Journal of Photoscience (2002), Vol. 9(2), pp. 293–295

Structural Stability of Bacteriorhodopsin Solubilized by Triton X-100

Takanori Sasaki*, Masashi Sonoyama, Yuri Mukai, Chieko Nakazawa and Shigeki Mitaku
Department of Biotechnology, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan

The structural stability of bacteriorhodopsin (bR) solubilized by Triton X-100 (TX-100) was studied by measuring the denaturation kinetics in the dark and under illumination, and compared with the structural stability of bR solubilized by octyl-β-glucoside (OG). In the dark, bR solubilized by TX-100 was more stable than bR solubilized by OG. Under illumination, bR solubilized by TX-100 showed light-induced denaturation in the same manner as bR solubilized by OG. These results in the dark well correlated with the experimental results of the visible CD band. Although solubilized bR in the TX-100 concentration range of 2-50 mM showed almost identical positive CD band and did not denature in the dark at 35 °C, the kinetic constant of the photobleaching increased with the increase of TX-100 concentration. These results suggested that photo-intermediates of solubilized bR are destabilized by TX-100 micelles.

Key words: bacteriorhodopsin, TritonX-100, solubilization, denaturation

INTRODUCTION

Bacteriorhodopsin (bR) is a typical intrinsic membrane protein whose biological function is a light-driven proton pump located in purple membrane of *Halobacterium salinarum*. BR has seven transmembrane helices and forms 2D crystalline structure of trimer as a unit. The 3D structure of bR has already been determined at high resolution [1]. On photo-excitation bR shows a linear cyclic photochemical reaction called a photocycle containing various photointermediates (K, L, M, N and O), each of which has different absorption maximum, implying the conformational change occurs during the photocycle. Diffraction images of intermediate states were also obtained recently, which give information of the structural basis of proton-pumping function of bR.

The stability of this protein was also studied by thermal and chemical denaturation experiments. It was revealed that the structure of bR in the ground state was preserved even when purple membrane was suspended in pure hexane, but complete denaturation occurred upon addition of alcohol. These observations strongly suggested that the tertiary structure is stabilized by polar interactions among helices, which is disrupted by hydroxyl group of alcohol [2]. Recent studies of photobleaching of bR at high temperatures showed that the stability is reduced in a high-temerature state activated by the light absorption. The structure of bR transformed from the ground state to the high temperature state above 60 °C in which the interaction responsible for the stability is weakened. The photobleaching is presumbly induced by the combination of the thermal and light effects. We also observed the light-induced denaturation for bR solubilized by a detergent, octyl-β-glucoside (OG) [3]. Namely, the OG solublized sample is in a state with reduced stability which is different from the ground state. However, this sample was not stable even in the dark. If we can obtain a solubilized sample which is stable in the dark but denature under light illumination, it will be possible to study the problem of the structural stability of bR in two steps: a solubilization step and a light effect.

In this work, we carried out denaturation experiments of bR solubilized by Triton X-100 (TX-100) in the dark and under illumination, using a detergent TX-100 for solubilization, and compared with the experimental results of OG-solubilized bR. We measured the kinetics of denaturation and photobleaching in the dark and under illumination in the TX-100 concentration range 6-200 mM at 35°C. The visible CD spectra were also measured for confirming the solubiliztion of bR.

METHODS

Purple membrane of Halobacterium salinarium, strain R1M1, was prepared according to the method established by Oesterhelt and Stoeckenius [4]. TX-100 was added to the suspension of purple membrane. After incubation for 2 h at 37 °C, the sample was centrifuged at 105,000 g for 60 min at 4 °C.

* To whom correspondence should be addressed.
E-mail: sasaki@proteome.bio.tuat.ac.jp

The absorption spectra of the supernatant after the centrifugation showed the absorption peak at 550 nm corresponding to the native state bR. The concentration of the solubilized bR was about 10 μM.

Denaturation was monitored using a diode-array spectrophotometer with a xenon lamp. In experiments examining the light-induced denaturation kinetics at temperature 35 °C, the absorption spectra were successively measured at intervals of 180 s for 1 h after the start of illumination.

RESULTS AND DISCUSSION

We solubilized purple membrane by TX-100 at various concentration and measured the absorbance of supernatant of centrifuged sample after the solubilization procedure. Fig. 1 shows the relative absorbance of bR in the supernatant normalized by the absorbance of original sample before solubilization. This value corresponds to the degree of solubilization. It saturated at around 5 mM TX-100 to the level of about 0.75. Because pellets of bR did not exist above 5 mM TX-100, the absorption coefficient of solubilized bR may be smaller than the sample in purple membrane.

Circular dichroism in the visible region was measured for samples solubilized by TX-100. Purple membrane suspensions show a bilobed exiton type CD spectra which change to a positive band in TX-100 solubilized bR. When the CD spectra were measured for solubilized bR at various TX-100 concentrationin the range between 6 and 200 mM, the same positive type spectra were obtained, indicating that the complex of bR with TX-100 is stable in wide range of detergent concentration. On the other hand, the CD band of bR solubilized by OG was flat (data not shown). The bR solubilized by OG has to be more relaxed than TX-100 solubilized sample.

Figures 2A and 2B shows the time-resolved absorption spectra of bR solubilized by 5 mM TX-100 at 35 °C in the dark and under visible light illumination. In the dark, the change in the absorption spectra was negligibly small. On the other hand, the absorption peak at 550 nm decayed under illumination and a concomitant increase in a peak at about 380 nm was observed. Consequently, bR molecule solubilized by TX-100 was stable in the dark, but it denatures by the activation by light absorption. Comparing the effect of solubilization on bR, we found that TX-100 was milder than OG. The solubilization by TX-100 did not give rise to the denaturaion of bR in the dark, but bR solubilized by OG denatured at 35 °C in any conditions.

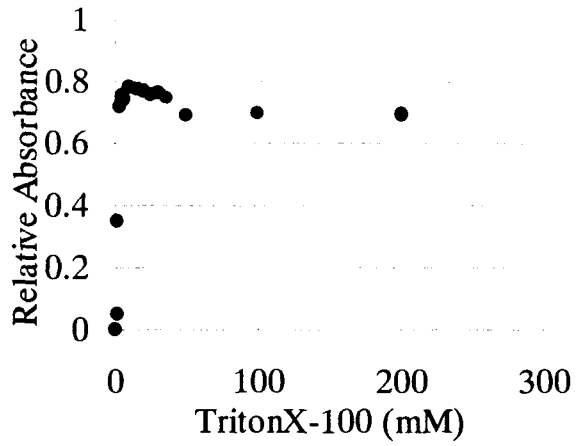

Figure 1. Relative absorbance of bacteriorhodopsin solubilized by Triton X-100. (Experimental conditions: pH 7.0, Triton X-100 concenntration of 1-200 mM, 37 °C)

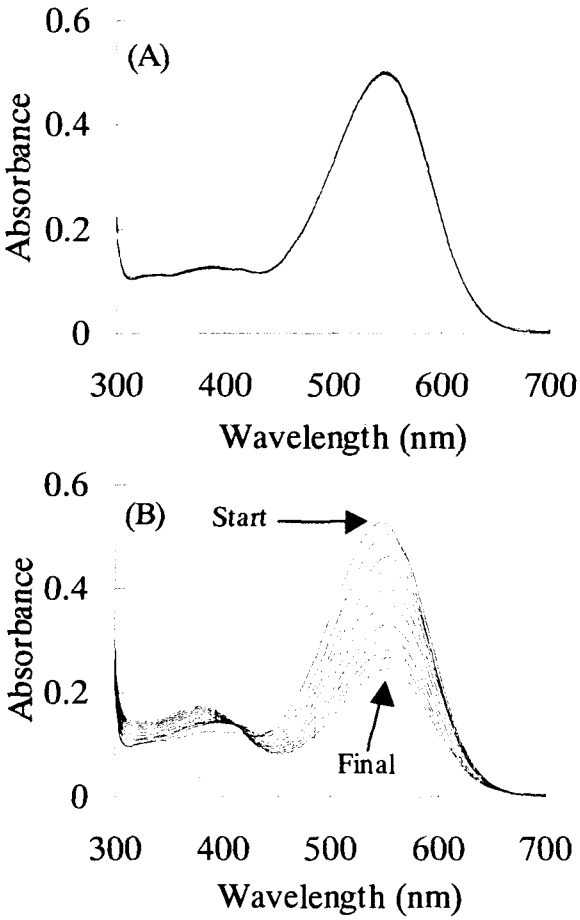

Figure 2. Absorption spectra of bacteriorhodopsin, solubilized by Triton X-100 in the dark (A) and under light irradiation (B) for an hour at 35 °C. (Experimental condisitons: pH 7.0, Triton X-100 concentration of 5 mM)

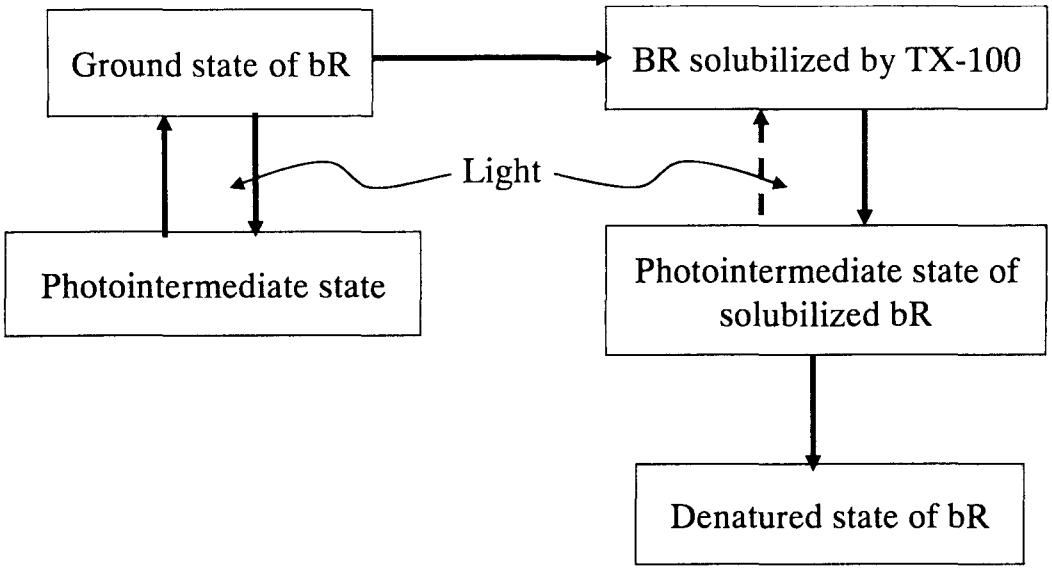

Fig. 3 Scheme of photobleaching of bR solublized by TX-100

The decay time constants of denaturation significantly changed according to the TX-100 concentration under illumination at 35 °C. The decay time constant was proportional to the concentration up to about 50 mM and showed saturation at higher concentration. The reason of this concentration dependence is still an open question.

The experimental results in this work lead to the scheme of the photobleaching of bR solubilized by TX-100. In the dark, bR solubilized by TX-100 is stable but different from the ground state in purple membrane. The photointermediate state recovers to the ground state in purple membrane, while solubilized bR denatures through the photointermediate state. Consequently, the combination of the weakening of intramolecular interaction by the solubilization and the light absorption is responsible for the stability of the molecular structure of this membrane protein.

REFERENCES

1. Luecke, H., B, Schobert, H.-T. Richter, J.-P. Cartailler and J.K. Lanyi (1999) Structure of bacteriorhodopsin at 1.55 Å resolution. *J. Mol. Biol.* 291, 899-911.
2. Mitaku, S., K. Suzuki, S. Odashima, K. Ikuta, M. Suwa, F. Kukita, M. Ishikawa and H. Itoh (1995) Interaction stabilizing tertiary structure of bacteriorhodopsin studied by denaturation experiments. *Proteins* 22, 350-362.
3. Mukai, Y., N. Kamo and S. Mitaku (1999) Light-induced denaturation of bacteriorhodopsin solubilized by octyu-β-glucoside. *Protein Eng.* 12, 755-759.
4. Oesterhelt, D. and W. Stoeckenius (1974) Isolation of the cell membrane of *Halobacterium halobium* and its fractionation into red and purple membrane. *Methods Enzymol.* 31,667-678.