Redox Potential of Bacteriorhodopsin in Purple Membrane Determined by Differential Pulse Voltammetry

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Differential pulse voltammograms of bacteriorhodopsin (BR) in a purple membrane showed one redox peak at −781 mV for light-adapted BR and two redox peaks at −484 and −781 mV for dark-adapted BR. The redox potential did not significantly depend on pH in the range of 3.0 to 11.0 (about 5 mV/pH), indicating that a change in the protonation state was not involved in this redox process. The C=N bond in the Schiff base of BR is responsible for the observed redox reactions.

Key words bacteriorhodopsin; differential pulse voltammetry; redox potential; retinal configuration

Bacteriorhodopsin (BR), the only pigment protein in purple membrane of *Halobacterium salinarium*, functions as an electrogenic light-driven proton pump, translocating two or more protons from the cytoplasmic to extracellular medium.[1] BR in the purple membrane is a chromophore-protein complex with a single molecule of retinal covalently bound to the protein as a Schiff base. It is of interest to determine whether or not the process including the redox transformations of the molecules is connected with light-driven proton pumping. It has been reported that BR undergoes redox transformations on the electrode.[2,3] Suponeva *et al.*[3] showed that only one polarographic reduction wave was observed for BR and that light-adapted and dark-adapted BRs gave polarographic waves of the same shape. We now report a differential pulse voltammetric study concerning the redox reaction of BR in the purple membrane. Our results showed different redox potentials for light-adapted and dark-adapted BRs.

The purple membrane was prepared from *H. salinarium* strain S-9 cells, according to the method of Oesterhelt and Stoeckenius.[4] The freeze-dried purple membrane fragments were suspended in 0.1 M KCl solution and the pH of the suspension was adjusted using 1 M HCl and 1 M NaOH. The redox potential was measured by the differential pulse voltammetric method. Voltammograms were recorded with a Yanako-P-1100 polarographic analyzer (Kyoto, Japan). For voltammetric measurements, a three-electrode circuit was employed (working electrode, Pt plate [1.0 cm×5.0 cm×0.1 cm]; counter electrode, Pt wire [0.1 cmϕ×5.0 cm]; reference, SCE). All potentials in this study are given *vs.* SCE.

Figure 1 shows the differential pulse voltammograms of BR in the purple membrane in the light-adapted state, together with the bleached BR which was treated with hydroxylamine under light irradiation. Light-adapted BR showed a redox peak at −781 mV and no redox peak was observed for bleached BR. Therefore the observed redox peak is attributable to the retinal chromophoric part of the molecule including Schiff base. In the light-adapted state, the retinal chromophore with an absorption maximum at 568 nm is in an all-*trans* configuration, and in the absence of light, the retinal converts to a mixture of the all-*trans* and 13-*cis* configuration,[5] absorbing at 557 nm. Figure 2 shows the effect of light irradiation on the redox potential of BR. Dark-adapted BR showed two peaks at −484 mV and −781 mV. The electric

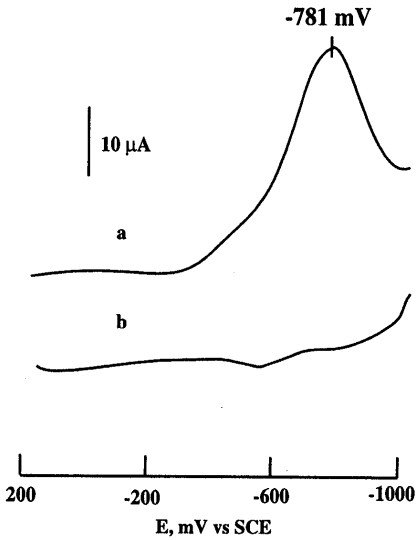

Fig. 1. Voltamgrams of BR (a) and Bleached BR (b) at pH 7.4.
Scan speed, 20 mV/sec; pulse amplitude, 50 mV.

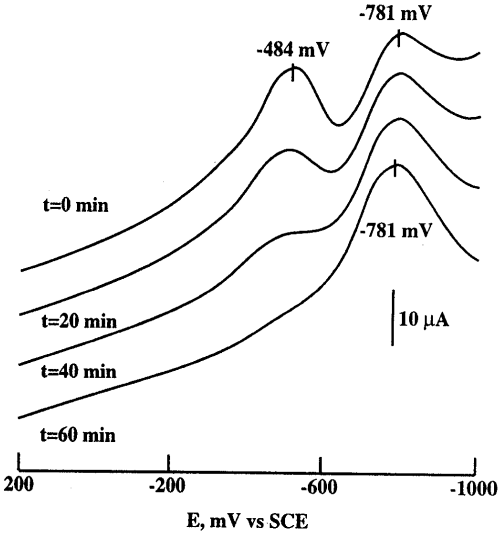

Fig. 2. Effects of Light Iirradiation Times of the Redox Potential of BR at pH 7.4
t=0 min, Dark-adapted BR; t=90 min, light-adapted BR. Scan speed, 20 mV/sec; pulse amplitude, 50 mV.

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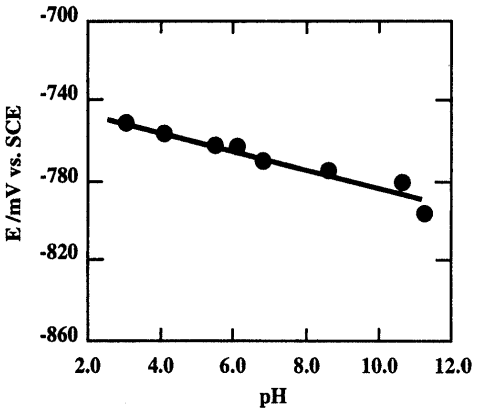

Fig. 3. pH Dependence of Redox Potential for BR in Purple Membrane

current at the higher potential peak of −484 mV decreased with irradiation time and completely disappeared at times longer than about 60 min. The elapsed time of 60 min is exactly equal to the time required for the transformation from the 557-nm species, consisting of the mixture of all-*trans* and 13-*cis* isomers of the retinal chromophore, to the 567-nm one, consisting only of the all-*trans* isomer. This indicates that the −781-mV peak, which is present in both the light- and dark-adapted states, is closely correlated to the all-*trans* retinal configuration and the −484-mV peak in the dark-adapted state is correlated to the 13-*cis* configuration. Suponeva *et al.*,[3] reporting results different from ours, found polarographic reduction waves of the same shape (peak potential −1.0 V) for both light-adapted and dark-adapted BRs. Figure 3 shows the relationship between pH and the redox potential in the pH range of 3.0 to 11.0. The redox potential did not significantly depend on pH in this range (about 5 mV/pH), indicating that a change in the protonation state was not involved in this redox process.

Taking into account the same redox potential for both the all-*trans* and 13-*cis* retinal in 60% aqueous ethanol,[2] the differences in the redox potentials between the all-*trans* (light-adapted form) and 13-*cis* configurations (dark-adapted form) of the retinal in BR (Fig. 2) are considered to be caused by the interaction between the chromophore part including the Schiff base and the amino acid residues in the apoprotein. A computer modeling study of the structure of BR by Sankararamakrishnan *et al.*[7] indicated that the distance between the Schiff base nitrogen and the carboxylate oxygen atoms of the Asp 96-residue, which is known to reprotonate the Schiff base during later stages of the photocycle, was 12—13 Å for the all-*trans* configuration of the retinal and 4—5 Å for the 13-*cis* configuration. Therefore the formation of the NH···O (Asp 96) hydrogen bond in the retinal binding pocket is possible for only the 13-*cis* configuration. The formation of the hydrogen bond and the conformational change of the retinal Shiff base by transition from the all-*trans* to the 13-*cis* form result in a lowering of electron density of the C=N bond in the 13-*cis* form. This a possible reason why the electroreduction potential (−484 mV) of the C=N bond in the Schiff base for the 13-*cis* form is significatly lower than that (−781 mV) for the all-*trans* form (Fig. 2). The effect of hydrogen bonds on redox potentials has been recognized, particularly in redox proteins.[8] We conclude that the different redox potentials of BR in the light-adapted and dark-adapted states are attributable to hydrogen bonding between the NH bond in the Schiff base and the amino acid residues around the Schiff base.

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