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Selective Detection and Assignment of the Solution NMR Signals of Bacteriochlorophyll *a* in a Reconstituted Subunit of a Light-Harvesting Complex

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Abstract: High-resolution solution NMR spectra have been obtained for bacteriochlorophyll (BChl) *a* molecules in a biologically functional subunit of a bacterial core light-harvesting complex based on a modified reconstitution method. The reconstituted subunit of pigment-integral membrane polypeptides is stable and homogeneous at high concentrations at room temperature and exhibits a Q_y absorption peak at 818 nm. ^{1}H and ^{13}C chemical shifts have been specifically assigned for BChl *a* using the fully and selectively ^{13}C-labeled pigments incorporated with natural abundance polypeptides in deuterated detergent solution. Remarkable signal broadening has been observed upon reconstitution, where the bacteriochlorin macrocycle is shown in a highly restricted molecular motion while the phytol side chain remains relatively mobile. Two sets of resonances are revealed for 3^2, 8^1, 10, 12^1, and 13^4 protons, and 8^2 methyl protons exhibit four resonances with large upfield complexation shifts. The result indicates a nonequivalent state for the two BChl *a* molecules in the subunit and can be best interpreted in terms of a parallel face-to-face configuration with partial overlap over the pyrrolic rings II, III, and V. In comparison with BChl *a* in acetone, 8^2, 13^2, and 13^4 protons are largely perturbed, and the propionic and phytol side chain may adopt a different conformation in the reconstituted subunit. The ^{13}C chemical shift of 3^1 carbonyl carbon shows a large change downfield, indicating strong hydrogen bonding for all the acetyl carbonyls. Carbonyl carbons at 13^1 give rise to two ^{13}C resonances with equal intensities, suggesting that the keto carbonyl in one BChl *a* molecule within a subunit forms a stronger hydrogen bond than that in another BChl *a* molecule.

Introduction

Despite intense interest and continuing effort, the molecular organization of bacteriochlorophyll (BChl) in the core light-harvesting complex (LH1) of purple photosynthetic bacteria has not been determined to atomic resolution. To date, the highest-resolution structure of LH1 is obtained as a 2D projection map at 8.5 Å produced by electron diffraction using 2D crystals of reconstituted LH1 complexes from a purple nonsulfur bacterium *Rhodospirillum* (*R.*) *rubrum*. In contrast, a number of high-resolution X-ray crystal structures have been determined for the reaction center (RC)[1−3] and peripheral light-harvesting complex (LH2).[4,5] The difficulty may be partially due to the unique in vivo location of LH1, where the integral membrane pigment−protein complex is sandwiched between the RC and LH2 components in most species, making it difficult to isolate intact LH1 complex and to prepare high-quality crystals. Hitherto, a large body of biochemical and spectroscopic data has been collected to provide information on the structure and function of the LH1 complex.[6,7] A resonance Raman study has utilized the carbonyl stretch region (1600−1700 cm^{-1}) to probe the interaction mode of carbonyl groups of the BChl *a* molecule with its polypeptide environment and revealed that acetyl carbonyl groups are hydrogen bonded in its native form.[8] At present, only a reconstitution methodology using various BChl analogues has been employed to examine the structural requirements of the BChl binding in the LH1 complex.[9] However, the molecular basis is not fully understood for the role of each individual functional group of BChl *a*. We describe here the first observation of high-resolution solution NMR signals from intact BChl *a* molecules in a reconstituted subunit of the LH1 complex and show several remarkable characteristic features of the complexation-induced chemical shift and intensity

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changes on the basis of the assigned resonances. This work provides complementary information to the ongoing effort of crystal structure determination of LH1, in terms of pigment−pigment and pigment−protein interactions. The system of this study, as a model of chromophore−protein complexes, also has potential applications in the peripheral areas, such as heme proteins and another type of photoreceptors: rhodopsin/bacteriorhodopsin.

LH1 of photosynthetic bacteria generally comprises two small polypeptides, α and β, with a ratio of 1:1, along with two BChl and one or two carotenoid molecules per $\alpha\beta$ pair. The α- and β-polypeptides used in this study were obtained from wild-type *R. rubrum*. The BChl *a* is believed to be ligated by a histidine residue to its central Mg atom and absorbs at 880 nm (Q_y band) in the near-infrared region for the native LH1 of *R. rubrum*, a substantial red shift (110 nm) compared to the absorption of its monomeric form in organic solvents. The LH1 complex is capable of forming a stable intermediate species, characterized by a Q_y absorption at approximately 820 nm (referred to as B820), by dissociation of the LH1 with detergent *n*-octyl β-D-glucopyranoside (OG).[10] The B820 species is considered to be a structural subunit of the LH1 complex and can be reassociated to form native LH1 as well as reversibly dissociated to its individual components by adjusting the detergent concentration. Furthermore, Parkes-Loach et al. have shown that both the B820 subunit and LH1 can be spontaneously reconstituted from the separately isolated polypeptides and BChl *a*.[11] The self-assembling feature and relatively small size of the B820 subunit provide an ideal target for the structural analysis by NMR in the solution state.

Attempts for NMR measurements of biologically active integral membrane pigment−protein complexes in solution state usually encounter at least three hurdles: (a) low spectral resolution due to the large molecular mass of the protein-detergent micelles, (b) poor selectivity in distinguishing the resonances between pigment molecule and protein, and (c) giant signals from the detergent molecules. Using an organic solvent system to solubilize the membrane protein is an alternative way to avoid the difficulties. However, this in most cases leads to a loss of functional property, implying a conformational change from its native form. Recently, Conroy et al.[12] determined a solution structure of β-polypeptide of the LH1 by NMR. They used a chloroform/methanol mixed solvent system and found that BChl *a* is unable to bind to the polypeptide in this solvent system. Therefore, they were unable to observe structural changes on BChl ligation. We show in this study that the difficulties mentioned above can be overcome by choosing a pigment−protein complex with a minimum size while maintaining its functionality and by developing a method of preparing a highly concentrated sample suitable for the NMR measurement. In addition, we replaced the pigment molecules in the complex by various ^{13}C-labeled equivalents, which give the same spectral features and significantly enhance the ^{1}H−^{13}C correlation signals. Furthermore, deuterated detergent has been used to suppress the strong resonances from the small molecules.

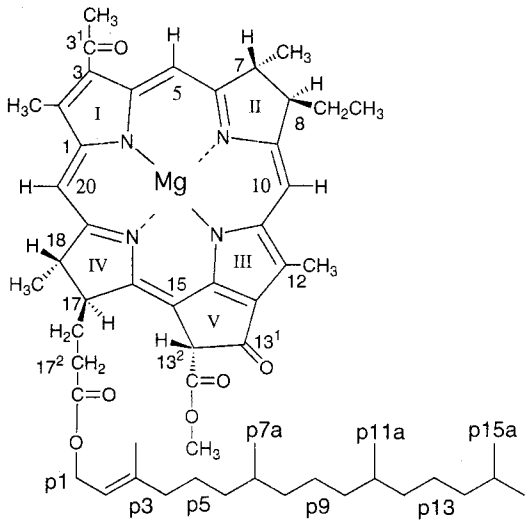

Figure 1. Structure and nomenclature of BChl a_p.

With all these efforts, we have succeeded in selectively detecting and specifically assigning the NMR resonances from the individual functional groups of BChl *a* molecule.

Materials and Methods

Preparation of ^{13}C-Labeled BChl a_p Molecules. Fully ^{13}C-labeled BChl *a* containing phytol as the esterifying alcohol (BChl a_p, Figure 1) was isolated from purple sulfur bacterium *Chromatium vinosum*. The cells were grown in a medium containing $NaH^{13}CO_3$ (^{13}C > 98% atom, Isotec Inc.) as the sole carbon source. Partially ^{13}C-labeled BChl a_p was isolated from purple sulfur bacterium *Chromatium tepidum*. The cells were grown in a medium containing $NaHCO_3$ and sodium acetate as carbon sources. Three combinations of the carbon-containing chemicals were made in the preparation of culture media: (1) $NaH^{13}CO_3$ and CH_3COONa, (2) $NaHCO_3$ and $^{13}CH_3COONa$ (^{13}C > 99%, Isotec Inc.), and (3) $NaHCO_3$ and $CH_3{}^{13}COONa$ (^{13}C > 99%, Isotec Inc.). All BChl a_p molecules were extracted following procedures based on Omata and Murata[13] and were purified on a reverse-phase HPLC column (ODS-80Ts, TOSOH) with solvent mixtures of acetonitrile/acetone/methanol/water (33:50:15:2, volume ratio).[14]

Isolation and Purification of LH1 Apopolypeptides. The LH1 complex was isolated and purified in its native form from wild-type *R. rubrum* according to method B of Picorel et al.[15] Fractions eluted from a DEAE column (25 mm × 250 mm, Toyopearl 650S, TOSOH) were monitored spectroscopically by measuring the absorbance between 250 and 900 nm. The ratios of absorbance at 880 nm (A_{880}) to those at 800 nm (A_{800}, due to the absorbance of RC) and at 280 nm (A_{280}, due to the absorbance of aromatic amino acids) were used as indicators of purity. Complexes which gave ratios A_{880}/A_{800} greater than 7.5 and A_{880}/A_{280} greater than 2.6 were considered pure enough for use in the reconstitution experiment. After dialysis and drying, the purified LH1 was extracted repeatedly, first with benzene and then with methanol to remove carotenoids and BChl *a*. At this stage, a small amount of the apopolypeptides was chromatographed on a reverse-phase HPLC system (TSKgel, Super ODS, 4.6 mm × 100 mm, TOSOH) to further check the purity using a three-stage linear gradient.[11] No other components other than the α- and β-polypeptides were detected.[14]

Reconstitution of B820 Subunit. Typically, 20 mg of lyophilized apopolypeptide was dissolved in 700 μL of phosphate buffer (D_2O, 50 mM, pH 7.0) containing 5% deuterated OG (w/v, Anatrace Inc., D > 97%) and lyophilized after thorough mixing. Aliquots of ^{13}C-BChl a_p

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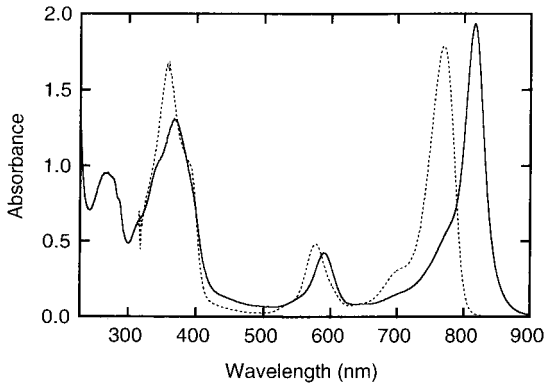

Figure 2. Absorption spectra of the reconstituted B820 complex (solid line) used for NMR measurements and BChl a_p in acetone (dotted line) at room temperature. The spectrum of the highly concentrated B820 subunit solution was recorded using a cuvette with a light path length of 0.12 mm.

dissolved in acetone-d_6 (D > 99.9%, Isotec Inc.) were added with a proportion of $ODV_{770} = 9.1$ per mg of apopolypeptides and then freeze-dried after thorough mixing. A 700 μL volume of deuterated water (D > 99.9%, Isotec Inc.) was added to the pigment−polypeptide−detergent mixture and then was vortexed thoroughly. The solution was centrifuged at 14 000 *g* for 10 min. The supernatant was checked with absorption spectroscopy (Beckman DU-640) and used for NMR measurement. Normally, the precipitate contained about 5% of the original amount of polypeptides. All operations of the reconstitution were conducted in dim light at room temperature (~22−23 °C).

NMR Measurements. NMR spectra were collected on Bruker DRX-400 and DRX-500 spectrometers at a temperature of 35 °C. One- and two-dimensional $^1H-^{13}C$ shift correlation spectra using ^1H-detected heteronuclear multiple-quantum coherence via direct coupling method (HMQC) were acquired with the pulse sequence described by Bax et al.[16] The spectral width for ^1H was 4800 Hz and for ^{13}C was 16 kHz. A total of 128 t_1 points of 2K data points were acquired. For each t_1 value, 320 or 400 transients were recorded, the number of scans depending on the experiment. During acquisition, the ^{13}C spectrum was decoupled using a broadband GARP modulation.[17] One-dimensional proton-decoupled ^{13}C spectra were recorded with 30° pulse, 8K data points and 1.0 s repetition time. Refocused insensitive nuclei enhanced by polarization transfer (INEPT)[18] spectra were recorded for the assignment of proton-bound carbons using selectively ^{13}C-enriched samples. Chemical shifts were referenced to 2,2-dimethyl-2-silapentane-5-sulfonate (DSS).

Results and Discussion

Overall Spectral Features. The reconstituted B820 subunit is fully functional even at high concentrations (~1.8−2.2 mM protomer), as can be confirmed from the spectroscopic homogeneity of its absorption spectrum (Figure 2). The subunit is stable at room temperature for several days and exhibits a Q_y band at 818 nm, whereas the BChl a_p in acetone has a Q_y absorbance at 770 nm. Figure 3 shows the two-dimensional $^1H-^{13}C$ HMQC spectrum of the reconstituted B820 subunit incorporated with fully ^{13}C-labeled BChl a_p and solubilized in phosphate buffer containing 5% deuterated OG at 35 °C, together with that of the corresponding apopolypeptides under the same conditions. Significant signal broadening was observed for all protons and proton-attached carbons of the BChl a_p molecule, indicating a largely reduced molecular motion upon introduction of the BChl a_p into the polypeptide−detergent micelles. Despite the broadening, most resonances were resolved in the 2D spectrum. To facilitate assignment, similar measurements were performed on the reconstituted B820 complexes using selectively ^{13}C-labeled BChl a_p. Such experiments not only help distinguish the signals due to trace contaminants of small molecules but also improve the spectral resolution by reducing dipole−dipole couplings that may occur significantly for the B820 subunit reconstituted with fully ^{13}C-labeled BChl a_p. Using the results of selective labeling and comparing with the spectrum of intact BChl a_p in acetone, we were able to specifically assign all the resonances of BChl a_p in the B820 complex.

One of the most significant features is that many proton resonances are multisplit with nonsymmetric spectral shapes. The result strongly suggests that BChl a_p molecules in the B820 subunit exist in a nonequivalent configuration where the corresponding functional groups are surrounded by different local environments.[19−21] The local effects may arise from the interaction with a neighboring BChl a_p molecule or with surrounding amino acid residues and are consequently reflected in the chemical shift changes of the individual protons. Another characteristic feature observed in the NMR spectra is the differing dynamic behavior between the macrocycle and phytol side chain. The most obvious evidence can be found from comparison of the intensities between the meso CH and P2-CH. While these methine groups showed similar intensities and line widths in the ^1H and ^{13}C spectra in acetone, considerably greater signal broadening was observed for the meso groups, particularly the protons, compared to that of P2 methine group (Figure 4). This corresponds to a much faster relaxation rate for the protons directly attached to the macrocycle and indicates that molecular motion of the macrocycle becomes highly restricted upon incorporation into the polypeptide environment, whereas the phytol chain remains in a relatively mobile mode as this portion is relatively distant from the Mg−His binding site.

^1H NMR Assignment of the BChl a_p in Reconstituted B820 Complex. Assignment of the ^1H NMR spectrum for the BChl a_p in the reconstituted B820 subunit largely relied on both the corresponding ^1H and proton-attached ^{13}C chemical shifts of BChl a_p in organic solvents. Although the ^1H chemical shifts of BChl a_p have been established, these values vary with coordination and aggregation states in different solvents.[22,23] Therefore, it was insufficient to use only the ^1H chemical shift values obtained from organic solvents for the assignment of BChl a_p in the reconstituted B820 complex, where the chemical shift changes induced by peptide binding were much greater than those observed from solvent effects. In contrast, complexation-induced ^{13}C chemical shift changes are relatively small for the proton-attached carbons.[19] This greatly assists the assignment of the ^1H spectrum of BChl a_p through H−C correlation. We have confirmed the assignment by 2D HMQC

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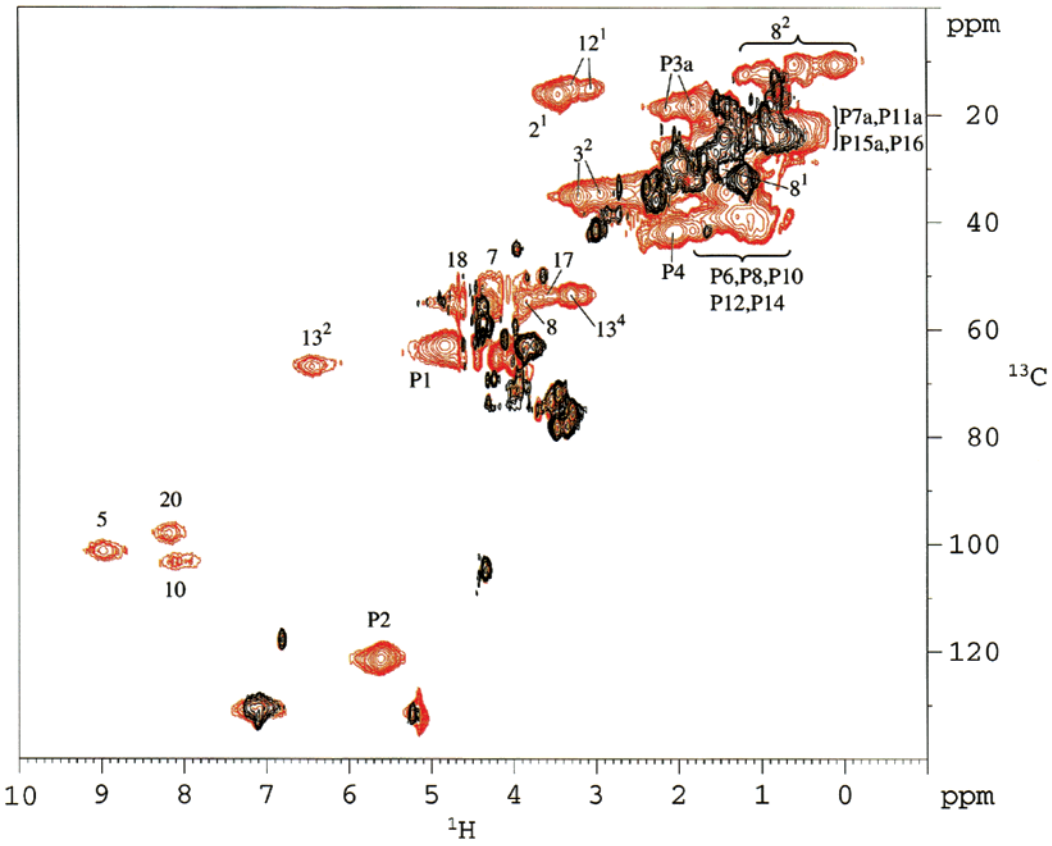

Figure 3. Two-dimensional ^{1}H−^{13}C shift correlation spectrum of the reconstituted B820 subunit (~2 mM protomers) using fully ^{13}C-labeled BChl a_p and natural abundance polypeptides solubilized in 5% deuterated OG-phosphate buffer (50 mM, D_2O, pH 7.0) at 35 °C (red), together with that of the corresponding apopolypeptides (black) under the same conditions.

experiment using acetone-d_6 as a solvent in which the BChl a_p is present in a five-coordinate monomeric form.[22] This spectrum (Supporting Information) was used as a reference in this study because BChl a_p in the B820 complex is also believed to exist in a five-coordinated form. Table 1 shows the full assignment of ^{1}H chemical shifts for the BChl a_p in the reconstituted B820 subunit together with the complexation shifts defined by $\Delta\delta_H = \delta^H_{B820} - \delta^H_{770}$(acetone-$d_6$).

Assignments of the meso protons, 5-, 20-, and 10-H, were straightforward, because these protons attach to the carbons whose chemical shifts in organic solvents have been well established.[24−26] It was of interest to note that the chemical shift of 10-H for the BChl a_p in B820 complex appeared at slightly higher field relative to that of 20-H, whereas the reverse is known for the BChl a_p in organic solvents regardless of its coordination number and aggregation state.[22] Assignments of 13^2-, P2-, and P1-H were also apparent, as their resonances were well separated from others in the 2D HMQC spectrum. These protons exhibited large downfield complexation shifts, up to 0.48 ppm, as shown in Figure 4. Four methine protons at 18, 7, 8, and 17 were assigned on the basis of 2D HMQC spectra using the selectively ^{13}C-labeled BChl a_p. Carbons at the 18 and 7 positions were found to be labeled in the medium containing $CH_3{}^{13}COONa$, while carbons at the 8 and 17 positions were labeled in the medium containing $^{13}CH_3COONa$. Methyl protons at 13,[4] 2^1, 12^1, and 3^2 were identified by their strong H−C correlation resonances together with the results of selective ^{13}C-labeling experiments. While chemical shifts of the 2^1- and 12^1-methyl protons remained almost the same values as those in acetone, large upfield and downfield complexation shifts were observed for the 13^4- and 3^2-methyl protons, respectively. In the propionic side chain, resonance of 17^2 protons appeared at lower field relative to that of 17^1 protons, in reverse order for those of the BChl a_p in acetone. Ethyl protons at 8^1 and 8^2 gave the largest upfield complexation shifts observed for the BChl a_p in the reconstituted B820 complex. The 8^2-H resonances can be distinguished by H−C correlation, because ^{13}C resonance of 8^2 carbon appears the most upfield. Because of structural similarity, phytol protons were less resolved on the 2D HMQC spectra except for P3a and P4 protons even using the selectively ^{13}C-labeled BChl a_p. Only P3a and P15 protons revealed relatively large downfield complexation shifts.

Several peripheral groups of BChl a_p in the reconstituted B820 subunit exhibit two sets of resonances. These are apparent for the 3^2, 12^1, and P3a methyl protons and are somewhat less apparent for the 13^4 methyl and 10 methine protons. Surprisingly, the 8^2 methyl protons exhibit four resonances at the high-field region of the 2D HMQC spectrum. Assignment of the four resonances of 8^2-H was confirmed using the selectively ^{13}C-labeled BChl a_p in which the 8^2 methyl carbon was efficiently labeled in the $^{13}CH_3COONa$-containing medium. There is a tendency for the protons in the peripheral groups around pyrrolic rings II and III, that is, 8^2-H, 8^1-H, 10-H, and 12^1-H, to give two resonances, whereas the protons on the opposite side, that

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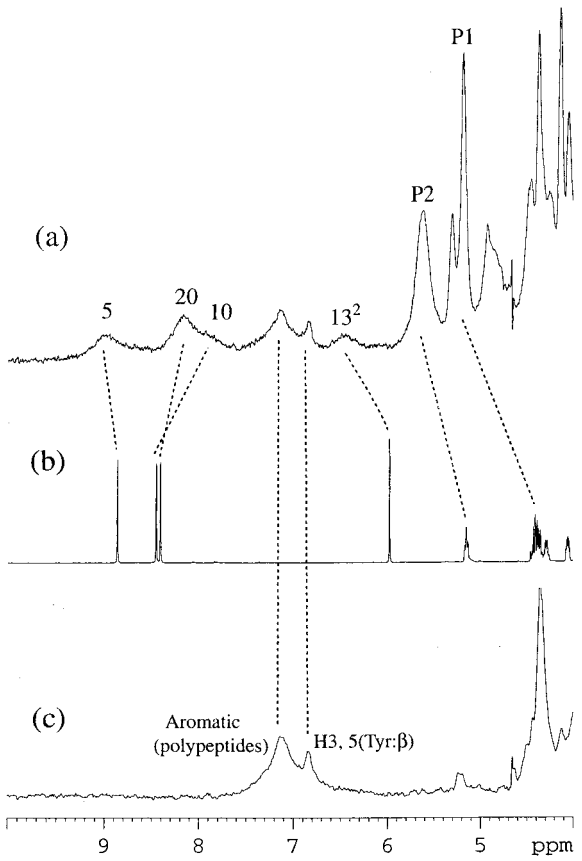

Figure 4. Downfield regions of ^{1}H NMR spectra. (a) One-dimensional HMQC spectrum of the reconstituted B820 subunit with fully ^{13}C-labeled BChl a_p in 5% deuterated OG-phosphate buffer (50 mM, D_2O, pH 7.0). (b) Conventional ^{1}H spectrum of natural abundance BChl a_p in acetone-d_6. (c) One-dimensional HMQC spectrum of natural abundance apopolypeptides in 5% deuterated OG-phosphate buffer (50 mM, D_2O, pH 7.0).

is, 2^1-H, 20-H, and 18^1-H, appear as single resonances. Two possibilities may be considered to interpret the different behavior. Because the B820 subunit has been determined to exist in the (BChl $a)_2\alpha\beta$ form by a small-angle neutron scattering experiment (unpublished result) and other techniques,[6] the two BChl *a* molecules may overlap each other over pyrrolic rings II and III to which the attached protons experience slightly different ring-current effects. In this case, the protons on the other side (rings I and IV) of BChl *a* macrocycle may be surrounded by polypeptides or detergent molecules in a similar environment. This pigment configuration is supported by the known LH2 crystal structure in which the two BChl *a* molecules in a subunit are present in a parallel face-to-face conformation with partial overlap over rings III and V.[4] Another explanation is that the pigment molecules may adopt a conformation in which the peripheral groups attached to rings II and III are exposed to polypeptides and interact in a different way with the side chains of amino acid residues.

In comparison with the BChl a_p in acetone, a variety of changes in the chemical shift were observed for the protons of BChl a_p in the reconstituted B820 subunit. Relatively large downfield complexation shifts ($\Delta\delta_H > 0.4$ ppm) were found for 13^2-H, 17^2-H, P1-H, P2-H, and one of the resonances from P3a-H. In contrast, 13^4-H, 8^1-H, and two of resonances from 8^2-H exhibited substantial upfield complexation shifts ($\Delta\delta_H \sim -0.4$ to -1.1 ppm), most of these protons being in the side chains attached to rings II and V. The result coincides with the observation of two resonances for the corresponding protons and indicates that peripheral groups of rings II, III, and V in the two BChl *a* molecules are involved in different interactions with either adjacent pigment molecule or side chains of amino acid residues. The large complexation shifts observed for the propionic side chain and the first two groups of phytol chains (P1-H and P2-H) may reflect very different conformations adopted for this portion of the long side chain from that in organic solvents. Similar behavior was also found for BChl *c* molecules upon dimer formation.[19]

Table 1. Assignments of ^{1}H Chemical Shifts for the BChl *a* in Reconstituted B820 Subunit

position	B820	$\Delta\delta_H$[a]	position	B820	$\Delta\delta_H$[a]
5	8.99	0.14	P3a	2.14	0.56
20	8.18	−0.26		1.81	0.23
10	8.12	−0.28	P15	1.87	0.35
	7.91	−0.49	P7	1.42	0.06
13^2	6.44	0.46	P11	1.42	0.06
P2	5.62	0.48	P5	1.15[b]	−0.21
P1	4.83	0.44	P9	1.15[b]	−0.17
18	4.37[b]	0.02	P13	1.31	0.12
7	4.27[b]	0.02	P14	1.13	0.01
8	3.85	0.20		1.00	−0.14
17	3.67	−0.29	P8	1.30	0.06
13^4	3.30	−0.48	P10	1.29	0.04
2^1	3.46	0.02	P12	1.14	0.09
12^1	3.31	−0.02		1.02	−0.03
	3.06	−0.27	P6	1.15	0.10
3^2	3.21	0.16	8^2	1.24	0.14
	2.93	−0.12		0.96	−0.14
17^1	2.28	−0.13		0.59	−0.51
8^1	1.43	−1.08		0.11	−0.99
	1.30	−0.68	P16	0.83	−0.03
17^2	2.64	0.54		0.72	−0.14
P4	2.02	0.11	P15a	0.82	−0.04
	1.83	−0.09		0.71	−0.15
7^1	1.53	−0.22	P11a	0.87[b]	0.05
18^1	1.53	−0.14	P7a	0.81[b]	0.00

[a] $\Delta\delta_H = \delta^H_{B820} - \delta^H_{770}$(acetone-$d_6$). [b] These assignments are provisional.

Dynamic properties revealed by the ^{1}H NMR spectra were striking for both pigment molecules and polypeptides. Protons on the macrocycle of BChl a_p exhibited significant signal broadening compared to those of the phytol side chain, as shown in Figure 4. It is worth noting that signals from the highly flexible part of polypeptides can also be resolved even for the natural abundance sample. In Figure 4c, the resonance at 6.85 ppm was assigned as 3-H and 5-H of the phenol ring of tyrosine in the β-polypeptide on the basis of 2D HMQC and COSY experiments where these protons were shown to correlate with neighboring 2-H and 6-H. The unique tyrosine (no tyrosine residue in α-polypeptide) is the second residue from the carboxyl terminus of β-polypeptide and gives rise to sharp signals without labeling. Because the shape and chemical shift of this resonance remained unchanged upon reconstitution, the carboxyl terminal domain of the β-polypeptide is considered to retain a highly mobile motion in the detergent-solubilized B820 subunit solution.

^{13}C NMR Assignment of the BChl a_p in Reconstituted B820 Complex. Figure 5 shows the one-dimensional ^{13}C spectrum of BChl a_p in the reconstituted B820 subunit using fractionally $NaH^{13}CO_3$-enriched pigments. The overall features are similar to those observed from the corresponding ^{1}H spectra. Signal broadening was remarkable for all carbons of the incorporated BChl a_p, and the upfield region was largely dominated by the signals from detergent molecules. Therefore,

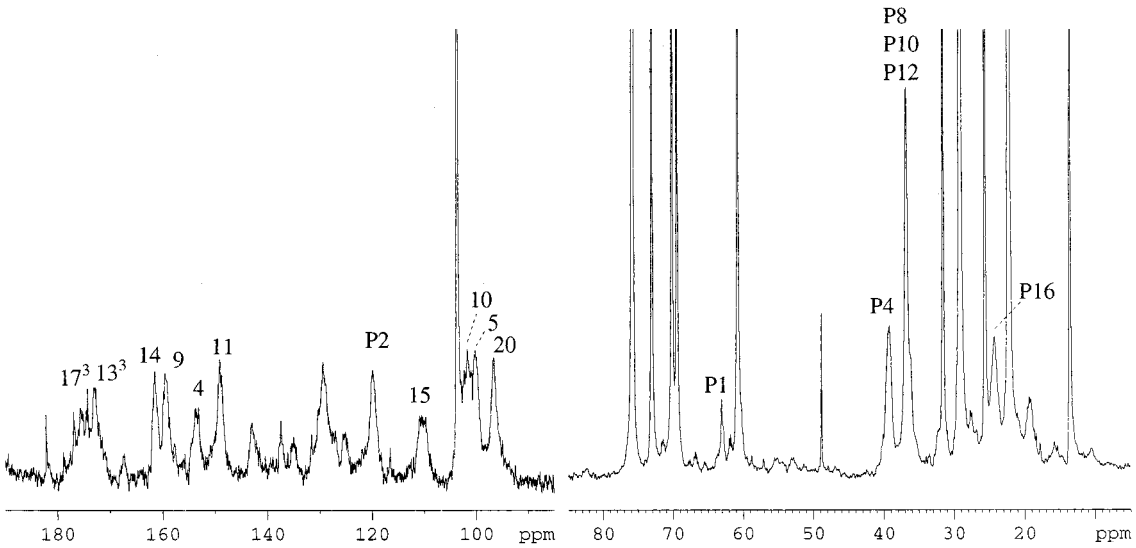

Figure 5. ^{13}C NMR spectrum of the reconstituted B820 subunit using fractionally ^{13}C-enriched BChl a_p with the $NaH^{13}CO_3$-containing medium. The spectrum was acquired in 5% deuterated OG-phosphate buffer (50 mM, D_2O, pH 7.0) at 35 °C.

Table 2. Assignments of ^{13}C Chemical Shifts of BChl *a* in the Reconstituted B820 Subunit

position	B820	$\Delta\delta_C$[a]	position	B820	$\Delta\delta_C$[a]
3^1	203.3	4.0	7	49.2	0.7
13^1	192.0	2.9	P4	41.5	1.1
	191.2	2.2	P14	41.3	1.2
17^3	174.3	0.8	P10	39.1[b]	1.0
13^3	173.4	2.0	P12	39.2	1.2
	173.3	0.0	P8	39.1	1.1
6	168.8	0.1	P6	38.6	1.3
19	166.9	−0.56	P11	34.7	1.2
	166.2	−1.3	P7	34.1	0.8
14	162.8	2.0	3^2	35.2	2.1
9	160.8	2.1	17^1	32.0	0.9
16	154.8	2.5	17^2	32.0	0.9
1	152.6	1.4	8^1	31.8	1.1
4	150.3	0.0	P15	30.0	1.3
11	151.1	1.4	P13	26.7	1.0
P3	144.3	1.7	P9	26.3	0.8
2	143.3	0.8	P5	26.0	0.9
3	137.2	−0.5	18^1	25.6[b]	2.1
13	130.6	0.2	7^1	25.0	1.7
12	128.6	2.0	P15a	24.7[b]	1.6
P2	121.1	1.7	P16	24.4	1.5
15	111.6	1.6	P7a	21.9	1.8
10	102.8	0.5	P11a	21.7	1.7
5	101.1	1.4	P3a	18.6	2.3
20	97.7	1.1	2^1	16.1	2.4
13^2	66.8	1.2	12^1	15.1	3.1
P1	62.7	1.2	8^2	13.0	2.1
8	56.7[b]	0.9		12.4	1.5
13^4	53.8	1.4		11.6	0.7
17	52.3[b]	1.6		10.7	−0.2
18	51.4[b]	1.7			

[a] $\Delta\delta_C = \delta^C_{B820} - \delta^C_{770}$(acetone-$d_6$). [b] These assignments are provisional, see text.

assignment of the upfield carbon resonances had to rely on 2D HMQC and INEPT experiments using the selectively ^{13}C-labeled BChl a_p in comparison with the corresponding spectra from apopolypeptides under the same conditions. Using the combined techniques, we have assigned the ^{13}C chemical shifts of BChl a_p in the reconstituted B820 subunit, which are shown in Table 2 together with the complexation shifts relative to those in acetone. It should be noted that the ^{13}C chemical shift is relatively insensitive to the ring-current effect compared to the ^{1}H chemical shift because it is generally governed by mixed effects of paramagnetic shielding, hydrogen bonding, polarity of the solvents, and coordination state, along with the ring-current effect.[19] In a specific case, change in the ^{13}C chemical shift for an individual carbon may be caused by a single effect.

Assignment of the downfield resonances of BChl a_p in the reconstituted B820 subunit was made by using the selectively ^{13}C-enriched pigments. Resonances of carbonyl carbons appeared the most downfield, followed by those of carboxyl, quaternary, and aromatic carbons. All carbonyl and carboxyl carbons of BChl a_p were found to be highly enriched in the $CH_3{}^{13}COONa$-containing medium with their neighboring carbons unlabeled, leading to a well-resolved spectrum even for the reconstituted complex as shown in Figure 6. The most remarkable features observed from the spectra are the large downfield complexation shifts for the carbonyl carbons and two resonances revealed by the 13^1 keto carbonyl carbon. The ^{13}C chemical shift of the carbonyl group can be used as a sensitive probe for detecting hydrogen bonding, as we have demonstrated that the formation of a hydrogen bond results in more than a 3 ppm downfield change in the ^{13}C chemical shift for the carbonyl carbon involved.[27] The BChl a_p molecules in the reconstituted B820 subunit showed a single resonance with a large downfield complexation shift ($\Delta\delta_C = 4.0$ ppm) for the 3^1-acetyl carbons, indicating strong hydrogen bonding for all these carbonyl groups. The two resonances of 13^1 keto carbonyl carbons with moderate downfield complexation shifts can be interpreted in terms of different strengths of hydrogen bond interaction. Similar conclusions have been derived from resonance Raman studies. Visschers et al.[8] showed that the 3^1-acetyl groups of all BChl *a* molecules in the LH1 complex were hydrogen bonded, and about half of the original hydrogen bonds were enhanced upon dissociation of the LH1 complex into the B820 subunits. They also observed two bands corresponding to the frequencies of the 13^1 keto vibration upon formation of the B820 subunit and proposed that a substantial fraction of the hydrogen bonds were weakened or may even be lost.[8] The result of this study is in

(27) Nozawa, T.; Ohtomo, K.; Suzuki, M.; Nakagawa, H.; Shikama, Y.; Konami, H.; Wang, Z.-Y. *Photosynth. Res.* **1994**, *41*, 211−223.

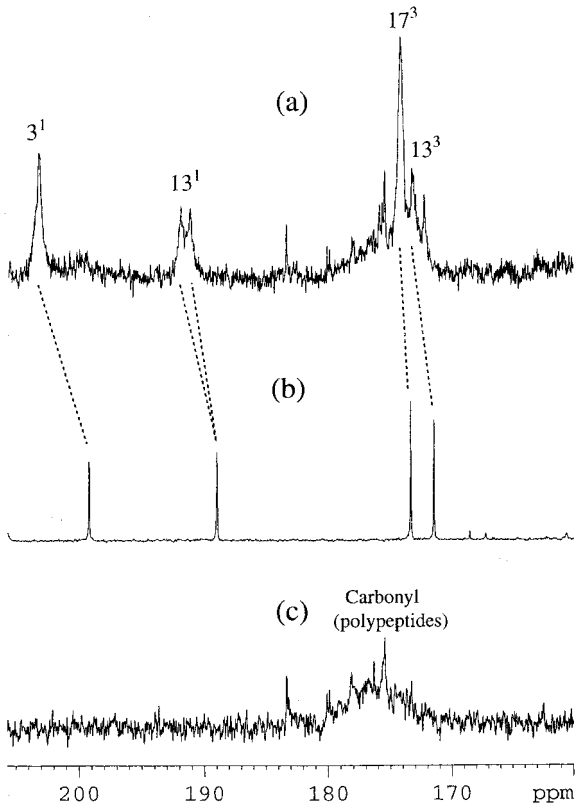

Figure 6. Downfield regions of ^{13}C NMR spectra from (a) the reconstituted B820 subunit using ^{13}C-BChl a_p labeled with $CH_3{}^{13}COONa$-containing medium, (b) ^{13}C-BChl a_p labeled with $CH_3{}^{13}COONa$-containing medium in acetone-d_6, and (c) natural abundance apopolypeptides. Both the spectra of the B820 subunit and the apopolypeptides were acquired in 5% deuterated OG-phosphate buffer (50 mM, D_2O, pH 7.0) at 35 °C.

good agreement with that of resonance Raman. The equal intensities of the two ^{13}C resonances of 13^1 keto carbons indicate that the 13^1 carbonyls involved in different interactions have an exact ratio of 1:1, or in other words, the 13^1 carbonyl group in one BChl a_p molecule within a B820 subunit forms a stronger hydrogen bond than that in another BChl a_p molecule.

Assignment of quaternary carbons of BChl a_p in the B820 complex was mainly made by using the selectively ^{13}C-labeled pigments. Because there is a discrepancy in the assignment of some of these carbons in organic solvents,[26] we have reassigned the ^{13}C signals of this region using acetone as a solvent by C−C correlation and long-range H−C correlation techniques as described previously.[19] Assignment of the upfield ^{13}C resonances was relatively easy, as chemical shifts of the BChl a_p in organic solvent have been well established for these carbons, and H−C correlation can be employed to assist the assignment. There was no peak splitting observed for these carbons upon reconstitution. All resonances in this region exhibited a downfield change in the chemical shift, and only a few closely spaced resonances interchanged their orders with respect to those in acetone. This may be mainly due to a solvent effect. Several resonances from carbons in pyrrolic rings II, IV, and the phytol group were heavily overlapped even using the selectively ^{13}C-labeled sample; therefore, their assignments remained provisional.

Conclusions

In summary, we have developed a reconstitution method for preparing a highly concentrated and homogeneous solution of the bacterial core light-harvesting complex subunit suitable for NMR measurement. The reconstituted pigment-membrane polypeptide subunit is fully functional and reveals spectroscopic features resembling those from native type. High-resolution NMR spectra have been obtained for the BChl a_p molecules in the reconstituted subunit, where fully and selectively ^{13}C-labeled pigments have been incorporated with natural abundance polypeptides in deuterated detergent solution. Molecular motion is significantly reduced for the BChl a_p molecules, particularly for the bacteriochlorin macrocycles, upon formation of the complex with polypeptides. The two BChl a_p molecules exist in a nonequivalent configuration in the subunit where peripheral groups attached to rings II, III, and IV in the respective molecules are surrounded with different local environments. It is highly likely that the two BChl *a* molecules adopt a parallel face-to-face conformation with a partial overlap over their pyrrolic rings II, III, and V. In comparison with the BChl a_p in acetone, 8^2, 13^2, and 13^4 protons are largely perturbed in the reconstituted subunit, and the propionic and phytol side chain may adopt a very different conformation. Both the 3^1-acetyl carbonyls in the BChl a_p dimer show strong hydrogen bonding with similar strength, whereas 13^1 keto carbonyl in one BChl a_p molecule forms a stronger hydrogen bond than that in another BChl a_p molecule.

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Supporting Information Available: $^1H-^{13}C$ HMQC spectra of the B820 subunit reconstituted from various selectively ^{13}C-labeled BChl a_p with natural abundance polypeptides along with that of BChl a_p in acetone-d_6 solution (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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