S1-016

Characterization of the polypeptides of core light-harvesting complex from purple-sulfur bacteria

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Keywords: antenna complex, membrane protein, self-assembly

Introduction

Purple photosynthetic bacteria can be classified into two groups based on their ability to use reduced sulfur compounds as an electron donor in photosynthesis. The photosynthetic complexes from purple-nonsulfur organisms have been well characterized both biochemically and biophysically at gene and atomic levels. In contrast, investigation on the photosynthetic apparatus of purple-sulfur organisms is largely delayed. Only recently, crystal structure of the reaction center (RC) from a purple-sulfur bacterium *Chromatium* (*Chr*.) *tepidum* has been determined to atomic resolution (Nogi et al. 2000), and a number of gene sequences encoding RC and core light-harvesting complexes (LH1) of purple-sulfur bacteria has become available (Fathir et al. 1997, 1998; Corson et al. 1999). To date, no full characterization of completely purified antenna polypeptides has been made for the purple-sulfur bacteria, and the property of their reconstitution with pigment molecules has not been examined. In a previous study, we reported spectroscopic features of the LH1 complex from *Chr. tepidum* in an associated form with RC (Fathir et al. 1998). Here, we present results on the characterization of LH1 polypeptides from two purple-sulfur bacteria, *Chr. tepidum* and *Chr. vinosum*, and the reconstitution behavior using HPLC, MALDI/TOF-MS, Edman analysis and UV spectroscopy. A study on RC-LH1 complex isolated from *Chr. purpuratum* by electrophoretic methods showed that the B820 subunit can be formed from LH1 in a mixed detergent solution and retains its carotenoid absorption properties (Kerfeld et al. 1994).

Materials and methods

Chr. tepidum cells were grown anaerobically at 48˚C as described previously (Fathir et al. 1998). Chromatophores were isolated by sonication of the whole cells suspended in 20 mM TRIS-HCl buffer (pH 8.5) followed by differential centrifugation. Unless otherwise stated, this buffer was used throughout the experiment. The chromatophores were first extracted with 0.35% w/v lauryldimethylamine N-oxide (LDAO) at room temperature for 90 min to remove the LH2-rich components, and then with 1% w/v OG under the same conditions to solubilize the LH1-containing components. The extract was further purified on a DEAE column (Toyopearl 650S, TOSOH) equilibrated with the buffer containing 1% w/v of OG. The LH1-RC fraction was eluted by a linear gradient of NaCl from 50 mM to 250 mM.

Isolation of LH1-RC complex from *Chr. vinosum* was conducted using chromatophores treated with 2% w/v Triton X-100 and 1% w/v OG at 4˚C for 60 min. The extract was loaded on the DEAE column and washed with 0.03% w/v Triton X-

100, 1M ascorbate and 100mM NaCl. After the LH2-riched components were removed, Triton X-100 in the elution buffer was replaced by 0.8% w/v OG. The LH1-RC fraction was then eluted by a linear gradient of NaCl from 100 mM to 200 mM.

LH1 polypeptides were isolated from the purified LH1-RC complexes by reverse-phase (RP) HPLC column with pretreatment by benzene and methanol to remove pigments. Mass spectra were measured on a REFLEX III MALDI-TOF MS spectrometer. Detailed procedures of these operations, along with that of reconstitution of B820 subunits, have been given elsewhere (Wang et al. 2001). Edman analysis for determination of the N-terminal amino acids were performed by the Biochemical analysis group of KISHIDA Chemical Co., LTD.

Results

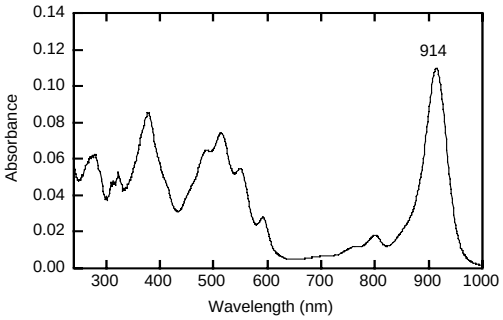

Fig. 1 Absorption spectrum of highly purified LH1-RC complex from *Chr. tepidum*.

Figure 1 shows the absorption spectrum of highly purified LH1-RC fraction of *Chr. tepidum* eluted from the DEAE column. The Q_y transition of LH1 is at 914 nm, about 30 nm red-shift compared with those from most other photosynthetic bacteria. Three fractions were eluted from the RP-HPLC. Edman analysis revealed that the first fraction corresponds to the β-polypeptide and both the second and third fractions correspond to the α-polypeptide. MALDI/TOF-MS measurement yielded a molecular weight of 5410 for the β-polypeptide. Further analysis of the N-terminal tryptic fragment by NMR spectroscopy revealed that the β-polypeptide has a *N*-methylated alanine as the N-terminal residue. The second HPLC fraction has a measured mass of 7025 Da, in agreement with that calculated from amino acid sequence of the α-polypeptide within experiment error. Mass spectrum of the third fraction gave a broad peak around 7060 to 7077 (m/z), making it difficult for precise determination, therefore this fraction is considered as a modified α-polypeptide.

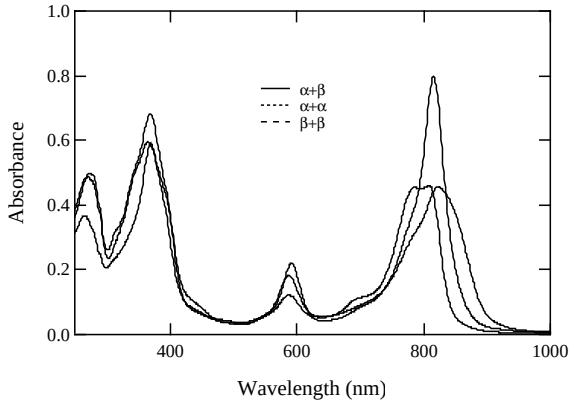

Fig. 2 Absorption spectra of the reconstituted B820 subunits at room temperature using the polypeptides from *Chr. tepidum* and BChl *a*.

Figure 2 shows the absorption spectra of reconstitution using α- and β-polypeptides of *Chr. tepidum* and BChl *a*. Only the combination of α- and β-polypeptides with BChl *a* can form the structural subunit with a Q_y transition at 816 nm, whereas neither the α-polypeptide only nor β-polypeptide only with pigment molecules are capable of reconstituting a high-quality subunit. Attempt for reconstitution of LH1-type complex from the subunits has not succeeded. Hybrid reconstitution of the LH1 polypeptides from *Chr. tepidum* with

those from purple-nonsulfur bacterium *R. rubrum* were examined. Any combination of α-polypeptide with β-polypeptide resulted in homogeneous B820 subunits, whereas mixture composed of α-polypeptides only or β-polypeptides only from both bacteria were unable to form the structural subunits.

The LH1-RC fraction of *Chr. vinosum* eluted from DEAE column shows an absorption spectrum similar to that of *Chr. tepidum* but with the Q_y transition of LH1 at 889 nm. Four fractions were eluted from the RP-HPLC as shown in Figure 3. TOF-MS measurements yielded mass values of 5394.8 Da (F1), 5133.0 Da (F2), 5128.5 Da (F3) and 5905.0 Da (F4) for these fractions. First cycle of Edman degradation of the F1 fraction liberated an unknown amino acid, following by Asp, Gln, Lys, Ser·····. This confirms that the F1 fraction corresponds to the third β-polypeptide (β3) as identified from its gene sequence (Nagashima, 2000). Further analysis of the N-terminal tryptic fragment by NMR spectroscopy revealed that the β3-polypeptide has a *N*-methylated alanine as the N-terminal residue. The result is completely consistent with the measured mass value of the F1 fraction. Edman analysis of the F2 fraction confirmed that this fraction corresponds to β1-polypetide with an amino acid sequence reported by Corson et al. (1999), and Ala is the N-terminal residue. F3 and F4 fractions were determined by Edman analysis to be α2- and α1-polypeptides, respectively. However, the calculated mass values are different from those measured by TOF-MS, especially for the F4 fraction. Further investigation on the differences is in progress.

Discussion

LH1 complex from *Chr. tepidum* was purified in a RC-associated form. Attempts for isolation of pure LH1 complex under various conditions were not successful. This may imply that strong interaction exist between the LH1 and RC complexes, and such interaction might also be responsible for the Q_y transition at 914 nm. The B820 subunit can be obtained by addition of octyl-glucoside. We have also observed an intermediate species with Q_y around 850 nm by addition of a cationic detergent, cetyl trimethylammonium bromide (CTAB). Considering that the Q_y transition can be reversibly converted between 914 nm and 885 nm by addition and removal of various salts (Fathir et al. 1998), ionic interaction may be involved between the LH1 and RC, or among the LH1 polypeptides. Although reconstitution of B820 subunit can be achieved using the separated α- and β-polypeptides with BChl *a*, we have not been able to reconstitute a LH1-type complex from its components. The result suggests that other components or factors may be necessary for the higher-order organization.

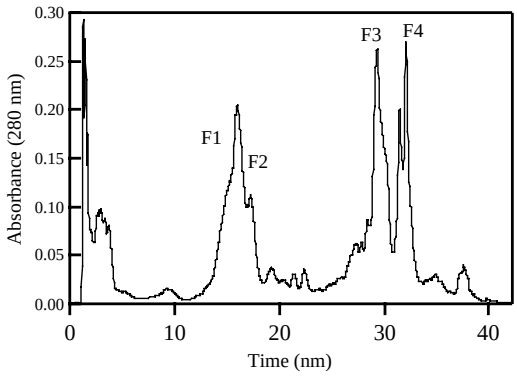

Fig. 3 RP-HPLC chromatogram of LH1 polypeptides from *Chr. vinosum*.

We have identified two polypeptides for each of the α- and β-polypeptides of LH1 from *Chr. vinosum*. The α3- and β2-polypeptides as reported from gene sequencing remain undiscovered. It is of interest to note that N-terminal residue (Ala) of the β3-polypeptide is *N*-methylated, as the same modification at the same position has been

found for the β-polypeptides from *Chr. tepidum* and *R. rubrum*. The α1-polypeptide seems to experience a carboxyl-terminal processing based on the mass difference, and probably 12 residues be truncated from the C-terminal domain.

Acknowledgments

This work was supported by Grant-in-aid for Scientific Research (No. 12878108 and 12450341), and Scientific Research of Priority Areas: Single-Cell Molecular Technology (No. 11227203), the Ministry of Education, Science, Sports and Culture, Japan.

References

Corson GE, Nagashima KVP, Matsuura K, Sakuragi Y, Wettasinghe R, Qin H, Allen R, Knaff DB (1999) *Photosynth. Res* **59**, 39-52.

Fathir I, Tanaka K, Yoza K, Kojima A, Kobayashi M, Wang Z-Y, Lottspeich F, Nozawa T (1997) *Photosynth. Res* **51**, 71-82.

Fathir I, Ashikaga M, Tanaka K, Katano T, Nirasawa T, Kobayashi M, Wang Z-Y, Nozawa T (1998) *Photosynth. Res* **58**, 193-302.

Kerfeld CA, Yeates TO, Thornber JP (1994) *Biochim. Biophys. Acta* **1185**, 193-202.

Nagashima, S. (2000) Acc. No:AB050620, DNA Data Bank of Japan (http://srs.ddbj.nig.ac.jp/index-e.html)

Nogi T, Fathir I, Kobayashi M, Nozawa T, Miki K (2000) *Proc. Natl. Acad. Sci. USA* **97**, 13561-13566.

Wang Z-Y, Shimonaga M, Muraoka Y, Kobayashi M, Nozawa T (2001) *Eur. J. Biochem.* **268**, 3375-3382.