Effects of low-molecular-weight polyols on the hydration status of the light-harvesting complex 2 from *Rhodobacter sphaeroides* 2.4.1

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Abstract

Low-molecular-weight (MW) polyols are organic osmolytes influencing water activity. We have investigated the effects of polyol molecules (glycerol and sorbitol) on the optical and triplet excitation dynamics of light-harvesting complex 2 (LH2) from *Rhodobacter (Rba.) sphaeroides* in buffer-detergent solutions. The resonance Raman spectroscopy demonstrated that, on increasing glycerol and sorbitol volume fractions ranging from 0 to 80% (v/v) (accompanied by the decreasing water activities), the planar and all-trans conformation of carotenoids (Crt) remained unchanged, and the bacteriochlorophyll a (BChl) Q_y absorption intensity decreased. The B850 fluorescence amplitude elevated in the 20–80% v/v sorbitol and 20–40% v/v glycerol solution, but decreased in 80% v/v glycerol solution. The change of $^3[\text{Crt}^*-\text{BChl}]$ interaction bands caused by $^3\text{Crt}^*-\text{BChl}$ interaction had no obvious correlation with water activities against polyol volume fractions, which are rationalized by the water activity sensitive of C- and N-termini of protein which binding with BChls. The results suggest that *Rba. sphaeroides* LH2 is more sensitive to low-molecular-weight polyols compared with that of the thermophiles purple bacterium *Thermochromatium (Tch.) tepidum* we had investigated before.

Graphic abstract

The C- and N-termini of protein, which binding with BChls are sensitive to water activity change.

Keywords *Rhodobacter sphaeroides* · Hydration · Glycerol · Sorbitol · Triplet excited state

1 Introduction

The peripheral light-harvesting complexes 2 (LH2) and the core light-harvesting complexes 1 (LH1) are encircling reaction centers (RC) arrangement orderly in the intracytoplasmic membranes (ICMs) of photosynthetic purple bacteria. These membrane proteins can harvest and utilize light

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efficiently by the cascading LH2 → LH1 → RC energy transfer and the subsequent primary charge separation in RCs [1]. Some researchers have successfully applied the energy transfer process of photosynthesis to artificial photosynthesis system, such as photoelectrochemical cells based on a bacterial photosynthetic pigment-protein and artificial photosynthetic cell producing energy for protein synthesis [2, 3]. Up to now, the high-resolution crystal structures of LH2s from *Rhodospseudomonas (Rps.) acidophila* 10,050 [4] and *Rhodospirillum (Rs.) molischianum* [5] have been determined. The crystal structures of LH2 complexes showed that LH2s are nanomeric/octameric assemblies with the heterodimer of repeating α - and β -apoproteins units. Each α, β -subunit includes one carotenoid (Crt) and three bacteriochlorophyll *a* (BChl) molecules. For the LH2 complex of *Rps. acidophila*, 18 BChl molecules (B850) perpendicular to the membrane plane and excitonically coupled form the ring aggregates, and 9 weakly coupled BChl molecules (B800) parallel to the membrane plane and excitonically coupled form loose ring aggregates. The B800s and B850s binding sites are close to the cytoplasmic and periplasmic of membrane surfaces, respectively. The carotenoid passes in close contact to the edge of the bacteriochlorin ring of a B800 (closest contact ~ 3.4 Å). It then passes into the next α, β -apoprotein pair and runs over the face of the bacteriochlorin ring of the α -bound B850 (the closest contact is ~ 3.7 Å) [6].

As membrane protein, LH2s are generally solubilized in aqueous buffer solution with assistance of detergent for further study. Therefore, the aqueous components, such as detergents (ionic, non-ionic, or zwitterionic), proton, and metal ions, are supposed to influence the properties of pigments embedded in complexes through interacting with the hydrophilic C- or N-terminal domains of LH2 complex, since they are exposed directly to the aqueous phase [4, 7–9]. The effect of aqueous factors on function of photosynthetic pigment–protein complexes could be observed conveniently through various spectroscopic methods to monitor the conformational change, electronic energy level variation, and excitation dynamics of Crt and BChls.

Some low-molecular-weight (MW) polyols, e.g., glycerol and sorbitol, usually used as bio-preservatives and cryoprotectants, are capable of maintaining structural integrity and activity of (macro) biomolecules in aqueous solution at low temperature, however, they might influence the tertiary or quaternary structures of biomolecules solubilized in it. Many works reported the mechanisms of glycerol affecting water-soluble proteins [10–17], e.g., the glycerol viscosity causing proteins deformation [18], the solvent-accessible surface area of protein [10], the formation of hydrogen-bond network between water and glycerol [19, 20], and the access of glycerol to the catalytic site [21]. Concerning the effects of low-molecular-weight polyols on photosynthetic membrane proteins, glycerol addition was found to facilitate Q_A -to- Q_B

electron transfer and consequently accelerate $P^+Q_B^-(P^+Q_A^-)$ charge recombination through changing protein conformational dynamics in bacterial RCs buffer-detergent solutions [16, 17]. These studies addressed the critical roles of the hydration shell, composed of the extended hydrogen-bond (HB) network among polyol, residual water, and protein surface residues, in the conformational dynamics and the structural stability of proteins [22, 23].

We have reported the effect of glycerol and sorbitol, as cosolvents, on the embedded pigment's excitation dynamics of LH2 from moderately thermophilic purple bacterium *Tch. tepidum*, in terms of the interference of hydration status of the whole pigment-protein complexes induced by glycerol and sorbitol [24]. In this study, we intend to examine the effects of glycerol and sorbitol on the hydration status of LH2s from *Rba. sphaeroides* 2.4.1 in aqueous phase. *Rba. sphaeroides* 2.4.1 owns *acidophila*-like LH2 based on their α, β -apoprotein sequences similarity [25]. We systematically adjusted the volume fractions of glycerol or sorbitol (0–80%) to tune the water activity around the LH2 α, β -apoproteins. Accordingly, the spectral changes of both ground-state absorption/fluorescence and triplet excited-state dynamics of the pigment cofactors had been observed. The results are discussed in terms of the water activity effects of C- and N-termini on the relative BChl–Crt and BChl orientations.

2 Materials and methods

2.1 LH2 preparation

The LH2 was prepared from *Rba. sphaeroides* 2.4.1 following the procedures reported in ref [26]. The LH2 preparation in 20 mM Tris–HCl buffer (pH 7.8) with 0.1% LDAO was used in the experiments. A series of LH2 samples in 20 mM Tris–HCl buffer (pH 7.8, 0.1% LDAO) with different glycerol or sorbitol contents, 20%, 40%, 60%, and 80% (v/v), were prepared for the subsequent spectroscopic measurements.

2.2 Steady-state spectroscopy

Visible-to-near-infrared absorption spectra were recorded on a Cary-50 absorption spectrometer (Varian). Near-infrared fluorescence spectra were recorded on the F-4600 fluorescence spectrometer (Hitachi), whose spectral response sensitivity was calibrated by the use of an HL-2000 CAL radiometric calibration standard (Ocean Optics Inc.). For Raman spectroscopy, the continuous-wave optical excitation at 488 nm was provided by an Ar⁺ laser (Reliant 300 M, Laser Physics Inc.), and the excitation power was 1.5 mW. A backward scattering geometry was used for collecting the scattering light, which was sent to a triple spectrograph (Trivista

555, Princeton Instruments, USA), and was detected by an intensified CCD detector (ICCD PI-MAX, Princeton Instruments). Before Raman measurements, the LH2 preparations admixed with glycerol or sorbitol at various fractions were incubated for 6 h. The total exposure time was 100 s for each measurement. Background spectra were recorded and subtracted from the Raman spectra of the same samples. All measurements were operated at room temperature.

2.3 Laser flash photolysis

The excitation pulses at 532 nm (1.4 mJ/pulse, 7 ns, 10 Hz) were the second harmonics from a Nd³⁺:YAG laser (Quanta-Ray Pro-Series, Spectra Physics Lasers Inc.). The visible-to-near-infrared probe light was provided by a laser-driven white light source (LDLS-EQ-1500, Energetiq), which was sent to a spectrograph (SP2500i, Princeton Instruments, USA). Appropriate optical filters were set in front of the entrance slit to avoid the interference from laser scattering and ambient stray light. The temporal evolution profile of difference absorption (ΔOD) was recorded with a Si-PIN photodiode (model S3071, Hamamatsu Photonics), and the transient output was fed to a digital storage oscilloscope (bandwidth 600 MHz; LeCroy WaveSurfer 64Xs, Chestnut Ridge). The time resolution was 100 ns as defined by the full width at half maximum (FWHM) of the instrumental response function (IRF). The kinetics trace at a specific probing wavelength was averaged for 250 shots, yielding a ΔOD detection sensitivity better than 10^{-4} . The LH2 preparations in a quartz glass cuvette with optical path length of 1 cm were adjusted to an optical density of $OD_{532nm} = 0.5$. To remove oxygen, the LH2 samples were bubbled with high purity nitrogen for 30 min before measurements. The measurements were carried out at room temperature (298 K). The least-square curve fitting programs for kinetics analyses were coded based on Matlab 5.2 (Mathworks Inc.).

3 Results

3.1 Resonance Raman spectroscopy

Resonance Raman spectroscopy is widely used to investigate the conformational change of Crt_s in solutions and in light-harvesting complexes [1, 27–32]. The Crt_s lie in the hydrophobic environment of membrane, which is apart from the aqueous phase. Moreover, in *Rba. sphaeroides* 2.4.1 LH2, the main Crt_s component is spheroidene ($n_{C=C} = 10$) in planar all-*trans* conformation [33]. Figure 1 shows the resonance Raman spectra of Crt_s in *Rba. sphaeroides* 2.4.1 LH2 complexes in buffer-detergent

Fig. 1 Resonance Raman spectra of carotenoids in the LH2 complexes from *Rba. sphaeroides* 2.4.1 in buffer-detergent solutions (20 mM Tris-HCl, 0.1% LDAO, pH 7.8) with the indicated volume fractions of **a** glycerol and **b** sorbitol. Raman excitation wavelength was 488 nm (1.5 mW). In each panel, the spectrum shown in thicker black is an average of the Raman spectra under the four different glycerol or sorbitol fractions

solutions with different glycerol and sorbitol volume ratio (0 → 80% v/v). The excitation wavelength is 488 nm, which is matched the $S_2(1B_u^+) \leftarrow S_0(1A_g^-)$ absorptive transitions of Crt_s (mainly spheroidene ($n_{C=C} = 10$)). There are two key Raman lines at ~ 1517 cm⁻¹ (ν_1) and ~ 1152 cm⁻¹ (ν_2), respectively, meaning the symmetric C=C stretching and the C-C stretching mixed with C-H bending at ~ 1194 cm⁻¹. The Crt_s in *Rba. sphaeroides* 2.4.1 LH2 remaining all-*trans* planar conformation could be proved by the lack of the characteristic non-planar twist signal of conjugated backbone [34], i.e., the out-of-plane C-H wagging at ~ 966 cm⁻¹.

In each panel of Fig. 1, both the sum-up spectrum (thicker solid) and each spectrum recorded under the various polyol fractions closely resemble the spectrum of the control (0%). Based on this constant Raman mode for all of the samples with various fractions of glycerol or sorbitol mixing, the conclusion that no obvious interference from the addition of these two polyols were performed on Crt_s conformation could be drawn.

3.2 UV–near-infrared absorption and fluorescence spectroscopies

Considering that glycerol may cause protein degradation at room temperature, steady-state spectroscopic experiments were carried out at different times for glycerol with a high volume ratio (80% v/v) in the sample, as shown in Figure S1 (Supporting Information), it was found that during the course of 2–6 h after mixing, the sample and glycerol reached a relatively stable state, and the protein was not degraded. In Fig. 2, each absorption spectrum is an average of two independent measurements for the LH2 preparations solubilized for 2 and 6 h, respectively. Due to the constant conformation adopted by Crt in all preparation (proved by Raman measurement and as shown as Fig. 1), the absorption spectra are normalized at the Crt absorption maxima (~480 nm, Fig. 2). The effects of polyols on the steady-state absorption and fluorescence spectra of BChl bands are characterized as the following. For steady-state absorption spectra, comparing with the control sample, upon the increase of glycerol and sorbitol fractions from 20 to 80% v/v, the Soret band (~376 nm) and the Q_x band (~590 nm) of BChls change slightly, while the Q_y bands of B800 absorption intensity decrease by 22% and 16% for glycerol and sorbitol, respectively, the Q_y bands of B850 absorption intensity decrease by 8% and 19% for glycerol and sorbitol, respectively. The 80%

v/v glycerol causes ~2 nm red shift for B850 Q_y band, ~1 nm blue shift for B800 Q_y band (Fig. 3). These results indicate that glycerol can affect the aggregation condition of both B800 and B850, and sorbitol only affects B800's. Besides,

Fig. 3 Steady-state absorption peak position of B800 (square) and B850 (triangle) of the LH2 complexes from *Rba. sphaeroides* 2.4.1 in buffer-detergent solutions (20 mM Tris-HCl, 0.1% LDAO, pH=7.8) with the increased volume fractions of glycerol (black) and sorbitol (red)

Fig. 2 Steady-state absorption (solid) and fluorescence (dashed) spectra of the LH2 complexes from *Rba. sphaeroides* 2.4.1 in buffer-detergent solutions (20 mM Tris-HCl, 0.1% LDAO, pH 7.8) with the indicated volume fractions of **a** glycerol and **b** sorbitol. In either

panels, the absorption spectra are normalized at the Crt absorption maxima (480 nm), while the fluorescence spectra are shown without normalization. Fluorescence excitation wavelength was 800 nm

we observed that 80% v/v glycerol causes a slight rise of the shorter wavelength side (< 820 nm) of the spectra (Fig. 2a), which comes from the free BChls. Such a rise indicated that the structural intactness of LH2 complexes is broken due to the addition of very high percentage of glycerol. For the fluorescence spectra, when glycerol volume fractions increase from 20-to-40% v/v, the intensity increase by ~25%, but from 60-to-80% v/v, decrease by ~21%. When sorbitol volume fractions increase from 20-to-80% v/v, the intensity increases by ~40%. The polyol induced spectral changes of the B800 and B850 Q_y absorption characterized above should be ascribed to the variation in the tertiary structures of the *Rba. sphaeroides* 2.4.1 LH2 complexes, which might be a result of the water activity changed in solution induced by polyols (see Discussion).

3.3 Excitation dynamics of *Rba. sphaeroides* 2.4.1 LH2 complexes in aqueous phase without polyols

In bacterial photosynthetic pigment–protein complexes, Crt can play photoprotective roles via rapid BChl-to-Crt triplet excitation energy transfer (EET). This process occurs in a few nanoseconds and can unitarily quench $^3\text{BChl}^*$ to avoid the formation of deleterious singlet oxygen ($^1\text{O}_2^*$) [25, 35]. For *Rba. sphaeroides* 2.4.1 LH2 complexes, we investigated the triplet excitation dynamics of Crt and BChls without polyols. Used laser flash photolysis with the actinic laser pulses at 532 nm, which corresponding to the $S_2(1B_u^+) \leftarrow S_0(1A_g^-)$ transition of Crt. The ultrafast Crt(S_2)-to-BChl(Q_x) singlet EET processes (~100 fs) account for an overall transfer efficiency > 90% [36] (whereas the Crt(S_1)-to-BChl(Q_y) channels of EET are

interrupted for energetics reasons). The subsequent BChl-to-Crt triplet EET takes place within 10 ns, and all of the excitation conversion and EET processes accomplished within the instrumental response time of 100 ns [37]. The sub-microsecond time-resolved absorption spectra are predominated by the triplet excitations of spheroidene ($n = 10$) in *Rba. sphaeroides* 2.4.1 LH2 complexes. To enhance the quality of spectral, we divided the whole spectra into some regions. Take the control sample in buffer solutions without polyols as an example (Fig. 4a): (i) Overall photoexcitation induces the ground-state absorption bleaching (BLC) of Crt with maximum vibronic features at 475 nm, shoulder peaks at 445 nm and 510 nm. Because Crt ESA and BLC come from $^3\text{Crt}^*$ generated by BChl-to-Crt triplet EET, the main peak of Crt excited-state absorption (ESA) at 530 nm and shoulder peak at 495 nm have the same decay time with Crt BLC. (ii) In the initial temporal time regime of 0–0.5 μs , B800 and B850 BLC signals appear at ~800 nm and ~850 nm, respectively. A negative signal at the region of 920–980 nm is the red wavelength side of spontaneous B850 fluorescence (~866 nm, Fig. 2), and the time regime partially coincides with the instrumental response time (~0.1 μs). We assigned this negative signal to the stimulated emission of B850. (iii) In the temporal regime of 0.5–1.0 μs and later, a long time after BChl-to-Crt triplet EET and yielding $^3\text{Crt}^*$, the distinct B800 and B850 BLC features appear (the inset of Fig. 4a). Compared with the time regime of 0–0.5 μs , the intensities of B800 and B850 BLC decrease. (iv) In the 3–7.5 μs time region (cover the lifetime of $^3\text{Crt}^*$), a weak 855 nm ESA accompanied by B850 BLC is shown in the inset of Fig. 4a and farther blowup is in Fig. 4b.

These weak BLC and ESA of BChl are not from free BChl pigment whose triplet excited state lives in several

Fig. 4 a Transient spectra for the LH2 complexes from *Rba. sphaeroides* 2.4.1 in buffer-detergent solution (20 mM Tris-HCl, 0.1% LDAO, pH 7.8) without polyol (the control). Inset shows the

blowup spectra in the near-infrared region. **b** Transient spectra at 3–7.5 μs and the simulated spectrum (red solid. See text for details)

tens of microseconds. Similar phenomena are observed for *Tch. tepidum* LH2 excited at Crt directly named *interaction bands* [38], which suggest originated from the interaction of $^3\text{Crt}^*$ excitation with the adjacent BChl molecules [4, 18]. Therefore, we ascribed this ESA features to the exciplex-like species owing to the $^3\text{Crt}^*\text{--BChl}$ electronic coupling. The near-infrared B850 BLC along with the ESA forms a derivative-type spectrum in 830–870 nm (Fig. 4a, b). This spectral feature could be described by the red-shifted B850 ground-state absorption spectrum $\text{OD}_{\text{B850}}(\lambda + \delta\lambda)$ to minus the original B850 ground-state absorption spectrum $\text{OD}_{\text{B850}}(\lambda)$ using the below equation [24]:

$$\Delta\text{OD}_{\text{obs}}(\lambda) = A_1 \times \text{OD}_{\text{B850}}(\lambda + \delta\lambda) - A_2 \cdot \text{OD}_{\text{B850}}(\lambda), \quad (1)$$

where $\delta\lambda$ represents the amount of band shift, and A_1 and A_2 are amplitude parameters. The first term of Eq. (1) stems from the dynamic Stark effect of $^3\text{Crt}^*$ on the adjacent B850 molecules caused B850 ground absorption red shift by $\delta\lambda$, while the second one originated from the depletion of B850 molecules ground absorption spectrum. In this scenario, the size of $\delta\lambda$ can be regarded as a metric of the $^3\text{Crt}^*\text{--B850}$ coupling strength, i.e., a larger $\delta\lambda$ implies a stronger coupling. Figure 4b exemplifies the simulation of the 3–7.5 μs transient spectrum, which yield B850 ground absorption red shift of $\delta\lambda \approx 12.2$ nm (Table 1). The positive amplitude is slightly larger than the negative one ($A_1/A_2 \approx 1.30$), which makes this derivative-type spectrum lightly asymmetric. The dynamic Stark effect between $^3\text{Crt}^*$ and B850 induces a red-shifted B850 ground-state absorption, which can be used to compare the $^3\text{Crt}^*\text{--B850}$ interaction level with solution

added glycerol and sorbitol. The weak B800 and B850 BLCs decay corresponding with the $^3\text{Crt}^*$ ESA/BLC decay time is ~ 4.5 μs (as determined by simultaneously fitting the Crt kinetics at 475 nm and 530 nm, B800 and B850 BLC kinetics to a mono-exponential model function, see Table 1 and Figure S2 for details). The lifetime is longer than $^3\text{Crt}^*$ in *Tch. tepidum* LH2 at a similar condition in our previous result (data not shown) [24], because Crt with longer conjugation length has longer $^3\text{Crt}^*$ lifetime (Crt with $n_{\text{C=C}} = 13$ dominated in *Tch. tepidum* LH2) [25].

3.4 Effects of glycerol and sorbitol on the triplet excitation dynamics of *Rba. sphaeroides* 2.4.1 LH2

Figure 5 shows the transient spectra of *Rba. sphaeroides* 2.4.1 LH2 complexes in buffer solution with glycerol or sorbitol in different volume fractions (40% and 80%, v/v). See Supporting Information Figure S3 for the other volume fractions (20% and 60%, v/v).

Compare the glycerol additions (Fig. 5a, b, 40% and 80% v/v) with the control one (Fig. 4a, 0% v/v), the BLC and ESA of Crts in the visible region remain the same, but the *interaction bands* are highly variable. It is interesting to see that 20% v/v and 80% v/v glycerol led to the disappearance of the $^3[\text{Crt} \cdots \text{BChl}]^*$ *interaction band* at ~ 860 nm (Fig. 5B, Figure S3A), Comparing with control one, the $^3[\text{Crt} \cdots \text{BChl}]^*$ *interaction band* (~ 860 nm) absent at 20% v/v sorbitol, but more prominent upon mixing with other sorbitol volume fractions (Fig. 4a, 0% v/v, Fig. 5, Figure S3). Besides, glycerol induced slight stronger molecular interactions between $^3\text{Crt}^*$

Table 1 Dynamic Stark shifts ($\delta\lambda$) derived by least-square spectral fitting the *interaction bands* at 3–7.5 μs to equation $\Delta\text{OD}_{\text{obs}}(\lambda) = A_1 \cdot \text{OD}_{\text{B850}}(\lambda + \delta\lambda) - A_2 \cdot \text{OD}_{\text{B850}}(\lambda)$

Concentration (v/v)	Glycerol			
	a_w^a	$\delta\lambda$ (nm)	A_1/A_2	τ_T (μs)
0%	1	12.23 ± 1.03	1.30	4.48 ± 0.02
20%	0.936	0.00 ± 0.00	2.09	3.84 ± 0.03
40%	0.828	14.07 ± 5.37	1.29	4.69 ± 0.03
60%	0.683	10.75 ± 3.17	1.12	6.11 ± 0.04
80%	0.399	0.00 ± 0.08	1.05	5.65 ± 0.03
	Sorbitol			
20%	0.955	0.00 ± 2.23	1.00	5.59 ± 0.02
40%	0.897	12.43 ± 6.00	0.40	5.59 ± 0.02
60%	0.843	3.03 ± 0.80	1.15	5.49 ± 0.02
80%	0.803	4.03 ± 2.36	0.80	6.41 ± 0.02

The amplitude ratio A_1/A_2 indicates the relative contribution of the dynamic Stark shift to the observed *interaction bands* (see Supporting Information S4 for details). The triplet excited state lifetime (τ_T) of $^3\text{Crt}^*$ obtained by global fitting of the four kinetics traces including the BLC and ESA of Crts and the BLC of B800 and B850 (see Supporting Information S2 for details), and water activity (a_w) of the binary solvents at 25 $^\circ\text{C}$, the B800/B850 BLC amplitude ratio (r)

^aFrom Reference[39]

Fig. 5 Transient spectra for the LH2 complexes from *Rba. sphaeroides* 2.4.1 in buffer-detergent solutions (20 mM Tris–HCl, 0.1% LDAO, pH 7.8) with the indicated volume fractions of **a, b** glycerol and **c, d** sorbitol. Insets show the blowup spectra in the near-infrared region

and BChl ($\delta\lambda = 11\text{--}14$ nm for glycerol and $3\text{--}12$ nm for sorbitol (Table 1, Supporting Information Figure S4)). The $^3\text{Crt}^*$ lifetimes varied in $3.8\text{--}6.1$ μs , showing no clear dependence on the polyol type and volume fractions (Table 1).

4 Discussion

The Q_y absorption spectra of purple photosynthetic bacteria light-harvest antenna LH2 are susceptible to the ionic properties of detergents, metal ions, temperature, pressure, pH, and grown situations [7, 8, 40–43]. In the present work, we investigated the structure and excitation dynamics of *Rba. sphaeroides* 2.4.1 LH2 complexes in aqueous phase mixed with glycerol or sorbitol by a series of steady-state and time-resolved spectroscopy. The results show profound variation in the near-infrared absorption and fluorescence spectra of BChls and dynamics of $^3\text{Crt}^*\text{--BChl}$ interactions upon the addition of glycerol and sorbitol. Based on the steady-state

and excited-state spectral properties (dynamics) dependence of polyol concentration and type, the discussion will be focused on the effect of water activity on the C- and N-termini of proteins, which binding with BChls and exposed to the aqueous phase.

4.1 Hydration susceptible protein-BChl hydrogen-bond strength

For bacterial LH2 complexes solubilized in buffers, the hydrophobic membrane-spanning section ($30\text{--}35$ Å) is surrounded by detergent molecules, while the relatively hydrophilic C- and N-termini are exposed to the aqueous phase [44, 45]. In the case of *Rps. acidophila* 10050, the high-resolution crystal structures support that the N-terminal domains of LH2 α,β -apoproteins, embracing $\alpha\text{-Met}_{30}$ as the ligand to B800 (Figure S5), are susceptible to hydrostatic pressure or pH [9, 46]. For example, under hydrostatic pressure, the B800 Q_y absorption decreases, which is due

to the destabilized tertiary or quaternary structures of the N-terminal domains owing to the loss of B800 cofactors and the access of water molecules to the B800 binding motifs. The C-terminal domains of LH2 α, β -apoproteins, α -Trp₊₁₄, and α -Tyr₊₁₃ form stronger H-bindings with B850, and they are sensitive to environmental change such as basic pH, which makes B850 remarkable spectral blue shift from 852 to 837 nm in *acidophila*-like *Rhodopseudomonas palustris* [41]. Note that the *Rba. sphaeroides* LH2 is considered to be *acidophila*-like, the α, β -apoprotein sequences resemble those of *Rps. acidophila* LH2 meaning similar B800 or B850 binding motifs between these LH2 complexes (See Supporting Information Figure S5 for a comparison of the LH2 α, β -apoprotein sequences). The crystal structure of *Rps. acidophila* LH2 reveals that there are some hydrogen bonds between the B800 C3 acetyl group and β -Arg₋₁₀. B850 C3 acetyl groups form hydrogen bonds with α -Trp₊₁₄ and α -Tyr₊₁₃, respectively. C13² ester group forms a weak hydrogen bond with α -Ala₋₄ [4]. *Rba. sphaeroides* 2.4.1 LH2 is *acidophila*-like and has a similar gene sequence with *Rps. acidophila* LH2, which suggests that some similar hydrogen bonds may be present in *Rba. sphaeroides* 2.4.1 LH2 [47], but α -Ala₋₄ is replaced by α -Ser₋₄, α -Trp₊₁₄ is replaced by α -Tyr₊₁₄. The above residues almost are weak hydrophobic, which are exquisitely sensitive to the water molecular [48]. Compared with the sequence of *Tch. tepidum* LH2, the BChls-protein hydrogen bonds are closer to the terminal of protein (Figure S5). The high-resolution neutron scattering analyses have shown that the numbers of water–glycerol hydrogen bonds compensate for the reduction in water–water and glycerol–glycerol hydrogen bonding, and glycerol molecules take the place of water network molecules in the hydrogen-bonded network, then impeded the extended water network [19]. The protein hydration interface is disturbed by the hydrogen-bonding network from the bulky water–polyol mixture. The synchrotron radiation wide-angle X-ray scattering analyses have revealed that at glycerol concentrations above 50% v/v, the penetration or replacement of glycerol into or with the hydration shell of water molecules surrounding the protein surface appears to depend on the glycerol concentration [49].

It infers that the polyol molecules additions lead to the change of the hydration of proteins, which influence the hydrogen-bond strength between BChls and residues and then affect the characteristics of the steady-state spectrum. In our experiments, glycerol and sorbitol additions changed the B800 & B850 Q_y absorption and the fluorescence spectra intensities and peak wavelengths; however, at the same volume fraction, glycerol–water binary solvents showed lower water activity generally [39] (Table 1). Therefore, comparing with sorbitol, glycerol is expected to be more influencing on the hydration status of LH2 complexes. The water activity effects on LH2s can be considered as that the polyol-water

hydrogen-bonding network causes a loss of activity water adjacent to the hydration layer of the N-terminal and C-terminal of protein which is close to BChls binding sites.

As the glycerol and sorbitol volume fractions increased from 20 to 40%, the changes in steady-state absorption and fluorescence-emission were similar. When the glycerol volume fractions reached to 60% and 80% volume fraction, the B850 absorption band had experienced ~2 nm red shift, and fluorescence-emission intensity had declined by ~21% with ~1 nm red shift. However, 60% and 80% v/v sorbitol remained the same fluorescence intensities without red shift. These spectral changes suggest that low water activities can compress the LH2 complexes and enhance molecular interaction among BChls. After all, at the volume fraction of 80%, the water activity of sorbitol solution (0.803) is about twofold of glycerol solution (0.399, Table 1).

4.2 Hydration susceptible BChl–Crt interaction

The effective ³Crt*–BChl interactions fall into van der Waals sphere, and it is sensitive to the BChl–Crt mutual orientation. Adding glycerol and sorbitol altered the shape of ³Crt*–BChl interaction bands of *Rba. sphaeroides* 2.4.1 LH2 complexes (Figs. 4, 5, and Figure S3). The resonance Raman spectroscopy had proved that the Crts remained all-trans and planar conformation, so the Crts are essentially unaffected by the water activity change. As discussed above, BChl binding with protein through hydrogen bond and sensitive to water activity, so the changes in interaction bands spectral are mainly due to the geometrical alteration of BChls relative to Crts.

The dynamic Stark shift ($\delta\lambda$) of B850 BLC varied with glycerol and sorbitol concentrations change. The sample without polyol caused a band shift of $\delta\lambda \approx 12.2$ nm at long delay time 3–7.5 μ s. For glycerol and sorbitol additions, the $\delta\lambda$ were 11–14 nm, and 3–12 nm, respectively, shown polyol volume fraction independent. Only the LH2 in 20%, 80% v/v glycerol and 20% v/v sorbitol solution, the interaction bands vanished (i.e., the ³[Crt⋯BChl]* interaction band at ~855 nm disappeared), which make none band shift of $\delta\lambda \approx 0.0$ nm (Table 1). Besides, the glycerol additions have a bit larger A_1/A_2 . However, the mechanism behind this phenomenon remains to be explored. A plain conjecture is that glycerol and sorbitol have different molecular weight, may cause different effects on the water hydrogen-bond network, then form different polyol-water and polyol-protein hydrogen-bond patterns, which led to different effects on BChl orientations change. Here, we note that the changes of intriguing fluorescence intensity can be connected to the drastic decrease of water activity. Glycerol addition with lower water activity reduces the fluorescence intensity. After all, research has shown that under 80% molar fraction of glycerol, majority water molecules exist as monomers owing

to the strong hydrogen bonding to glycerol, so the original hydrogen-bonded network of water is disrupted [50]. However, the refined explanation about this phenomenon effect on protein and binding BChls needs more elaborate experiments.

5 Conclusions

Used steady-state optical spectroscopies and sub-microsecond laser flash photolysis, we investigated the impacts of glycerol and sorbitol on the near-infrared absorption/fluorescence, and the triplet excitation dynamics of the detergent-solubilized *Rba. sphaeroides* 2.4.1 LH2 complexes. The BChl Q_y absorption/fluorescence experienced changes in glycerol and sorbitol additions, which correlated to the effect of water activities of the water-polyol binary solvents on the hydration status of the LH2 C- and N-termini. The dynamic spectra of the $^3\text{Crt}^*$ -BChl interaction bands were found been influenced by glycerol and sorbitol additions, which were interpreted to BChl-Crt orientation respond to polyols addition. The *Rba. sphaeroides* 2.4.1 LH2 are found to retain the photoprotective functionality via efficient BChl-to-Crt triplet excitation energy transfer, and reference the thermophiles purple bacterium *Tch. tepidum*, *Rba. sphaeroides* 2.4.1 LH2 is more sensitive to low-molecular-weight polyols.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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