginine protonation or the compensating effect of negatively charged groups were advanced as possible explanations. Both of these possibilities inspired us to check the photoelectric behaviour of BR at such a low pH, where the arginines and the negatively charged groups which could compensate for the positive charges of the arginine residues are surely protonated.

When planning the experiments we took into account some analogue features of BR and HR. Namely, if the anion-binding sites of BR behave similarly to those of HR, they should have a much higher affinity for Cl[−] than for SO₄^{2−} ions [8]. The pH was, therefore, lowered to pH 0.55 either by H₂SO₄ or by HCl. In H₂SO₄ the purple membranes turned blue, with an absorption maximum of 603 nm (BR₆₀₃), while in HCl, after turning blue, they almost regained their original purple colour ($\lambda_{\max}$ = 564 nm; BR₅₆₄). This difference was attributed to Cl[−]-binding to the protein in the neighbourhood of the retina, in agreement with other investigations [9,10].

Pumping activity of the acidic forms of BR was checked by the gel method [7]. Transient electric signals of the blue and purple acidic forms of BR are shown in fig.1. The time integral of the photocurrent (i.e. the area under the curve) is proportional to the transported

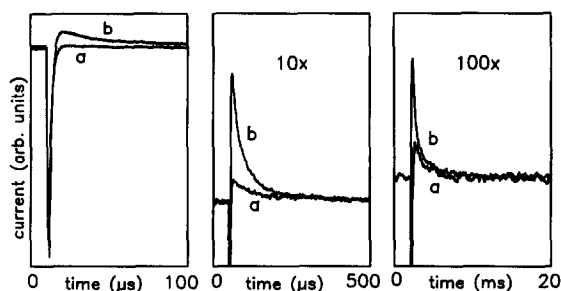


Fig.1. Transient photocurrent signals of the acidic forms of BR measured with three different time resolutions. The time integral of the signal is zero for BR₆₀₃ (a) and positive for BR₅₆₄ (b). The curves which are not normalized are the averages of 40 traces. Note the different amplitude and time scales.

charge [11]. This quantity was zero within the error of the measurement in the case of BR₆₀₃, but not for BR₅₆₄, suggesting no pumping activity in the former, and a net charge transport in the latter case. It could not be excluded, however, that there are further components (with longer lifetime) in the photoelectric signal. Hence quasi-stationary measurements were carried out to reveal the real pumping activities (figs 2 and 3).

Fig.2a,b shows the stationary photocurrent signals measured on samples containing BR₆₀₃ and BR₅₆₄, respectively. These results clearly demonstrate the existence of pumping activity in BR₅₆₄, and the lack of it in BR₆₀₃.

Two saturation curves are depicted in fig.3, where the ratio of Cl⁻ and SO₄²⁻ concentrations is changed at constant pH. The pumping activity and the amount of BR₅₆₄ show a similar tendency for saturation.

The amplitude of the maximal photocurrent is some 4 times less than that of BR in distilled water at pH 6 (data not shown). This difference may be explained by a lowered quantum efficiency of BR₅₆₄ compared to that of normal BR [10]. Pumping activity reaches half-saturation at as high X_{Cl} values as 0.3 (which cor-

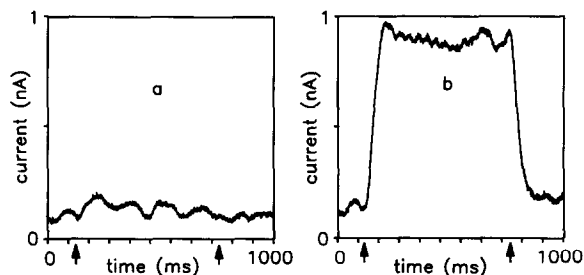


Fig.2. Quasi-stationary photocurrent signals of BR₆₀₃ (a) and BR₅₆₄ (b) at 24°C, pH 0.55 set by H₂SO₄ for BR₆₀₃ and HCl for BR₅₆₄. The curves depicted are the average of 64 traces. Arrow tips indicate timing of the first and the last laser pulse in the series. Transmission changes of BR₅₆₄ and BR₆₀₃ measured at 675 and 500 nm under the same conditions as the electric signals showed that stationary amplitude has been approximated to 90% within 30 ms (data not shown).

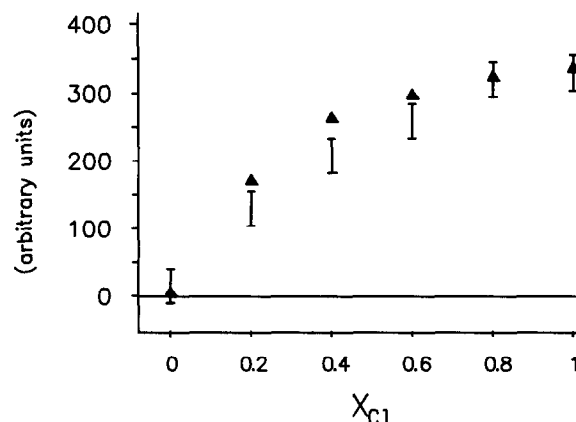


Fig.3. Pumping activity (error bars) and concentration of BR₅₆₄ (▲) versus Cl⁻ concentration given in molar ratio of the anions (X_{Cl}) at pH 0.55. The data are normalized at X_{Cl} = 1. Gel samples were incubated in solutions containing HCl and H₂SO₄ in different ratios, while pH was kept constant (0.55). The amplitude of the quasi-stationary photocurrent signal served as a measure of pumping activity. Absorption spectra of the samples were fitted with two Gaussians centered to 603 and 564 nm, and the amplitude of the latter one is shown in the figure.

responds to some 100 mM Cl⁻ concentration), i.e. the presence of a considerable amount of Cl⁻ is necessary to detect a pumping activity. This behaviour may explain the finding of a well-known paper, in which transient photoelectric signals of BR measured also at low pH by a different method were presented [12]. The photovoltage signal detected in that experiment lacked a positive phase at pH 0.9, suggesting no transport activity under the experimental conditions given. The Cl⁻ concentration in question, however, might have been too small to allow detection of net charge transport at this pH. (See also [10] for the pH and Cl⁻ concentration dependence of BR₅₆₄.)

In order to discuss, whether the transported charge is Cl⁻ or proton, absorption kinetic measurements were carried out on the acidic forms of BR at pH 0.55 (λ_{meas} = 400 nm). No sign of the M intermediate was found in either the photocycle of BR₆₀₃ or of BR₅₆₄ (data not shown), in agreement with other investigations [13]. Since the proton transport activity in BR seemingly requires an M-like intermediate [13,14], the lack of such a form and the appearance of a stationary current in the presence of Cl⁻ suggest the possibility of a Cl⁻-pump in BR₅₆₄. It is sure, however, that further experiments are needed to confirm this possibility.

Accepting the idea of a Cl⁻ pump then, by comparison of the sign of the electric signals of normal BR and BR₅₆₄, it is concluded that BR₅₆₄ transports Cl⁻ ions in the direction opposite to that in which normal BR pumps protons. Such a behaviour is expected also based on the postulate that the direction of Cl⁻ transport in cells is the opposite to that of proton transport [15].

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