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Editor's Choice

Highly Stable Solubilization of Membrane Protein Bacteriorhodopsin with a Short-chain Phospholipid Diheptanoylphosphatidylcholine

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A short-chain phosphatidylcholine, diheptanoyl PC (diC7 PC), was employed for solubilizing a membrane protein bacteriorhodopsin (bR). Highly stable and efficient solubilization of bR was accomplished above the surfactant concentration of ca. 15 mM. Denaturation experiments for the solubilized bR showed that diC7 PC provided a more stable environment with bR than *n*-octyl-β-glucosides, especially in the functional state under light irradiation, suggesting that the short chain PC is a promising surfactant for solubilizing membrane proteins.

Solubilizing surfactants are indispensable in the isolation and purification of membrane proteins.[1] Although it is crucial to preserve structural and functional properties of native membrane proteins in the surfactant-solubilized state for further biochemical and biophysical studies, it is well known that even mild surfactants often induce serious denaturation upon solubilization. In the present study, solubilization of a membrane protein, bacteriorhodopsin (bR) from the purple membrane (PM) of *H. salinarum*, has been attempted with a short-chain phospholipid, diheptanoyl phosphatidylcholine (diC7 PC), and structural stability of the solubilized bR against heat and visible light were examined, since it has high potential for solubilization of membrane proteins[2] in that it has amphiphilic properties as usual surfactants have and the same basic chemical structure as native long chain phospholipid.

DiC7 PC was purchased from Avanti Polar Lipids, Inc. Other chemicals were purchased from Wako Pure Chemical Industries, Ltd. All materials were used without further purification. PM from *H. salinarum*, strain R1M1, was isolated and purified by using an established procedure by Oesterhelt and Stoeckenius.[3] The purified PM was suspended in 100 mM phosphate buffer (pH 7.0). Solubilization of bR with diC7 PC was performed as follows: After mixing PM suspension with the surfactant suspension at the final bR concentration of 8 μM and gentle stirring at 4 °C for 24 h in the dark, the solubilized sample was obtained as the supernatant by 1 h ultracentrifugation at 100000 g. The solubilization degree of bR was examined at surfactant concentration of 2–40 mM. UV–visible absorption and visible circular dichroism spectra were recorded with a Beckmann Coulter DU-7500 spectrophotometer and a JASCO J-820 spectropolarimeter, respectively. For investigation of structural stability of the solubilized bR in the dark and under continuous visible light irradiation, UV–visible spectroscopic measurements were performed at 2 min intervals for 2 h. The denaturation kinetics measurements were initiated by mixing the solubilized sample and phosphate buffer preheated at desired temperatures (25–50 °C). The final concentration of bR and diC7 PC in the denaturation experiments was 4 μM and 15 mM, respectively. Light illumination was performed with a Xe lamp (average light power 200 mW cm^{-2}), Y52 color filter, and heat-cut filter.

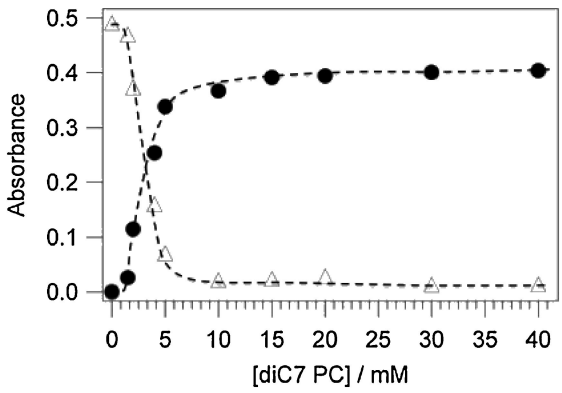

Figure 1. Absorbance at the maximum wavelengh for solubilized (filled circles) and unsolubilized (open triangles) bR as a function of diC7 PC concentration.

UV–visible absorption maxima of the solubilized samples were observed at ca. 550 nm irrespective of the surfactant concentration. The blue shift for the solubilized samples was very similar to that for previously reported detergent-solubilized bR[4,5] and monomeric bR reconstituted in dimyristoylphosphatidylcholine (DMPC) vesicles.[6,7] The solubilized bR studied here has no significant absorption at ca. 380 nm, indicating no remarkable denaturation of bR during solubilization with diC7 PC. The intensity of the absorption maxima of the solubilized bR is plotted as a function of diC7 PC concentration in Figure 1 with the absorbance of the corresponding precipitation resuspended with the same volume as the supernatant. The absorbance of the solubilized bR showed a sharp rise at 3 mM and reached a plateau at 0.4 in the concentration range of 15–40 mM, while the precipitate dramatically started to decrease in absorbance above ca. 3 mM and reached almost zero above ca. 15 mM. These experimental results demonstrate that above the diC7 PC concentration of ca. 15 mM, solubilization of bR molecules in PM is complete without serious denaturation. Differences in absorbance between the completely solubilized bR and the original bR in the PM may be due to changes in molar extinction coefficient upon solubilization.[4,5] The visible CD spectrum of the solubilized bR demonstrated a single positive pattern, showing disassembly of bR molecules into monomer.

Denaturation experiments on the solubilized bR were performed in the dark and under continuous visible light illumination for 2 h. No significant spectral changes in the dark were observed below 30 °C; however, the band at ca. 550 nm showed gradual decrease with concomitant increase in absorbance at ca. 380 nm above this temperature, indicating slow thermal denaturation of the solubilized bR. Under continuous light illumination, on the other hand, acceleration of denatura-

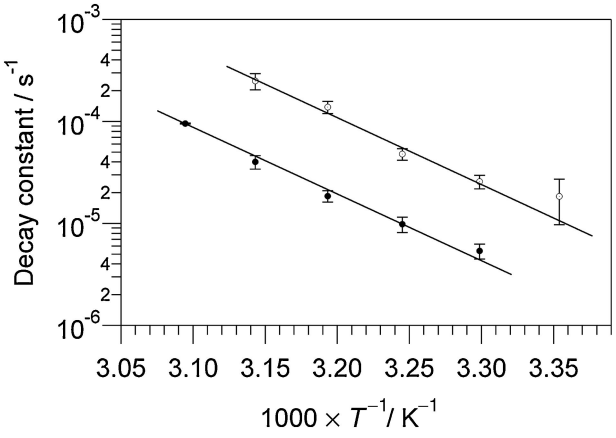

Figure 2. Arrhenius plots of heat (closed circles) and light-induced (open circles) denaturation of diC7 PC-solubilized bR.

tion by light was clearly observed. The light enhancement effect on denaturation was previously reported for bR in the solubilized state with other detergents,[4] at high temperature[8] and under alkaline conditions of the purple membrane,[9] and in the reconstituted state into DMPC vesicles (unpublished results). To perform denaturation kinetics analysis, the absorbance at ca. 550 nm was plotted as a function of time. Contributions from thermal denaturation were subtracted for the data under light illumination to examine real light-induced denaturation. Time variations of the absorbance at ca. 550 nm were well expressed as a single exponential function both in the dark and under light illumination. Decay constants were obtained by fitting the experimental data to a single exponential function. The decay constants are plotted as a function of the inverse of the absolute temperature in Figure 2. Although diC7 PC-solubilized bR undergoes thermal and light-induced denaturation, the decay constants are remarkably smaller than that of solubilized bR with other useful surfactants such as octyl-β-glucoside (OG)[4a] and Triton X-100 (TX100),[4b] indicating higher structural stability of bR solubilized with the short-chain phospholipid. More stable solubilization was observed at low temperature, in comparison with solubilization with OG[4a] and TX100.[4b] This is very advantageous for practical isolation and purification of membrane proteins.

It is of note that the Arrhenius plot demonstrates that the slopes for thermal and light-induced denaturation are very similar, although light-induced denaturation is approximately 10 times faster than thermal denaturation in the temperature range studied. Actually, there was no significant difference in the apparent activation energy between the two different denaturation processes, as show in Table 1. It is, therefore, thought that the light enhancement effect for bR solubilized with diC7 PC is attributable to increase in frequency factor of denaturation. This situation is in contrast to previous reports stating that light-induced denaturation of bR was accelerated by a decrease in apparent activation energy of denaturation in the solubilized state[4] and in the purple membrane at high temperateure[8] and alkaline pH.[9] Furthermore, in the case of TX100-solubilized bR, Sasaki et al. reported a similar value of activation energy for light-induced denaturation, as shown in Table 1.[4b] Based on the kinetic parameters obtained by the present and the previous

Table 1. Apparent activation energy E_a for denaturation of surfactant-solubilized bR[a]

Surfactant	E_a/kJ mol^{-1}	
	Heat denaturation	Light-induced denaturation
diC7 PC	125.1 ± 5.8	125.5 ± 12.0
Octyl glucoside*	110.2	50.3
Triton X-100**	—	70.7

[a]The values for octyl-β-glucoside* and Triton X-100** are taken from ref 4.

studies, it can be said that the short-chain phospholipid diC7 PC provides solubilized bR with a remarkably stable environment in the functional state as well. What is responsible for the highly stable solubilized state of bR with diC7 PC is not clear at present; however, dynamic structural fluctuation of bR molecules in the surfactant–protein complex may be a clue. Recently, Santonicola et al. have reported that in solubilized bR with alkyl polyglucoside, protein stability is closely related to the thickness of the surfactant shell and the lateral pressure on the transmembrane helices.[10] Our previous findings that OG-solubilized bR undergoes much faster hydrogen–deuterium exchange of the peptide hydrogen than in the native purple membrane strongly suggested destabilization of helical assembly in the OG-solubilized state.[11] The origin of the high stability of the diC7 PC-solubilized bR will be discussed based on structural characterization with dynamic light scattering and other spectroscopic methods.

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