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Thermal denaturation of photosynthetic membrane proteins from *Rhodobacter sphaeroides* ☆

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Abstract

The thermal stability of detergent-solubilized reaction centers (RC) and light-harvesting B800–850 complex from *Rhodobacter sphaeroides* was studied by the temperature-scanning (T-scan) method in the absorbance mode and circular dichroism (CD), and by DSC. The denaturation temperatures of RC solubilized with *n*-octyl β-D-glucoside (OG) and lauryldimethylamine *N*-oxide (LDAO) obtained by the T-scan method did not depend on wavelengths or the methods of measurement. The denaturation temperature T_d of B800–850 complex was higher than that of RC in all measurements. The values of T_d measured at various wavelengths for RC in OG was about 10 K higher than those in LDAO. The value of T_d of B800–850 complex in OG obtained by T-scan CD at 290 nm was about 7 K higher than that in LDAO. Compared with LDAO, OG has a stabilizing effect on both RC and B800–850 complex against heat denaturation.

Keywords: Denaturation; Photosynthesis; Protein; *Rhodobacter sphaeroides*

Abbreviations

CD, circular dichroism; DSC, differential scanning calorimetry; RC, reaction center; BChl, bacteriochlorophyll; BChl800, BChl850 and BChl875, bacteriochlorophyll molecules with their near-infrared absorption maxima; B800–850 complex and B870 complex, light-harvesting complexes identified by their near-infrared absorption

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☆ Dedicated to Hiroshi Suga on the Occasion of his 65th Birthday.

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maxima; T-scan, temperature-scanning; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane; LDAO, lauryldimethylamine *N*-oxide; OG, *n*-octyl β-D-glucoside.

1. Introduction

Bacterial photosynthetic systems are composed of integral membrane proteins, and a light-driven electron transfer reaction across the membrane is generated in this system. For *Rhodobacter sphaeroides*, the excitation energy transfers from B800–850 light-harvesting complex (B800–850 complex) through B870 light-harvesting complex (B870 complex) to the reaction center (RC). In the chromatophore membrane, RC-B870 complex is surrounded by B800–850 complexes [1]. The three-dimensional structures of RC from *Rhodopseudomonas viridis* [2] and *Rb. sphaeroides* [3] were determined by X-ray analysis and it is shown that the RC of the latter, which contains H, M and L subunits, has almost the same structure as that from *Rps. viridis*, except for a missing cytochrome-C subunit [3]. These reaction centers have a similar electron transfer system to photosystem II in plants [4]. The B800–850 complex from *Rb. sphaeroides* is composed of $\alpha_2\beta_2$-type subunits, four bacteriochlorophyll (BChl) 850 molecules, two BChl 800 molecules and three carotenoids [5].

The thermal denaturation of these photosynthetic membrane proteins has been investigated to provide insight into their structural organization and to analyze the thermal stability of each subunit. The thermal behavior of the photosystem II membrane proteins was investigated by differential scanning calorimetry (DSC) and DSC peaks were assigned by thermal gel analysis and electron paramagnetic resonance method [6, 7].

There have been several investigations on the thermal stability of bacterial photosynthetic reaction systems, namely, the reaction unit of *Rps. viridis* [8] and the reaction centers of *Chloroflexus aurantiacus*, *Chromatium tepidum*, *Rb. sphaeroides* and *Rb. sphaeroides* R-26 [9–11]. Two of these investigations were based on measurements of CD and absorbance spectra at various temperatures and the change in absorbance with heating period at individual temperatures [9, 10]. In other studies [8, 11], the absorbance was measured on cooled samples after heat treatment and the relative intensity to that of untreated samples was plotted against the temperature of heat treatment. Because of the different experimental conditions such as the temperature and heating period in the above methods, careful consideration is necessary to compare data for the evaluation of the thermal stability of the system containing irreversible processes. In other words, the thermal denaturation depends on both the temperature of heat treatment and the heating period, especially in the case of membrane proteins.

In contrast to the above methods, the temperature-scanning (T-scan) method, in which physical properties (such as CD or absorbance) of the samples are observed during heating at a constant rate, provides a more general determination of the denaturation temperature, T_d. The method has an advantage in evaluating the thermal behavior of proteins, as we can compare transition profiles which reflect different aspects of the conformational change of proteins [12].

In this paper we report the thermal properties of RC and B800–850 complex of *Rb. sphaeroides* using a CD spectrometer and a UV-VIS spectrometer with a T-scan system, as developed by our group [13], and DSC. We also studied the effect of detergents on the thermal stability of RC and B800–850 complex.

2. Experimental

2.1. Preparation of the detergent-solubilized RC and B800–850 complex solution

Photosynthetic membrane of *Rb. sphaeroides* 2.4.1 was solubilized in 1% lauryldimethylamine *N*-oxide (LDAO) and centrifuged at 40,000 rpm (Hitachi RP 50-2) for 1 h. RC and B800–850 complex were purified from the supernatant using molecular-sieve chromatography (CL-6B) and anion exchange chromatography (DEAE-Sephacel) in the presence of 0.1% LDAO [14]. Finally, purified proteins were solubilized with 10 mM Tris buffer solution (pH 8.0) containing 0.05% NaN_3, 1 mM EDTA and 0.1% LDAO. To replace LDAO with *n*-octyl β-D-glucoside (OG), protein complexes were adsorbed on a DEAE-Sephacel anion exchange column and washed with 10 mM Tris buffer (pH 8.0) containing 0.05% NaN_3, 1 mM EDTA and 0.8% OG. The detergent concentrations were selected to be higher than the critical micelle concentrations of LDAO and OG at 25°C, which are 0.025% and 0.73%, respectively. All chemicals used were reagent grade.

The molecular weight and the number of amino acid residues of RC were 93.5 kDa and 850, respectively [10]. The RC concentration was calculated by measuring the absorbance at 802 nm with that molar extinction coefficient 288 $cm^{-1}\,mM^{-1}$ [10]. RC concentrations used for measurements were 1.27×10^{-2} mM in LDAO–buffer and 0.67×10^{-2} mM in OG–buffer. DSC experiments for RC in LDAO were carried out at an RC concentration of 1.27×10^{-2} mM. The values of the absorbance (850 nm, 1 cm) of the B800–850 complex used in this study were 1.98 in LDAO and 2.71 in OG, respectively.

2.2. CD and absorbance spectra

CD and absorbance spectra were measured with a Jasco J-600 spectropolarimeter and a Shimazu UV-1200 UV-VIS spectrometer, respectively. The temperature of the sample was controlled with the thermostatic circulating bath. The CD spectropolarimeter was calibrated with ammonium *d*-10-camphorsulfonate [15]. The detailed conditions of the CD measurements were described previously [16]. The CD spectra were expressed as the mean residue molar ellipticity, $[\theta]$.

2.3. Temperature-scanning CD and UV-VIS measurements

Changes in molar ellipticity and absorbance with temperature were recorded with a temperature-scanning CD system (T-scan CD) or temperature-scanning absorbance

system (T-scan UV-VIS). Both systems were developed by our group and are composed of a CD or UV-VIS spectrometer, temperature-controlling units, and a computer [13]. In these systems, the sample temperature can be controlled at any heating rate. The output signals from the spectrometer and thermocouple at the cell were recorded simultaneously as digital data every second using in-house original software. The methodological basis of these measurements is almost the same as that proposed by A. Wada et al. [12]. The heating rate in these measurements was 1.0°C min^{-1}.

2.4. DSC measurement

The DSC experiments were conducted using a Microcal MCS calorimeter at a heating rate of 1.0°C min^{-1}. Data were analyzed with Microcal Origin software.

3. Results and discussion

3.1. The denaturation profile and reversibility of RC and B800–850 complex

The absorbance and CD spectra of RC and B800–850 complex in LDAO and OG were measured at 10 and 90°C. As typical examples, the absorbance spectra of RC in LDAO and the far-UV CD spectra of RC in OG are shown in Figs. 1 and 2, respectively. The CD spectra at 10°C for RC agreed with the results of Nicolini et al. [10], who reported that the secondary structure composition of RC at 25°C is α-helix (54%), β-sheet (11%) and coil (35%). Similarly, the curve at 10°C for B800–850 complex agreed with the results of Breton and Nabedryk [17], who gave the contents of α-helix, β-sheet and coil of the B800–850 complex at 25°C as 46, 12 and 42%, respectively.

When solutions were cooled immediately to 10°C after heating up to 90°C, native state was slightly recovered from denatured states (data are not shown). After the measurements, turbidity was observed in the solutions. The aggregation of denatured proteins occurs at high temperatures and seems to cause the turbidity of the solution. This phenomenon is reflected in the DSC curves (Fig. 3). The decrease in heat capacity at higher temperatures in this figure seems to be caused by the aggregation of the denatured proteins. As shown in Fig. 3, the second DSC run has no endothermic peak.

The T-scan curves of the molar ellipticity of RC solution at 222 nm in LDAO and OG and their temperature differential curves are shown in Fig. 4a and b, respectively. Similarly, those of B800–850 complex solution at 290 nm are shown in Fig. 5a and b. RC is composed of L, M, and H subunits which have 5, 5 and 1 transmembrane α-helices, respectively. The values of molar ellipticity at 222 nm in Fig. 4a are mainly affected by the total change in the conformation of polypeptide chains of these three subunits. Those at 290 nm in Fig. 5a mainly reflect the change in the anisotropic circumstance of the aromatic amino acid residues. From T-scan curves such as those

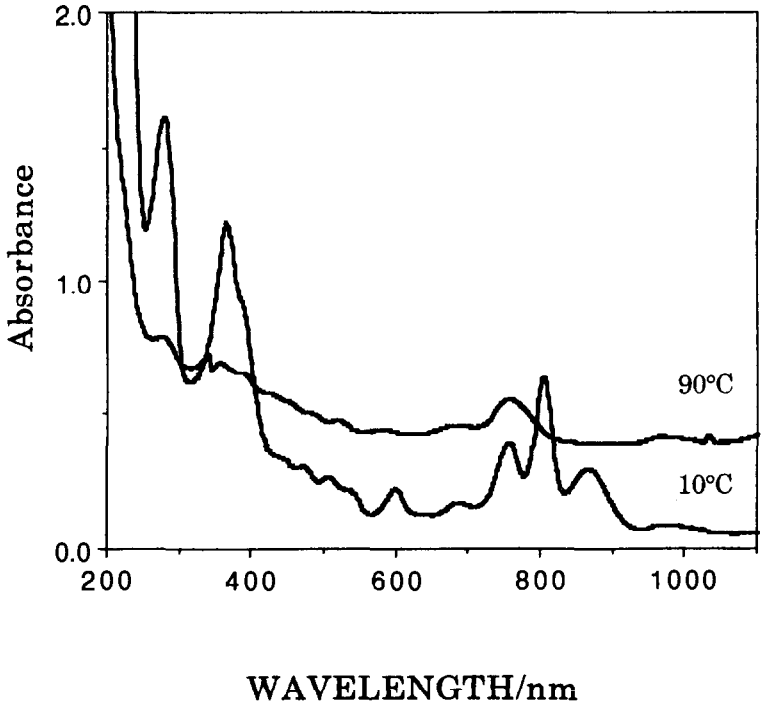

Fig. 1. The absorbance spectra of RC from *Rb. sphaeroides* in LDAO at 10 and 90°C.

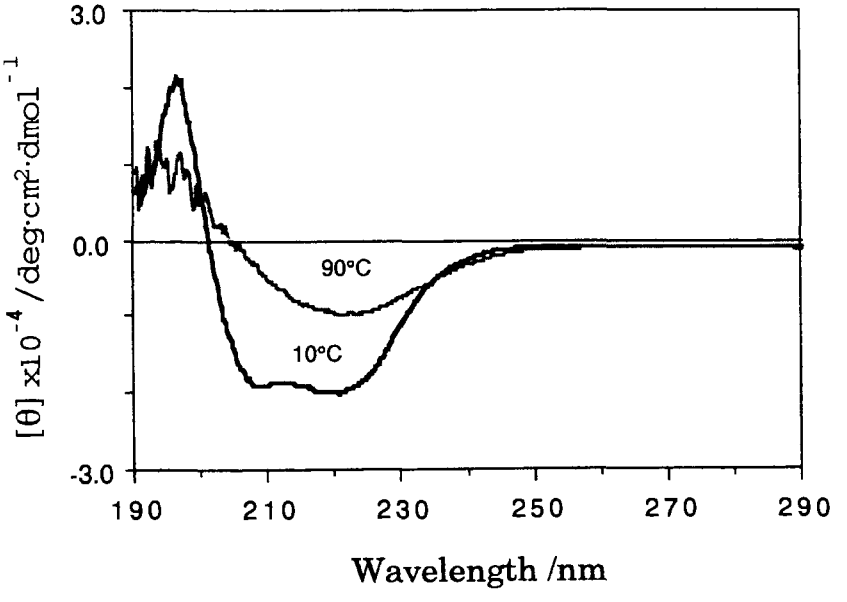

Fig. 2. The far-UV CD spectra of RC from *Rb. sphaeroides* in OG at 10 and 90°C.

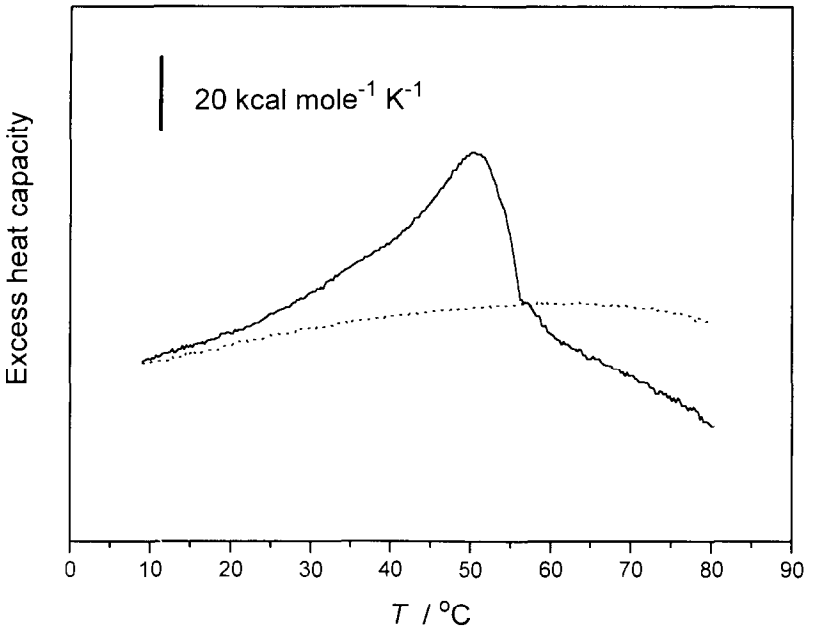

Fig. 3. DSC curves of RC from *Rb. sphaeroides* in LDAO at 1.27×10^{-2} mM and at 1 °C min^{-1}. The solid and dotted lines denote the first and second runs, respectively.

shown in Figs. 4a and 5a, we can estimate the van't Hoff enthalpy and denaturation temperature, T_d, by assuming the reversible two-state transition. However, the thermal transition of the protein complex was not completely reversible under the experimental conditions. Therefore, we used T_d as a measure of the thermal stability of these membrane proteins without discussing the van't Hoff enthalpy.

The temperature differentials of the curves in Figs. 4a and 5a were calculated in order to obtain the temperature at which the helix content or the anisotropy of the aromatic amino acid residues decreases most drastically (Figs. 4b and 5b). The peak temperatures, T_p, of the differential curves in Fig. 4b in OG and LDAO are 66 and 51 °C, respectively, and these values are almost coincident with the values of T_d from Fig. 4a in OG and in LDAO, 63 and 52 °C, respectively. Similarly, the values of T_d from Fig. 5a coincide with those of T_p from Fig. 5b.

3.2. The thermal denaturation temperature of RC and B800–850 complex obtained at various wavelengths

The values of T_d obtained by various methods are summarized in Table 1. The absorbance of RC at 800 nm and of B800–850 complex at 800 and 850 nm comes from BChl, and the absorbance within the wavelength range from 400 to 500 nm comes from carotenoids.

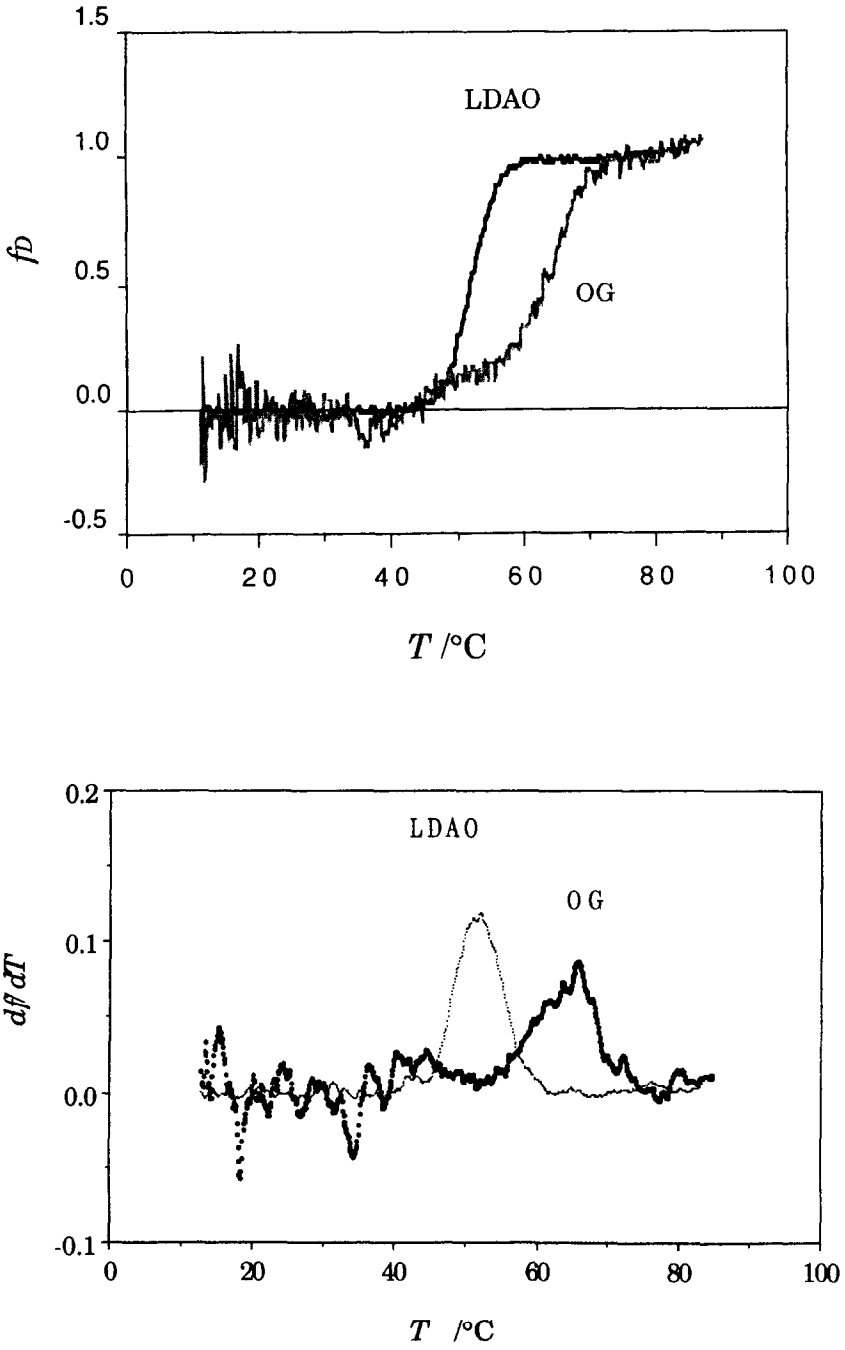

Fig. 4. a. T-scan CD curves of RC from *Rb. sphaeroides* in LDAO and in OG at 222 nm and at 1 °C min^{-1}
b. Temperature differential curves of the T-scan CD curves in a.

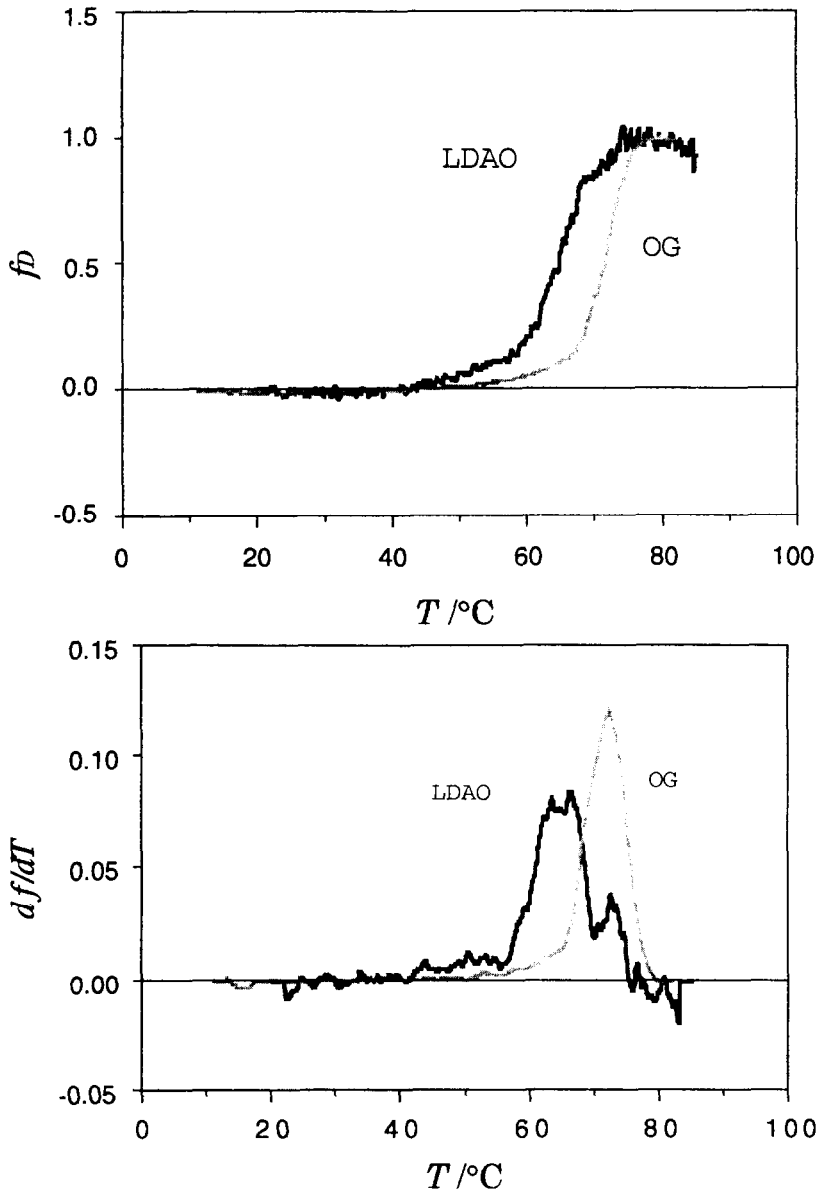

Fig. 5 a. T-scan CD curves of B800–850 complex from *Rb. sphaeroides* in LDAO and in OG at 290 nm and at 1 °C min^{-1}. b. Temperature differential curves of the T-scan CD curves in a.

In the case of RC in LDAO and OG, the values of T_d obtained by T-scan CD and T-scan UV-VIS at various wavelengths are coincident, as shown in Table 1. The T_p obtained by DSC for RC in LDAO also agrees with those values of T_d. The observed DSC curve of RC did not show the separated peaks corresponding to subunits in RC. It

Table 1
T_d of RC and B800–850 in OG and LDAO

	CD					Absorbance 802 nm	DSC
	222 nm	290 nm	368 nm	482 nm	512 nm		
RC							
LDAO	52	52				~ 52	50
OG	63	62	62[a]				
B800–850 complex							
LDAO	70	64	64				
OG	70	71	70–75	70–75	65–70		

[a] Measured at 370 nm.

is possible to explain these results in two ways: one is that RC denatures in a single step, and the other is that these subunits denature at the same temperature or within a very narrow temperature range. We cannot exclude the second possibility for the results obtained.

A similar denaturation pattern was observed for B800–850 complex in OG. However, the T_d values of B800–850 complex in LDAO at 290 and 368 nm are 5–6 K lower than that obtained at 222 nm. This suggests that the structural change around the hydrophobic side chains and pigments occurs at lower temperatures than for the polypeptide backbones of B800–850 complex under the influence of LDAO.

3.3. *Comparison of* T_d *for RC and B800–850 complex and the effect of detergents*

The value of T_d for B800–850 complex is higher than that of RC by about 18 K in LDAO and 7 K in OG. RC is surrounded by B870 complexes (photo reaction unit), and further surrounded by B800–850 complexes in the native membrane [1]. The result that the T_d of B800–850 complex is higher than that of RC suggests that the photo reaction unit is surrounded by more thermally stable complexes in the native membrane. A similar organization of membrane proteins was found in the case of photosystem II [6], in which the T_p of LHCP fraction (corresponding to a light-harvesting protein) was higher than that of the core preparation (corresponding to RC).

From Table 1, all the values of T_d in OG solution are higher than those in LDAO solution, except for the case of B800–850 complex at 222 nm, in which the two T_d values are the same. As the hydrophobic alkyl chains in the two detergents are similar, this difference seems to be related to the effect of the head groups of the two detergents. For globular proteins, it is known that sugars increase the denaturation temperature [18, 19].Therefore the glucoside group, the head of OG, is considered to have a similar effect on membrane proteins against thermal denaturation.

4. Conclusion

The temperature-scanning methods applied in this study, DSC, T-scan CD and T-scan UV-VIS, were found to be useful for the evaluation of the thermal behavior of membrane proteins. The T_d observed by these methods were almost the same for RC from *Rb. sphaeroides* both in LDAO or OG. It was also revealed that the thermal transition of both RC and B800–850 complex in OG occurred at higher temperatures than that in LDAO, which reflects the difference in the head groups of the detergents.

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