Bacteriorhodopsin as a possible chloride pump

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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 H_2SO_4, 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 H_2SO_4 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).

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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 SO_4^- ions [8]. The pH was, therefore, lowered to pH 0.55 either by H_2SO_4 or by HCl. In H_2SO_4 the purple membranes turned blue, with an absorption maximum of 603 nm (BR_{603}), while in HCl, after turning blue, they almost regained their original purple colour (\(\lambda_{max} = 564\) nm; BR_{564}). 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 BR$_{603}$ (a) and positive for BR$_{564}$ (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 BR$_{603}$, but not for BR$_{564}$, 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 BR$_{603}$ and BR$_{564}$, respectively. These results clearly demonstrate the existence of pumping activity in BR$_{564}$, and the lack of it in BR$_{603}$.

Two saturation curves are depicted in fig. 3, where the ratio of Cl$^-$ and SO$_4^{2-}$ concentrations is changed at constant pH. The pumping activity and the amount of BR$_{564}$ 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 BR$_{564}$ compared to that of normal BR [10]. Pumping activity reaches half-saturation at as high X$_{Cl^-}$ values as 0.3 (which corresponds 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 BR$_{564}$.)

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 ($\lambda$$_{meas}$ = 400 nm). No sign of the M intermediate was found in either the photocycle of BR$_{603}$ or of BR$_{564}$ (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 BR$_{564}$. 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 BR$_{564}$, it is concluded that BR$_{564}$ 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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REFERENCES