

Effect of metal ion exchange on the photocurrent response from bacteriorhodopsin on tin oxide electrodes

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Abstract

The transient photocurrent response from bacteriorhodopsin (bR) on tin oxide electrodes was strongly influenced by metal ions bound to bR molecules. The photocurrent polarity reversal pH, which corresponded to the pH value for the reversal of the proton release/uptake sequence in the bR photocycle, of cation-substituted purple membrane (PM) was shifted to lower pH with the increase in the cation affinities to carboxyl groups and a close correlation was noted between the two values. This suggests that the metal ion present in the extracellular region of a bR molecule modulates the pK_a of proton release groups of bR by stabilizing the ionized state of the proton-releasing glutamic acids. The behavior of photocurrents at light-off in alkaline media, reflecting the proton uptake by bR, was unchanged by binding monovalent (Na^+ and K^+) or divalent cations (Mg^{2+} and Ca^{2+}), but was drastically changed by binding La^{3+} ions. This can be explained by invoking a substantial slowing of the proton uptake process in the presence of La^{3+} . © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Bacteriorhodopsin (bR), the retinal-containing protein in the purple membrane (PM) of *Halobacterium salinarum*, functions as a light-driven proton pump. Photoisomerization of the retinal chromophore drives the proton transport from the cytoplasmic side to the extracellular side of the membrane. The proton transport in bR is a cyclic process consisting of a series of optical intermediates, with lifetimes varying from picoseconds to milliseconds [1,2].

bR is normally purple ($\lambda_{max} = 568$ nm) and turns blue ($\lambda_{max} = 603$ nm) by removal of metal ions [3,4]. Metal ions bound to a bR molecule (Ca^{2+} and/or Mg^{2+} in nature) would affect the proton transport activity of bR. The binding sites of metal ions in a bR molecule have been studied extensively [5–15] following a report by Kimura et al. [3]. A variety of experimental evidence suggests that there are two high-affinity cation-binding sites and several low-affinity binding sites in a bR molecule [8–11]. It was recently reported that one of the high-affinity cation-binding sites

was located on a loop between the helix F and G on the extracellular surface by means of ^{13}C solid state NMR [14]. The role of metal ions on the proton transport process, however, has not been unraveled.

Photoelectric measurements have been employed to study the charge movements in bR molecules [16,17]. The flash-induced photoelectric signal from oriented PM, probably due to internal and/or external charge movements of bR, has at least three components with duration times lasting from picoseconds to milliseconds [18–27]. The duration times of these components correspond to interconversions of intermediate states along the bR photocycle. Therefore, the photoelectric signals could be a good probe to unravel the conformational changes and the proton translocations taking place at the transitions of bR intermediate states.

Miyasaka et al. [28–30] have reported another type of photoelectrochemical signal upon continuous illumination, where the time-differential photocurrents are generated from bR immobilized on tin oxide (SnO_2) electrodes by turning on and off the incident light. The origin of the unique photocurrent response has not been thoroughly understood yet, and extensive studies for unraveling its mechanism are still going on. Recent works suggest that the transient photocurrent responses originate from the interfacial pH

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change due to proton release and uptake by bR molecules [31–36]. This photoelectrochemical response has been successfully applied to investigate the bR proton transfer mechanism [37–42].

Recently, we reported that lanthanum ions (La^{3+}) drastically changed the pH dependence of the photocurrent responses from bR [42]. By binding La^{3+} to bR molecules, the photocurrent polarity reversal pH was lowered and by turning off the incident light, the photocurrent intensity was significantly decreased, as compared to wild-type bR. These could be rationalized by invoking the effect of La^{3+} on the bR proton pump activity.

Following the preliminary work, we examine here the effect of metal ions bound to bR molecules on the photoelectrochemical responses of bR and discuss the role of metal ions on the proton release and uptake of bR molecules.

2. Experimental

2.1. Preparation of cation-substituted purple membrane

Purple membranes (PM) were prepared from cultured *H. salinarium* S9 according to the method of Oesterhelt and Stoeckenius [43] and were deionized by passage through a cation exchange column (BioRad AG-50W) as described by Kimura et al. [3]. The blue membranes thus obtained were washed with distilled water and concentrated by centrifugation. Cation-substituted PMs were prepared by adding an aqueous solution of a metal chloride salt ($[\text{cation}]/[\text{bR}] = \text{ca. } 200, 50 \text{ and } 5$ for monovalent, divalent and trivalent cations, respectively) with a micropipette to the blue membranes, followed by immobilization on SnO_2 electrodes. Visible absorption spectra of purple and blue membrane were measured with a Jasco spectrophotometer Ubest-50.

2.2. Immobilization of purple membrane on SnO_2 electrode

SnO_2 electrodes (Nippon Sheet Glass, with a 450-nm thick SnO_2 layer) were washed first with hot methanol, then treated with hot concentrated sulfuric acid and rinsed thoroughly with distilled water. A 50- μl aliquot of the cation-substituted PM suspension, with an optical density of 1 at 570 nm for a 1-cm path length, was deposited on a 1- cm^2 area of the electrode, and then dried at room temperature and humidity.

2.3. Photocurrent measurements

The photocurrent measurement setup was essentially the same as the one reported elsewhere [34]. An Ushio Electric 500-W xenon arc lamp Model UXL-500D-O served as the light source. Infrared radiation was removed with an 18-cm path length water cell, and Toshiba Glass cut-off filters L-39 and G-55S were used to extract green light. A PM-immobilized electrode was mounted as a window (1 cm in

diameter) of a photoelectrochemical cell. The potential of the electrode was controlled with a Toho Technical Research potentiostat Model 2000 and a Ag/AgCl and a platinum wire served as reference and counter electrode, respectively. In order to avoid the exchange of metal ions in electrolyte solutions for cation-substituted PMs, metal chloride salts corresponding to the binding cations were used as supporting electrolyte. The photocurrents were measured with a Sony Tektronix oscilloscope Model TDS-340. A NF Electric Instruments low-pass filter Model E-3201B was used for photocurrent measurements in a pH region near the photocurrent polarity reversal.

3. Results and discussion

3.1. Visible absorption spectra of blue and cation-substituted purple membrane

Addition of metal ions reconverted the blue membrane into purple. Fig. 1 depicts the visible absorption spectra of the blue membrane and a typical cation-substituted PM (Ca^{2+} -substituted PM). The deionized blue membrane had an absorption maximum at 603 nm, while the Ca^{2+} -substituted PM, at 568 nm. The latter spectrum was essentially the same as that of wild-type PM, indicating that bR was completely regenerated. All the other cation-substituted PMs used in the present work exhibited almost the same visible absorption spectra as the Ca^{2+} -substituted PM.

3.2. Photocurrent patterns

Fig. 2 depicts typical photocurrent response patterns from Na^+ -substituted PM on SnO_2 electrodes at neutral, low and high pH. Hereafter, the photocurrent, by turning on and off the incident light, is denoted, respectively, as the light-on and light-off photocurrent. As compared with the pattern at neutral pH, the photoelectrochemical response

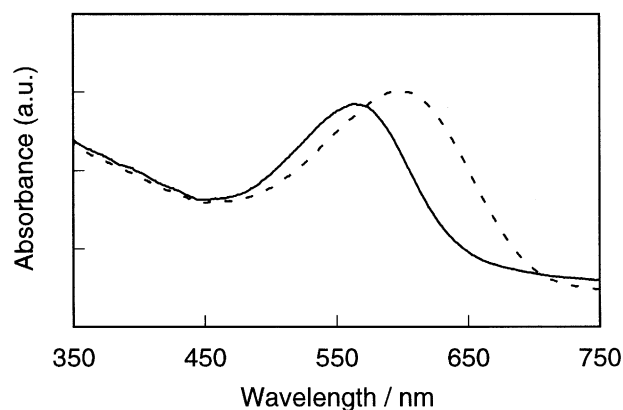


Fig. 1. Visible absorption spectra of Ca^{2+} -substituted purple membrane (solid curve) and blue membrane (dashed curve).

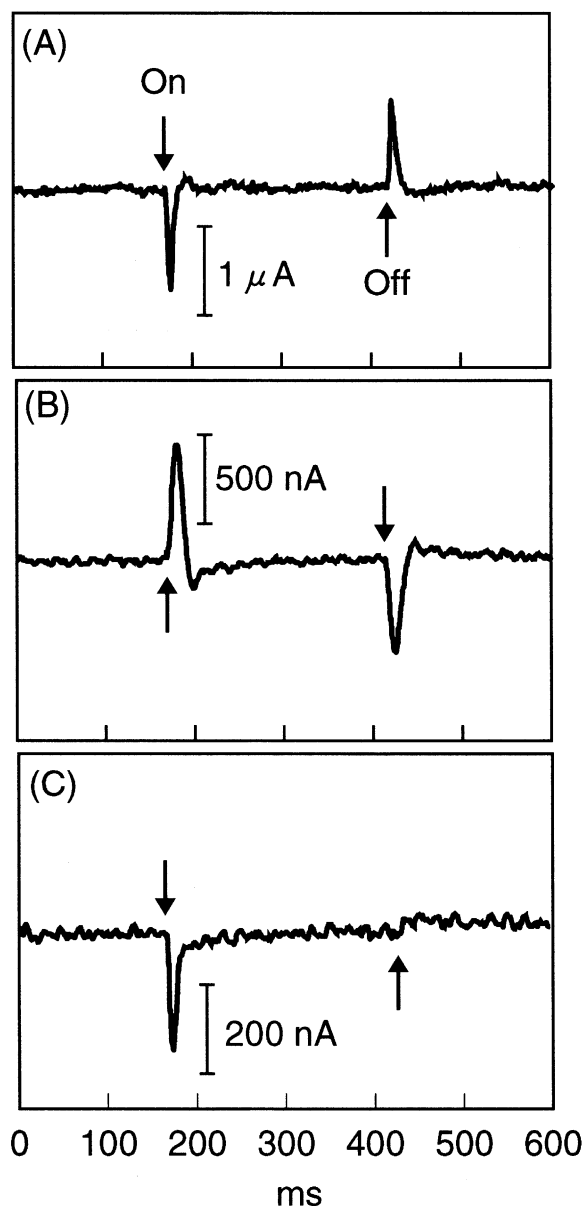


Fig. 2. Photocurrent response patterns from Na^+ -substituted PM on a SnO_2 electrode. (A) pH 7.0, (B) pH 3.4, (C) pH 9.6. Light intensity, $1.3 \times 10^2 \text{ mW cm}^{-2}$. Electrode potential, 0 V vs. Ag/AgCl.

was reversed at low pH and the light-off photocurrent lost its intensity at high pH.

Recent studies suggest the mechanism of the transient photocurrent generation from bR on SnO_2 electrodes as follows [31–36], although its origin has not been thoroughly unraveled yet. The transient photocurrent generation from bR on an oxide electrode is due to the shifting of proton dissociation equilibrium at the surface of an oxide electrode, arising from proton release/uptake by bR molecules [31,34]. At neutral pH, the interfacial pH is lowered at the onset of irradiation because the proton release precedes the proton uptake in the bR photocycle. This induces an anodic shift of the surface potential, which is governed by

the proton dissociation equilibrium of the surface hydroxide, of an oxide electrode. Under potentiostatic conditions as in the present work, such a potential shift is equivalent, in terms of the potential profile in the space charge layer, to a cathodic polarization of the oxide electrode, giving rise to a transient capacitive current. The transient light-on photocurrent could be interpreted in this manner. This is followed by a photostationary state of the bR photocycle during continuous irradiation, where no capacitive current is to be expected because the surface potential remains the same. At the termination of irradiation, the slower process (proton uptake) entails transiently, giving rise to an anodic capacitive current. Thus, the reversal of the proton release/uptake sequence causes the photocurrent polarity reversal at lower pHs. At higher pH, the proton uptake process is naturally slowed down. This reduces the value of $\Delta\text{pH}/\Delta t$ near the surface of an electrode, and therefore the light-off photocurrent loses its intensity.

The patterns shown in Fig. 2 were essentially the same as those from all the other cation-substituted PMs examined in the present study. However, the photocurrent polarity reversal pH was changed by metal ions bound to PM and the light-off photocurrent intensities from La^{3+} -substituted PM were significantly weak at neutral and high pH. The photocurrent behaviors of cation-substituted PMs in acidic and alkaline media are to be discussed in detail in the following sections.

3.3. Effect of metal ions on the photocurrent polarity reversal pH

The light-on photocurrent peak intensities of cation-substituted PMs at low pH are plotted in Fig. 3 as a function of electrolyte pH. As seen, the photocurrent polarity reversal pH depends on the nature of metal ions bound to bR molecules. This pH value was 5.1–5.3, 4.2–4.7 and 3.9 by binding monovalent, divalent and trivalent cations, respectively.

According to the photocurrent generation mechanism described above, the photocurrent polarity reversal pH is consistent with the pH value for the reversal of proton release/uptake sequences in bR. Hence, the results displayed in Fig. 3 indicate that metal ions bound to a bR molecule change the pH value at which the proton release/uptake steps are reversed.

In order to examine more closely the effect of metal ions, the photocurrent polarity reversal pHs for cation-substituted PMs are plotted in Fig. 4 against the logarithm of the equilibrium constant between acetic acid and metal ion (from Ref. [44]). The pH values of the photoresponse reversal were shifted to acidic with the increase in the equilibrium constant and were closely correlated. This indicates that the photocurrent polarity reversal pH is dependent on the cation affinities to carboxyl groups.

A hydrogen-bond network formed in the extracellular domain of a bR molecule, comprising several key amino acid residues and water molecules, has recently been the

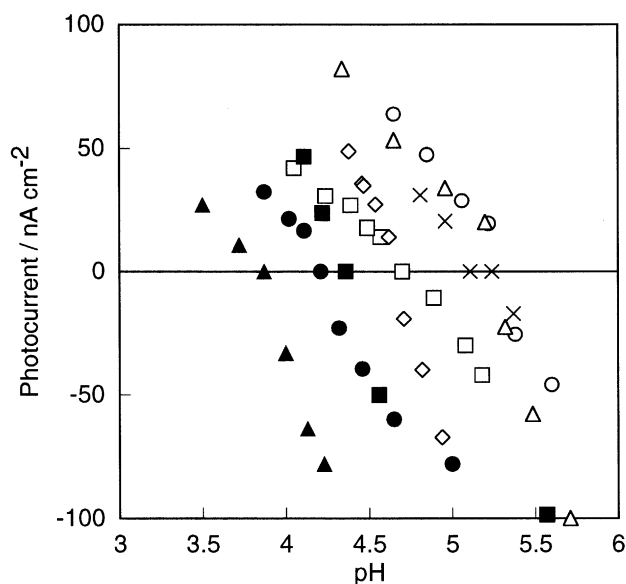


Fig. 3. pH-dependence of peak photocurrent by turning the light on for Li^+ (\times), Na^+ (\circ), K^+ (\triangle), Mg^{2+} (\square), Ca^{2+} (\diamond), Zn^{2+} (\bullet), Ba^{2+} (\blacksquare), and La^{3+} (\blacktriangle)-substituted PM. Electrolyte is 0.1 M metal chloride salt of the binding cation. The pH value was controlled with HCl.

subject of intense research with regard to the proton release by bR. The mechanism of proton release from bR is currently explained in terms of the coupling of pK_a between Asp85 which accepts a proton from the Schiff base and proton releasing groups at extracellular surface [45–48], although the proton releasing groups have not been finally identified. Upon protonation of Asp85, the pK_a of the proton release groups is lowered by about 5-pH units, which would cause the release of a proton if the external solution were at neutral pH. Thus, the pH for the reversal of proton release and uptake sequences in bR would be determined by the pK_a of the proton-releasing glutamic acids.

One of the high-affinity cation-binding sites exists on the loop between helix F and G on the extracellular surface, close to proton release groups [14], although it is not clear whether the cation binds to carboxyl groups of amino acid residues or lipid molecules. The metal ion at this site would stabilize the dissociated state of carboxyl groups of the proton-releasing glutamic acids, resulting in the lowering of pK_a of the proton release groups. We propose that the metal ion present in the extracellular region of a bR molecule modulates the pK_a of the proton release groups.

The slope in Fig. 4 is not unity, probably because of the difference in the environmental conditions around the carboxyl groups. Namely, the proton-releasing carboxyl groups are located inside the proteins, whereas the equilibrium constants represented in Fig. 4 have been estimated in aqueous solutions.

Local structural changes or surface potential change on the extracellular surface by binding metal ions may be additional possibilities of the pK_a shift of the proton release groups in bR molecules. Metal ions would bind to PM in

association with negative groups of the membrane surface. This may change the surface potential of PM and hence indirectly affect the proton-releasing complex at the extracellular surface. Further structural and spectroscopic studies on the extracellular region of cation-binding bR is needed to elucidate the effect of metal ions on proton release groups in bR.

3.4. Effect of metal ions on light-off photocurrents at high pH

Light-off photocurrents from PM on SnO_2 electrodes lose their intensities at high pHs [39]. The interfacial pH is raised at the termination of irradiation by bR proton uptake in neutral and alkaline media, and this generates an anodic transient photocurrent. The bR proton uptake is naturally slowed at higher pHs [49–50–51] and this reduces the value of $\Delta\text{pH}/\Delta t$ near the surface of SnO_2 electrodes. Therefore, the light-off photocurrent loses its intensity in alkaline media. The behavior of light-off photocurrents thus reflects the proton uptake process in the bR photocycle. The bR mutants which slowed the proton uptake process showed the similar suppression of the light-off photocurrents [38,40,41], indicating that the delay of the proton uptake step caused the loss of the bR light-off photocurrent intensities at neutral and high pHs.

The ratio of the light-off to the light-on photocurrent peak intensities, $I_{\text{off}}/I_{\text{on}}$, from cation-substituted PMs were examined at neutral and high pHs, and the results are depicted in Fig. 5. Among them, monovalent and divalent

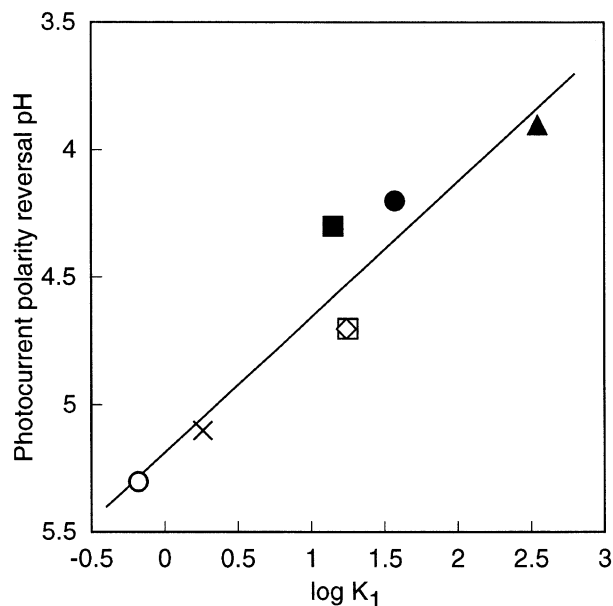


Fig. 4. Plots for the photocurrent polarity reversal pH against the logarithm of the equilibrium constant between metal ion and acetic acid (from Ref. [44]). Li^+ (\times), Na^+ (\circ), Mg^{2+} (\square), Ca^{2+} (\diamond), Zn^{2+} (\bullet), Ba^{2+} (\blacksquare) and La^{3+} (\blacktriangle)-substituted PM.

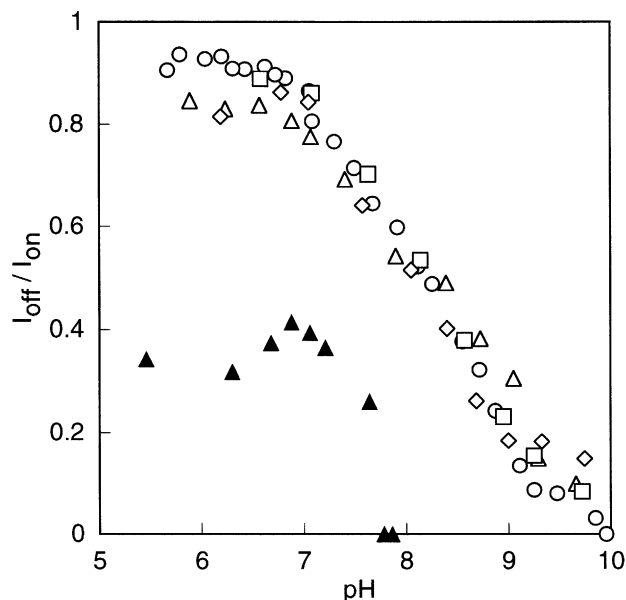


Fig. 5. pH-dependence of amplitude ratio of the photocurrent at light-off (I_{off}) to that at light-on (I_{on}) for Na^+ (\circ), K^+ (\triangle), Mg^{2+} (\square), Ca^{2+} (\diamond) and La^{3+} (\blacktriangle)-substituted PM. Electrolyte, 0.1 M metal chloride salt of the binding cation. The pH value was controlled with HCl or NaOH.

cation-substituted PMs exhibited almost the same behavior, where the amplitude ratio decreased systematically from ca. 0.9 to 0 by raising pH. The curves of $I_{\text{off}}/I_{\text{on}}$ plots by raising pH coincided with those by lowering pH (data not shown), demonstrating that the results in Fig. 5 reflect an inherent photochemistry in the cation-substituted bR and not their denaturation. The results obtained here thus indicate that the binding of monovalent or divalent cations would not influence the proton uptake delay of bR in alkaline media.

In sharp contrast, La^{3+} ions significantly decreased the light-off intensities. The $I_{\text{off}}/I_{\text{on}}$ ratio from La^{3+} -substituted PM was small even at neutral pH, and abruptly decreased at higher pHs. It was reported that La^{3+} substantially slowed down the proton uptake process of bR [52,53]. The slowing of proton uptake suppresses the interfacial pH change at the termination of irradiation and therefore, the light-off photocurrents from La^{3+} -substituted PM lose their intensities. The unique behavior of the light-off photocurrents from La^{3+} -substituted PM is therefore in line with the La^{3+} effect on the proton uptake process of bR.

4. Summary

Metal ions bound to bR molecules had a pronounced influence on the photocurrent responses from bR on SnO_2 electrodes at low and high pHs. The pH of photocurrent polarity reversal was shifted to lower pH with the cation affinity to carboxyl groups. This suggests that metal ions at the extracellular region affect the proton release step in the bR photocycle. Light-off photocurrent intensities were

much weaker by the binding of La^{3+} compared with other cation-substituted PMs because of the substantial slowing of the proton uptake step in the bR photocycle in the presence of La^{3+} ions. The present photoelectrochemical system could be a powerful tool for unraveling the proton transport mechanism of bR.

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