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Letter to the editor

Re-identification of the N-terminal amino acid residue and its modification of β-polypeptide of light-harvesting complex I from *Rhodospirillum rubrum*

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Recently, we have reported an oxidative modification of α-polypeptide of core light-harvesting complex (LH 1) from purple nonsulfur photosynthetic bacterium *Rhodospirillum* (*R.*) *rubrum* and its consequence for the stability of a reconstituted subunit of the LH 1 (Wang et al. 2001). Here, we would like to address a long-standing uncertainty about the N-terminal amino acid residue of β-polypeptide in the LH 1, as the incomplete amino acid sequence has been widely cited in the literature since it was published. The result of this study clarified a discrepancy between the measured and calculated molecular weights based on the published amino acid sequence, and led to a discovery of *N*-methylation as a novel post-translational modification in photosynthetic bacteria.

The primary structure of the β-polypeptide of LH 1 from wild-type and carotenoidless mutant G-9$^+$ strains of *R. rubrum* was first reported by Brunisholz et al. (1984) using Edman degradation. In the first cycle of analysis, about 50% of glutamic acid was found to be liberated. Concomitant with the partial liberation of the PTH-Glu, they also observed two peaks derived from unknown substances, one close to PTH-Leu and another close to PTH-Ala. The authors postulated that the Glu was partly inaccessible because of blockage or protection by a non-peptide substance. Since then, the Glu has been believed to be the N-terminal residue of the β-polypeptide. Based on this sequence, molecular weight is calculated to be 6077.8. However, the N-terminal Glu was once questioned when gene sequence encoding the β-polypeptide was subsequently determined by Berard et al. (1986) The gene sequence corresponds to a polypeptide product beginning with Met-Ala-Glu-· · ·, and predicts Ala for the N-terminal residue instead of Glu. We have measured the molecular weights of highly purified β-polypeptides (Tonn et al. 1977; Wang et al. 2001) from both wild-type and G-9$^+$ strains by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS), which give a same value of m/z = 6162.4 (supplementary material). This value is about 85 Da greater than that calculated from the amino acid sequence as given above and still cannot be accounted for even by taking into account an additional alanine residue (+71.1 Da).

In order to find out where the mass difference occurred, the β-polypeptide was digested with trypsin (5 μg/ml, Wako Pure Chem. Industries Ltd) in Tris-buffer (50 mM, pH 8.0) containing 2% *n*-octyl β-D-glucopyranoside (OG). Fragments were separated by a reverse-phase HPLC column (Source, 5RPC ST 4.6/150, Pharmacia) with a gradient program consisting of aqueous and mixed organic solvents as described elsewhere (Wang et al. 2001). MALDI-TOF/MS measurements of each fragment revealed that the mass difference was due to the N-terminal tryptic fragment. A calculated molecular weight for the cleaved N-terminal peptide Glu-Val-Lys is 374.4, whereas the measured mass is 459.2, indicating a mass difference of about 85 Da. We further used nuclear magnetic resonance (NMR) spectroscopy to characterize this fragment. The ^{1}H NMR spectra recorded with

D_2O confirmed an Ala residue together with Glu, Val and Lys residues (supplementary material). In addition, a singlet resonance was observed around $\delta(^1H)$ = 2.5 ppm, and was identified as a CH_3 group by ^1H-^{13}C correlation spectra. The Ala residue is bound to the Glu through a peptide bond as confirmed by a correlation observed between the carbonyl carbon of Ala and amide proton of Glu from long-range ^1H-^{13}C correlation spectra. To identify the position of the newly found CH_3 group, we measured ^1H-^1H correlated spectroscopy (COSY) of the N-terminal fragment dissolved in deuterated dimethyl sulfoxide. A strong correlation was observed between the methyl protons and the amino proton of Ala, indicating that the CH_3 group is attached to amino nitrogen of the terminal alanine residue. Therefore, structure of the N-terminal fragment is determined as *N*-(CH_3)Ala-Glu-Val-Lys. The corrected amino acid sequence gives a molecular weight completely consistent with that measured by TOF/MS spectroscopy.

The result of Ala as the N-terminal residue can be interpreted in terms of stability of proteins in a living cell. According to the N-end rule (Tobias et al. 1991; Varshavsky 1992), both Ala and Glu in bacteria are stabilizing residues that protect proteins against intracellular proteolytic degradation. The Ala residue does not prevent the removal of N-terminal Met by Met aminopeptidase after the initiation of translation and, therefore, can be exposed at the N-termini of proteins that initially bear this residue at the second position. However, detection of the N-terminal alanine in the β-polypeptide has been obviously complicated by the modification with a methyl group. The methylation at N-terminal α-amino group appears to be widely distributed in nature from bacteria to mammalia, although much less common compared to acetylation and formylation (Stock et al. 1987). Most N-terminal methylated proteins, such as ribosomes, nucleosomes, pilins and flagella, are found to have large macromolecular structures. A number of abundant intracellular proteins including calmodulin, actin, myosin and some nuclear proteins are also known to be *N*-methylated on the side chain nitrogens of Lys, Arg, or His residues (Kim et al. 1998). The occurrence of *N*-methylation in a protein is often apparent only after amino acid composition analysis and sequencing of the purified protein. Monomethylated proteins at their N-termini were shown to be accessible to the Edman degradation process but with a different elution time from that of its corresponding unmodified amino acid (Chen et al. 1977). Its identification largely involves utilization of radiolabeled *S*-adenosyl-L-methionine as the methyl donor. Although an increasing number of proteins modified by methylation has been identified, very few protein methyltransferases have been purified and characterized. The *N*-methylation is believed to occur during or shortly after polypeptide chain synthesis and is generally regarded as irreversible. Little is known about the physiological function of this modification. N-terminal monomethylation may have chemical consequences of a small change in pK_a of the α-amino group and a slight reduction in its reactivity due to the steric effects of the methyl group (Stock et al. 1987). For the β-polypeptide of this study, other effects on membrane assembly, solubility of the polar N-terminal region in the cytoplasm can also be considered. We are extending our investigation to the light-harvesting membrane polypeptides from other photosynthetic bacteria.

Supplementary material available

MALDI-TOF/MS spectrum of β-polypeptide; 1D ^1H-NMR spectrum of the N-terminal tryptic fragment in D_2O; 2D ^1H-^{13}C long-range correlation spectrum of the N-terminal tryptic fragment in D_2O; COSY spectrum of the N-terminal tryptic fragment in DMSO-d_6. Supplementary material can be obtained from the corresponding author.

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References

Berard J, Belanger G, Corriveau P and Gingras G (1986) Molecular cloning and sequence of the B880 holochrome gene from *Rhodospirillum rubrum*. J Biol Chem 261: 82–87

Brunisholz RA, Suter F and Zuber H (1984) The light-harvesting polypeptides of *Rhodospirillum rubrum* I. The amino-acid sequence of the second light-harvesting polypeptide B880-β (B870-β) of *Rhodospirillum rubrum* S1 and the carotenoidless mutant G-9$^+$. Hoppe-Seyler's Z. Physiol Chem 365: 675–688

Chen R, Brosius J and Wittmann–Liebold B (1977) Occurrence of methylated amino acids as N-termini of proteins from *Escherichia coli* ribosomes. J Mol Biol 111: 173–181

Kim S, Park GH and Paik WK (1998) Recent advances in protein methylation: Enzymatic methylation of nucleic acid binding proteins. Amino Acids 15: 291–306

Stock A, Clarke S, Clarke C and Stock J (1987) N-terminal methylation of proteins: structure, function and specificity. FEBS Lett 220: 8–14

Tobias JW, Shrader TE, Rocap G and Varshavsky A (1991) The N-end rule in bacteria. Science 254: 1374–1377

Tonn SJ, Gogel GE and Loach PA (1977) Isolation and characterization of an organic solvent soluble polypeptide component from photoreceptor complexes of *Rhodospirillum rubrum*. Biochemistry 16: 877–885

Varshavsky A (1992) The N-end rule. Cell 69: 725–735

Wang Z-Y, Shimonaga M, Muraoka Y, Kobayashi M and Nozawa T (2001) Methionine oxidation and its effect on the stability of reconstituted subunit of light-harvesting complex from *Rhodospirillum rubrum*. Eur J Biochem 268: 3375–3382