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Methionine oxidation and its effect on the stability of a reconstituted subunit of the light-harvesting complex from *Rhodospirillum rubrum*

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An additional component in the purified core light-harvesting complex (LH1) from wild-type purple photosynthetic bacterium *Rhodospirillum rubrum* has been identified as an oxidized species of α-polypeptide by MALDI-TOF mass spectrometry. This component appears as a slightly earlier-eluting peak in the RP-HPLC chromatogram compared with the authentic α-polypeptide. The oxidation site has been determined to be the N-terminal methionine residue by high-resolution NMR spectroscopy, where the methionine is oxidized to methionine sulfoxide in a diastereoisomeric form. Interconversion between the oxidized and authentic α-polypeptides has been confirmed by selective oxidation and reduction. The oxidative modification of methionine is shown to have discernible effects on the ability to form B820 subunit with β-polypeptide and bacteriochlorophyll *a*, and on the stability of the reconstituted B820 subunit. Both the ability and the stability for the samples using the oxidized α-polypeptide are moderately reduced, indicating that the oxidation-induced conformational change in the N-terminal domain of α-polypeptide may affect the pigment-binding environment through a long-range interaction. The MALDI-TOF mass results also reveal that the N-terminus of α-polypeptide is formylated and no phosphorylation has occurred in this polypeptide.

Keywords: antenna complex; membrane protein; methionine sulfoxide; self-assembly; TOF-MS.

Light-harvesting (LH) complex serves as highly efficient molecular machinery for the collection and transfer of solar energy to photochemical reaction centers in photosynthetic organisms. In purple photosynthetic bacteria, the LH complexes are classified into either of two major types depending on their *in vivo* locations to the reaction center, namely, the core (LH1) and peripheral (LH2) complexes, both comprising two small polypeptides α and β (plus γ in some species) along with bacteriochlorophyll (BChl) and carotenoid molecules [1]. In contrast to most wild-type species that have both LH1 and LH2, purple nonsulfur photosynthetic bacterium *Rhodospirillum rubrum* has only LH1 with a Q_y absorbance at about 880 nm and a stoichiometry of 1 : 1 : 2 : 1 for the α and , β polypeptides, BChl *a* and spirilloxanthin molecules for the wild-type [2,3]. The LH1 complex from *R. rubrum* is one of the most commonly studied integral pigment-membrane protein complexes and possesses a unique property, i.e. the ability to form a structural subunit characterized by a Q_y absorption band at 820 nm by dissociation of the LH1 complex with detergents [4]. In addition, both the subunit (referred to as B820) and LH1 complexes can be reversibly dissociated and reconstituted from the individual components [5]. Biochemical and spectroscopic properties of the subunit and LH1 complexes from various species have been extensively investigated by using a wide variety of modified and synthesized polypeptides and pigment molecules in order to correlate the functions with the structural features [6–17]. Despite an increase in our knowledge on the structure–function relationship, no atomic resolution structure for the LH1 complex is available at present whereas a couple of crystal structures have been determined for the LH2 complex [18,19]. This may be partly due to the difficulty of preparing high-quality crystal samples of LH1 with the high stability required for the experiment of structural determination.

During the isolation and purification process of LH1 or α-polypeptide and β-polypeptide from the wild-type *R. rubrum*, it has been frequently observed that there exists an additional component with a slightly faster elution time than the α-polypeptide from RP-HPLC (Fig. 1). This fraction was first found in the early experiments of reconstitution of LH1 complex from separately isolated α-polypeptide and β-polypeptide and BChl *a*, and was confirmed to have identical amino-acid composition with that of α-polypeptide [4,5]. Therefore, it is designated by α2 in this study to distinguish from the authentic α-polypeptide, designated by α1. Two possibilities then can be considered for the difference between α2 and α1: one is that the α-polypeptide may adopt two structurally different conformations in solution; and another may be due to any modification in amino-acid residues which could not be detected by conventional amino-acid sequencing and

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Abbreviations: BChl, bacteriochlorophyll; CCA, α-cyano-4-hydroxycinnamic acid; GARP, globally optimized alternating phase rectangular pulse; HMQC, heteronuclear multiple-quantum coherence; LDAO, lauryldimethylamine *N*-oxide; LH, light-harvesting; OG, *n*-octyl β-D-glucopyranoside.

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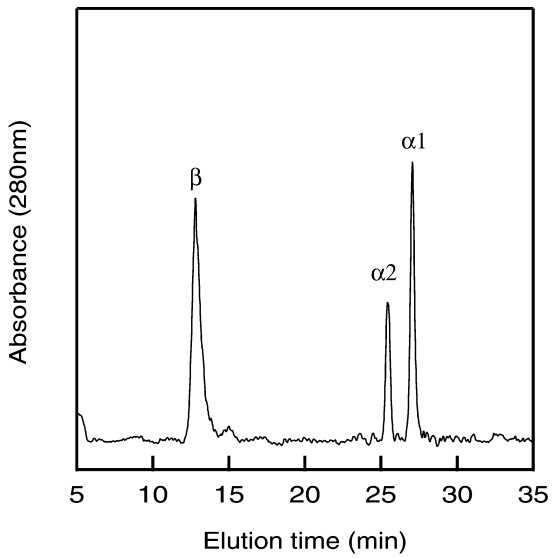

Fig. 1. Reverse-phase HPLC chromatogram of purified LH1 apopolypeptides prepared from wild-type *R. rubrum*. A linear gradient from 60% to 90% of buffer B over 30 min was started 5 min after sample injection, and the flow rate was 0.7 mL·min^{-1} at 20 °C (see text for details). Samples were detected by monitoring the absorbance at 280 nm.

composition analysis. In a series of preliminary experiments, we have found that the proportion of α2 to α1 can vary up to 1 : 1 depending on the storage condition and isolation procedure, and that reconstitution with α2-polypeptide, β-polypeptide and BChl *a* resulted in somewhat inhomogeneous and less stable B820 subunit compared with use of α1. These results prompted us to attempt to clarify the identity of the α2 component. In this study, we address the long-standing issue by presenting results of MALDI-TOF mass spectrometry and high-resolution NMR along with RP-HPLC to demonstrate that the α2 component is actually an N-terminal methionine-oxidized α-polypeptide. Furthermore, we examine effects of the methionine oxidation on ability to form structural subunits and the stability of the reconstituted B820 subunit.

MATERIALS AND METHODS

Materials

Wild-type *R. rubrum* cells were grown phototrophically in modified Hutner's media [20]. BChl *a* containing phytol as the esterifying alcohol (BChl a_p) was isolated from thermophilic purple sulfur photosynthetic bacterium *Chromatium tepidum* following procedures based on Omata and Murata [21], and was purified on a RP-HPLC column (ODS-80Ts, TOSOH) with mixed solvents of acetonitrile/acetone/methanol/water (33 : 50 : 15 : 2, volume ratio). *n*-Octyl β-D-glucopyranoside (OG) was obtained from Dojindo Laboratories. *N*-Methyl-mercaptoacetamide was from Fluka Chemie, and hydrogen peroxide (H_2O_2, 31% solution) was from Mitsubishi Gas Chemical Co. α-Cyano-4-hydroxycinnamic acid (CCA) and all standard molecular mass samples were purchased from Sigma Chemical Company. Deuterated solvents were from Isotec Inc. HPLC solvents were all high purity HPLC grade and obtained from Wako Pure Chemical Industries Ltd.

Isolation and purification of α-polypeptide and β-polypeptide

Chromatophores were isolated by sonication of the whole cells suspended in 50 mM phosphate buffer (pH 7.0) followed by differential centrifugation. LH1 complexes were extracted from the chromatophores by detergent lauryldimethylamine *N*-oxide (LDAO) and were purified according to method B of Picorel *et al.* [3]. The purified LH1 complexes were first treated with benzene and then methanol to remove carotenoid and BChl *a*. The pigment-depleted material was dissolved in hexafluoroacetone (hydrate) and injected onto a RP-HPLC column (TSKgel, Super ODS, 4.6 × 100 mm, TOSOH) for collection of the individual components of α1-polypeptide, α2-polypeptide and β-polypeptide. The flow rate was 0.7 mL·min^{-1} and column temperature was 20 °C. A gradient program used to elute the polypeptides consists of 0.1% trifluoroacetic acid in water as the aqueous solvent (eluent A) and acetonitrile/2-propanol (2 : 1) containing 0.1% trifluoroacetic acid as the organic solvent (eluent B). All the components were eluted within a range of 60–90% of the organic solvent and separated into distinct peaks as seen in Fig. 1.

Mass spectrometry

Mass spectra were measured on a REFLEX III MALDI-TOF MS spectrometer (Bruker Analytische, GMBH, Germany) equipped with a nitrogen laser (337 nm). Recrystallized CCA was used as matrix and was dissolved to saturation in water/acetonitrile (2 : 1, v/v) containing 0.067% trifluoroacetic acid. Polypeptide samples dissolved in the trifluoroacetic acid buffer were mixed with the CCA-saturated solution in a ratio of 1 : 1 (v/v) and then loaded onto the sample stage for cocrystallization. Analysis was performed in positive and linear modes with an accelerating voltage of 25 kV, and 110 scans were averaged. The spectra obtained were calibrated externally using the $[M + H^+]$ ions from three protein standards: adrenocorticotropic hormone fragments (ACTH-CLIP, *m/z* 2465.72), bovine insulin (*m/z* 5733.55) and cytochrome *c* from horse heart (*m/z* 12360.08). Typically, the amount of polypeptides analyzed was of 10–30 pmol.

NMR measurements

NMR spectra were acquired at 45 °C on Bruker DRX-400 spectrometer equipped with an inverse probe (TXI). Purified polypeptide samples were dissolved in $CDCl_3$/methanol-d_4(1 : 1, v/v) solutions at concentration of 1–2 mM. One-dimensional ^{1}H spectra were recorded with 8 K data points, sweep width 5590 Hz giving a digital resolution of 0.683 Hz·point^{-1}, acquisition time 1.7 s, and 480 accumulations. Two-dimensional ^{1}H-^{13}C shift correlation spectra using ^{1}H-detected heteronuclear multiple-quantum coherence via direct coupling method (HMQC) were obtained as described elsewhere [22]. The spectral width for ^{1}H was 4400 Hz and for ^{13}C was 17 kHz. A total of 256 t_1 points of 2K data points was acquired. For each t_1 value 160 and 400 transients were recorded for the

α1-polypeptide and α2-polypeptide, respectively. During acquisition, the ^{13}C was decoupled using a broadband GARP (globally optimized alternating phase rectangular pulse) modulation [22]. Chemical shifts were referenced to tetramethyl silane.

Oxidation and reduction of α-polypeptides

The freeze-dried α1-polypeptide sample was dissolved in a mixture of eluent A/eluent B at 1 : 9 and treated with 20 mM H_2O_2 in an Eppendorf tube at 37 °C for 4 h. The oxidized sample was then lyophilized and analyzed by HPLC with the same procedure and system as that used in separation of individual polypeptides. Reduction of α2-polypeptide was carried out in aqueous solution of 1.5 M *N*-methyl-mercaptoacetamide at 37 °C for 12 h. The initial volume of the reaction solution was 300 μL and was concentrated to 30 μL after the reaction. The organic solvents (eluent B) was used to dissolve the reaction products followed by HPLC analysis without removal of the *N*-methyl-mercaptoacetamide.

Reconstitution of the B820 subunit

The procedure used in this study was essentially that of Parkes-Loach *et al*. [5] with modification. Pure α1-polypeptide, α2-polypeptide and β-polypeptide were solubilized separately in hexafluoroacetone for quantitative determination of their amounts by using the molar absorption coefficient ($\varepsilon_{290} = 10\ 200\ M^{-1}{\cdot}cm^{-1}$). Two sets of combination, α1 + β and α2 + β, were prepared under completely identical condition by mixing the polypeptides (25 nmol each) in a 1 : 1 molar ratio and lyophilized to remove the organic solvent. The samples were redissolved in 540 μL of 50 mM phosphate buffer (pH 7.0) containing 1% OG and then freeze-dried. Aliquots of BChl a_p dissolved in acetone with an absorbance of 1.5 (2-mm light-path length) at 770 nm were added onto the polypeptide mixtures and lyophilized again after mixing to remove the acetone. Finally, 600 μL of distilled water was added to the polypeptide-pigment mixture, producing the reconstituted B820 subunit. The final concentrations of protein and OG were 41 μM and 0.9%, respectively. All operations were conducted at room temperature (≈ 23 °C). Absorption spectra were recorded with a Beckman DU-640 spectrophotometer.

RESULTS

Mass evidence for the oxidation of α-polypeptide

Because α1-polypeptide and α2-polypeptide were identified to have the same amino-acid sequence [5], the two possibilities mentioned before can be examined by mass measurement. If the two polypeptides are only different in conformation, they should give the same mass value; or if the α2-polypeptide is a modified species of α1-polypeptide, different values of mass should be observed. Figure 2 shows the MALDI-TOF mass spectra of the purified α1-polypeptide and α2-polypeptide. The α2-polypeptide has a single [M + H^+] *m/z* value of 6120.0, which is 16.2 Da larger than that of the α1-polypeptide. The mass

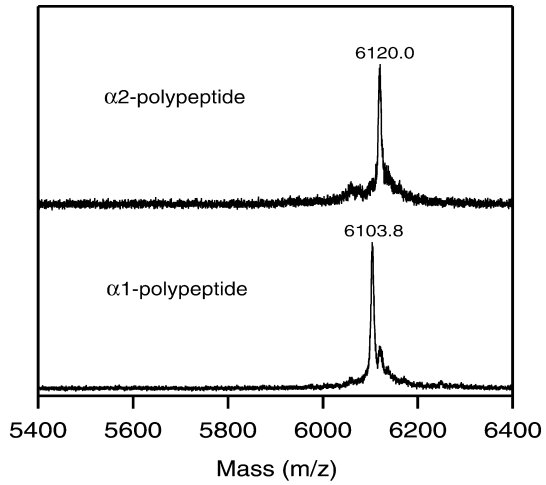

Fig. 2. MALDI-TOF mass spectra of individual α1-polypeptide and α2-polypeptide collected from HPLC fractions. CCA was used as the matrix. The spectra were acquired in positive and linear modes and 110 scans were averaged.

difference corresponds to one oxygen atom, and this was the first sign that α2-polypeptide must be an oxidized form of the α1-polypeptide. Although at this stage we could not definitely determine the oxidation site, both methionine and tryptophan residues are well known as potential oxidation targets. According to the amino-acid sequence, α1-polypeptide contains one methionine and three tryptophan residues. Oxidation of tryptophan would result in an increase of 32 Da, while oxidation of methionine would result in an increase of 16 Da (methionine sulfoxide). The presence of a +16-Da adduct observed can therefore be viewed as a strong indication of the presence of an oxidized methionine residue.

Another revelation from the mass spectra was an absolute molecular mass of the α1-polypeptide. This provided evidence for the N-terminal blocking with a formyl group. In the early work of determination of amino-acid sequence for the α-polypeptide, the N-terminal methionine was found to be blocked, presumably by a formyl group [23,24]. The observed mass of the α1-polypeptide in this study was 27.7 Da greater than the calculated molecular mass (6076.1 Da) of unmodified peptide, confirming the formylation (+28 Da) within experimental error.

Identification of methionine oxidation by NMR

To identify the oxidized residue, we carried out high-resolution NMR measurement with the intact polypeptides. Figure 3 shows high-field regions of the 2-D ^1H-^{13}C HMQC spectra of the purified α1-polypeptide and α2-polypeptide in chloroform/methanol (1 : 1) solution at 45 °C. Resonance of the methionine methyl group of α1-polypeptide was identified as a singlet in the one-dimensional ^1H spectrum and at $\delta(^1H) = 1.89$ppm and $\delta(^{13}C) = 14.7$ppm on the two-dimensional spectrum. This signal disappeared completely from the two-dimensional spectrum of the α2-polypeptide; instead, two very close singlets at about 2.4 p.p.m. of one-dimensional ^1H spectrum were observed. These protons attached to the

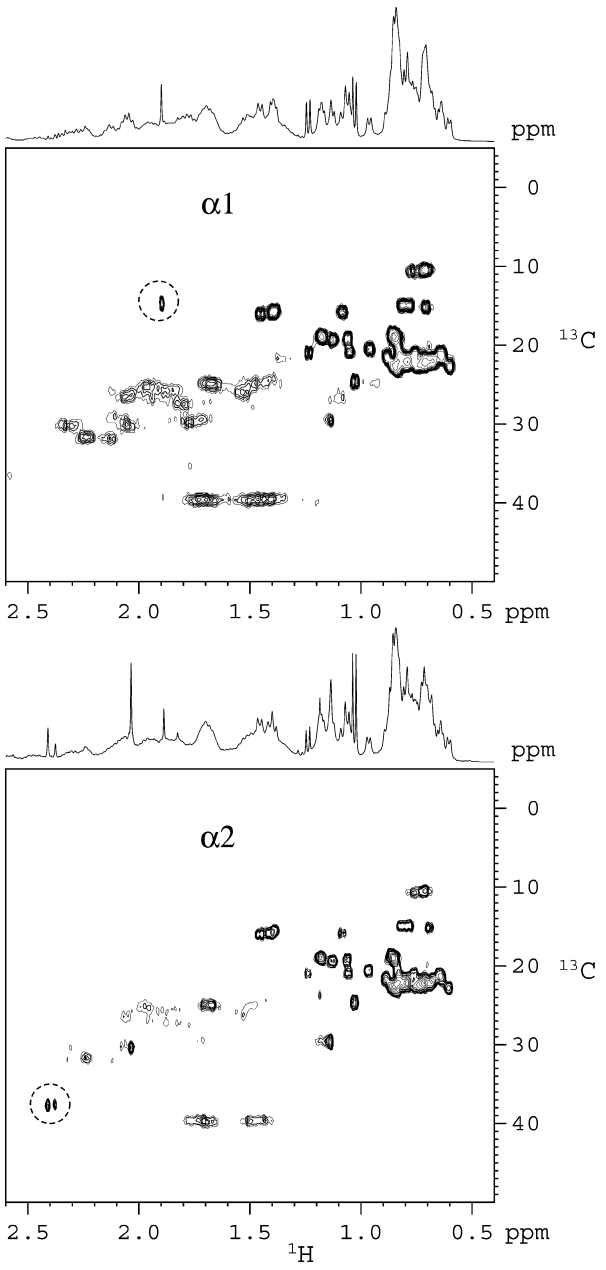

Fig. 3. Two-dimensional ^{1}H-^{13}C correlation NMR spectra of intact α1-polypeptide and α2-polypeptide in $CDCl_3$/methanol-d_4 (1 : 1, v/v) solution at 45 °C. Corresponding one-dimensional ^{1}H-NMR spectra are attached at the top of each two-dimensional plot. The signals surrounded by dashed circles indicate the resonances from the side-chain methyl groups of methionine or methionine sulfoxide. The two resonances of methyl group in the two-dimensional plot of α2-polypeptide correspond to two diastereoisomeric forms.

carbons that have almost the same ^{13}C chemical shifts of 37 p.p.m. as can be seen from the two-dimensional ^{1}H-^{13}C correlation spectrum. Similar measurements with authentic L-methionine sulfoxide (Sigma) confirmed that the resonances were from the side-chain methyl groups of oxidized methionine residue [-S(O)CH_3]. The presence of the two singlets on ^{1}H spectrum corresponds to two diastereoisomers of methionine sulfoxide species [25,26]. We were not able to assign these resonances to their stereochemical configuration by the resolution of this study as only 0.2 p.p.m. ^{13}C chemical shift difference and nearly the same ^{1}H chemical shifts were reported for the *R* and *S* forms of methionine sulfoxide [27,28]. However, the diastereoisomeric ratio in α2-polypeptide can be evaluated on the basis of integration of the resonances from one-dimensional and two-dimensional spectra, and was determined to be about 5 : 3. It was of interest to note that the ^{1}H chemical shifts associated with the stereochemical configuration of methionine sulfoxide residue can be clearly resolved using the intact polypeptide. No essential differences were found for other residues between the α1-polypeptide and α2-polypeptide.

Oxidation of α1-polypeptide and reduction of α2-polypeptide

Formation of the methionine sulfoxide residue in α2-polypeptide can be further confirmed by interconversion between α1-polypeptide and α2-polypeptide through chemical oxidation and reduction. Methionine residue is known to be specifically oxidized by hydrogen peroxide at low pH. Figure 4A shows the RP-HPLC chromatograms of α1-polypeptide before and 4 h after oxidation at 37 °C in the mixed solution of eluent A/eluent B at 1 : 9 containing 20 mM H_2O_2. The peak of α1-polypeptide (27.3 min) decreased largely upon the oxidation and an earlier-eluting peak appeared with an elution time of 25.5 min same as that of α2-polypeptide. The earlier elution time for the methionine-oxidized species may be interpreted in terms of slightly increased hydrophilicity or polarity compared with the unmodified methionine residue. No methionine sulfone was formed under the experimental conditions of this study. However, it was found that the methionine residue was completely oxidized to methionine sulfone within 2 h when treated with 100 mM H_2O_2, as characterized by an elution time of 23.9 min (data not shown). On the other hand, it is also possible to convert methionine sulfoxide to methionine under relatively mild conditions. This can be accomplished by a number of reducing reagents, but all requiring long reaction times and/or elevated temperatures. Among them, *N*-methylmercaptoacetamide was demonstrated most effective and having no adverse interaction with other residues in peptides and proteins [29]. Figure 4B shows the RP-HPLC chromatograms of α2-polypeptide before and 12 h after reduction with the *N*-methylmercaptoacetamide at 37 °C. About 70% of the α2-polypeptide was converted to α1-polypeptide as can be judged from elution time and peak area. As the reaction was conducted in aqueous solution in which the membrane protein was essentially insoluble, the result suggested that methionine sulfoxide residues may locate on the surface and hence be accessible to the reducing reagent.

Comparisons of the ability of reconstitution and the stability of reconstituted B820 subunits using α1-polypeptide and α2-polypeptide

Effect of the methionine oxidation on the functions of the antenna complex was investigated by measuring the

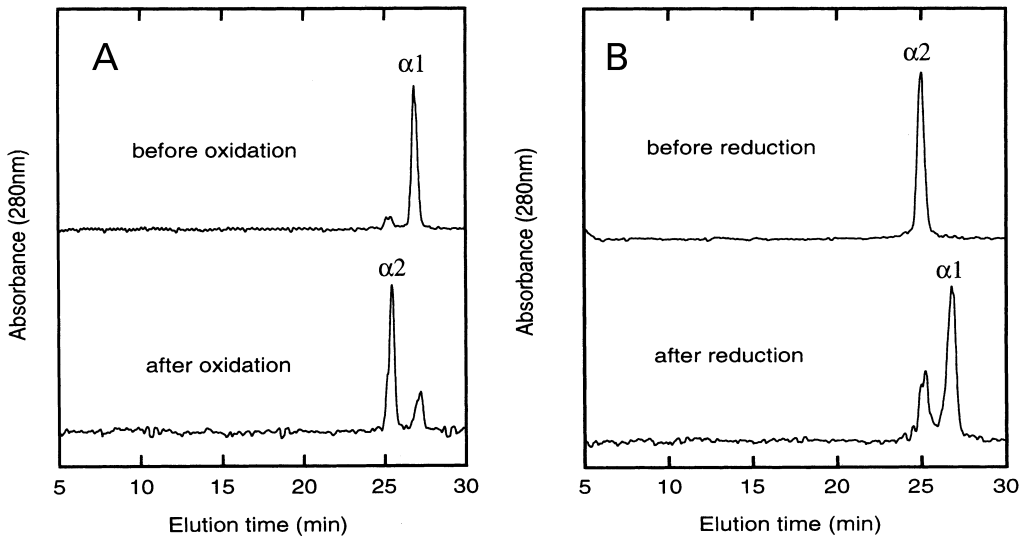

Fig. 4. Reverse-phase HPLC chromatograms illustrating the interconversion between α1-polypeptide and α2-polypeptide through selective chemical oxidation and reduction. (A) Chromatograms before and after oxidation of α1-polypeptide treated with 20 mM H_2O_2 at 37 °C for 4 h. (B) Chromatograms before and after reduction of α2-polypeptide treated with 1.5 M *N*-methyl-mercaptoacetamide at 37 °C for 12 h. The conditions for the HPLC analysis were the same as those used in Fig. 1.

absorption and CD spectrum and its time variation of the B820 subunits reconstituted from α1, β, BChl a_p components and α2, β, BChl a_p components under completely identical conditions. The typical results obtained with 0.9% OG are shown in Fig. 5. Several differences were observed and can be summarized as follows. Firstly, freshly reconstituted B820 with α1-polypeptide showed a highly homogeneous spectral characteristic (Fig. 5A); whereas the B820 prepared with α2-polypeptide gave a shoulder at 770 nm in the absorption spectrum (solid line in Fig. 5B) and the absorbance at 820 nm was somewhat lower than that of its counterpart. This indicated that BChl a_p molecules were not completely incorporated into the polypeptides at the beginning when α2-polypeptide was used as a component. The unbound BChl a_p may exist in either pigment-only or peptide-attached monomeric forms. Secondly, on incubation at room temperature (≈ 23 °C), distinct changes with respect to their initial spectra were observed after five hours. Absorption spectra of the reconstituted B820 with α1-polypeptide remained almost the same shape and the absorbance at 820 nm only decreased slowly with time; whereas the B820 made by α2-polypeptide showed a more drastic change in spectra and the relative intensity at 820 nm decreased to about 50% after 15 h (Fig. 5C). The result demonstrated that the N-terminal methionine oxidation brought about a discernible effect on the stability of the B820 subunit despite the distant position of the methionine residue from the pigment-binding site. It may occur by an oxidation-induced conformational change or through a long-range interaction with the BChl *a* binding environment as reported for the reconstituted B820 using the truncated polypeptides [13]. Thirdly, a broad peak around 920 nm appeared frequently in the absorption spectra for the α2-containing B820 (Fig. 5B). The 920-nm component is considered to contain some higher aggregates with the disordered form of the pigment-polypeptide complex as can be confirmed by a large CD signal and by the fact that the absorbance at 920 nm increased as the absorbance at 820 nm decreased. This component cannot be removed by ultracentrifuging at 14 000 ***g*** for 10 min. It was found that both α1-polypeptide and α2-polypeptide were able to form LH1-type complex with a maximum absorbance at 871 nm when mixed with β-polypeptide and BChl a_p at OG concentrations less than 0.6%, but the absorption spectra obtained with α2-polypeptide revealed a higher inhomogeneity than that of its α1-counterpart.

DISCUSSION

The oxidation of methionine to methionine sulfoxide constitutes one of the many post-translational modifications that proteins undergo. This nonenzymatic reaction has been shown to occur both *in vivo* and *in vitro*, and has been associated with the loss of biological activity in a wide variety of proteins and polypeptides. Comprehensive reviews of this field can be found in the earlier literature [30,31]. Studies of the structural features and biological functions, where the methionine residues have been partly or fully oxidized, include *Escherichia coli* ribosomal protein L12 [32], α1-protein inhibitor [33], chemotactic peptide *N*-formyl Met-Leu-Phe [34,35], parathyroid hormone [36], calmodulin [37], coagulation factor VIIa [38] and A-type potassium channel [39]. Replacement of the methionine residue in f-Met-Leu-Phe by leucine was showed to result in an active chemotaxin that was resistant to attack by myeloperoxidase products. Hence, it was the oxidation of methionine that prevented the activity. In most cases, extent of oxidation of the surface-exposed methionine residues is correlated with the time during which the proteins and peptides are exposed to oxidants. In this study, four processes are considered to affect the content of α2-polypeptide: (a) storage of the cells in culture after the growth reached saturation; (b) storage of the dried cells in refrigerator; (c) isolation and purification; and (d) storage of the purified and lyophilized LH1 samples at −20 °C.

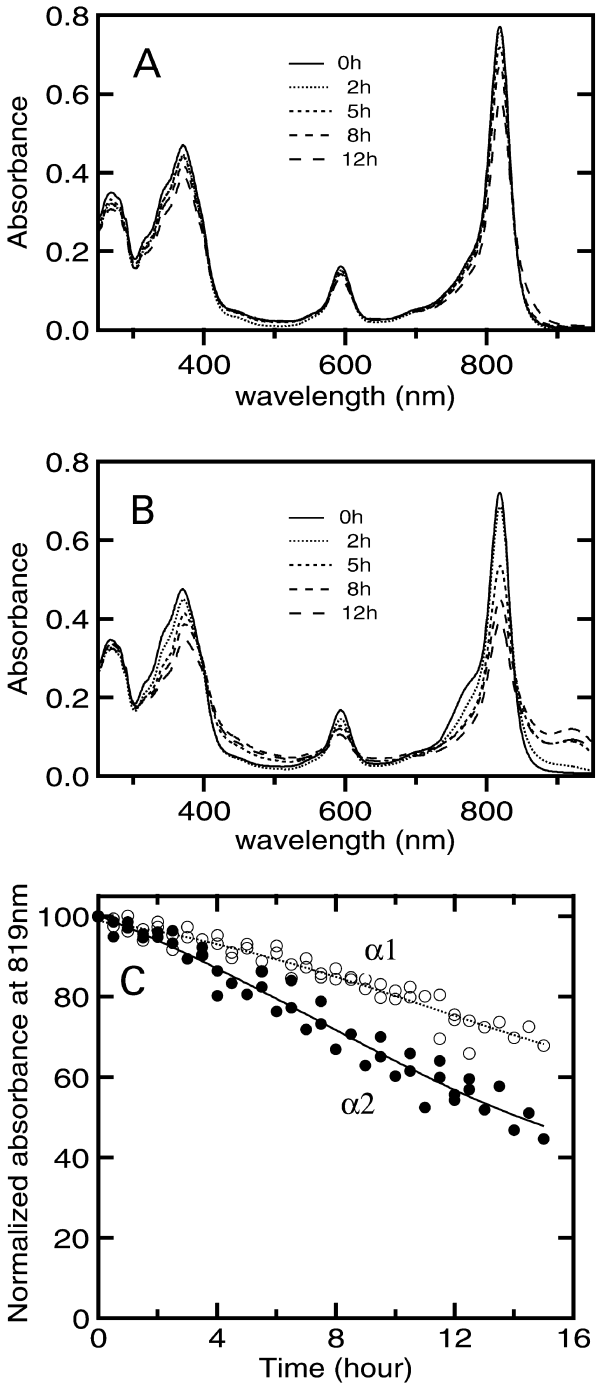

Fig. 5. Change-with-time plots. (A) Change with time of absorption spectra for the reconstituted B820 subunit using α1-polypeptide and β-polypeptide with BChl a_p. (B) Change with time of absorption spectra for the reconstituted B820 subunit using α2-polypeptide and β-polypeptide with BChl a_p. (c) Change with time of the normalized absorbances at 820 nm, each set was obtained from four reconstituted B820 samples using α1 + β (open circles) or α2 + β (filled circles) polypeptides with BChl a_p. The reconstitution experiments were conducted at room temperature under optimized and completely identical conditions for each pair of samples.

Extension of the time period of each of these processes could increase the proportion of α2-polypeptide. Although we have not precisely evaluated the contribution of each process to the final content, the time period of dialysis at 4 °C during the purification was found as one of the most significant factors, and generally, aged cells and old LH1 samples appeared to contain more methionine-oxidized polypeptides. LH1 samples prepared from fresh cells in logarithmic growth period and with the shortest purification time were found to yield less than 4% of α2-polypeptide using the procedure of this study. This percentage may be further minimized by shortening the purification time or by using alternative purification procedures.

As the cells used in this study were cultured under anaerobic photosynthetic condition, any extensive oxidation is thought unlikely to occur *in vivo* during the growth. However, *R. rubrum* is also known to be able to grow under semiaerobic conditions and still synthesize the photosynthetic apparatus. Although we have not investigated whether oxidized α-polypeptide is present in the native state of the LH1 complex under such conditions and this possibility cannot be excluded, an increasing number of studies provide evidence that the methionine sulfoxide residues, if they exist, can be reduced back to methionine by the enzyme peptide methionine sulfoxide reductase. This enzyme is widely distributed in nature and has been found in both prokaryotes and eukaryotes from *E. coli*, plants to rat tissues and human lens [30]. The ubiquitous distribution of the enzyme suggests that it has a fundamental role in all cells where methionine oxidation and reduction can be viewed as a regulatory reaction of cell functions [40]. In this respect, methionine residues may represent an endogenous antioxidant defense that protects proteins from the oxidation of other critically important residues [41].

The unique methionine residue at the N-terminus of α-polypeptide enabled us to quickly identify it as the likeliest oxidation target from the MALDI-TOF mass spectra and subsequently to confirm unequivocally by NMR. Unlike most other proteins and polypeptides used in the methionine oxidation studies, the α-polypeptide of the LH1 has a relatively small number of amino acids (52 residues) and this allows various characterizations to be carried out in its intact form. The mass spectra not only revealed information on the oxidation, but also confirmed and clarified several results on the α-polypeptide reported previously. First, an increase of 28 Da in molecular mass indicated the existence of a formyl group and therefore supported a previous observation from Edman degradation that the α-polypeptide of LH1 from *R. rubrum* seemed to be blocked by a formyl group at the N-terminus [23,24]. Second, the mass spectra showed single peaks for both α1-polypeptide and α2-polypeptide, indicating that the purified components with well-defined molecular masses were homogeneous and that no modifications occurred to other residues except for the formylation and oxidation at the N-terminal methionine residue. This is in contrast to a previous report that both α-polypeptide and β-polypeptide were significantly phosphorylated (one-fifth and one-seventh of the total, respectively) in the purified complexes from the carotenoidless mutant *R. rubrum* G9 [42]. The phosphorylated species were shown to increase up to one-third of the complexes by *in vitro* phosphorylation using a

purified B873 kinase. Such phosphorylation, however, was not observed for the α-polypeptide of this study for the wild-type strain. The discrepancy might originate from the use of different strains, as a third polypeptide, Ω, was found in the LH1 complex of *R. rubrum* G9 [42] but has not been reported for wild-type *R. rubrum*.

Methionine and cysteine are the only two amino acids commonly found in proteins that contain sulfur. No cysteine residue is contained in the LH1 complex and only one methionine residue is at the N-terminus of α-polypeptide. This facilitated the detection of structural changes due to the oxidation by NMR in its intact form. The methyl group attached to sulfur in the methionine residue gave a singlet peak in the ^{1}H-NMR spectra and can be readily assigned. Oxidation of the sulfur atom led to large downfield shifts of both ^{1}H and ^{13}C nuclei of the methyl group. Formation of the methionine sulfoxide resulted in the formation of an asymmetric sulfur atom and consequently two diastereoisomers of methionine sulfoxide were formed. Two singlet peaks with a ratio of 5 : 3 appeared in the ^{1}H spectrum, corresponding to the two diastereoisomers of sulfoxide. The two diastereoisomeric species cannot be separated by RP-HPLC.

Oxidation of the N-terminal methionine residue in α-polypeptide was shown in this study to have apparent influences on both the ability to form homogeneous subunit complex and the stability of the reconstituted subunit. Similar phenomena have been observed in the reconstitution using N-terminal domain-truncated α-polypeptide from wild-type *R. rubrum* [13]. When nine amino-acid residues were removed from the N-terminus, the truncated α-polypeptide was found still to be capable of forming both subunit and LH1 complex with native β-polypeptide and BChl *a*. However, a significant absorbance at 780 nm remained, and the reconstituted LH1 complex was not stable upon incubation at 4 °C overnight as the absorbance of 862 nm decreased significantly. A reduction in ion-pairing and/or hydrogen bonding, brought about by the removal of a portion of the N-terminal domain of the α-polypeptide, was suggested as the cause. In contrast, methionine oxidation results in an increase in polarity for the side chain of methionine residue, and this could provide a potential site for the hydrogen bonding. The result of this study demonstrated that the additional interaction may also have negative consequence on the local conformation, leading to subtle conformational changes in the environment of the pigment-binding site of the reconstituted B820 subunit through long-range structural perturbation. Although no crystal structure at atomic resolution is currently available for the LH1 complex, comparison with the solved three-dimensional structures of LH2 complexes is useful because the two complexes share many structural and functional similarities, and some features may be relevant in discussing the role of the N-terminal methionine residue. The α−polypeptide of LH2 complex from *Rhodopseudomonas acidophila* has 53 residues with a formylated methionine at the N-terminus. The only direct interaction between the helices was found at the N-terminal and C-terminal ends [19]. Turns in the N-terminal domain of α-polypeptide are responsible for oligomerization, and this portion, submerged in the membrane, is involved in binding of a BChl *a* pigment, B800, by the formyl oxygen atom [43]. The position of the sulfur atom of methionine residue restricts the possible movement of the terminal amine towards the Mg at the center of the bacteriochlorin [19]. The information from an X-ray crystal study on LH2 complex provided the structural basis for the role of the N-terminal domain, in particular the methionine residue, of α-polypeptide. All these behaviors can be understood by the unique structure of the side chain of methionine. The thioether moiety of methionine is hydrophobic, but unlike the side chains of other hydrophobic amino acids, the methionine side chain is rather flexible. This allows the thioether group to adapt its shape to nonpolar structures of recognition regions in other proteins, and this may explain why methionine residues are so often involved in the formation of active sites. However, because the side chain is easily changed and the sulfur atom is chemically unsaturated, its oxidation leads to the less flexible and more hydrophilic sulfoxide, and consequently brings about detectable structural and functional changes.

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SUPPLEMENTARY MATERIAL

The following material is available from http://www.ejbiochem.com/

Fig. S1. Absorption spectra of reconstituted B873 complexes using α1/β and α2/β polypeptides. The time indicates the dialysis period after the B820 subunit was formed.

Fig. S2. Absorption and CD spectra of reconstituted B820 subunits using α1/β and α2/β polypeptides.