Volume 285, number 1, 80–84 FEBS 09877 July 1991

ADONIS 0014579391005836

Possible role of the highly conserved amino acids Trp-8 and Pro-13 in the N-terminal segment of the pigment-binding polypeptide LHI α of *Rhodobacter capsulatus*

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Received 19 April 1991

Trp-8 and Pro-13 of the *Rhodobacter capsulatus* light-harvesting (LH) I α polypeptide are highly conserved among LHI and LHII α proteins of several species of the *Rhodospirillaceae*. Exchange of Trp-8 and Pro-13 to other amino acyl residues similar in structure and/or hydrophobicity indicates that Trp-8 is involved in the insertion of the LHI α polypeptide into the intracytoplasmic membrane (ICM). Pro-13, however, seems not to participate in the integration process of the LHI α protein but seems to be important for stable insertion of the LHI β partner protein in the ICM.

LHI α polypeptide; Membrane insertion; Signal peptide; Site-directed mutagenesis; *Rhodobacter capsulatus*

1. INTRODUCTION

Light-harvesting (LH) complex I of the facultative phototrophic bacterium *Rhodobacter capsulatus* is involved in harvesting photons and transfer of excitation energy to the photosynthetic reaction center (RC) (for review see [1]). Both complexes are located in the intracytoplasmic membrane (ICM). Bacteriochlorophyll (Bchl) and carotenoids are bound non-covalently in stoichiometric ratios to different pigment-binding polypeptides [1]. The synthesis of the pigment-binding proteins is regulated on transcriptional level mainly upon changes in light intensity and oxygen partial pressure [2,3].

The LHI complex is composed of two pigment-binding integral membrane proteins, αI and βI, which are encoded by the *puf*A (αI) and the *puf*B (βI) gene of the *puf* operon [4]. Both polypeptides are amphiphilic and span the ICM once by an α-helical central domain. The N-termini of both LHI polypeptides are oppositely charged and exposed on the cytoplasmic surface of the membrane [5,6] whereas the C-termini face to the periplasmic space. It was supposed that the charged N-terminal segments of the LHI proteins stabilize the LHI complex and play a role in the insertion of both LHI polypeptides into the ICM [7,8].

Abbreviations: SDS, sodium dodecyl sulfate; LH, light-harvesting; RC, reaction center; Bchl, bacteriochlorophyll; ICM, intracytoplasmic membrane; PSU, photosynthetic unit.

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Recently it has been shown that the efficient integration of the LHI α polypeptide into the membrane is dependent on the presence of the LHI β partner polypeptide (unpublished data). The N-terminal segment of the LHI α polypeptide is similarly structured as a typical bacterial signal sequence (for review see [9,10]). Two amino acyl residues, Trp-8 and Pro-13, of the N-terminal domain of the LHI α protein are highly conserved in LHI and LHII α polypeptides among several species of the *Rhodospirillaceae* (for review see [10]). It was proposed that these conserved residues play a role in targeting and insertion of the LHI α protein into the ICM [10]. Here we report substitutions of Trp-8 or Pro-13 by other amino acyl residues similar in structure and/or hydrophobicity [11] to clarify their possible role in the insertion process.

2. MATERIALS AND METHODS

2.1. *Plasmid construction; strains of* Rhodobacter capsulatus

A 912 bp *Eco*RI-*Pst*I DNA fragment carrying the *puf*B, the *puf*A and the first part of the *puf*L gene of the *puf* operon of *R. capsulatus* (for restriction map see [2,12]) was subcloned into M13mp18. Site-directed mutagenesis was carried out with the mutagenesis kit from Amersham International, following the protocol of the manufacturer. Synthetic oligonucleotides (oligos) for changing the Trp and Pro codons in the *puf*A gene were designed (Fig. 1) by establishing the codon usage of *R. capsulatus* [12], synthesized at the University of Bielefeld, and purified following a protocol of Applied Biosystems (California, USA). The oligos were annealed in the mutagenesis reactions to the wild type *puf*A gene (Fig. 1). The mutated single stranded DNA was sequenced according to the method of Sanger et al. [13] using appropriate synthetic primers (not shown). The mutated fragments were isolated from the replicative form of the M13mp18 constructions and used to replace the 912 bp *Eco*RI-*Pst*I DNA fragment of the wild type *puf* operon expressed in a derivative of plasmid

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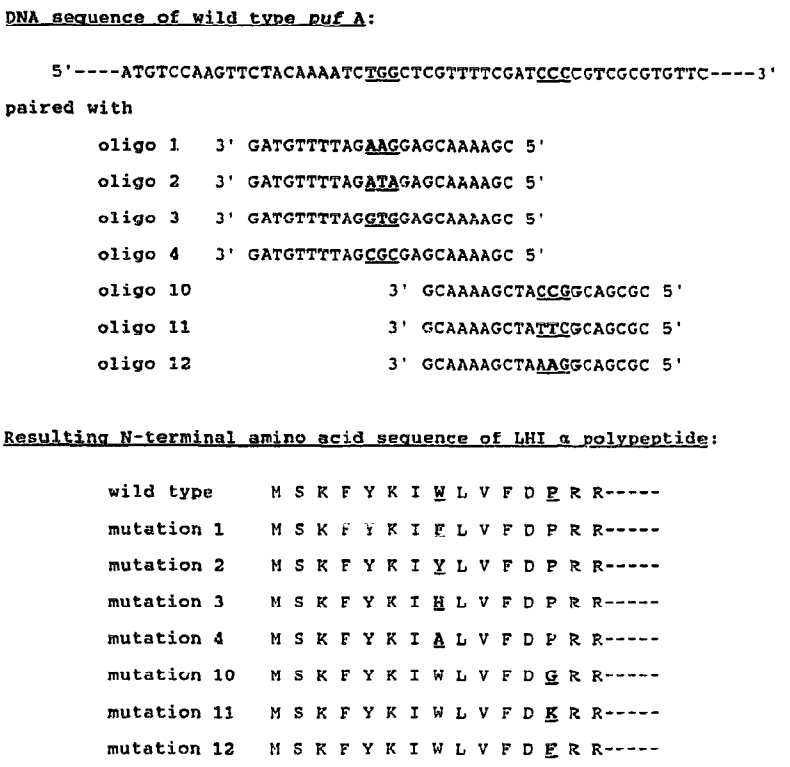

Fig. 1. Sequences of synthetic oligonucleotides used for exchange of the Trp and Pro codons in the *puf*A gene and pairing with wild type *puf* DNA of *R. capsulatus*; N-terminal amino acid sequence of wild type and mutated LHI α polypeptides.

pTJS133 [14]. The constructed plasmids containing the mutated *puf* operon were called pTPR1 (αI Trp-8→Phe), pTPR2 (αI Trp-8→Tyr), pTPR3 (αI Trp-8→His), pTPR4 (αI Trp-8→Ala), pTPR10 (αI Pro-13→Gly), pTPR11 (αI Pro-13→Lys) and pTPR12 (αI Pro-13→Phe). These plasmids were transferred from *Escherichia coli* to *R. capsulatus* strain U43 by triparental mating using pRK2013 as conjugative helper plasmid and were established in *trans* as described [14]. The phenotype of U43 is LHI⁻ LHII⁻ RC⁻ because of a point mutation in the *puc*C gene (Tichy, H.-V., pers. comm.) and a deletion in the *puf* operon [15]. For control experiments strain U43 (pTX35) was used which expresses the wild type *puf* operon in *trans* [14].

2.2. *Analytical methods; quantification of RC*

ICM from French-press broken cells were isolated as described elsewhere [8]. Membrane absorption spectra were measured with a Kontron spectrophotometer UVICON 860 and plotted with the MSPEC computer program. The bacteriochlorophyll (Bchl) content and protein concentrations were determined according to Clayton [16] and Lowry [17]. The quantification of the reaction center by photooxidation has been described recently [8]. The size of the photosynthetic unit (PSU) was calculated from the molar ratio of total Bchl per RC.

3. RESULTS

Transconjugants of *Rhodobacter capsulatus* U43 containing the *puf* operon with a mutated *puf*A gene in *trans* were grown semiaerobically in the dark to induce the formation of the pigment-protein complexes. ICMs were isolated and absorption spectra were measured. Absorption spectra of purified ICM from mutants with exchanges of αI Trp-8 to Phe or Tyr, and of Pro-13 to Gly or Phe, respectively, were similar to the absorption spectrum taken from membranes of the control strain U43(pTX35) (Fig. 2A,B). The height of the peak at 872 nm, however, typical for the LHI antenna complex, was reduced after substitution of αI Trp-8 by His to about one third (Fig. 2A) and even more drastically in the αI Pro-13→Lys mutant (Fig. 2B) in comparison with the control. Membranes from the αI-TrP-8→Ala mutant did not contain any LH complex but the RC (Fig. 2A). The PSU of the αI Pro-13→Lys mutant was determined to be 6, and that of the αI Trp-8→Ala mutant was about 4, showing that no LHI complex was formed in the latter mutant. All other mutant strains have PSU similar to that of the control strain U43(pTX35) (ranging from 25 to 35) including the αI Trp-8→His mutant strain with the reduced LHI spectrum. All mutant strains were able to grow under low light and the mutations were stable under these growth conditions.

The LHI α and β polypeptides were present in about wild type amounts ([8] and unpublished data) in purified membranes of all mutant strains besides U43(pTPR11) which contained only trace amounts of both LHI polypeptides and U43(pTPR4) lacking the LHI polypeptides (not shown).

The integration of the mutated αI polypeptides into the ICM and their stability in the membrane of the αI Trp-8→His, αI Trp-8→Ala and αI Pro-13→Lys

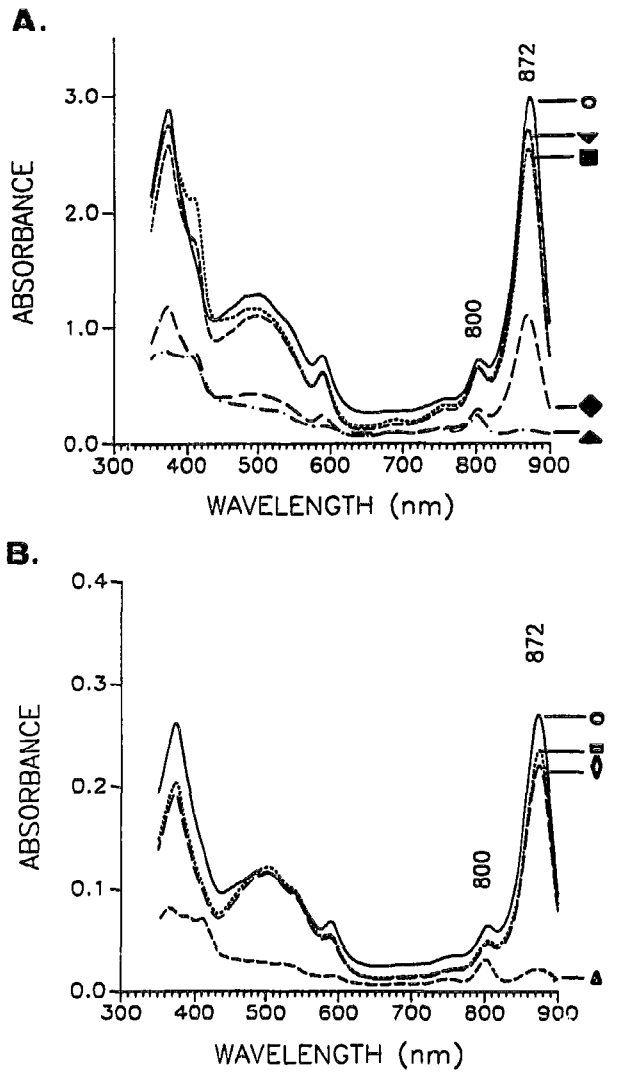

Fig. 2. Absorption spectra of purified membranes isolated from semiaerobically grown mutant strains of *R. capsulatus*. The membrane suspensions were adjusted to 1 mg (A) or 100 μg (B) protein per ml in 20 mM Tris-HCl, pH 7.8. (A) Spectra after exchange of αI Trp-8 to Phe (▼ U43(pTPR1)), Tyr (■ U43(pTPR2)), His (◆ U43 (pTPR3)) or Ala (▲ U43(pTPR4)). (B) Spectra after substitution of αI Pro-13 by Gly (□ U43(pTPR10)), Lys (△ U43(pTPR11)) or Phe (◇ U43(pTPR12)). ○ indicates in (A) and (B) the spectrum taken from purified membranes of the control strain U43(pTX35).

mutants were examined in vivo after pulse labeling with [^{35}S]methionine and chase of the radioactivity [8]. Membrane proteins were isolated and subjected to gel electrophoresis [18] and autoradiography. Both LHI polypeptides were stably inserted into membranes of the αI Trp-8→His mutant at a lower concentration level (Fig. 3A) than in the control strain U43(pTX35) [8]. After replacement of Trp-8 by Ala, a small amount of the mutated LHI α protein was integrated into ICM, but the label disappeared within 10 min during the chase (Fig. 3B). The βI protein of this mutant, however, was inserted in normal amounts and the label disappeared

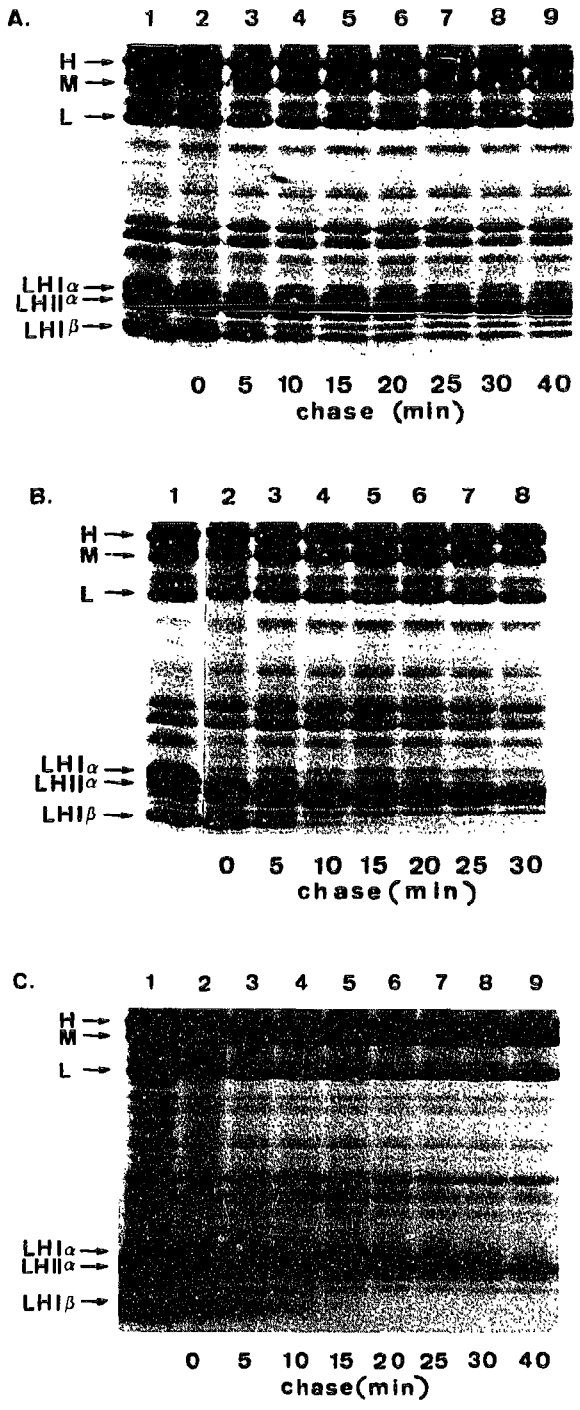

Fig. 3. Membrane proteins isolated after pulse-chase experiments. After 25 min of semiaerobic induction, cells were pulse labeled with [^{35}S]methionine for 2 min and chased with cold methionine as described [8]. Membrane proteins of samples were isolated as described elsewhere [8], subjected to SDS gels according to Laemmli [18] and exposed to X-ray films. Only the lower parts of the autoradiographs are shown. H, M and L indicate the RC subunits; the LHI α/β and LHII α proteins are also marked. The first panels (A1, B1, C1) show membrane proteins from U43(pTX35) as controls. The other panels show membrane proteins isolated from samples taken at time points (in min) after chase (see abscissa) of (A) U43(pTPR3), αI Trp-8→His; (B) U43(pTPR4), αI Trp-8→Ala; and (C) U43(pTPR11), αI Pro-13→Lys.

from membranes later than that of the mutated αI protein (Fig. 3B). After substitution of Pro-13 in the αI protein by Lys, normal amounts of both LHI polypeptides were integrated into the membrane but this insertion was not stable. During chase experiments the non-mutated βI partner polypeptide disappeared earlier from membranes than the mutated LHI α protein (Fig. 3C).

4. DISCUSSION

The N-terminal domain of the pigment-binding polypeptide α of the light-harvesting complex I is amphiphilic like typical signal sequences of bacterial membrane proteins: positively charged amino acids are located at both ends of the N-terminal segment, interspaced by a short sequence of hydrophobic amino acids [9,10]. It has been shown that an exchange of the positively charged amino acids of the LHI α polypeptide to negatively charged amino acids inhibits completely the stable insertion and the assembly of both LHI proteins [8]. This might be caused by a disturbed interaction of the αI polypeptide with the negatively charged N-terminus of the βI partner protein [7] and/or with the negatively charged headgroups of phospholipids. It is also possible that these mutations disturbed the interaction of the LHI proteins with a putative receptor. An alignment of the amino acid sequences of LHI and LHII α polypeptides from several purple bacteria indicated that the general primary structure and some amino acyl residues are conserved [10]. The two residues Trp-8 and Pro-13 of the LHI α polypeptide are highly conserved among light-harvesting α proteins [10]. Proline and tryptophan residues seem to have an important role in the interaction of proteins with the phospholipids of bacterial membranes [19,20]. Therefore Trp-8 and Pro-13, respectively, of the αI polypeptide were substituted by various residues with decreasing similarity in structure and hydrophobicity [11] in order to get more information of their possible role in the membrane insertion process.

Trp is a highly hydrophobic aromatic amino acid [11]. Substitution of Trp-8 of the LHI α protein by alanine, similar to tryptophan in hydrophobicity [11] but not in structure, abolished the LHI formation (Fig. 2A) and had a drastic effect on the membrane insertion (Fig. 3B) of the mutated protein. This result indicated that there is not simply a requirement for hydrophobicity at position 8 of the LHI α polypeptide. Replacement of Trp-8 by Phe or Tyr, similar in structure and hydrophobicity to tryptophan [11], did not alter considerably the formation of the LHI complex (Fig. 2A). Therefore the aromatic nature of Trp seems to be of special importance at position 8 of the αI polypeptide. The exchange of Trp-8 to histidine, similar in structure but not in hydrophobicity to Trp [11], resulted in formation of a only slightly reduced PSU but in a less stable insertion of both LHI polypeptides into the ICM (Fig. 3A) than in the control strain U43(pTX35) [8]. It has to be determined whether this newly introduced His-8 is capable of interacting with additional Bchl molecules in the ICM as supposed for the His present in the N-terminus of light-harvesting β polypeptides [10]. In conclusion, it appears that the aromatic substructure of tryptophan is of greater importance than the hydrophobicity at position 8 of the LHI α polypeptide.

Proline, known as a helix-breaking residue, is often found at the C-terminal end of signal sequences of bacterial membrane proteins [19,20]. Replacement of Pro-13 near the membrane-spanning segment of the αI polypeptide of *R. capsulatus* by Gly, also known as a helix-breaking residue (for review see [20]), and by the hydrophobic Phe [11] only slightly reduced the absorption peak of purified membranes at 872 nm typical for the LHI complex (Fig. 2B). Exchange of Pro-13 for the charged, hydrophilic lysine [11] resulted in formation of a very small LHI complex (Fig. 2B). However, this mutation did not prevent the insertion of the mutated LHI α protein into the membrane, but the stability of the non-mutated βI polypeptide was strongly reduced (Fig. 3C). The label of the LHI β protein disappeared earlier from the membrane than the labeled LHI α protein. Taken together, our results indicate that a hydrophobic residue with helix-breaking properties is necessary at position 13 of the LHI α polypeptide for stable insertion of the LHI β partner protein and for proper finding of α–β pairs in the membrane.

Our knowledge is not sufficient to explain exactly the function of Trp-8 and Pro-13 in the N-terminal segment of the LHI α polypeptide. All results, however, support the idea that targeting and stable insertion into ICM of both LHI proteins are dependent on a specific structure of the N-termini of the LHI proteins.

Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft (grant Dr 29/31-6A) and Fonds der Chemischen Industrie.

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