Characterization of the Light-Harvesting Polypeptide/ Bacteriochlorophyll *a* Complex Isolated from Photosynthetic Bacteria by the Linear Dichroism Spectra

Kouji Iida,* Nobuyasu Ohya,† Ayumi Kashiwada,†† Mamoru Mimuro,††† and Mamoru Nango*,††

Nagoya Municipal Industrial Research Institute, Rokuban 3-4-41, Atsuta-ku, Nagoya 456-0058

†Department of Applied Chemistry, Aichi Institute of Technology, Yachigusa, Yakusa-cho, Toyota 470-0356

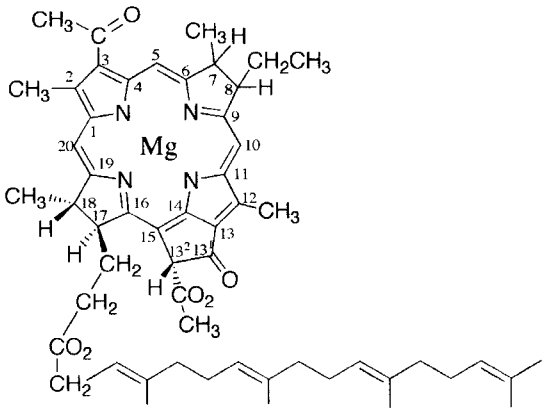
††Department of Applied Chemistry, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466-8555

†††Department of Physics, Biology and Informatics, Faculty of Science, Yamaguchi University, Yoshida 1677-1, Yamaguchi 753-8512

(Received August 11, 1999)

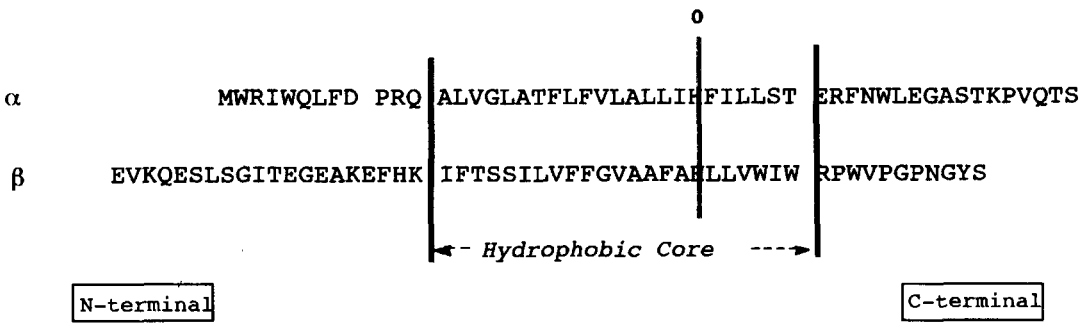
The linear dichroism (LD) spectra of the light-harvesting polypeptide (LH)/bacteriochlorophyll (BChl *a*) complex, isolated from photosynthetic bacteria, *Rhodospirillum rubrum*, were measured to provide insight into the pigment orientation in the LH/BChl *a* complex. The LD spectrum for the Qy band of BChl *a* in the complex in a squeezed polyacrylamide gel was positive, while that for the Qx band was negative, indicating that the dipole moment of the Qy band was membrane-parallel while that of the Qx band was membrane-perpendicular. The anisotropy ratio, the LD/A value for the Qy transition of BChl *a* in the complex, was 0.18±0.02 at a 0.5 compression-ratio, consistent with that for the Qy band of a special pair of BChl *a* in the reaction center (RC) complex isolated from *R. rubrum*. This result implies that the orientation of the Qy band in the LH/BChl *a* complex is perpendicular for the transmembrane axis of the LH in the complex as well as that of the special pair of BChl *a* in the RC. Furthermore, the LD/A spectrum for the Qy band of BChl *a* in the LH/BChl *a* complex indicated that the Qy band was geometrically homogeneous in the direction to the transmembrane axis in the complex.

The orientation of pigments plays a crucial role in energy transfer and electron transfer in biological energy conversion, such as that which occurs in photosynthesis. The primary reactions of photosynthesis, in general, are ultrafast reactions.[1] This reaction rate is realized by diffusion-less processes, that is, the geometry of all reactants is fixed relative to each other. The molecular geometry and electronic states of pigments are organized by polypeptides which form supramolecular assemblies, such as the reaction center (RC) and light-harvesting (LH) antenna complexes in photosynthetic bacteria.[2,3] Thus, the molecular structure at atomic resolution is primarily important for understanding the reaction mechanism. Recently, the molecular structure of the LH 2 complex from *Rhodopseudomonas acidophila* was reported;[2,3] it shows a ring-like structure consisting of nine identical units, each of which contains α- and β-subunits. There are two kinds of bacteriochlorophyll BChl *a* (Scheme 1) molecules in the subunit complex: One is the B850 complex, whose chlorin rings are perpendicular to the membrane surface; the other is the B800 complex, whose chlorin rings are parallel to the membrane surface. The B850 complex shows a periodical structure of nine dimers to the central symmetry axis; the center-to-center distance within the dimer is 0.89 nm and that between BChl *a*'s or adjacent dimers is 0.95 nm. Essentially, the same ring-like structure was reported for the LH 1 complex isolated from *Rhodospirillum rubrum*.[4] In this



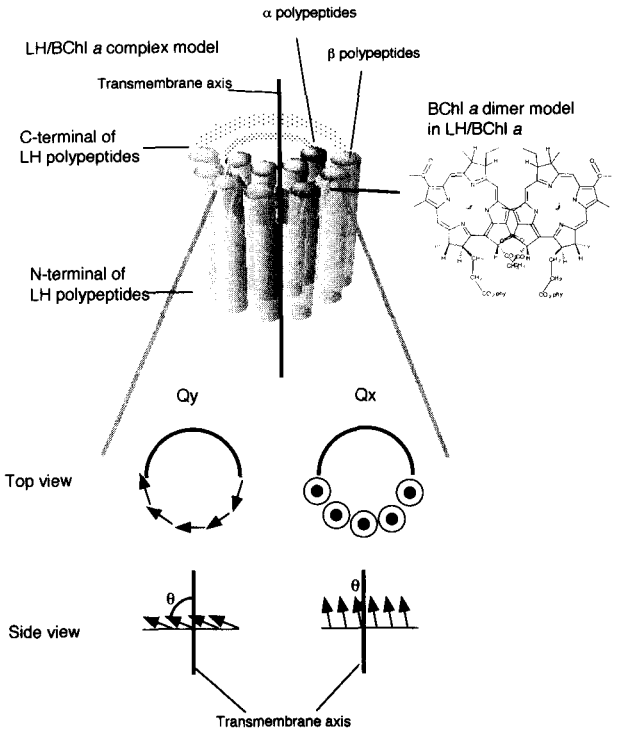
Scheme 1. Bacteriochlorophyll *a* (BChl *a*).

case, the crystal used for analyses was a two-dimensional type obtained by reconstitution from RC-depleted samples. Because of this preparation method, the molecular structure of the LH 1 complex is still being debated. The LH 1 complex is likely to be built up from two short polypeptides, α and β (Scheme 2),[5—10] each of which binds one BChl *a*. The BChl *a*'s in the LH complex from *R. rubrum* have a unique Qy-electron transition absorption, while in acetone the λ_{max} of the Qy band is located at 777 nm. The core complex has a subunit form called the B820 complex in the presence of the detergent, octyl glycoside, and BChl *a* dimer



Scheme 2. Amino acid sequence of LH α and β polypeptides of *R. rubrum*.[5—10] For ease of comparison, the common His residue nearest the C-terminus was used for alignment and defined as the zero position. BChl *a* is bound to imidazole moiety of His (0), and the α and β polypeptides–BChl *a* complex are copmosed of hetero dimer. The hetero dimer is called as subunit form B820 in the presence of OG at CMC, while the hetero dimer becomes reassociated to a large core LH complex below CMC of OG.

in the B820 complex can be reaggregated into a large antenna complex.[11] The observed red-shift upon aggregation from 820 to 870 nm is probably due to increasing excitonic interactions between BChl *a* dimers induced by the light-harvesting polypeptides.[12] The orientation and distance of the BChl *a* dimer are crucially important for energy transfer within the BChl *a* dimer.[13] In the dimer, the Qy-electron transition moments are strongly coupled due to their associations with the light-harvesting polypeptides (Scheme 3).[14,15] Based on analogy with an X-ray analysis of the LH 2 complex of photosynthetic bacteria and from reconstitution model studies of the LH 1 complex,[16,17] it is concluded that the Mg atom in BChl *a* coordinates with the histidine residue in the hydrophobic core of the LH polypeptides and that the C_3 acetyl carbonyl of BChl *a* binds with the Trp or polar amino acid residue in the C-terminal of the α- or β-polypeptide through hydrogen bonding to form the LH 1 complex.[18] Thus, it is interesting to note that the LH polypeptides of photosynthetic bacteria organize BChl *a*'s complex according to cooperative interactions between the LH polypeptide and BChl *a* so that an efficient energy-transfer involving BChl *a* may occur. However, information on the orientation of the red-shifted Qy band of BChl *a* in the polypeptides is still uncertain (Scheme 3). Recently, Visschers has noted that a distinct increase of the fluorescence anisotropy occurred upon the red wing of the Qy band of B870 complex from *Rhodobacter sphaeroides*.[15] The fluorescence anisotropy reflects the relative angle between the absorption and emitting of BChl *a*'s, while linear dichroism (LD) measure the absolute angle between the transition dipole and the axis of orientation. Thus, the methodology of LD is useful for investigating the pigment orientation in the LH/BChl *a* complex as well as in the RC complex.[19—21]



Scheme 3. The orientation model of BChl *a* in LH/BChl *a* complex. The dipole moment of BChl *a* is represented by the arrows and θ is the angle between the dipole moment and transmembrane axis which is the direction of normal vector of photosynthetic membrane.

In this work, we measured the LD spectra of BChl *a* in the LH/BChl *a* complex isolated from *R. rubrum* to provide insight into the pigment orientation in the complex. This study will be relevant to estimate the molecular structure of the LH 1 complex recently proposed based on the two-dimensional crystal.[4] The isolated LH/BChl *a* complex is recovered from a partially loosened structure after extraction of carotenoids from the chromatophore of *R. rubrum*,[5,8—10] since the LH/BChl *a* complex has been used to elucidate the molecular organization of the substituted LH complex[22] and other artificial units.[23—25] Thus, systematic measurements of the LD spectra for the LH/BChl *a* complex may provide insight into the orientation (θ) of BChl *a* for the transmembrane axis of the LH complex and the electron state of BChl *a* in the LH/BChl *a* complex, as shown in Scheme 3.

Experimental

Octyl β-D-glucopyranoside (OG), ethylenediaminetetraacetic acid (EDTA), acetic acid, and high-performance liquid chromatography (HPLC) solvents (acetnitrile, 2-propanol, and acetone) were obtained from Nacalai Tesque. Sephadex G-100 was obtained from Sigma Chemical Co.

The Growth of *Rhodospirillum rubrum* Wild Type and G-9. *R. rubrum* wild type was grown anaerobically in modified Hunter's media under low-intensity fluorescent light as previously described.[11] The cells were harvested during logarithmic growth (usually in 3—5 d), centrifuged, washed once in 50 mM (1 M = 1 mol dm^{-3}) phosphate buffer, pH 7.5, repelleted, and stored at −20 °C as a pellet until use.

Reassociation Procedure. Chromatophores of *R. rubrum* were prepared as previously described.[11] Carotenoid was extracted by benzene from the chromatophore. As shown in Scheme 4, OG was added to dissolve chromatophore containing the LH 1 and RC complexes until the sample's far-red absorption band shifted from 873 to 820 nm, usually about 1% OG (50 mM KPi buffer, pH 7.5). The aqueous solution was then applied to a Sephadex G-100 column (1.5 i.d. ×75 cm) to separate RC and the subunit LH-1 (B820 complex) complexes. The RC complex was eluted immediately behind the void volume and was usually nearly completely separated from the B820 complex. Figure 1 shows the absorption spectrum of the B820 complex from the peak fraction after purification on a G-100 column. The λ_{max} was shifted from 820 nm to a longer wavelength (860—870 nm) by diluting the OG concentration to about 10 mM (Fig. 1). The reassociation between the LH/BChl *a* complex and the RC complex was mixed at 1 : 1 for BChl *a* and stored at 4 °C in 10 mM OG.[26] Each concentration of BChl *a* of the LH complex and RC complex is 2 μM.

CD Spectrum. The CD spectra were recorded with a JASCO J600 spectropolarimeter. The samples were measured in an aqueous 0.75% OG buffer (50 mM KPi buffer, pH 7.5).

LD Spectrum. LD was measured by the gel-squeezing method.[27,28] The buffer suspension of the sample was mixed with the components of the polyacrylamide gel (acrylamide, 10—15% (w/v); *N,N'*-methylenebisacrylamide, 0.4% (w/v); glycerol, 50% (v/v)). The samples were polymerized by the addition of 0.03% (v/v) piperazine and 0.05% (w/v) ammonium persulfate in 1 × 1 cm cuvette (Scheme 4). The gels were compressed stepwise in a press with a home-made gel holder from an initial width of 10.0 mm a final width of 5.0 mm (Scheme 5). The spectra were measured using a Hitachi 3410 spectrophotometer at room temperature equipped for LD measurements. A Glan–Thompson polarizer was used. All data were transferred to a personal computer and then analyzed.

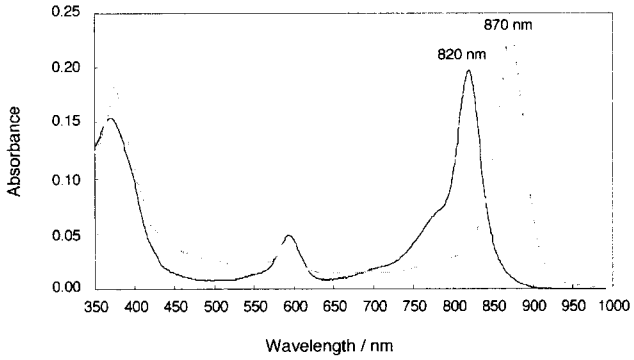
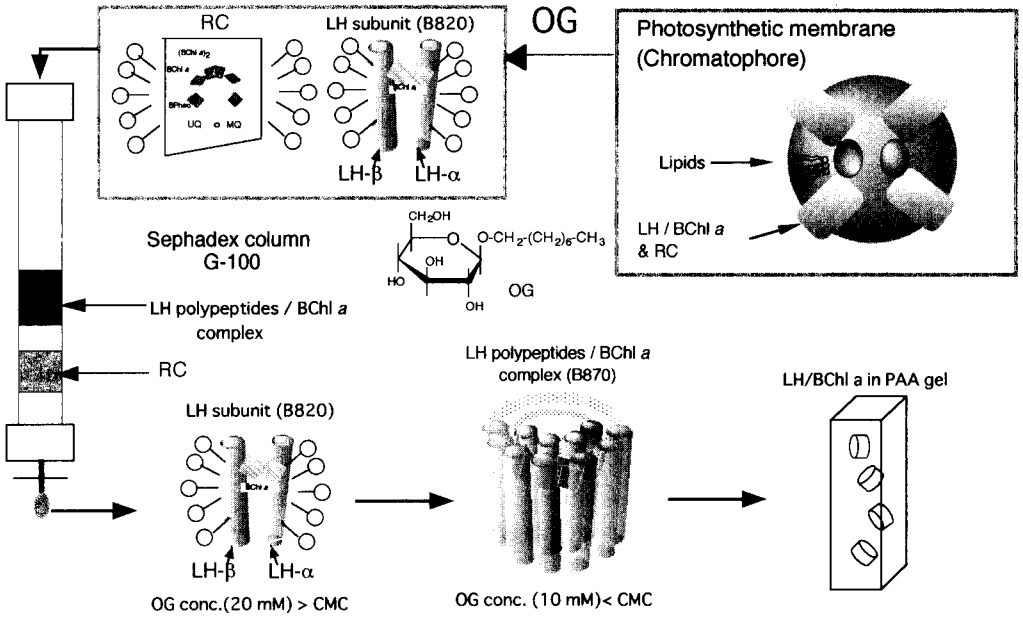
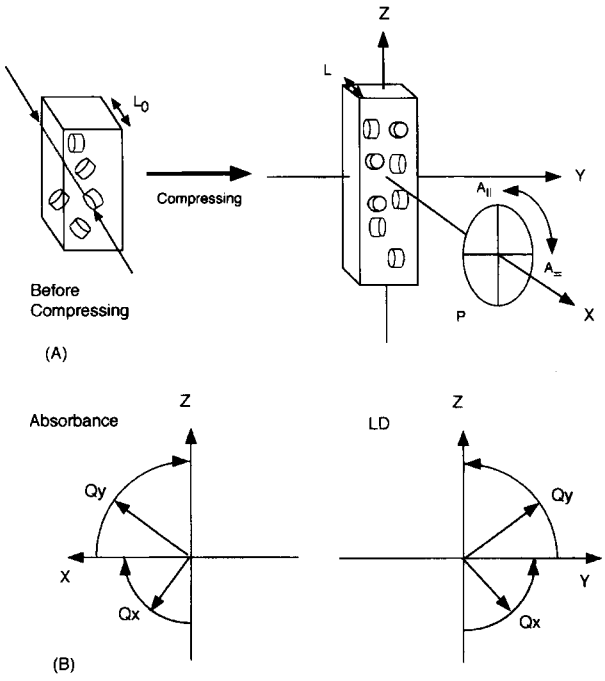


Fig. 1. UV-vis absorption spectra of reassociated LH/BChl *a* complex from *R. rubrum* at room temperature in the dark room (0.05 M phosphate buffer, pH 7.5). LH/BChl *a* complex in 20 mM OG (— B820) and in 10 mM OG (— B870). The concentration of LH/BChl *a* was 1.6 μM. The LH/BChl *a* was purified by sephadex G-100 column (1.5 cm i.d. ×80 cm) from chromatophore. The absorbance of Qy (870 nm) band of B870 is very close to that of chromatophore. Spectra were recorded in 1 cm cuvettes.



Scheme 4. Purification and gelation procedure of LH polypeptides/BChl *a*. Photosynthetic membrane from *R. rubrum* was dissolved into OG (22 mM) buffer (50 mM KPi buffer, pH = 7.5). The λ_{max} of LH 1 complex was shifted from 870 to 820 nm. The aqueous solution was applied to Sephadex G-100 column (1.5 i.d. ×75 cm) to separate the RC and the subunit form complex of LH polypeptides/BChl *a* complex (B820). RC were eluted immediately behind the void volume and were usually nearly completely separated from the B820 complex. The subunit form (B820) was reassociated to LH polypeptides/BChl *a* complex (B870) when OG concentration was lower than 10 mM (the CMC of OG is about 20 mM) (see Fig. 1), where the subunit complex is presumed to comprise either a single $\alpha\beta$ pair or a $(\alpha\beta)_2$ unit and the LH polypeptides/BChl *a* complex (B870) is build up from the subunit complex (B820) by hydrophobic interaction. We made polyacrylamide (PAA) gels containing LH/BChl *a* complex.



Scheme 5. Linear Dichroism (A) definition of *X*, *Y*, *Z*-axis. L_0 is original path length of the gel (1 cm) and *L* is current path length when the gel is compressed (0.9, 0.8, 0.7, 0.6, 0.5 cm). P is a Glan–Thompson polarizer. $A_{||}$ and $A_=$ are absorption spectra as the electric vector of polarized light is parallel to *Z* axis and *Y* axis, respectively. LH/BChl *a* complexes were oriented in compressed PAA gel. (B) The moving of the dipole moment when the compressing. Each dipole moment is randam distribution in the gel brfore compressing. Absorption spectra shows that the Qy band directed to *Z*-axis and the Qx band directed to *X*-axis when the gel is compressed. LD spectra shows that the Qy band directed to *Z*-axis and Ox band directed to *Y*-axis.

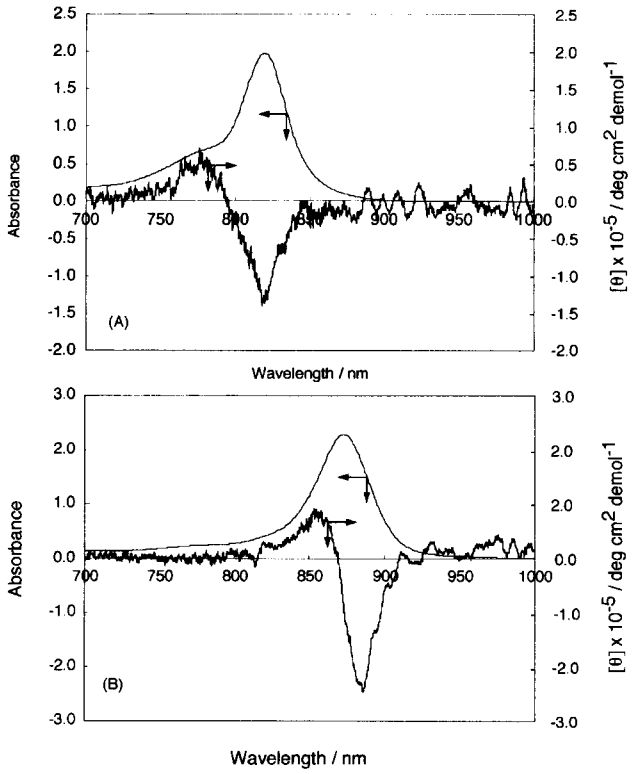


Fig. 2. Circular dichroism and UV-vis absorption spectra of the LH/BChl *a* complex from *R. rubrum* (0.05 M phosphate buffer, pH 7.5). (A) B820 and (B) B870. The concentration of LH/BChl *a* complex was 16 μM. CD (—) and absorbance (—). The baseline of CD & UV-vis. absorption spectra were corrected. Spectra were recorded in 1 cm cuvettes.

Results and Discussion

UV-vis and CD Spectra of the Reassociated LH/BChl *a* Complex from *R. rubrum*. Figures 1 and 2 show UV-vis and CD spectra of the reassociation for the subunit B820 complex and core B870 complex, respectively. Reassociation of the LH/BChl *a* complex from *R. rubrum* was performed as described in the methodology.[16] The Qy absorption band of BChl *a* is red-shifted from 777 to near 870 nm via 820 nm. The B820 complex is thought to be a subunit form which is dissolved from the LH 1 complex by OG at upper cmc of OG (20 mM), while the B870 complex is thought to be a core complex due to the aggregation of the subunit below the cmc of OG, as shown in Scheme 4. The CD spectrum of the B820 complex has a simple peak and trough in the Qy band with relatively broad positive and negative bands centered between 776 and 820 nm, respectively, and a crossover point at about 796 nm, which is far from the absorption maximum (821 nm). The CD spectra of the Qy bands may be interpreted as being due to two excitonically coupled BChl *a* molecules, giving rise the absorption band from 777 to 820 nm. Excitonic interactions between two BChl *a* molecules is a plausible explanation where the positive and negative lobes at 777 and 820 nm of the B820 complex are assigned to the high- and low-energy exciton transitions. If the CD signal at B777 of B820 is that from induced CD between BChl *a* and LH polypeptide, the corresponding absorbance at 777 nm should be 2—3-fold larger. Also, B820 has the spectroscopic properties of a strongly coupled dimer of BChl *a*.[1,14,17] Thus, the CD spectra of B820 should be interpreted as being excitonic interactions of the BChl *a* dimer. The CD spectrum of the B870 complex has a split at near 870 nm with relatively broad positive and negative bands centered between 850 and 890 nm, respectively. The reassociation of the core LH/BChl *a* complex had been characterized by fluorescence decay,[17] fluorescence polarization[14,15] and resonance Raman.[17] These results suggest that the LH 1 complex can be reversibly reassociated from a minimal subunit form, B820 complex, which has the spectroscopic properties of a strongly coupled dimer of BChl *a*. The orientation of the electron transition moment of the B870 complex is not sufficiently characterized, although the orientation is important for energy transfer. Thus, the orientation of BChl *a* in the LH/BChl *a* complex in OG should be revealed by measuring the LD spectra.

LD Spectra of the Reassociated LH/BChl *a* Complex

from *R. rubrum*. The reassociated LH/BChl *a* complex isolated from *R. rubrum* was fixed in the polyacrylamide gel to measure the LD spectra. The polarized absorption spectra of the reassociated LH/BChl *a* complex in the squeezed polyacrylamide gel were measured with decreasing compression-ratio (L/L_0, L: length of the compressed gel) of the gel as shown in Scheme 5. The gel is compressed in the X-direction and expands along the Z-axis. The measuring light-beam is incident along the X-axis. The LH/BChl *a* complexes were oriented in the compressed polyacrylamide gels. The LD spectra showed the orientation of the dipole moment of BChl *a* in the LH/BChl *a* complex oriented in the gel.[21] We recorded absorption spectra $A_{\parallel}$ and $A_{=}$ versus linearly polarized light with the electric vector direction parallel and perpendicular to the stretching direction (Z-axis) of the sample, respectively (see Scheme 5). The absorbance of the sample is the largest when the pigment dipole moment is the same direction as the electric vector of the polarized light. Thus, when the dipole moment is directed parallel and perpendicular to the squeezing direction (Z-axis), respectively, the LD ($A_{\parallel} - A_{=}$) is positive and negative, respectively. The anisotropy of the dipole moment of the pigment is calculated to be LD/A, where A is the isotropic absorbance of the LH/BChl *a* complex in the current compressed gel. The orientation of disc-like particles and the correponding linear dichroism in the case of uniaxial compressing of the gels were mathematically described by Amerongen et al.[27] The angles of the dipole moment of the pigment in LH/BChl *a* complex as disc-like particle are estimated by using the following equation:

$$1/2 \cdot k \cdot (3 \cos^2 \theta - 1) = \mathrm{LD/A}, \quad (1)$$

where k is the macro-orientation factor of the LH/BChl *a* complex and theta (θ) is the angle degree for the LH transmembrane axis (Scheme 3). The k value is determined by a calibration using the pigment orientation in the RC complex isolated from *R. rubrum*.

Figure 3 shows the LD and LD/A spectra of the reassociated LH/BChl *a* complex when the gel is compressed. The peak-shift and the change in the peak-shape of the polarized absorption spectra were not observed with decreasing compression-ratio, indicating that the LH/BChl *a* complex was stable during compression of the gel. Figure 3(A) shows the isotropic absorption spectra of BChl *a* in the complexes at the magic angle in the squeezed gel. The absorbance for the Qy and Soret bands of BChl *a* increased with decreasing the compression-ratio and, in contrast, that for the Qx band decreased. This result implies that when the gel is compressed, BChl *a* in the LH/BChl *a* complex is macro-oriented in the gel and that the dipole moment of the Qy transition is perpendicular to that of the Qx band, where the Qy band orients to the Z–Y plane and the Qx band orients to the X–Y plane (see Scheme 5). Thus, the degree of the pigment-direction in the LH/BChl *a* complex is determined from that of the special pair of BChl *a* in the RC complex, as described in the following section (see Table 1). However, we could not measure the LD spectra of the B820 subunit complex because the B820 complex is converted to the B870 complex, even in 2% OG, in the presence of acrylamide monomer before its polymerization, where the LH/BChl *a* complex dissociated completely to the B777 complex (BChl *a* monomer) in 2% OG in the absence of acrylamide. Figure 3(B) shows the LD spectra of BChl *a* in the reassociated LH/BChl *a* complex when the gel is compressed, in which with decreasing compression-ratio the LD spectra for the Qy band become positive; in contrast, the LD spectra for the Qx band become negative. This result indicates that the dipole moment of the Qy bands becomes more parallel to the stretching direction (Z-axis) while that of the Qx bands becomes perpendicular (Y-axis) with decreasing compression-ratio. A small LD spectrum is observed in an uncompressed gel due to setting of the gel in the cell holder. Interestingly, the LD spectrum at the Soret band splits at 376 and 412 nm to positive and negative signs, respectively, showing that the Soret band comprises of two electron-transition moments. The component of the LD spectrum at 376 nm is likely to generate due to π–π electron-transition between the symmetrical rings of the chlorin structure. Figure 3(C) shows the LD/A spectra of BChl *a* in the reassociated LH/BChl *a* complex when the gel is compressed. Interestingly, the LD/A spectra for the Qx band become sharp and negative with decreasing compression ratio. However, the change of LD/A for the Qy band is small, indicating that the dipole moment of the Qy band has a geometrically homogeneous orientation for the LH-polypeptide axis. The LD/A values for the Qy, Qx, and Soret bands at a compression ratio of 0.5 and the angle (θ) of the dipole moment for BChl *a* in the complex are summarized in Table 1.

Figure 4 shows plots of the relative changes of the UV-vis absorption (A/A_0), LD and LD/A spectra of the reassociated LH/BChl *a* complex at the maxima of the Qy, Qx, and Soret bands of BChl *a*, 870, 589, 412, and 376 nm, respectively as a function of the compression-ratio. As is apparent from Fig. 4(A), the A/A_0 changes with decreasing the compression-ratio at the maxima of those bands in the following order: Qx > Qy > Soret. The increase for the Qy band-absorption is likely to be due to the orientation of the Qy transition moment from random to the Y–Z plane with gel-compression (Scheme 5B). In contrast, the Qx transition moment orients along the X-direction, which is the direction along light-beam. As is apparent from Fig. 4(B), the LD values at the Qy band (870 nm) and the Soret band (376 nm) increased with decreasing the compression-ratio, and then became saturated. While the LD values at both the Qx (589 nm) and Soret bands (412 nm) decreased with decreasing compression-ratio until 0.8—0.7 of the compression ratio. The increase in the LD value at 870 nm indicates that the Qy transition may rotate from a random state to the Z-axis along with decreasing the compression ratio (Scheme 5B), while the decrease in LD at 589 nm indicates that the Qx transition may rotate to the Y-axis. The absorption change, (A/A_0) with decreasing compression-ratio does not correspond to that of the LD spectra (Scheme 5B). As is apparent from

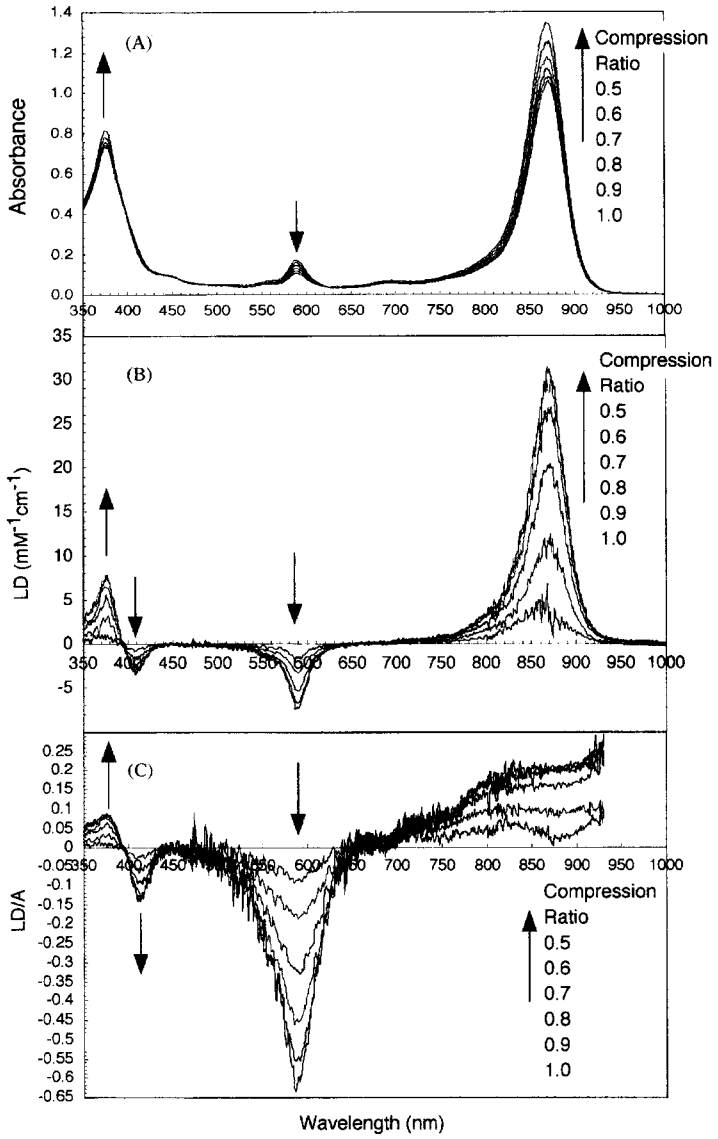


Fig. 3. (A) Isotropic absorbance, (B) linear dichroism ($A_{\parallel} - A_{=}$) and (C) LD/A spectra of reassociated LH/BChl *a* complex from *R. rubrum* in polyacrylamide gel in various compression ratios. The concentration of LH/BChl *a* complex was 8 μM. The gel was squeezed by a hand-made cell. The compression ratio is L/L_0, where L_0 is the length of original gel (1 cm) and L is the current gel length when the gel compressed, respectively.

Table 1. LD/A Value and the Angle (θ) of Dipole Moment at Various BChl *a* and Carotenoid for the LH/BChl *a* Complex and the LH/BChl *a* Complex with RC Isolated from *R. rubrum*

	Soret band		Carotenoid	Qx band	Qy band		
	375 (nm)	415 (nm)	500 (nm)	589 (nm)	BPheo 750 (nm)	BChl *a* 800 (nm)	BChl *a* 870 (nm)
LH / BChl *a* complex	0.08 (65°)	−0.14 (39°)	—[a)]	−0.6 (0°)	—[b)]	—[b)]	0.18 (90°)
LH / BChl *a* complex with RC	0.025 (58°)	−0.02 (53°)	0.05 (61°)	−0.13 (41°)	−0.065 (48°)	0.1 (68°)	0.18 (90°)[c)]

a) Carotenoid in LH/BChl *a* complex was extracted by benzene from the chromatophore. b) BPheo and accessory BChl *a* are not contained in LH/BChl *a* complex. c) This angle is from X-ray crystallography of RC from *R. viridis*.[19]

Fig. 4(C), the anisotropy ratio for the Qy band (at 870 nm) was saturated at around 0.7 of the compression ratio, while that of the Qx band (at 589 nm) decreased with decreasing the compression-ratio. This result implies that the LD/A for the Qx band is more sensitive than that for the Qy band, and that the dipole moment of the Qx band has a random distribution in the *X*–*Y* plane. When the LD of the Qy band is linear before reaching saturation, the anisotropy ratio for

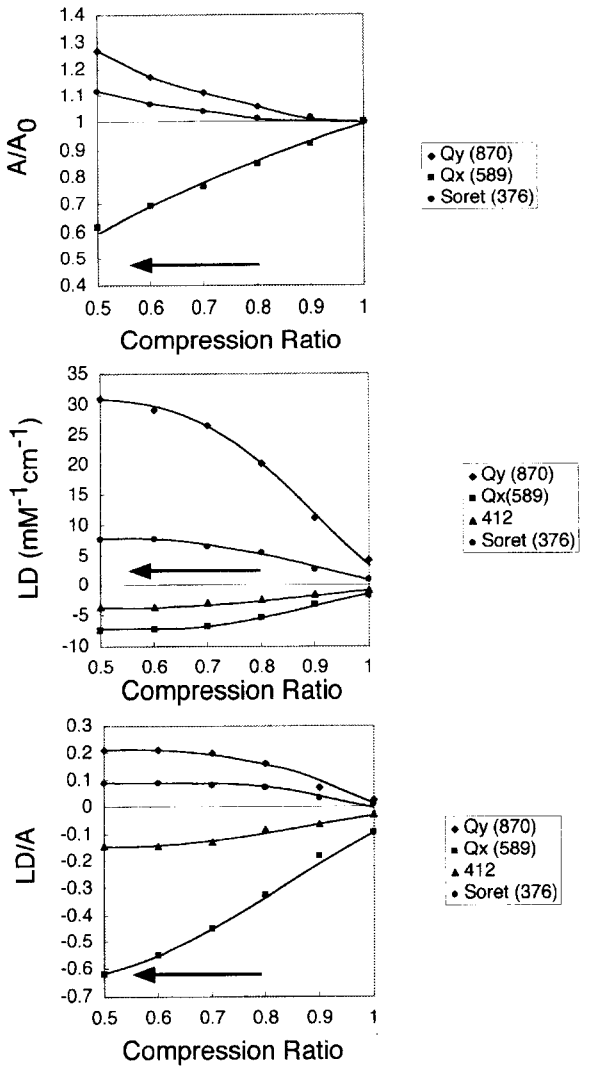
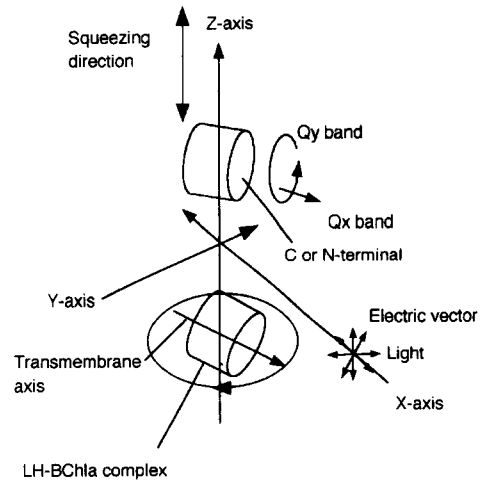


Fig. 4. The plots of (A) isotropic absorbance, (B) LD, and (C) LD/A value at λ maxima (minimum) in the various compression ratios of the gel (Fig. 3). The gels were compressed stepwise in a press with a home-made gel holder from an initial width of 10.0 mm a final width of 5.0 mm. Arrows (—) show the compression ratio order. The values of the absorbance (A) are normalized as each absorption maxima at non-compressed absorbance (A_0).

the Qx band can be estimated to −0.4 at 0.8 of the compression ratio. These results show that the polypeptides axis of the LH/BChl *a* complex is distributed around the squeezing direction (Scheme 6).

LD Spectra of the Reassociated the LH/BChl *a* Complex with RC Complex Isolated from *R. rubrum*. Figure 5 shows UV-vis, LD, and LD/A spectra for a mixture of the LH/BChl *a* complex and RC (1 : 1) in the gel at 0.5 of the compression-ratio to determine the orientation factor (k) in Eq. 1. The degree of the dipole moment for the Qy band of BChl *a* in the reassociated LH/BChl *a* complex is determined by using the degree of the Qy band of BChl *a* in the special pair of RC.[19] The shape of the association with RC and the LH/BChl *a* complex is similar to that of the LH/BChl



Scheme 6. The reassociated LH/BChl *a* complex is assumed as ring like structure in the squeezed gel. The LH/BChl *a* complex is in distributing around the squeezing direction. The dipole moment of Qy band is circle in LH/BChl *a* complex and the Qx band is in X–Y plane. N or C-terminal of the complex is faced by X axis. The electric vector of polarized light is in Y–Z plane.

a complex alone, since RC is likely to be positioned inside of the LH/BChl *a* complex ring.[4] Thus, the orientation of the complexes of LH/BChl *a* and RC in the squeezed gel is the same as that of the LH/BChl *a* alone in the gel, indicating that the pigment orientation in the LH/BChl *a* complex may be calibrated by using the orientation of BChl *a* in the special pair of RC. The Qy band of BChl *a* in the special pair of the RC or the LH/BChl *a* complex is at 865 and 870 nm, respectively. The anisotropy ratios (LD/A) of the Qy band of BChl *a* in the LH/BChl *a* complex are almost similar to that in the special pair of RC, as shown in Fig. 5. Thus, the orientation of the Qy dipole moment of BChl *a* in the LH/BChl *a* complex is very close to that in the special pair. The angle of the Qy band of the special pair of the RC is assumed to be 90° according to analogy of that from *R. sphaeroides* or *Rhodopseudomonas viridis*.[19] Assuming that θ for the Qy of a special pair for the protein axis is 90° and LD/A = 0.18, the k value is determined to be −0.36. The anisotropy ratio can be converted to θ. The Qy band of the accessary BChl *a* in the RC is 70° and the Qy band of BPheo is 47°, consistent with the data for the RC from *R. viridis*.[19] This result implies that 0.18 of the LD/A corresponds to 90° of the Qy dipole moment of BChl *a* in the complex. These LD/A values at the Qx, Qy, and Soret bands determined by Eq. 1 are summarized in Table 1. The degree of the dipole moment of the Qy or Qx band of BChl *a* in the LH/BChl *a* complex is 90 or 0° for the protein axis, respectively (Scheme 3). Interestingly, λ_{max} of the Qy band of BChl *a* in the reassociated LH/BChl *a* complex is red-shifted, as mentioned above, where the Qy band may be an excitonically coupled dimer of BChl *a* in the complex.[14,15,29,30] The LH/BChl *a* complex can be reversibly dissociated to a minimal subunit form, B820 complex in the presence of octyl glucoside micelle,[11] where the spectroscopic properties of BChl *a* in the complex imply a strongly

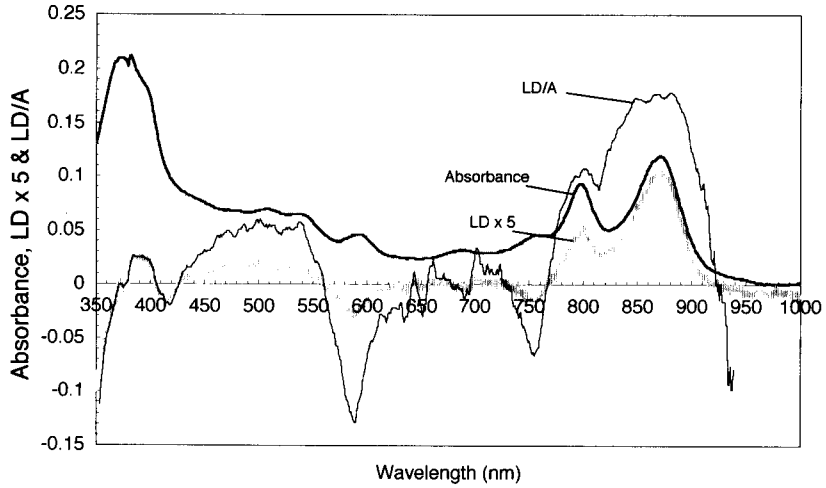


Fig. 5. Isotropic absorbance, LD and LD/A spectra of LH/BChl *a* complex with RC (1 : 1) from *R. rubrum* when the gel was at 0.5 of the compression ratio. The LH/BChl *a* complex (2 μM of BChl *a*) and RC (2 μM of Special Pair BChl *a*) were mixed at 22 mM OG and then diluted to 10 mM OG. The solution was gelled by polyacrylamide.

coupled dimer.[14,15,29,30] Despite the fact that the absorption maximum of the B820 subunit complex is blue shifted by about 55 nm relative to the LH 1 complex, the triplet–singlet (T–S)[14,29] and excited-state difference spectra[30] between B820 and the LH 1 complex are very similar. This is a strong indication that the dimeric nature of the B820 complex is maintained in the fully assembled LH 1 complex. Based on an analogy from an X-ray analysis of the LH 2 complex, the distance between the Mg–Mg atoms of BChl *a*'s in the dimer is 0.89 nm, and the charge transfer between the BChl *a*'s is an important contributor to the coupling.[31] It is being debated whether the dimer of BChl *a* in the complex is more electronic coupling due to its aggregation. Recently, Kobayashi et al. have noted that the Qy-component of BChl *a* in the complex is the dominant character of the dimer of BChl *a* according to an analysis of the MCD measurement.[32] Thus, the angle of the dipole moment of the Qy band in the complex is likely to be that of the BChl *a* dimer.

The Electron State of BChl *a* in the LH/BChl *a* Complex. The molecular structure of the LH 1 complex of photosynthetic bacteria was recently proposed based on the two-dimensional crystal, where the structure illustrates just the packing of the polypeptides.[4] However, the electronic state of the complex, which has an expanded electron cloud, is not revealed. Visscher et al. reported the anisotropy of the complex in OG micelle for a polarized fluorescence technique.[12] This technique shows the relative angle as the neighboring dipole moment of the pigment, and the angle is less 30°, indicating that the angle between the BChl *a* molecules in the dimer might be approximately 30°. Thus, the two BChl *a*'s are coupled by an angle of 30°, so that the orientation of the Qy dipole moment of the dimer is membrane-parallel. Measurements of the transient absorbance spectrum indicate that the heterogeneity from aggregation of the Qy band is crucial for energy transfer between the BChl *a*'s.[1,30,35] A hole-burning measurement also shows that an inhomogeneous distribution of BChl *a* in the LH complex and energy transfer between pigments occur with a different energy level between B875 and B896 complexes.[36,37] Our results concerning the LD spectra show that the Qy transition of BChl *a* in the reassociated LH/BChl *a* complex is homogeneous to the direction perpendicular to the transmembrane axis. The LD spectra show the angle of the transition for the transmembrane axis, although the spectra do not show the tangent angle around the ring of the LH/BChl *a* complex. The tangent angle between the Qy transition of BChl *a* must be measured by polarized fluorescence spectroscopy.[38] It is implied that the heterogeneous transition moment of the Qy transition is not in the direction of the protein axis, but that the moment is in the plane on a ring-like structure e.g. the tangent angle of the Qy band around the circle of BChl *a*. This homogeneous orientation may cause an efficient energy transfer between BChl *a*'s in the LH complex and from the LH/BChl *a* complex to the RC special pair. Thus, it is interesting to note that the LH polypeptides of photosynthetic bacteria organize the BChl *a* complex according to cooperative interactions between the LH polypeptide and BChl *a* so that an efficient energy-transfer processes between BChl *a*'s may occur.

Conclusion

The reassociated LH/BChl *a* complex in OG micelle is macro-orientated by a gel-squeezed method. The orientation of the dipole moment for the Qy, Qx, and Soret bands of BChl *a* in the LH complex could be observed by the LD spectra and the anisotropy of the dipole moment of BChl *a* was determined. The Qy transition moment, which is interpreted as the BChl *a* dimer, is perpendicularly directed for the transmembrane axis. The Qy-transition moment is homogeneous for the polypeptide axis in the complex. Thus, the origin of the heterogeneity of the Qy transition may be in the tangent angle of the Qy transition around the ring of BChl *a*. This result implies that the Qy band of the LH/BChl *a* complex from *R. rubrum* is membrane parallel in the photosynthetic membrane, and that the energy transfer between BChl *a*'s occurs in a homogeneous electronic state in the LH complex.

We thank Mr. K. Itoh and Mr. M Takeuchi of NMIRI for useful discussions. We also thank Mr. M. Hashii (NMIRI) for computer programming the data transport from the UV-vis spectrophotometer to a personal computer. We thank Dr. T. Takeo and Dr. K. Hirano (NMIRI) for using the polarizer and UV-vis spectra. M. N. thanks to Prof. P. A. Loach, Northwestern University, U.S.A. for a kind gift of photosynthetic bacteria, *R. rubrum* and helpful suggestions on the preparation of the reassociated LH/BChl *a* complex.

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