Journal of Photoscience (2002), Vol. 9(2), pp. 118–121

Structural Stability of High-Temperature State of Bacteriorhodopsin: A Model of Multi-state Membrane Proteins

Shigeki Mitaku*, Yasunori Yokoyama and Masashi Sonoyama

Department of Biotechnology, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan

A state of bacteriorhodopsin at high temperature was studied by various spectral measurements. The stability measurements indicated that the onset temperature of the denaturation was 70 °C in the dark and 60 °C under illumination. The reactivity of hydroxylamine with the Schiff's base also significantly increased in the temperature range between 60 and 70 °C. A spectral band at about 470 nm appeared in the temperature range higher than 60 °C. The circular dichroism spectra in the visible region started to change from a bilobed exiton type to a positive band at about 60 °C, suggesting that the two-dimensional configuration of bacteriorhodopsin molecules changed from crystalline to amorphous. All the measurements suggested a new state between 60 and 70 °C in which bacteriorhodopsin is stable only in the dark.

Key words: bacteriorhodopsin, intermediate state, high temperature state, photobleaching, denaturation kinetics

INTRODUCTION

Bacteriorhodopsin has been extensively studied as a model membrane protein, having seven transmembrane helices [1]. It was used as a structural template for the homology modeling of receptor proteins as well as archeal retinal proteins, when the 3D structure of other membrane proteins with seven transmembrane helices were not available [2]. The stage of the investigation of membrane protein structures is now shifting to the problem of the structural change related with the functioning mechanism [3].

It was recently established that the 3D structure of bacteriorhodopsin changes during the photocycle, as shown in Fig. 1. The essence of the structural change by the light energy absorbed by a retinal is the conversion of the direction of a proton channel from the Schiff's base to the aqueous phase [4]. The Schiff's base is open to the external side in the ground state, while it is open to the cytoplasmic side in the photo-intermediate states, M and N (Fig. 1b). Similar bistable structural switching also occurs in other types of membrane proteins: a channel and a receptor. An ion channel has two structures, open and close, leading to the generation of the action potential of membrane. A receptor changes the 3D structure upon the specific binding a ligand molecule and triggers the next step of the signal transduction. These examples indicate that many membrane proteins are molecular machines, having two stable or metastable structures during the function processes. Bacteriorhodopsin is again a good model system for the investigation of the structural change in a membrane proteins because much information is available for this protein.

In this work, we focused on a high temperature state just below the thermal denaturation (Fig. 2). Previous works about the thermal denaturation suggested that there is a state different from the ground state at room temperature [5, 7]. Denaturation point of bacteriorhodopsin by DSC and absorption spec-

*To whom correspondence should be addressed.
E-mail : mitaku@cc.tuat.ac.jp

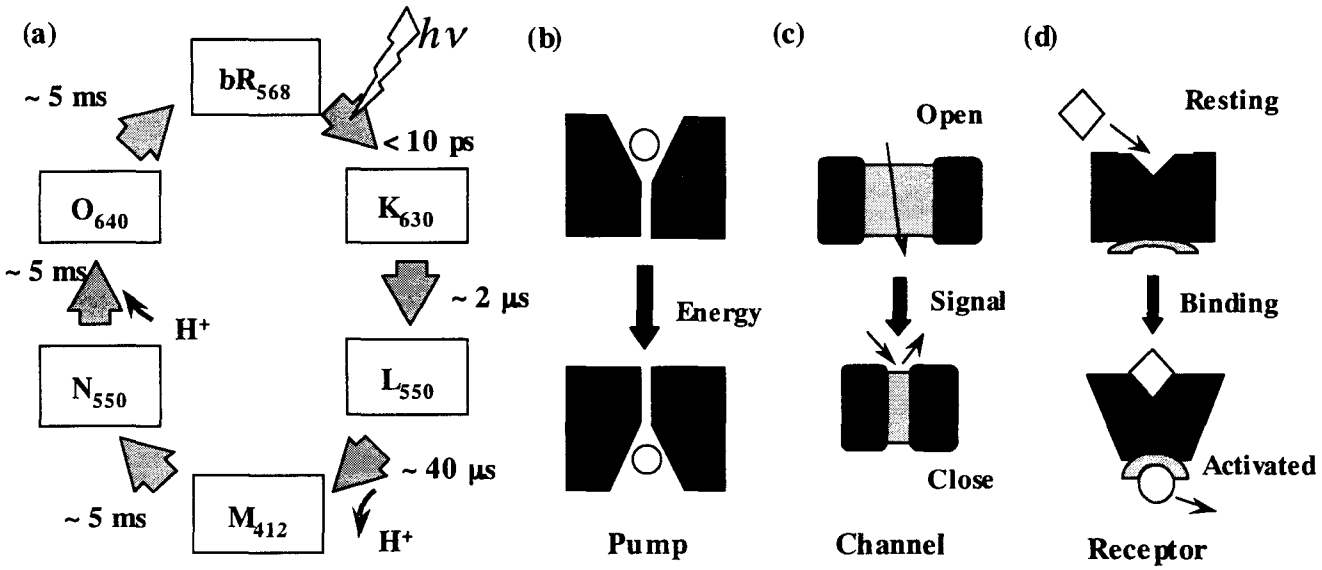

Fig.1. Photocycle of bacteriorhodopsin and bistable structural changes of various membrane proteins: (a) Photocycle of bacteriorhodopsin, (b) structural change of a pump by energy absorption, (c) Open-close change of a channel and (d) propagation of structural change in a receptor.

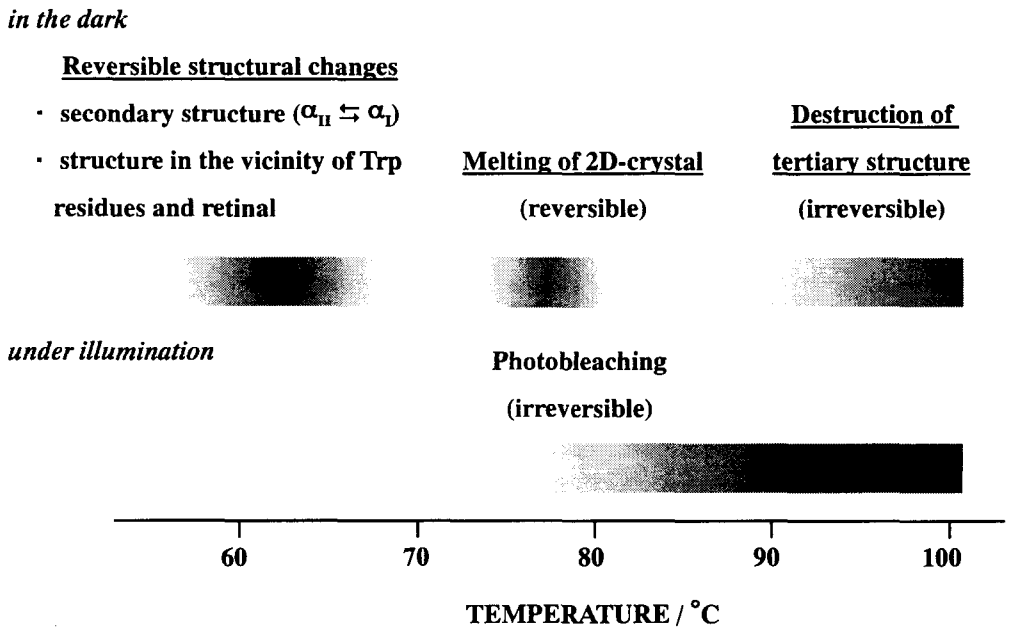

Fig. 2. Structural change of bacteriorhodopsin at high temperature in the dark and under illumination.

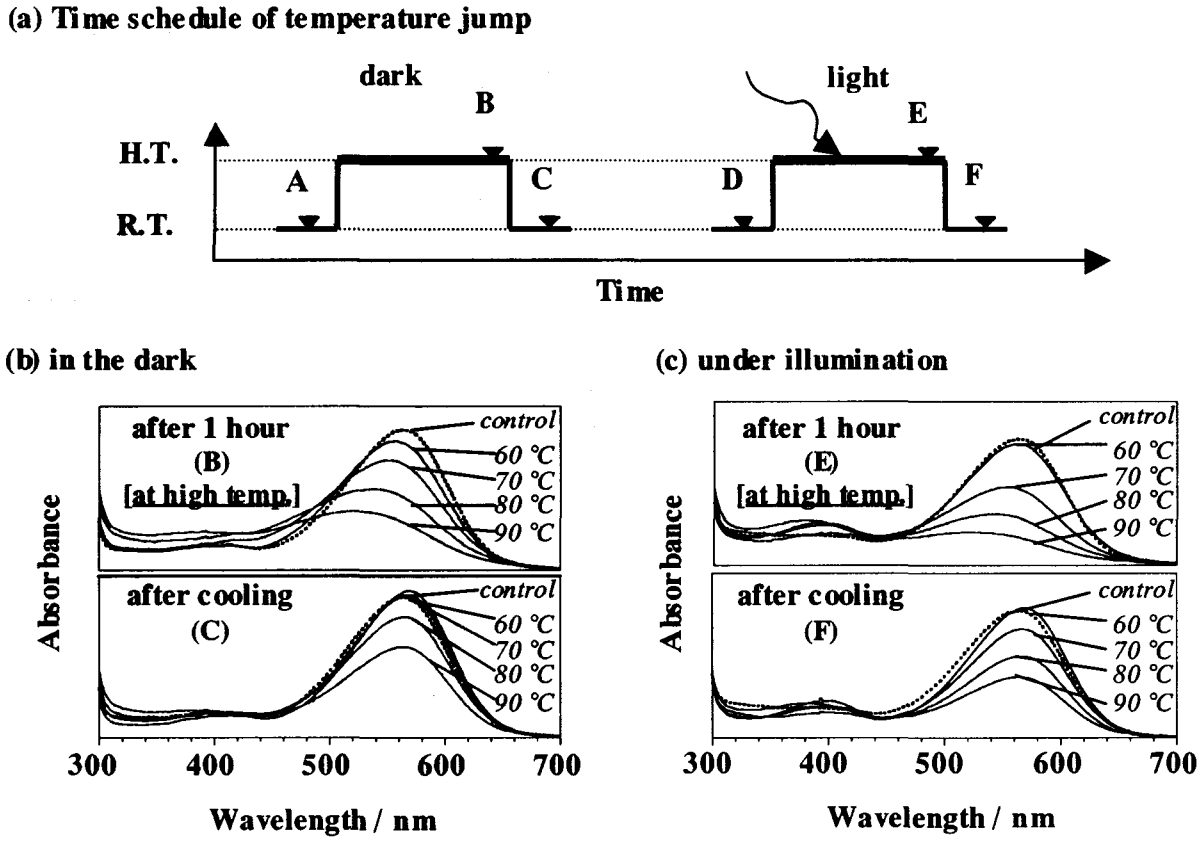

Fig. 3. Reversibility of structural change of bacteriorhodopsin in the dark and under illumination at high temperature was estimated by the time schedule (a). The effect of light illumination was observed from the results after cooling.

tra was above 90 °C [5]. Melting phenomenon was observed by X-ray diffraction around 80 °C [6]. Spectral shift of optical absorption has been reported in the temperature range above about 60 °C [7]. In addition to these phenomena in the dark, photobleaching was also observable even at the temperature below the denaturation point [8]. These experimental results strongly suggested that there is a stable state different from the ground state. We characterized the high temperature state by several techniques.

METHODS

We used bacteriorhodopsin, purified from the cytoplasmic membrane fragments of *halobacterium salinarum* [9].

The thermal stability was monitored by the absorption spectra according to the time course of the temperature jump of Fig. 3a. A sample was first incubated for an hour at high temperature, and then it was cooled down to the room temperature. The absorption spectra just before and after the cooling down of the sample were compared for estimating the irreversible component of the spectral change. The properties of the state was also studied by hydroxylamine reactivity with the Schiff's base and circular dichroism.

RESULTS AND DISCUSSION

The absorption spectra of bacteriorhodopsin on four conditions are shown in Figs. 3b and 3c. When bacteriorhodopsin is incubated at high temperature, the spectra showed small blue shift and decrease of maximum absorbance. After the

cooling down of samples, the wavelength of absorptions maxima completely recovered. However, the recovery of the maximum absorbance was incomplete, indicating the denaturation of bacteriorhodopsin. The change in the absorbance in the dark represents the thermal effect on bacteriorhodopsin. Whereas, the chang under illumination has to be the combination of the thermal and the light effets. In fact, the spectral change under illumination was significantly larger than the thermal effect in the dark. More detailed studies showed that the onset temperature of the denaturation in the dark was about 70 °C, whereas the photobleaching occurred above 60 °C.

Kinetics of hydroxylamine reaction with the Schiff's base in the dark were measured for evaluating the accessibility of molecules to the central portion of the protein. Arrhenius plot of reaction kinetic constant showed that there are three temperature ranges: T< 60 °C, 60 °C < T < 70 °C, T > 70 °C. The activation energies in these ranges were similar, and there were jumps of the frequency factor aroud 60 and 70 °C. These facts indicated that bacteriorhodopsin has a state in the temperature range between 60 and 70 °C, in which the structre and the stability is different from the ground state.

Temperature dependence of circular dichroism (CD) was measured in visible and UV range. Visible CD represents the configuration of bacteriorhodopsin. The change from the bilobed exiton band to the positive band started around 60 °C.

These results lead to the conclusion that bacteriorhodopsin has a subtle but stable state just below the denaturation temperature in the dark. The biological meaning of this state is not yet obscure, some intramolecular interaction which is responsible for the recovery to the ground state from the photointermediate states has to be weakened in the the high temperature state. A membrane protein generally may have multistates, and the stability mechanism of multi-states of bacteriorhodopsin will serve for the understanding of structural formation of various membrane proteins.

REFERENCES

1. Henerson, R., J.M. Baldwin, T.A. Ceska, F. Zemlin, E. Beckman and K.H. Downing (1990) Model for the structure of bacteriorhodopsin based on high-resolution electron cryo-microscopy. *J. Mol. Biol.* 213, 899-929.
2. Baldwin, J.M, G.F. Schertler and V.M. Unger (1997) An alpha-carbon template for the transmembrane helices in the rhodopsin family of G-protein-coupled receptors. *J. Mol. Biol.* 272, 144-164.
3. Luecke, H. (2000) Atomic resolution structures of bacteriorhodopsin photocycle intermediates: the role of discrete water molecules in the function of this light-driven ion pump. Biochim. Biophys. Acta 1460, 133-156.
4. Spudich, J.L. and J.K. Lanyi (1996) Shuttling between two protein conformations: the common mechanism for sensory transduction and ion transport. *Curr. Opin. Cell Biol.* 8, 452-457.
5. Jackson, M.B. and J.M. Sturtevant (1978) Phase transitions of the purple membrane of *Halobacterium halobium*. *Biochemistry* 17, 911-915.
6. Hiraki, K., T. Hamanaka, T. Mitsui and Y. Kito (1981) Phase transitions of the purple membrane and the brown holomembrane x-ray diffraction, circular dichroism spectrum and absorption spectrum studies. *Biochim. Biophys. Acta* 647, 18-28.
7. Wang, J.-P. and M.A. El-Sayed (2000) The effect of protein conformational change from α_{II} to α_{I} on the bacteriorhodopsin photocycle. *Biophys. J.* 78, 2031-2036.
8. Etoh, A., H. Itoh and S. Mitaku (1997) Light-induced denaturation of bacteriorhodopsin just above melting point of two-dimensional crystal. *J. Phys. Soc. Japan* 66, 975-978.
9. Oesterhelt, D. and W. Stoeckenius (1974) Isolation of thecell membrane of *Halobacterium halobium* and its fractionation into red and purple membrane. *Methods Enzymol.* 31, 667-678.