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ORIGINAL CONTRIBUTION

Effect of denaturants on the stability of light-harvesting complex

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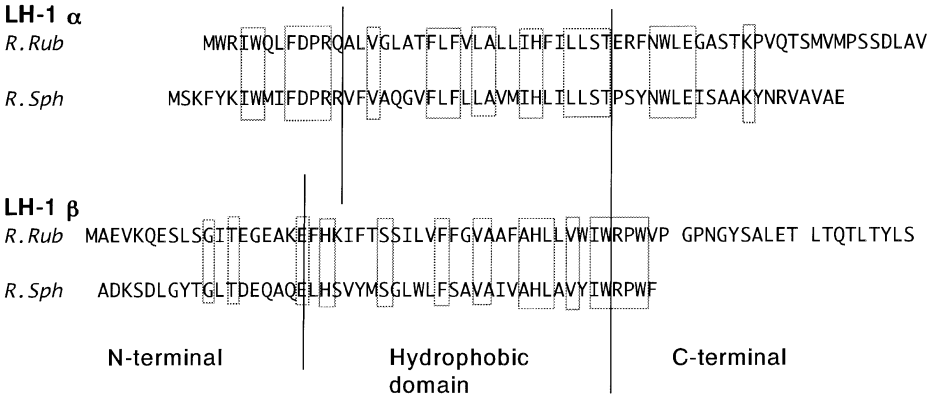
Abstract The effect of denaturants such as urea and normal alcohols on the formation of light-harvesting (LH) polypeptides/bacteriochlorophyll *a* (BChl*a*) complex (LH1 complex) in *n*-octyl-*β*-D-glucopyranoside (OG) micelle was examined to provide an insight into stability of the complex. The stabilities of the LH1 complex in OG micelle and of the complex in the chromatophore of photosynthetic bacteria were compared by addition of denaturants. The extent of stability of these complexes was monitored by the change in absorbance of Qy band of BChl*a* in these complexes, resulting generally in the blue-shifting of the Qy band from near 870 nm to about 777 nm upon addition of these denaturants. Urea and guanidium hydrochloride (Gnd) showed a relatively weak denaturing effect. Normal alcohols showed stronger denaturing effect, depending on the hydrophobicity of the alcohols. These results imply that the stability of LH1 complex in OG micelle can be largely attributed to the hydrophobic interactions in the complex as well as that of the complex in the chromatophore of photosynthetic bacteria.

Key words Denaturants – OG micelle – bacteriochlorophyll – reconstitution – light-harvesting complex

Introduction

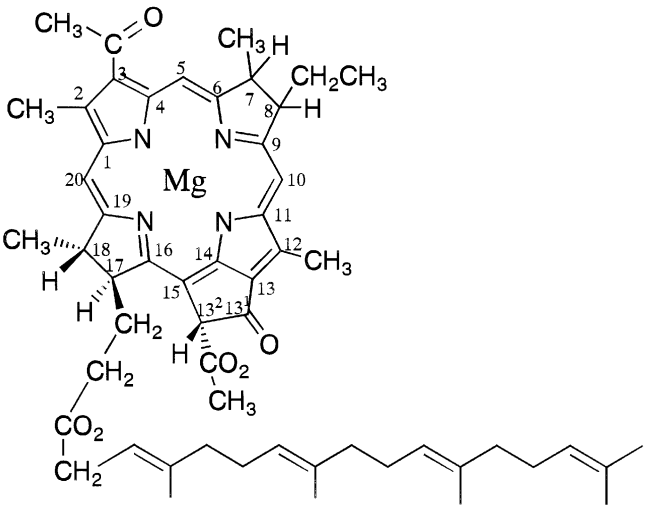
In photosynthetic bacteria, light-harvesting (LH) polypeptides/bacteriochlorophyll *a* (BChl*a*) complexes catch photon and transfer it to adjacent reaction center (RC), in which the LH polypeptides/BChl*a* complexes play an initial event in photosynthesis [1–5]. The special pair of bacteriochlorophyll derivatives in RC accepts photon and induces charge separation [6–8]. The cyclic transmembrane electron transfer by RC and metalloprotein such as cytochrome bc_1 generates proton gradient across the membrane as photon is converted to chemical energy. Purple photosynthetic bacteria *R. rubrum* has only one type LH polypeptides (LH1), while *R. sphaeroides*, *R. capsulatas*, *R. viridis* and *R. Acidophia* and others have two types of LH polypeptides, LH1 (core) and LH2 (peripheral). Recently, the crystal structure of LH2 for *R. acidphila* was revealed by X-ray analysis [9]. In photosynthetic bacteria, the structure of the LH2 complex is similar to that of LH1, which includes *α*- and *β*-polypeptides. Their molecular weights are about 6000 Da [10–15]. Each polypeptide has a central hydrophobic core to allow the LH polypeptides taking transmembrane topology. The topographical analysis of the arrangement has revealed that C- and N-terminals of both polypeptides are found on the periplasmic and cytoplasmic sides of the membrane [1–5]. By analogy from LH2 of *acidphila* [9, 10], the

Scheme 1 Amino acid residues of LH-1 of photosynthetic bacteria, *R. rubrum* (*R. Rub*) and *R. sphaeroides* (*R. Sph*)



transmembrane helices of α-polypeptides are packed side by side to form a hollow cylinder. The β-polypeptides are also arranged radially with the α-polypeptides to form an outer cylinder. BChl*a* is bound at the histidine site (His) of α- and β-polypeptides, which faces outwards and inwards, respectively, and the faces are parallel to the membrane. It is interesting to note that some non-covalent bonds organize these LH polypeptides/BChl*a* complexes (LH1) and the helices can form intersubunits between radial and adjacent α- and β-polypeptides through several hydrophobic interactions and hydrogen bonds at C-terminal aromatic residues. Recently, the results of site-directed mutagenesis indicate that LH1 formation requires certain amino acids. In particular, the hydrogen bond between tryptophan (Trp) on the C-terminal of α-polypeptide and BChl*a* is likely to play an important role for orientation and aggregation of BChl*a* [5, 16]. Furthermore, Meadows et al. reported that some of the N-terminals are important for LH1 formation [17]. However, the hydrophobic core and the C-terminal hydrophobic segment also play an important role on packing together to produce a well-defined three-dimensional LH–BChl*a* complex. The LH1 complex of purple photosynthetic bacteria can be reconstituted from separately isolated α- and β-polypeptides and BChl*a* [18, 19]. In the formation of the reconstituted LH1 complex in OG micelle, the Qy band of BChl*a* is shifted from 777 nm (monomeric form) to near 820 nm (B820 complex) as a subunit form, and fully shifted to near 870 nm (B870 complex), indicating that the purified LH polypeptides have a strong activity for binding and orientation of BChl*a*. Loach characterized the formation of LH1 complex in OG micelle spectroscopically [17–19], although the stability of the complex in OG micelle was little investigated.

In this paper, we report the effect of denaturants such as urea and normal alcohols on the formation of LH1 complex in OG micelle. The results were expected to provide an insight into the stability of the complex. The extent of stability of the formation of LH polypeptides (Scheme 1) and BChl*a* (Scheme 2) complex in OG micelle was monitored by the change in absorbance of Qy band of BChl*a*. λ_{max} was blue-shifted from near 870 nm (LH1 complex form) to about 777 nm (monomeric form) upon addition of denaturants. The comparison of stabilities of LH1 complex in OG micelle and the complex in the chromatophore of photosynthetic bacteria was made by the addition of various denaturants.



Scheme 2 Bacteriochloropyll (BChl*a*)

Experimental

Materials

n-Octyl-β-D-glucopyranoside (OG), ethylenediamine tetraacetic acid (EDTA), acetic acid, and high-performance liquid chromatography (HPLC) solvents (acetonitrile, 2-propanol, and acetone) were obtained from Nakarai Co.

Sephadex LH-60 was purchased from Sigma Co. Hexafluoro acetone (HFA) and trifluoroacetic acid (TFA) were obtained from Tokyo Kasei Co. Endoproteinase Glu-C was purchased from Boehringer Mannheim. Halothane ($CHClBrCF_3$) was a gift from Prof. Tadayoshi Yoshida, Nagoya Institute of Technology.

The growth of *R. rubrum* wild type, G-9 and *R. sphaeroides*

R. rubrum wild type was anaerobically grown in modified Hunter's media [20] under low-intensity fluorescent lights as previously described [21]. *R. rubrum* G-9, the carotenoidless mutant, was grown anaerobically in modified Hunter's media with yeast extract and illuminated with tungsten light. *R. sphaeroides* puc-705 BA, LH-2-less mutant, was grown anaerobically in Sistrom media. The cells were harvested during logarithmic growth (usually in 4–6 days), centrifuged, washed once in 50 mM phosphate buffer, pH 7.5, repelleted and stored at $-20\,^{\circ}C$ as a pellet until use.

Chromatophore preparation

Chromatophores were isolated from whole cells as previously described [21]. The whole cells were resuspended in 50 mM potassium phosphate buffer, pH 7.5, sonicated for 10–12 min, 50% pulse, and centrifuged to separate the chromatophores from the cell debris and unbroken whole cells. The chromatophores were then washed twice in phosphate buffer and twice in deionized water.

Isolation of α- and β-polypeptides

LH α- and β-polypeptides of *R. sphaeroides* or *R. rubrum* (Scheme 1) were isolated as described previously [17–19]. LH α-polypeptide was isolated by extracting 150 mg of chromatophores with 5 ml of $CHCl_3/CH_3OH$ [1 : 1 (v : v)] and running the extract four times on a Sephadex LH-60 column equilibrated in $CHCl_3/CH_3OH$ [1 : 1 (v : v)] containing 0.1 M ammonium acetate to separate it from other proteins, pigments, and lipid. Then the precipitate was washed four times by 5 ml of $CHCl_3/CH_3OH$ [1 : 1 (v : v)] containing 0.1 M ammonium acetate. The LH-β protein was extracted four times from the resultant precipitate by $CHCl_3/CH_3OH$ [1 : 1 (v : v)] containing 0.1 M ammonium acetate and 10% acetic acid. The extracted LH-β was then passed through an LH-60 column equilibrated with $CHCl_3/CH_3OH$ [1 : 1 (v : v)] containing 0.1 M ammonium acetate and 10% acetic acid. For further purification, the polypeptides were chromatographed on a reverse-phase HPLC system.

HPLC system

α- and β-polypeptides, and BChl*a* were purified by HPLC [17]. The polypeptides were dissolved in HFA trihydrate and injected into Perkin–Elmer Integral 100 with GL Science C-18 column (0.5×12 cm). A gradient program was used to elute the protein. It employed 0.1% TFA as the organic solvent [acetonitrile : 2-propanol = 2 : 1 (v : v)]. BChl*a* was purified by using $(CH_3)_2C{=}O : CH_3OH : H_2O$ = 64 : 20 : 16 (v : v) as single-phase eluent, and monitored at 360 nm.

Purification of bacteriochlorophyll *a*

The BChl*a* purification was carried out by literature method [22]. The pellet of whole cells (2L growth medium) from the carotenoide-less mutant, *R. rubrum* G-9 was suspended in 5 ml of CH_3OH, and centrifuged. The supernatant was collected three times, adding water to 50% CH_3OH. The resulting solution was passed through C-18 sep-pack (Waters), and the column was washed by 30 ml of CH_3OH : water = 70 : 30. The pigment was eluted with ethanol, and the solvent was removed in vacuo. The crude BChl*a* was analyzed and purified by HPLC (see HPLC section). The solvent of BChl*a* solution eluted from HPLC was removed in vacuo.

Reconstitution assay

The reconstitution was examined according to the method of Parkes–Loach [18, 19]. The polypeptide was dissolved in 0.5 ml of 4.5% OG (KPi buffer), and 2 ml buffer was added (0.9% OG concentration). BChl*a* dissolved in 2 μl was added to the solution and measured by absorption spectra. The OG concentration was further reduced in approximately 0.05% increments until an optimal absorbance near 820 nm was obtained (B820 complex condition). Finally, the resulting solution was chilled in an ice-bath or refrigerator overnight, during which the absorbance at near 870 nm (B870 complex condition) was obtained. Visible absorption spectra were measured with a Hitachi U-2000 spectrophotometer. CD spectra were measured with a Jasco J 600 spectropolarimeter.

Results

Reconstitution of LH polypeptides/BChl*a* complex

Figure 1 illustrates the visible absorption spectra of LH α- and β-polypeptides from *R. sphaeroides* and BChl*a* complex in OG solution. At 0.90% OG, the Qy absorption band of BChl*a* was split at 777 and 822 nm, then at 0.67% OG, in which 0.90% OG is above its cmc, while 0.67% OG is around its cmc [23]. The Qy band at 777 nm was shifted to 822 nm (B820 subunit-condition), which is the subunit structure of LH-polypeptides and BChl*a* complex as have been observed in the previous papers [18]. The Qy band was further red-shifted to 873 nm at 4 °C for 2 days (LH1 complex-condition) [18, 19], in which the absorbance is similar to the LH1 complex in the chromatophore of *R. sphaeroides*. Similar results of the absorption spectra were obtained for LH1 complex from LH α- and β-polypeptides of *R. rubrum* with BChl*a* in OG micelle, in which the homology of the sequence of amino acids between *R. rubrum* and *R. sphaeroides* is about 30–50%, depending on α- or β-polypeptide (Scheme 1). Near-IR CD spectra of LH α- and β-polypeptides from *R. sphaeroides* and BChl*a* complex were similar to that of the LH polypeptides from *R. rubrum* with BChl*a* in OG micelle and also to the complex in these chromatophores [18, 19], in which CD maxima at 870–880 nm and minima at 845–855 nm were observed. The detailed analysis of the CD spectra is described elsewhere [18, 19].

Effects of denaturants

Urea and guanidine hydrochloride (Gnd)

Urea or Gnd was added to the LH1 complex in OG micelle and also to the chromatophore of *R. rubrum* in buffer solution. Figure 2 shows that the absorbance at 870 nm for LH1 complex from LH α- and β-polypeptides of *R. rubrum* with BChl*a* and for the chromatophore decreased with increasing concentration of urea or Gnd, respectively. The absorbance at 870 nm (B870) for the chromatophore decreased to 80% while the absorbance for the complex in OG micelle decreased to about 60% when 8 M of urea or Gnd was added. However, the change of the split CD signal at the Qy absorption band of BChl*a* in the LH1 complex was not observed with addition of 8 M urea or Gnd. Furthermore, the CD spectra at 220–240 nm (near-IR region) indicated that the difference in α-helicities of these LH polypeptides between the LH1 complex in OG micelle and the complex in the chromatophore was not observed even in the presence of 8 M urea or Gnd (data not shown). These results indicated that LH1 complex in OG micelle and the complex in the chromatophore were not completely denatured even in the presence of 8 M urea or Gnd, in which Gnd was slightly more effective than urea.

Normal alcohols

Alcohols such as methanol, ethanol, *n*-propanol and *n*-butanol are added to the chromatophore from *R. rubrum* or *R. sphaeroides* in aqueous solution and their LH1 complexes in OG micelle. Figure 3 shows the absorption spectra of BChl*a* for the chromatophore of *R. rubrum* and for the LH1 complex in OG micelle when *n*-propanol was added. The absorbance at 879 nm (B870) for the

Fig. 1 Visible absorption spectra of the reconstitutions of B820 and B870 complexes from separately isolated LH α- and β-polypeptide from *R. sphaeroides* and BChl*a* in OG micelles. The spectra are diluted from 0.9% OG (- - - -) to B820 complex-forming condition of 0.67% OG at 25 °C (– – –) or to B870 complex-forming condition of 0.67% OG after storage at 4 °C overnight (———)

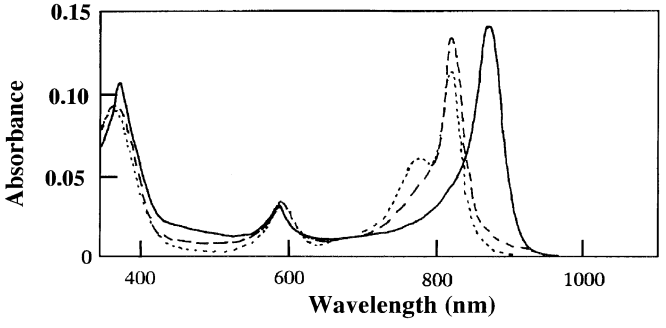
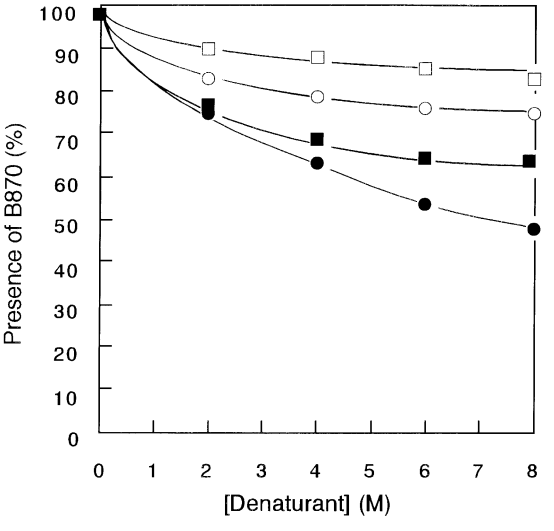


Fig. 2 The ratio of B870 complex remainder (%) for the chromatophore of *R. rubrum* in the presence of urea (□) and of guanidine (○), and for the reconstituted LH1 complex in the presence of urea (■) and of guanidine (○) at 0.67% OG after storage at 4 °C



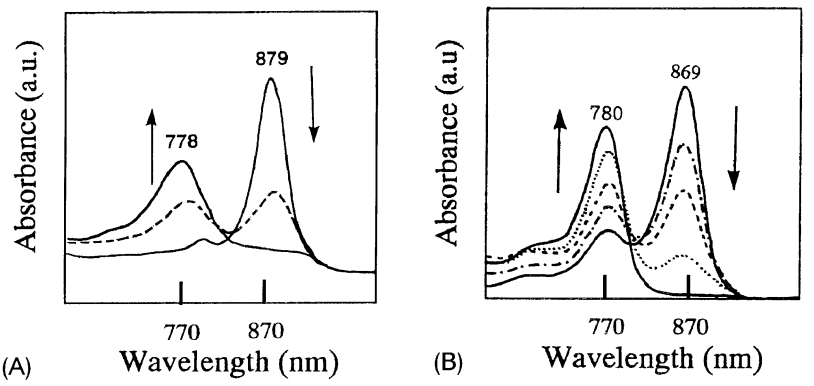


Fig. 3 The changes of absorption spectra upon addition of increasing amount of *n*-propanol at 0.67% OG after storage at 4 °C: (A) the chromatophore of *R. rubrum*; (B) the reconstituted LH1 complex from *R. rubrum*. Propanol concentrations are 0 M (——), 3.65 M (– – –), and 4.20 M (——) for (A) and 0 M (——), 1.74 M (- - -), 2.46 M (– – –), 3.09 M (–·–·–), and 3.65 M (————) for (B), respectively

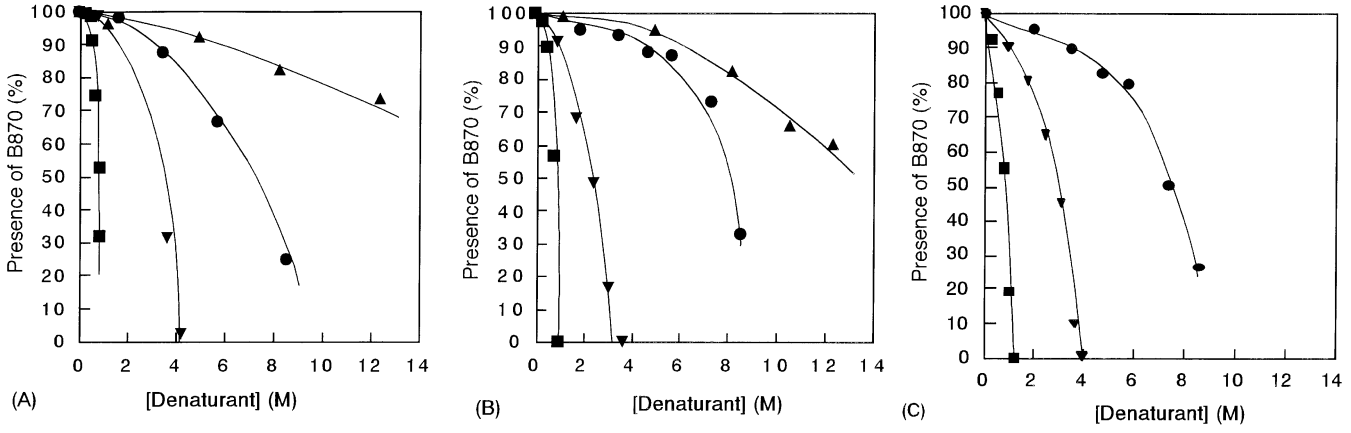


Fig. 4 The plots of B870 complex remainder (%) upon addition of various alcohols at 0.67% OG after storage at 4 °C: (A) the chromatophore of *R. rubrum*; (B) the reconstituted LH1 complex from *R. rubrum*; (C) the reconstituted LH1 complex from *R. sphaeroides*. (▲) MeOH, (●) EtOH, (▼) PrOH, (■) BuOH

chromatophore or at 869 nm for LH1 complex in OG micelle decreases with increasing concentration of *n*-propanol while the absorbance either at 778 or 780 nm increases, respectively, where the absorption of Qy band of BChl*a* is drastically blue-shifted upon addition of *n*-propanol. Similar addition effects of *n*-propanol were observed on the chromatophore of *R. sphaeroides* and the LH1 complex in OG micelle. These changes in the absorbance as shown in Fig. 3 were also observed when other alchohols were added. Figure 4 shows the change of absorbance at 870 nm (B870) for the chromatophore of *R. rubrum* or *R. sphaeroides* in aqueous solution and for the LH1 complex in OG micelle with increasing concentration of various alcohols. As is apparent from Fig. 4, the change of absorbance at 870 nm decreases with increasing concentration of alcohols for all cases. The order of the change becomes large as follows; methanol < ethanol < *n*-propanol < *n*-butanol for both samples, indicating that the change largely depends upon the hydrophobicity of the alcohols. To further examine the extent of stability of the LH1 complex in OG micelle, alcohols, halothane, and chloroform at low concentration were added as shown in Fig. 5. As is apparent from Fig. 5, the change of absorbance at 870 nm in the presence and absence of the LH polypeptides increased with increasing hydrophobicity of these alcohols except when ethanol was added. The Qy absorption band of BChl*a* is blue-shifted from near 870 nm in the presence of LH polypeptides (LH1 complex, B870) or 850 nm in the absence of polypeptides (aggregation form of BChl*a*, B850) to about 777 nm (monomeric form), respectively. Interestingly, the difference of the change due to the presence of LH polypeptide was observed with increasing concentration of butanol or hexanol, in which larger changes were observed in the absence of LH polypeptide than those in the presence of LH polypeptides. Moreover, large changes in the presence and absence of LH polypeptides were observed when halothane or chloroform was added.

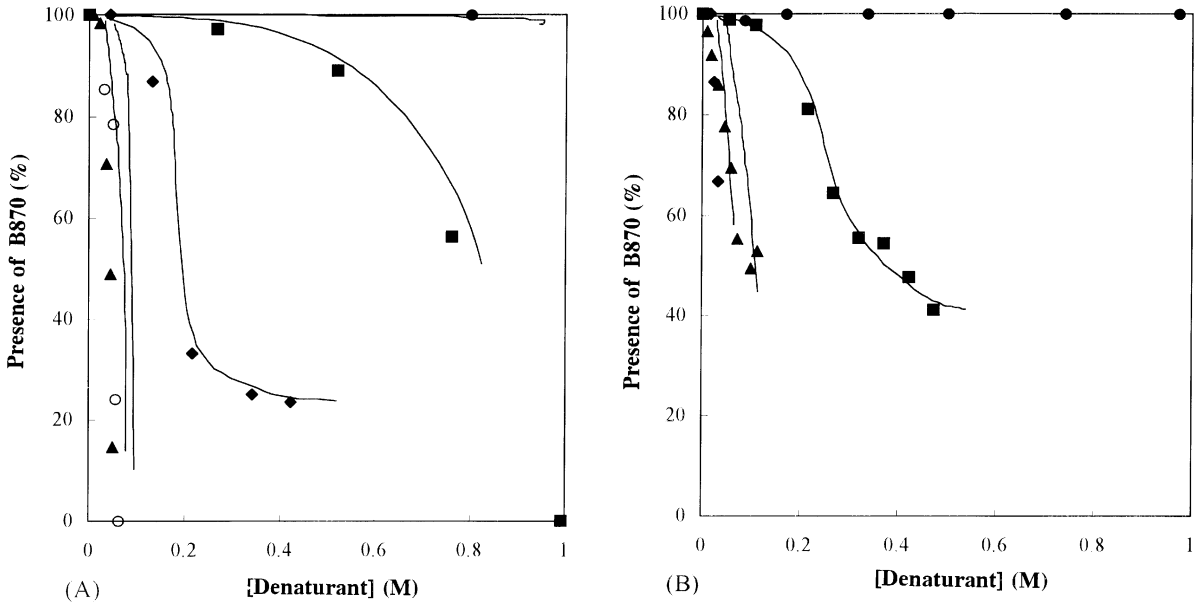


Fig. 5 The plots of B870 or B850 complex remainder (%) upon addition of low concentration of various alcohols, halothane and chloroform at 0.67% OG after storage at 4 °C: (A) the plots of B870 complex remainder (%) of the reconstituted LH1 complex from *R. rubrum*; (B) B850 complex remainder (%) of BChl*a* complex. (●) EtOH, (■) BuOH, (♦) HexOH, (○) $CHCl_3$, (▲) Halothane

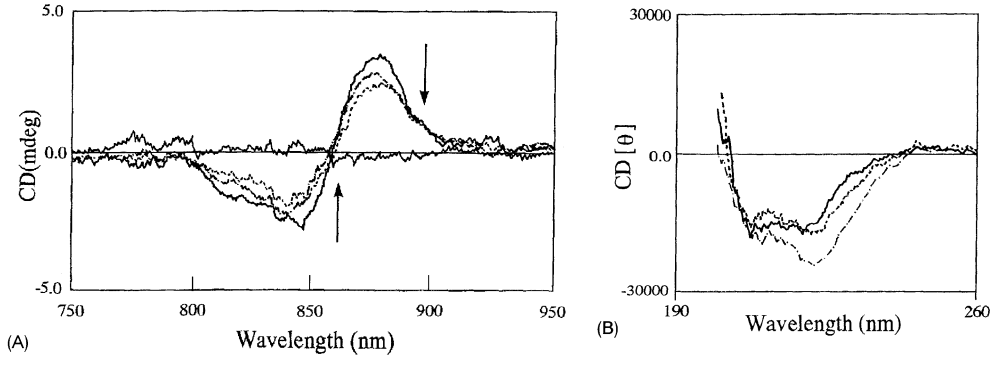


Fig. 6 CD spectra of the reconstituted LH1 complex from *R. rubrum* in the various concentrations of *n*-propanol at 0.67% OG after storage at 4 °C: (A) near-IR; (B) far-UV region. 0 M (———), 0.97 M (–·–·–), 1.74 M (- - -), 3.65 M (——)

Alternatively, Fig. 6 shows near-IR and far-UV CD spectra for LH α- and β-polypeptides from *R. rubrum* and BChl*a* complex in OG micelle when *n*-propanol was added. A split CD signal at 800–900 nm decreased with increasing concentration of *n*-propanol and then disappeared at 3.65 M propanol. Interestingly, the minus CD signal at 230 nm became large with increasing concentration of *n*-propanol, where the decrease of minus CD signal at 230 nm implies that the change in the packing mode of α-helix polypeptide with BChl*a* rather than destroying the α-helical conformation of these polypeptides for the LH1 complex in OG micelle. Similar CD results were observed when other alcohols were added. These CD spectra showed that a split CD signal induced by exciton coupling between BChl*a*s disappeared with increasing concentration of propanol and that, the α-helical conformation of LH polypeptides was restored as observed when urea or Gnd was added.

Discussion

Possible interactions contributing to the stabilization of the LH1 complex might include the following: (1) coordination of the ligand of Mg atom of BChl*a*; (2) hydrogen bonding between the polypeptides and BChl*a*; (3) hydrogen bonding between amino acid side chains of the polypeptides; (4) knob–hole (packing) interaction between

polypeptide α-helices. Since the Qy absorption band of BChl*a* shifts from 777 to 870 nm with cooperative interactions with the LH-polypeptides and BChl*a* in OG micelle as described in Figs. 1 and 6, the Qy transition moment of BChl*a* is a sensitive probe for the extent of stability and formation of the LH1 complex in OG micelle as well as the complex in the chromatophore. It is considered that the change of the Qy absorption band at 870 nm represents a dissociation of LH polypeptide and BChl*a* complex in OG micelle or in the chromatophore. Thus, the effect of denaturants on the extent of the stabilities of LH1 complex in OG micelle and the complex in the chromatophore can be estimated by the resulting Qy band-shift. Alcohols showed larger Qy band shifts than those for addition of urea or Gnd (Figs. 2 and 4), revealing that alcohols are effective denaturants to these complexes in comparison to urea or Gnd especially when hydrophobic alcohols are used. Itoh et al. indicated that the interior of OG micelle becomes very hydrophobic once the structure is formed [23]. These results suggest that urea and Gnd would not penetrate these complexes in the OG micelle, while hydrophobic denaturants such as alcohols having hydrophobic alkyl chains, halothane, and chloroform can penetrate the hydrophobic domains in these complexes, causing a strong undoing effect to these complexes. Interestingly, the Qy band-shift for LH1 complex in OG micelle increased with increasing hydrophobicity of various alcohols or denaturants such as halothane and chloroform except ethanol at low concentration (Figs. 4 and 5), in which halothane or chloroform is more effective to the complex than alcohols as shown in Fig. 5(A). These results indicate that hydrophobic interactions play an important role in the formation of the LH1 complex in OG micelle. Furthermore, large differences of the Qy band-shifts between the LH1 complex in OG micelle and that in the chromatophore were not observed for the addition of various alcohols (Fig. 4), implying that the extent of stability of the LH1 complex in OG micelle is similar to that in the chromatophore. However, differences for the band-shifts between the LH1 complex and BChl*a* alone in OG micelle were observed especially at low concentration of butanol or hexanol (Fig. 5), indicating that the shift of the Qy band is suppressed due to the presence of LH polypeptides in OG micelle. These results reveal that the extent of stability of BChl*a* complex in OG micelle increases due to the presence of LH polypeptides. It is considered that the N-terminal hydrophilic segment of the LH-polypeptides does not play an important role but the hydrophobic core and the C-terminal hydrophobic segment play a crucial role in packing together to form a three-dimensional complex of LH1 [17]. Thus, the C-terminal segments are probably affected by hydrophobic denaturants rather than the N-terminal hydrophilic segment. Olsen also indicated that the hydrogen-bond interaction between C_3 acetyl carbonyl of BChl*a* and Trp in C-terminal of β-polypeptide in the chromatophore of *R. sphaeroides* is observed while the Mg complex in BCh1*a* is bound by axial coordination with the imidazole moiety on the histidine residue of LH polypeptides [5, 16]. Thus, hydrophobic alcohols are likely to affect the hydrogen-bond interaction and also the axial coordination between LH polypeptides and BChl*a* in OG micelle as well as in the conformational change of OG micelle alone [23].

Conclusions

The extent of stability of the LH1 complex in OG micelle and the complex in the chromatophore of photosynthetic bacteria was monitored by the change in absorbance of the Qy absorption band of BChl*a* in these complexes, generally resulting in the blue-shifting of the Qy band from near 870 to about 777 nm upon addition of these denaturants. Alcohols showed a denaturing effect on both LH1 complex in the OG micelle and the complex in the chromatophores, depending upon the hydrophobicity of the alcohols, while urea and Gnd showed a relatively weak undoing effect in comparison to alcohols. These results indicated that hydrophobic interactions in the LH1 complex play an important role in the formation of the LH1 complex in the OG micelle as well as that in the chromatophore. The stability of BChl*a* complex in OG micelle due to the presence of LH polypeptides is also observed.

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References

1. Okamura MY, Feher G, Nelson N, Govindjee (1982) Photosynthesis. Academic Press, New York, p 197
2. Van Grondelle R (1985) Biochim Biophys Acta 811:147
3. Thornber JP (1986) Encycl Plant Physiol New Ser 19:85
4. Cogdell RJ (1986) Encycl Plant Physiol (New Ser) 19:252
5. Olsen JD, Hunter CN (1994) Photochem Photobiol 60:521
6. Deisenhofer J, Epp O, Miki K, Huber R, Michel H (1985) Nature 318:618

7. Chang C-H, El-Kabbani O, Tied D, Norris J, Schiffer M (1991) Biochemistry 30:5352
8. El-Kabbani O, Chang C-H, Tied D, Norris J, Schiffer M (1991) Biochemistry 30:5361
9. McDermott G, Prince SM, Freer AA, Hawthornthwaite-Lawless AM, Papiz MZ, Cogdell RJ, Isaacs NW (1995) Nature 374:517
10. Karrasch S, Bullough P, Ghosh R (1995) EMBO J 14:631
11. Brunisholz RA, Suter F, Zuber H (1984) Hoppe-Seyer's Z Physiol Chem 365:675
12. Brunisholz RA, Wiemken V, Suter F, Bachofen R, Zuber H (1984) Hoppe-Seyer's Z Physiol Chem 365:689
13. Brunisholz RA, Zuber H, Valentine J, Lindsay JG, Wooley KG, Cogdell RA (1986) Biochim Biophys Acta 849:29
14. Zuber H (1986) Trends Biochem Sci 11:414
15. Tonn SJ, Gogel GE, Loach PA (1977) Biochemistry 16:877
16. Olsen JD, Sockalingum GD, Robert B, Hunter CN (1994) Proc Natl Acad Sci USA 91:712
17. Meadows KA, Iida K, Tsuda K, Recchia PA, Heller BA, Antonio B, Nango M, Loach PA (1995) Biochemistry 34:1559
18. Miller JF, Hinchigeri SB, Parkes-Loach PS, Callahan PM, Sprinkle JR, Riccobono JR, Loach PA (1987) Biochemistry 26:5055
19. Parkes-Loach PS, Sprinkle JR, Loach PA (1988) Biochemistry 27:2718
20. Cohen-Bazire G, Sistrom WR, Stanier RY (1957) J Cell Comp Physiol 49:25
21. Loach PA, Androes GM, Maksim AF, Calvin M (1963) Photochem Photobiol 2:443
22. Pace CN (1986) Meth Enzymol 131:266
23. Itoh H, Ishido S, Nomura M, Hayakawa T, Mitaku S (1996) J Phys Chem 100:9047
24. Berger G, Kleo J, Andrianambinintsoa S, Neumann JM, Leonhard M (1990) J Liquid Chromatography 13:333
25. Schejter A, Luntz TL, Koshy TI, Margoliash E (1992) Biochemistry 31:8336
26. Monera OD, Kay CM, Hodges RS (1994) Protein Sci 3:1984