Effect of Lanthanum Ions on the Photoelectrochemical Response of Bacteriorhodopsin

Yoshitaka Saga, Tomoko Ishikawa, and Tadashi Watanabe*
Institute of Industrial Science, The University of Tokyo, Roppongi, Minato-ku, Tokyo 106-8558

(Received October 27, 2000; CL-000984)

Lanthanum ions (La^{3+}) drastically changed the pH dependence of the photocurrent responses from bacteriorhodopsin (bR). The pH value of the photocurrent polarity reversal was shifted to lower pH and the ratio of the light-off to the light-on photocurrent peak intensities, I_{off}/I_{on}, was significantly decreased, compared with the photoresponse from the wild-type bR. These results are explained by invoking the effect of La^{3+} on the bR proton pump activity.

Bacteriorhodopsin (bR), the light-driven proton pump of *Halobacterium salinarium*, is normally purple (λ_{max}=568 nm), and turns blue (λ_{max}=603 nm) by removal of metal ions.[1–3] Metal ions bound to a bR molecule (Ca^{2+} and Mg^{2+} in nature) would contribute to the color regulation and proton pump function of bR. The role of metal ions on the proton transport of bR, however, still remains ambiguous. It was reported that lanthanum ions (La^{3+}) changed the bR photocycle drastically,[4,5] although the mechanism at molecular level has not been unraveled. A study on the proton pump activity of La^{3+}-substituted bR, compared with wild-type, will lead to an elucidation of the role of metal ions on the proton transfer process of bR.

We have proposed that photoelectrochemical response from bR at electrode-electrolyte interfaces, which originates in the interfacial pH change arising from proton release/uptake by bR molecules,[6–10] is useful in probing the proton pump of bR. Here we report the effect of La^{3+} on the photoelectrochemical response from bR.

Purple membrane suspensions, purified from *Halobacterium salinarium* S9 according to the conventional method of Oesterhelt and Stoeckenius,[11] were deionized by passage through a cation exchange column (BioRad AG-50W) as described by Kimura et al.[2] La^{3+}-substituted bR was prepared by adding $LaCl_3$ solution with a micropipette to the deionized bR. The optical density at absorbance maximum of La^{3+}-substituted bR suspension was adjusted to 1 by adding pure water, and a 50-µL aliquot of the bR suspension was deposited on a 1-cm^2 area of an SnO_2 plate (Nippon Sheet Glass), followed by drying at room temperature and humidity. Photocurrent measurements were conducted in a manner similar to that described eleswhere.[8] A band-pass filter (Toshiba Glass, G-55S) was used to extract green light from an Ushio Electric xenon arc lump Model UXL-500D-O. The electrode potential was controlled with a Toho Technical Research potentiostat Model 2000, and an Ag/AgCl and a platinum wire served as the reference and the counter electrode, respectively. Photocurrents were recorded with a Sony Tetronix oscilloscope Model TDS-340. An NF Electric Instruments low-pass filter Model E-3201B was used for photocurrent measurements in the pH region near the photocurrent polarity reversal.

Figure 1 depicts typical photocurrent response patterns from a La^{3+}-substituted bR on an SnO_2 electrode at neutral and low pH.[12] Hereafter the photocurrent by turning on and off of incident light is denoted as the light-on and light-off photocurrent, respectively. The decay time of the photocurrent from a La^{3+}-substituted bR is about 50 ms, much slower than that from wild-type bR. The photoelectrochemical response is reversed at lower pH, compared with the pattern at neutral pH. The pH of photocurrent polarity reversal for La^{3+}-substituted bR is ca. 3.9, and is much lower than that of wild-type bR (ca. 5.2). We have reported that the polarity reversal pH coincided with the pH value at which the proton release/uptake steps were reversed.[9] Thus the result obtained here indicates that La^{3+} lowers the pH value for the reversal of the proton release/uptake sequence. This suggests that La^{3+} bound to bR lowers the pK_a of the proton release group existing in the extracellular region of bR molecules, because the sequence of the proton release and uptake would be determined by the pK_a of the proton release

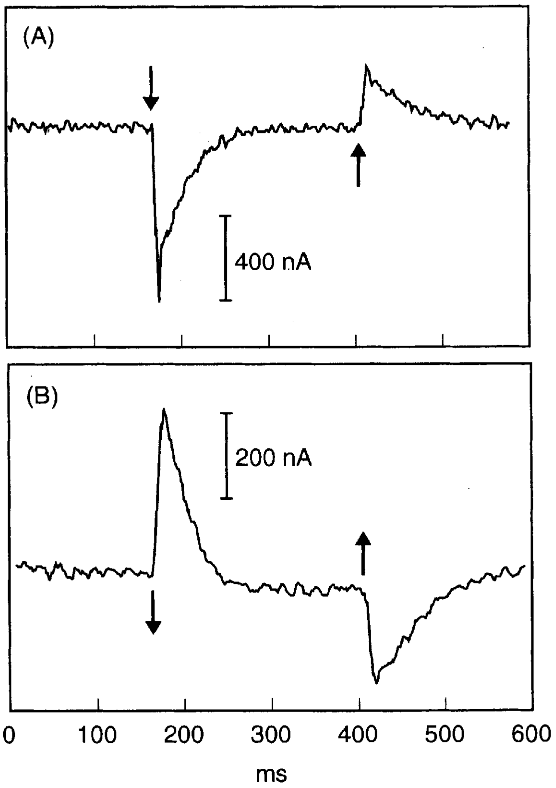

Figure 1. Photocurrent response patterns from La^{3+}-substituted bR on SnO_2 electrode. (A) pH 7.1: (B) pH 2.3. The down and up arrows denote light on and light off, respectively. Electrode potential, 0.0 V vs Ag/AgCl. Light intensity, 1.2×10^2 mW cm^{-2}. Electrolyte, 50 mM $LaCl_3$ with 10 mM Tris buffer. The pH value was controlled with HCl.

Copyright © 2001 The Chemical Society of Japan

group.[13,14] Recently, it was reported that one of the high affinity cation-binding sites was located around the loop between the helix F and G on the extracellular surface,[15] close to the proton release groups (Glu-204 and/or Glu-194). The deprotonated state of these groups would be more stable by binding a trivalent La^{3+} ion at this site, relative to Ca^{2+} or Mg^{2+}. We propose here that the metal ion at this high affinity cation-binding site stabilizes the ionized state of the carboxyl groups of the proton-releasing glutamic acids, resulting in the lowering of pK_a of the proton-releasing groups.

The light-off photocurrent intensity from a La^{3+}-substituted bR is much weaker than the light-on photocurrent, and this pattern is similar to that from the wild-type bR at alkaline pH. The ratio of the light-off to the light-on photocurrent peak intensities, I_{off}/I_{on}, was examined as a function of pH, and the results are depicted in Figure 2. The amplitude ratio from La^{3+}-substituted bR was nearly constant between pH 5 and 7, and abruptly decreased at higher pH. This could be explained as follows. As reported previously, the interfacial pH is raised at the termination of illumination by bR proton uptake in neutral and alkaline media, and this generates an anodic capacitive photocurrent.[8] The proton uptake by wild-type bR is naturally slowed at higher pHs,[16,17] and therefore the light-off peak photocurrent loses its intensity.[9] La^{3+} ions significantly slowed down the proton uptake process even at neutral pH.[4,5] Thus, the unique behavior of light-off photocurrent from a La^{3+}-substituted bR is viewed as neatly reflecting the substantial slowing of the proton uptake when La^{3+} ions are bound to a bR molecule.

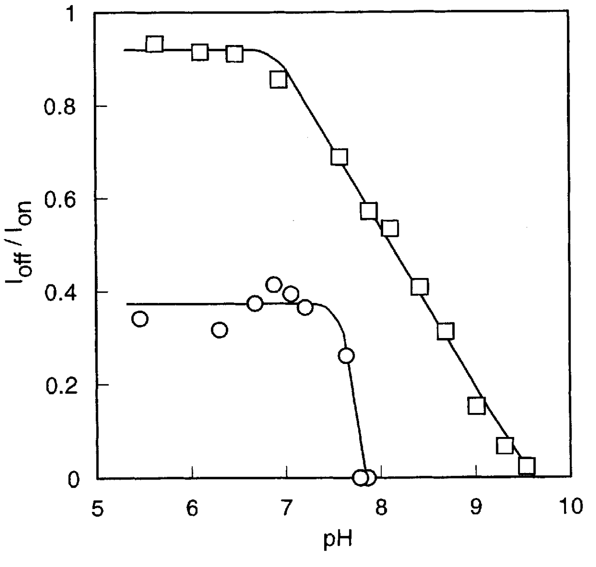

Figure 2. pH dependencies of amplitude ratio of the light-off photocurrent (I_{off}) to light-on photocurrent (I_{on}) for La^{3+}-substituted bR (○) and wild-type bR (□).

To summarize, the photoelectrochemical response from bR at electrode–electrolyte interfaces was strongly influenced by La^{3+} ions, and these are in line with the effects of La^{3+} on the proton release/uptake process of bR. These results suggest that metal ions bound to a bR molecule regulate the proton pump activity at molecular level.

This work was supported in part by a Grant-in-Aid for Scientific Research (No. 11875185) from Ministry of Education, Science, Sports and Culture of Japan, to T.W.

References and Notes

1 R. Jonas, Y. Koutalos, and T. G. Ebrey, *Photochem. Photobiol.*, **52**, 1163 (1990).
2 Y. Kimura, A. Ikegami, and W. Stoeckenius, *Photochem. Photobiol.*, **40**, 641 (1984).
3 C. -H. Chang, J. -G. Chen, R. Govindjee, and T. G. Ebrey, *Proc. Natl. Acad. Sci. U.S.A.*, **82**, 396 (1985).
4 L. A. Drachev, V. N. Frolov, A. D. Kaulen, E. A. Liberman, S. A. Ostroumov, V. J. Plakunova, A. Y. Semenov, and V. P. Skulachev, *J. Biol. Chem.*, **251**, 7059 (1976).
5 A. L. Drachev, L. A. Drachev, A. D. Kaulen, and L. V. Khitrina, *Eur. J. Biochem.*, **138**, 349 (1984).
6 B. Robertson and E. P. Lukashev, *Biophys. J.*, **68**, 1507 (1995).
7 J. -P. Wang, L. Song, S. -K. Yoo, and M. A. El-Sayed, *J. Phys. Chem. B*, **101**, 10599 (1997).
8 Y. Saga, T. Watanabe, K. Koyama, and T. Miyasaka, *J. Phys. Chem. B*, **103**, 234 (1999).
9 Y. Saga, T. Watanabe, K. Koyama, and T. Miyasaka, *Chem. Lett.*, **1998**, 961.
10 Y. Saga, T. Watanabe, K. Koyama, and T. Miyasaka, *Anal. Sci.*, **15**, 365 (1999).
11 D. Oesterhelt and W. Stoeckenius, *Methods Enzymol.*, **31**, 667 (1974).
12 According to the custom in electrochemistry, the cathodic and anodic currents are given here in the downward and upward directions, respectively, in a manner opposite to the one taken in previous works.[6,7]
13 H. -T. Richter, L. S. Brown, R. Needleman, and J. K. Lanyi, *Biochemistry*, **35**, 4054 (1996).
14 S. P. Balashov, E. S. Imasheva, T. G. Ebrey, N. Chen, D. R. Menick, and R. K. Crouch, *Biochemistry*, **36**, 8671 (1997).
15 S. Tuji, S. Yamaguchi, M. Tanio, H. Konishi, S. Inoue, A. Naito, R. Needleman, J. K. Lanyi, and H. Saito, *Biophys. J.*, **76**, 1523 (2000).
16 H. Otto, T. Marti, M. Holz, T. Mogi, M. Lindau, H. G. Khorana, and M. P. Heyn, *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 9228 (1989).
17 Y. Cao, L. S. Brown, R. Needleman, and J. K. Lanyi, *Biochemistry*, **32**, 10239 (1993).