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A NMR study on the bacteriochlorophyll(BChl) *a* in a reconstituted subunit of light-harvesting complex

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Introduction

Although a number of X-ray crystal structures have been determined for the reaction center and peripheral light-harvesting complex(LH2), molecular organization of the BChl *a* in core light-harvesting complex (LH1) of purple photosynthetic bacteria has not been determined to atomic resolution. LH1 comprises two small polypeptides, α and β, along with two BChl *a* and one or two carotenoid molecules per αβ pair. The BChl *a* is believed to be ligated by a histidine residue to its central Mg atom and absorbs around 880 nm (Q_y band) in the near infrared region. 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 *n*-octyl β-D-glucopyranoside (OG). 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. To date, discussion on the LH1 function and mechanism remains superficial. This is largely due to the lack of high-resolution structure available. As a step toward analyzing the LH1 suprastructure, we first focus on the pigment arrangement in the B820 complex. The self-assembling feature and relatively small size of the B820 subunit provide an ideal target for the structural analysis by NMR in solution state. Resonance Raman has been mainly used to probe the interaction of carbonyl groups of the BChl *a* molecule with its polypeptide environment. We show in this study that all functional groups of BChl *a* in the B820 subunit can be resolved at atomic level in the NMR spectra and several conformational features can be obtained from complexation-induced chemical shift and intensity changes based on the assigned resonances.

Materials and methods

LH1 complex was isolated and purified in its native form from wild-type *R. rubrum* as reported previously(Wang et al. 2001). All pigments were removed by benzene and methanol to yield apopolypeptides. ^{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 ^{13}C-labeled sodium acetate ($^{13}CH_3COONa$) as carbon sources. BChl a_p molecules were purified on a reverse-phase HPLC column (ODS-80Ts, TOSOH) with mixed solvents as described elsewhere(Wang et al. 2001).

Reconstitution of B820 subunit was conducted as follows. About 20mg of the lyophilized apopolypeptides were dissolved in 700 μl phosphate buffer (D_2O, 50mM, pH7.0) containing 5% deuterated OG and then lyophilized. Aliquots of ^{13}C-BChl a_p dissolved in acetone were

added with a proportion of ODV_{770}=9.1 per mg of apopolypeptides and then freeze-dried. Deuterated water of 700 µl (D>99.9%, Isotec Inc.) was added to the pigment-polypeptide-detergent mixture and was vortexed thoroughly. The solution was centrifuged for 10 minutes. Supernatant was checked with absorption spectrum and used for NMR measurement. All operations of the reconstitution were conducted in dim light at room temperature (22~23˚C).

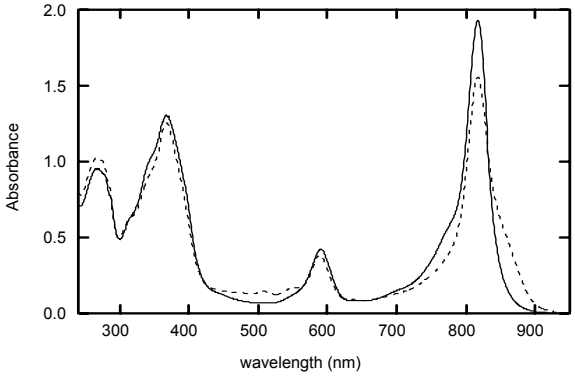

Fig. 1 Absorption spectra of the reconstituted B820 subunit (solid line) together with that of the subunit prepared from LH1 complex (dashed line) at OG concentration of 5%. The spectra of the highly concentrated B820 subunit solution was recorded using a cuvette with a light-path length of 0.12mm.

NMR spectra were collected on Bruker DRX-500 and DRX-400 spectrometers at 35˚C. One- and two-dimensional ^{1}H-^{13}C shift correlation spectra were acquired using ^{1}H-detected heteronuclear multiple-quantum coherence via direct coupling method(HMQC). The spectral width for ^{1}H was 4800 Hz and for ^{13}C was 16kHz. 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 was decoupled. One-dimensional proton-decoupled ^{13}C spectra were recorded with 30˚ pulse, 8 K data points and repetition time 1.0 s. Refocussed insensitive nuclei enhanced by polarization transfer (INEPT) 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

Figure 1 shows the absorption spectra of B820 subunits obtained by reconstitution and dissociation of B873 complex. The reconstituted B820 subunit is fully functional at high concentrations (1.8~2.2 mM protomer), as can be confirmed from its Q_y band at 818nm. Spectroscopic homogeneity of the reconstituted subunit is even better than that prepared from native LH1 complex.

Figure 2 shows the down-field region of a two-dimensional ^{1}H-^{13}C HMQC spectrum of the reconstituted B820 subunit using the partially ^{13}C-labeled BChl a_p. Remarkable signal broadening was observed for all protons and carbons of the BChl a_p molecule, indicating a highly

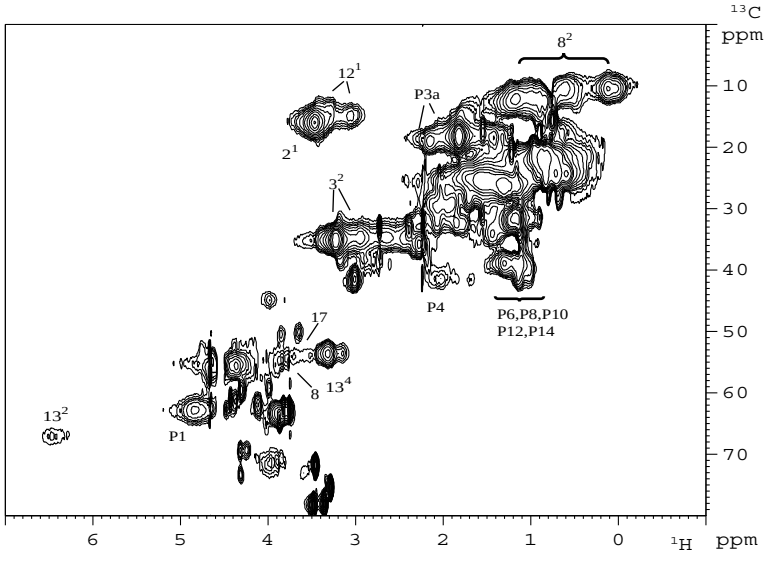

Fig. 2 Two dimensional HMQC spectrum of the B820 subunit reconstituted from natural abundance polypeptides and selectively ^{13}C-labeled BChl a_p in 5% deuterated OG solution. The BChl a_p was obtained using $NaHCO_3$ and $^{13}CH_3COONa$ as the carbon source in the medium.

reduced molecular motion upon incorporation of the BChl a_p into polypeptide-detergent micelles. 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.

The most significant features is that many proton resonances are multi-split 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(Wang et al. 1999a, b). The local effects may arise from the interaction with neighboring BChl a_p molecule or with surrounding amino acid residues. 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 phytol side chain. 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 ($\Delta\delta_H$: -0.4 ~ -1.1ppm), most of these protons being in the side chains attached to the rings II and V.

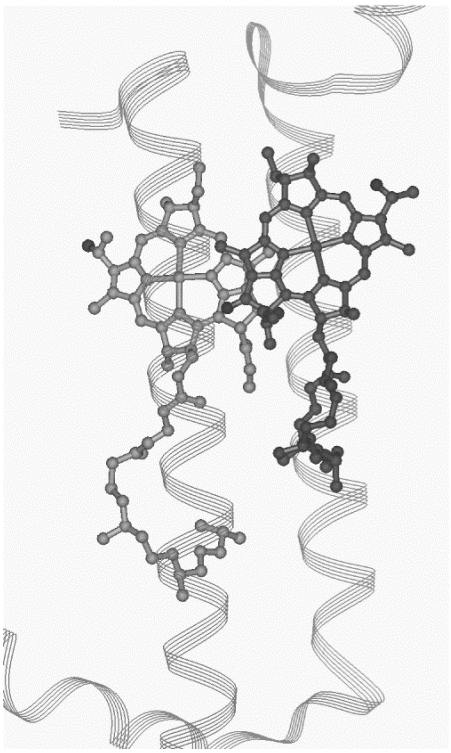

Fig. 3 A schematic model illustrating the two BChl a_p molecules in a reconstituted B820 subunit.

Discussion

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 somewhat less apparent for the 13^4 methyl and 10 methine protons. The 8^2 methyl protons exhibit four resonances at the high-field region of the 2D HMQC spectrum. There is a tendency for the protons in the peripheral groups around pyrrolic rings II and III, i.e. 8^2-H, 8^1-H, 10-H, and 12^1-H, to give two resonances, whereas the protons on the opposite side, i.e. 2^1-H, 20-H and 18^1-H, appear as single resonances. Since the B820 subunit can be considered to exist in a form of (BChl $a)_2\alpha\beta$, the two BChl a molecules may overlap each other over their pyrrolic rings II and III to which the protons attached experience slightly different ring-current effects(Fig. 3). 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 a partial overlap over the rings III and V(McDermott et al. 1995). The result coincides with the observation of large complexation shifts for the peripheral groups attached to rings II and V. The large complexation shifts observed for 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.

Acknowledgments

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