FEBS Letters 519 (2002) 164–168 FEBS 26100

# N-terminal methylation of the core light-harvesting complex in purple photosynthetic bacteria

Zheng-Yu Wang*, Masahiro Shimonaga, Masayuki Kobayashi, Tsunenori Nozawa

*Department of Biomolecular Engineering, Faculty of Engineering, Center for Interdisciplinary Research, Tohoku University, Sendai 980-8579, Japan*

Received 5 April 2002; accepted 17 April 2002

First published online 26 April 2002

Edited by Gunnar von Heijne

**Abstract Several core light-harvesting complexes from both sulfur and non-sulfur purple photosynthetic bacteria have been identified to be methylated at the N-terminal α-amino group of β-polypeptides by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and nuclear magnetic resonance. Monomethylation has been confirmed for the N-terminal alanine residues of the β-polypeptides from *Rhodospirillum rubrum*, *Thermochromatium tepidum* and *Chromatium vinosum*, but not for the β-polypeptide from *Rhodobacter sphaeroides*. The modification appears to be related with the amino acid sequence and charge distribution in the N-terminal end. Some common features and possible functions are discussed.** 

*Key words:* Bacterial antenna complex; N-terminal methylation; Mass spectrometry

## 1. Introduction

Core light-harvesting complex (LH1) of purple photosynthetic bacteria is located in close proximity to the photochemical reaction center (RC) in intracytoplasmic membrane. Its main role is to collect light energy and transfer it to the RC for the primary charge separation. Although there is abundant high-resolution structural information about various RCs and another accessory form of light-harvesting complex (LH2), this type of information about LH1 is missing [1]. An electron diffraction study using two-dimensional (2D) crystals of reconstituted LH1 complexes from *Rhodospirillum* (*Rs.*) *rubrum* has provided a 2D projection map at a resolution of 8.5 Å, showing a ring structure consisting of 16 pairs of α- and β-polypeptides [2]. The two small polypeptides have been demonstrated by biochemical analysis to orient in the intracytoplasmic membrane with their N-terminal regions exposed on the cytoplasmic surface and C-terminal regions exposed on periplasmic surface [3]. A brief examination of the primary structures of LH1 α- and β-polypeptides from many species exhibits some common features. Both the polypeptide chains can be topologically divided into three domains: the N-terminus, the C-terminus and the central hydrophobic domain. The N-terminal domains of both polypeptides have several conserved amino acid residues. The α-polypeptides are characterized by a large number of basic amino acid residues while the β-polypeptides have many acidic amino acid residues in the N-terminal region [4]. These charged amino acid residues are supposed to be involved in the process of insertion of the polypeptides into the membrane and assembly of the pigment complexes in various species of photosynthetic bacteria [5].

In a preliminary study, we have shown that the N-terminal amino acid residue (corrected to be alanine) of the LH1 β-polypeptide from *Rs. rubrum* appears to be modified by a methyl group [6]. Such methylation is for the first time found in the photosynthetic bacteria, although it has been reported for a wide range of organisms from bacteria to mammalian. Different from modifications such as formylation and acetylation, methylation at N-terminal α-amino group is accessible to the conventional Edman degradation, therefore it is difficult to be detected or is often attributed as an unknown substance in the sequencing analysis. The modification is believed to occur during or shortly after polypeptide chain synthesis and is generally regarded as irreversible. However, still little is known about its physiological function. In this work, we provide structural evidence for the N-terminal methylation of the LH1 β-polypeptides purified from a number of typical purple photosynthetic bacteria. Two well studied non-sulfur bacteria, *Rs. rubrum* and *Rhodobacter* (*Rb.*) *sphaeroides*, and two sulfur bacteria, *Thermochromatium* (*Tch.*) *tepidum* and *Chromatium* (*Ch.*) *vinosum*, have been used for the experiments. Re-evaluation of the primary structures by modern protein chemical micromethods, such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) and nuclear magnetic resonance (NMR), shows that four of six β-polypeptides from four species are methylated at their N-terminal alanine residues. Our result suggests that this modification is more widespread in the purple photosynthetic bacteria and may have a correlation with the charge distribution in the N-terminal region of LH1 β-polypeptides.

## 2. Materials and methods

### 2.1. Purification of LH1 β-polypeptides from purple photosynthetic bacteria

Isolation and purification of LH1 complexes from wild-type *Rs. rubrum* and a carotenoid-less mutant *Rs. rubrum* G9 essentially followed the method B of Picorel et al. [7], and some details were given elsewhere [8]. LH1 complex from *Rb. sphaeroides* 2.4.1 were isolated

*Corresponding author: Fax: (81)-22-217 7278.
*E-mail address:* wang@biophys.che.tohoku.ac.jp (Z.-Y. Wang).

*Abbreviations:* LH, light-harvesting; MALDI-TOF/MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; NMR, nuclear magnetic resonance; HPLC, high performance liquid chromatography; RC, reaction center; COSY, homonuclear shift correlation spectra; OG, *n*-octyl β-D-glucopyranoside; TFA, trifluoroacetic acid

0014-5793/02/$22.00 
PII: S0014-5793(02)02744-8

as follows. All operations were conducted at 4°C. Chromatophores were first suspended ($A_{850}$ = 40) in 50 mM Tris–HCl buffer (pH 8.5) containing 10 mM sodium ascorbate, and then were treated with 0.3% (w/v) Triton X-100 and 1% (w/v) *n*-octyl β-D-glucopyranoside (OG) for 60 min. Centrifugation of the suspension at 150 000 × *g* for 90 min yielded a pellet and the supernatant was discarded. The pellet was resuspended in the same buffer and treated with the mixed detergents under the completely same conditions as mentioned above. The suspension was then subjected to a two-step gradient of 0.6 and 1 M sucrose, each containing 0.8% OG and 50 mM Tris–HCl (pH 8.5), and centrifuged at 150 000 × *g* for 12 h. A highly purified LH1–RC complex was found around the interface of the two layers and was used for further purification. LH1 complexes from the two sulfur bacteria were isolated in a LH1–RC form [9]. The *Tch. tepidum* chromatophores were first extracted with 0.35% w/v lauryldimethylamine *N*-oxide in 20 mM Tris–HCl buffer (pH 8.5) at room temperature for 90 min to remove the LH2-rich components, and then with 1% 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 to 250 mM. Isolation of the LH1–RC complex from *Ch. vinosum* was conducted using chromatophores treated with 2% Triton X-100 and 1% OG at 0°C for 60 min. The extract was loaded on the DEAE column and washed with 0.03% Triton X-100, 1 mM sodium ascorbate and 100 mM NaCl. After the LH2-rich 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 to 200 mM. The β-polypeptides from LH1 and LH1–RC complexes were obtained using a reverse-phase high performance liquid chromatography (HPLC) column with a mixed organic solvent system as described previously [8]. Edman degradation analyses for determination of the N-terminal amino acids of the β-polypeptides from *Tch. tepidum* and *Ch. vinosum* were performed by the Biochemical Analysis group of Kishida Chemical Co.

### 2.2. Trypsin treatment and fragment separation

The purified β-polypeptides were digested with trypsin (5 μg/ml) in 50 mM Tris–HCl buffer (pH 8.0) containing 2% (w/v) OG at 37°C for 8 h. The reaction mixture was centrifuged at 14 000 × *g* for 5 min. The supernatant was subjected to separation by a reverse-phase HPLC column (Source 5RPC ST 4.6/150, Pharmacia). Column temperature: 20°C. Flow rate: 0.7 ml/min. Detection: 230 nm. Eluent A: 0.1% (v/v) trifluoroacetic acid (TFA). Eluent B: acetonitrile/2-propanol (2:1 v/v), 0.1% (v/v) TFA. N-Terminal fragments were eluted within a range of 0–25% of the organic solvent in 30 min.

### 2.3. Mass analysis

Masses of the β-polypeptides and their N-terminal fragments were determined by MALDI-TOF/MS (REFLEX III, Bruker Analytische, Germany) as described previously [8]. The only exceptions were that 2,5-dihydroxybenzoic acid (Sigma) was used as matrix and dimer of α-cyano-4-hydroxycinnamic acid, angiotensin II and adrenocorticotropic hormone fragments (ACTH-CLIP) were used for calibration in the measurement of the low molecular weight N-terminal fragments.

### 2.4. NMR spectroscopy

NMR spectra were acquired at 25°C on a Bruker DRX-400 spectrometer. One-dimensional $^1$H spectra of the purified N-terminal fragments in $D_2O$ (99.9%, Isotec) were recorded with 8 K data points, sweep width 5590 Hz giving a digital resolution of 0.683 Hz/point and acquisition time 1.7 s. 2D homonuclear ($^1$H–$^1$H) shift correlation spectra (COSY) of the purified N-terminal fragments in dimethyl sulfoxide-$d_6$ (99.96%, Aldrich Chemical Co.) were recorded using a 45° read pulse [10]. The spectral width was 4400 Hz, a total of 256$t_1$ points of 1 K data points was acquired. For each $t_1$ value 96 transients were recorded.

## 3. Results

All LH1 β-polypeptides from the purple photosynthetic bacteria were purified to homogeneity, as can be confirmed from their TOF/MS spectra (Fig. 1). Both *Rs. rubrum* wild-type and G9 strains gave essentially the same molecular mass within the experimental errors. The value is about 14 Da greater than that calculated from their amino acid sequences with alanine as the N-terminal residues [6]. The measured

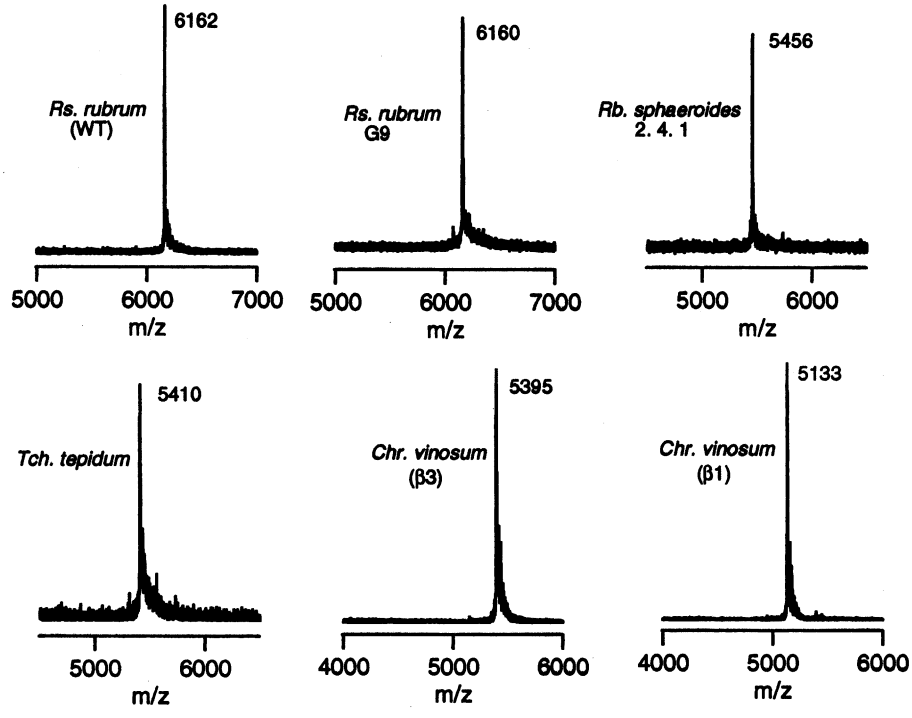


Fig. 1. Molecular mass determinations of the HPLC purified LH1 β-polypeptides from purple photosynthetic bacteria by MALDI-TOF/MS analysis.

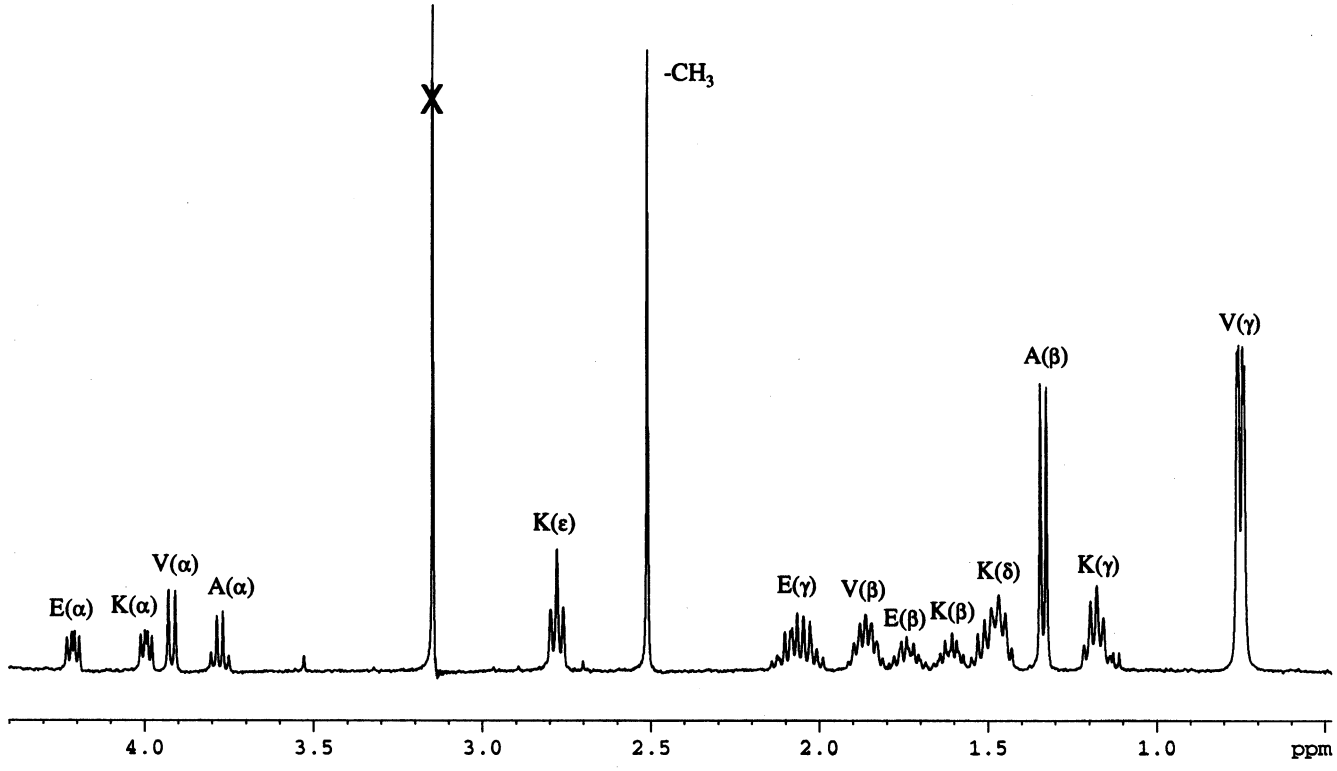


Fig. 2. $^{1}$H-NMR spectrum of the N-terminal fragment of LH1 β-polypeptide from *Rs. rubrum* obtained by trypsin digestion. The sample was dissolved in $D_2O$ and the spectrum was acquired at 25°C. The peak marked by × is due to impurity in $D_2O$. Amino acid residues are indicated by single letters, Greeks represent positions of protons.

mass of β-polypeptide from *Rb. sphaeroides* 2.4.1 was consistent with the amino acid sequence (5457 Da) determined by Edman degradation [11]. Therefore, there is no modification on this polypeptide. Molecular mass measured for the β-polypeptide of *Tch. tepidum* was 5410 Da. This is different from that calculated on the basis of its gene sequence [12], which predicts a value of 5529.4 Da and has a N-terminal domain of Met-Ala-Glu-Gln-···. First-cycle Edman degradation of the N-terminal domain released two peaks, one with a very close elution time to that of phenylthiohydantoin-Glu and another appeared as unknown substance. The sequence from second to tenth amino acid residue was completely consistent with that predicted from the gene sequence. The results indicate that the first methionine residue was removed and the second alanine residue was not identified. Two β-polypeptides were isolated from *Ch. vinosum* [9]. One was assigned to β1-polypeptide based on its N-terminal sequence and has the same molecular weight calculated from gene sequence [13] by taking into account the removal of N-terminal methionine residue. Another, designated as β3, has the same N-terminal amino acid sequence as previously reported from Edman degradation (B890-2) [14] and gene sequencing (β3) [15], except that the first-cycle Edman degradation in our analysis again released a strong unknown peak with the same elution time as that for the β-polypeptide of *Tch. tepidum*. The measured mass was also different from that calculated from its amino acid sequence. The third β-polypeptide corresponding to the gene sequence (β2) [13] was not found in our experiment.

In order to identify where the mass differences occurred, β-polypeptides from *Rs. rubrum*, *Tch. tepidum* and *Ch. vinosum* (β3) were digested with trypsin and all fragments were collected after separation by a reverse-phase HPLC column. TOF/MS measurements of each fragment revealed that all mass differences were due to the N-terminal tryptic fragments and were about 14 Da larger than those calculated from the corresponding amino acid sequences if taking into account the removal of N-terminal methionine residue. Exact determination of the unusual modification of the trypsin-digested fragments was achieved by NMR spectroscopy. $^{1}$H NMR spectra were acquired for the samples first dissolved in $D_2O$. A typical spectrum of the N-terminal fragment from the wild-type *Rs. rubrum* is shown in Fig. 2. A common feature for the four samples is that a singlet peak is observed at about 2.5 ppm and corresponds to three protons as determined from integration of its peak area. This resonance was further identified as $CH_3$-group by $^{1}H-^{13}C$ correlation measurement, however, such group cannot be assigned as a component to any amino acid residue. To determine the position of the newly found methyl group, we measured $^{1}H-^{1}H$ COSY spectroscopy for the N-terminal fragments dissolved in deuterated dimethyl sulfoxide. Such a spectrum obtained from wild-type *Rs. rubrum* is shown in Fig. 3. A strong correlation was observed between the methyl protons and the amino proton of Ala, indicating that the $CH_3$-group is attached to the amino nitrogen of the N-terminal alanine residue. Same correlations were also found for other three samples examined. Therefore, structure of the N-terminal residue is determined as *N*-($CH_3$)Ala- for the four β-polypeptides. Calculated molecular masses including this methyl group are completely consistent with those measured by MALDI-TOF/MS spectroscopy.

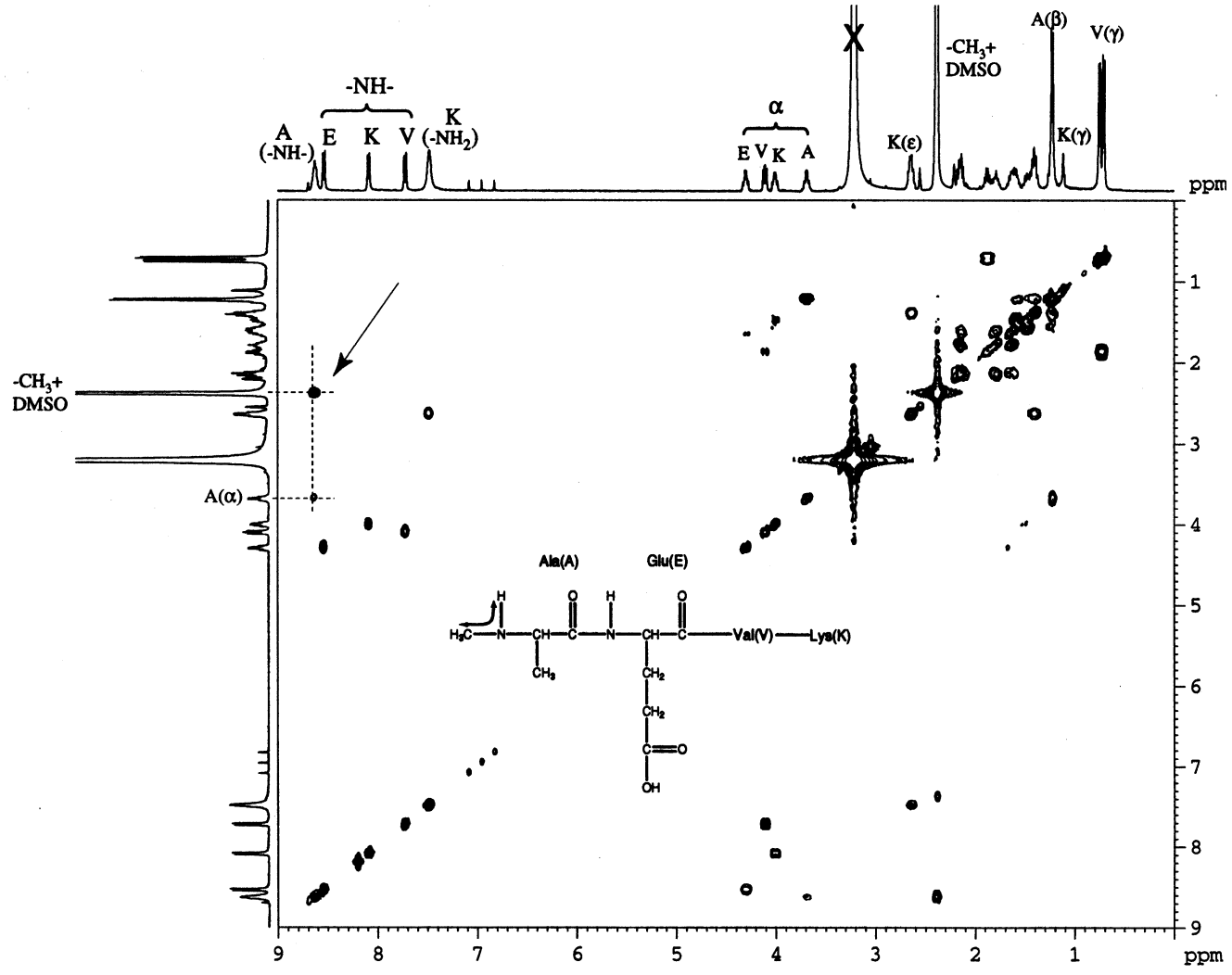


Fig. 3. 2D COSY spectrum of the N-terminal fragment of LH1 β-polypeptide from *Rs. rubrum* obtained by trypsin digestion. The sample was dissolved in deuterated dimethyl sulfoxide (DMSO-$d_6$) and the spectrum was acquired at 25°C. The peak marked by × is due to residual $H_2O$.

## 4. Discussion

Protein methylation is widely distributed in nature from prokaryotes to eukaryotes, although much less common compared to protein phosphorylation. The methylation reactions can be classified in two groups based on the substrate-specificity of protein methyltransferase [16]. The first group involves reversible modification of carboxyl groups to form methyl ester, which is considered to regulate activities of the protein. The second group generally results in irreversible methyl transfer to nitrogen and sulfur atoms and are often found in structural proteins. Such methylation may play a role in stabilization of polypeptides in complex cellular structures. Existence of both types of the methyltransferases has been reported for photosynthetic organisms. In most cases, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and LH complexes were identified as methyl acceptors. Light-regulated carboxyl methylation was observed in chloroplast proteins [17], where the extent of the methylation was stimulated by light indicating a dependence of the methyltransferase activity on photosynthetic electron transport and the transmembrane ΔpH. A post-translationally methylated asparagine residue, γ-*N*-methylasparagine, has been found at the β subunit position 72 of many phycobiliproteins from prokaryotic cyanobacteria and eukaryotic red algae. Evidence has been presented that this methylation serves to enhance efficiency of energy transfer in the LH complexes through Photosystem II [18,19]. A trimethyllysyl residue at position 14 has been identified in the large subunit of Rubisco [20], where the N-terminal methionine residue of the small subunit is post-translationally modified to *N*-methyl-methionine in many higher plants [21]. The result of this work adds purple bacteria to the list of protein methylation in photosynthetic organisms and indicates that this modification may have its ancient roots in sulfur bacteria from an evolutionary point of view.

Although protein methylation on carboxyl groups or on the side-chain nitrogen of glutamic acid residue has been known as a sensory adaptation (chemotaxis) to chemical stimuli in bacteria [22], the physiological role of methylation at N-terminal α-amino group is not well understood. At present, one can only speculate that this modification may serve for signal recognition during the membrane protein insertion and assem-

bly with pigment molecules, regulation of stability and solubility of the N-terminal region, and protection of the exposed sequences to attack by aminopeptidases within the cell. In LH1 complexes of purple photosynthetic bacteria, the β-subunit N-terminal end is exposed to the cytoplasm and is negatively charged. This segment is supposed to stabilize the LH1 structure by opposite-charge interaction with the α-subunit N-terminal segment [23]. We have found that the methylated β-polypeptides examined in this study all have the same charge distribution in their N-terminal end as shown below, whereas different charge distributions are found for those unmodified β-polypeptides.

| | | − | + |
|---|---|---|---|
| *Rs. rubrum* | $N(CH_3)$ – Ala – Glu – Val – Lys– | | |
| *Tch. tepidum* | $N(CH_3)$ – Ala – Glu – Gln – Lys– | | |
| *Ch. vinosum* (β1) | $N(CH_3)$ – Ala – Asp – Gln – Lys– | | |
| *Rb. sphaeroides* | Ala – Asp – Lys – Ser– | | |
| *Ch. vinosum* (β1) | Ala – Asn – Ser – Ser– | | |

The regularities in charge distribution and amino acid sequence may be related with the N-terminal methylation as sequence comparison of proteins containing similar N-terminal modifications suggests that the first few N-terminal residues play an important role in the specificity of the methylating enzymes [24]. Beside the methylation, no other modifications have been observed for the β-polypeptides examined in this work on the basis of the TOF/MS results. A number of studies reported that the LH1 polypeptides were phosphorylated in vivo and in vitro [25–27]. A soluble protein kinase was purified from *Rs. rubrum*, which was shown to significantly phosphorylate both LH1 α- and β-polypeptides [26]. But we have confirmed in a previous study that the α-polypeptide is also not phosphorylated for the wild-type *Rs. rubrum* [8]. Further investigations are needed to clarify the discrepancy.

*Acknowledgements:* 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

[1] Wang, Z.-Y., Muraoka, Y., Shimonaga, M., Kobayashi, M. and Nozawa, T. (2002) J. Am. Chem. Soc. 124, 1072–1078.
[2] Karrasch, S., Bullough, P.A. and Ghosh, R. (1995) EMBO J. 14, 631–638.
[3] Brunisholz, R.A., Zuber, H., Valentine, J., Lindsay, J.G., Woolley, K.J. and Cogdell, R.J. (1986) Biochim. Biophys. Acta 849, 295–303.
[4] Zuber, H. and Cogdell, R.J. (1995) in: Anoxygenic Photosynthetic Bacteria (Blankenship, R.E., Madigan, M.T. and Bauer, C.D., Eds.), pp. 315–348, Kluwer, Dordrecht.
[5] Drews, G. (1996) Arch. Microbiol. 166, 151–159.
[6] Wang, Z.-Y., Shimonaga, M., Muraoka, Y., Kobayashi, M. and Nozawa, T. (2001) Photosynth. Res. 70, 321–323.
[7] Picorel, R., Belanger, G. and Gingras, G. (1983) Biochemistry 22, 2491–2497.
[8] Wang, Z.-Y., Shimonaga, M., Muraoka, Y., Kobayashi, M. and Nozawa, T. (2001) Eur. J. Biochem. 268, 3375–3382.
[9] Shimonaga, M., Wang, Z.-Y., Muraoka, Y., Kobayashi, M. and Nozawa, T. (2001) Proceedings of the 12th International Congress on Photosynthesis, pp. S1–016.
[10] Nagayama, K., Kumar, A., Wüthrich, K. and Ernst, R.R. (1980) J. Magn. Reson. 40, 321–334.
[11] Theiler, R., Suter, F., Pennoyer, J.D., Zuber, H. and Niederman, R.A. (1985) FEBS Lett. 184, 231–236.
[12] Fathir, I., Ashikaga, M., Tanaka, K., Katano, T., Nirasawa, T., Kobayashi, M., Wang, Z.-Y. and Nozawa, T. (1998) Photosynth. Res. 58, 193–302.
[13] Corson, G.E., Nagashima, K.V.P., Matsuura, K., Sakuragi, Y., Wettasinghe, R., Qin, H., Allen, R. and Knaff, D.B. (1999) Photosynth. Res. 59, 39–52.
[14] Brunisholz, R.A. and Zuber, H. (1992) J. Photochem. Photobiol. B Biol. 15, 113–140.
[15] Nagashima, S. (2000) Acc. No: AB050620, DNA Data Bank of Japan (http://srs.ddbj.nig.ac.jp/index-e.html).
[16] Clarke, S. (1993) Curr. Opin. Cell Biol. 5, 977–983.
[17] Black, M.T., Meyer, D., Widger, W.R. and Cramer, W.A. (1987) J. Biol. Chem. 262, 9803–9807.
[18] Swanson, R.V. and Glazer, A.N. (1990) J. Mol. Biol. 214, 787–796.
[19] Thomas, B.A., Bricker, T.M. and Klotz, A.V. (1993) Biochim. Biophys. Acta 1143, 104–108.
[20] Houtz, R.L., Poneleit, L., Jones, S.B., Royer, M. and Stults, J.T. (1992) Plant Physiol. 98, 1170–1174.
[21] Grimm, R., Grimm, M., Eckerskorn, C., Pohlmeyer, K., Röhl, T. and Soll, J. (1997) FEBS Lett. 408, 350–354.
[22] Stock, J. and Simms, S. (1988) Adv. Exp. Med. Biol. 231, 201–212.
[23] Dörge, B., Klug, G., Gad'on, N., Cohen, S.N. and Drews, G. (1990) Biochemistry 29, 7754–7758.
[24] Stock, A., Clarke, S., Clarke, C. and Stock, J. (1987) FEBS Lett. 220, 8–14.
[25] Holmes, N.G. and Allen, J.F. (1988) Biochim. Biophys. Acta 935, 72–78.
[26] Ghosh, R., Ghosh-Eicher, S., DiBerardino, M. and Bachofen, R. (1994) Biochim. Biophys. Acta 1184, 28–36.
[27] Brand, M., Garcia, A.F., Pucheu, N., Meryandini, A., Kerber, N., Tadros, M.H. and Drews, G. (1995) Biochim. Biophys. Acta 1231, 169–175.