Complete amino acid sequence of the B875 light-harvesting protein of *Rhodopseudomonas sphaeroides* strain 2.4.1

Comparison with R26.1 carotenoidless-mutant strain

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The complete amino acid sequence was determined for the α- and β-chains of the B875 light-harvesting protein purified from photosynthetic membranes of *Rhodopseudomonas sphaeroides* 2.4.1. The sequence of the B875-α-polypeptide was identical to that reported for the R26.1 carotenoidless mutant [(1985) Biochim. Biophys. Acta 806, 185-186] and contained 58 amino acid residues with a blocked methionine and a glutamic acid at the N- and C-termini, respectively. The B875-β-polypeptide contained 48 amino acid residues with alanine and phenylalanine as respective N- and C-termini; although otherwise identical, the leucine at position 29 in the wild-type strain was replaced by proline in the mutant. This radical amino acid substitution occurred within the central hydrophobic domain of the β-polypeptide chain and is thought to result in a weakening of the structure of the α/β heterodimer since it was not possible to isolate the intact pigment-protein complex from the R26.1 mutant strain.

Amino acid sequence *Light-harvesting protein* *Photosynthetic membrane* *Polyacrylamide gel electrophoresis* Rhodopseudomonas sphaeroides

1. INTRODUCTION

The facultative photoheterotrophic bacterium *Rhodopseudomonas sphaeroides* contains two light-harvesting BChl-protein complexes designated as B800-850 and B875 on the basis of near-IR absorption maxima. Although spectroscopic studies [1–3] have suggested that B800-850 forms large 'lakes' within the photosynthetic (chromatophore) membrane and functions as a peripheral antenna while B875 serves as a core antenna which surrounds and interconnects the photochemical reaction centers, a detailed understanding of their arrangement and associations within the bilayer requires detailed characterization of their protein components. Spectrally intact preparations of B800-850 and B875 have been isolated with the aid of appropriate detergents [4–6] and electrophoretic procedures indicated that each contained two low-M_r polypeptide subunits [6,7]. (According to the nomenclature recommendations in [8], these polypeptides are designated as the respective α- and β-subunits of the apoproteins of these light-harvesting complexes on the basis of considerations discussed in the text.)

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Abbreviations: BChl, bacteriochlorophyll *a*; B800-850, B875, peripheral and core light-harvesting BChl-protein complexes, respectively, identified by near-IR absorption maxima; LDS, lithium dodecyl sulfate

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Recently, 4 organic-solvent-soluble polypeptides identified in [9] as the respective α- and β-subunits of the spectrally altered 'B850' [10–12] and 'B870' [11–13] complexes of *R. sphaeroides* carotenoidless mutant R26.1 have been purified and sequenced [9]. In addition, the primary structure was also reported recently for the α- and β-chains of the B800-850 apoprotein from the 2.4.1 parent strain [14]. To elucidate further the structural and functional properties of the B875 core antenna and to characterize differences between the wild-type and mutant strains, we have determined the complete amino acid sequence for the B875-α- and -β-polypeptide subunits from the 2.4.1 strain. Although the primary structure of the α-subunit was identical to that of R26.1, the replacement of the leucine at position 29 of the 2.4.1 β-subunit by a proline in R26.1 has suggested a basis for the decreased stability of the 'B870'-α/β heterodimer of the latter.

2. MATERIALS AND METHODS

Chromatophores were isolated from photoheterotropically grown *R. sphaeroides* 2.4.1 (wild-type) as described in [9]. The methods used for the isolation and purification of the α- and β-polypeptides of the B875 complex as well as the procedures for sequence analysis, were those described for the blue-green mutant R26.1 [9]. The light-harvesting polypeptides were extracted from freeze-dried chromatophores with 1:1 (v/v) chloroform/methanol containing 0.1 M ammonium acetate and purified on gel-filtration and ion-exchange columns in the presence of these organic solvents. Methods used for sequence analysis included automated Edman degradation, manual Edman degradation of C-terminal fragments and hydrazinolysis and carboxypeptidase digestion of C-termini; for the B875-α-chain, deblocking of the N-terminus was necessary. Manual Edman degradation [9] was also used to identify the polypeptide constituents of a B875 complex purified from *R. sphaeroides* 2.4.1 by two cycles of LDS-polyacrylamide gel electrophoresis [6]. SDS-polyacrylamide gel electrophoresis and scanning of the stained gel indicated a purity of 90% for the isolated complex and about a 1:1 ratio for the LH-1 and LH-3A polypeptide components as in [6].

3. RESULTS AND DISCUSSION

After deblocking of the B875 preparation isolated from *R. sphaeroides* 2.4.1, cycles 1, 2 and 4, respectively, of manual Edman degradation yielded two major phenylthiohydantoid derivatives at each step. Their molar ratios were Met/Ala, 0.84; Ser/Asp, 0.87; and Phe/Ser, 1.15; only Lys was found after the third cycle. Despite the usual difficulties in recoveries of the methionine and serine derivatives, these data permit identification of the LH-1 and LH-3A polypeptide components of this complex as the B875-α and -β-subunits, respectively. In addition, two of the polypeptides isolated directly from the strain 2.4.1 chromatophores and subjected to sequence analysis (figs 1 and 2) can be assigned to this complex. The other two were identified as the α- and β-polypeptide subunits of the B800-850 complex and their primary structure was reported in [14]. Since the average molar ratios of the residues in the 3 cycles of N-terminal sequence analysis was 0.95, this also establishes a 1:1 ratio for the α- and β-chains of the B875 apoprotein to which two molecules each of BChl and carotenoid are bound [6].

The sequence determined for the B875-α-chain from strain 2.4.1 is presented in fig.1. It consists of 58 amino acid residues with a molecular mass of 6809 Da and is identical to that reported for the corresponding α-subunit of the R16.1-mutant strain [9]. It shows the characteristic tripartite structure [9,14–18] with a central hydrophobic domain (residues 16–36) that separates polar N- and

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                              5                  10                  15
A: (HCO)-Met-Ser-Lys-Phe-Tyr-Lys-Ile-Trp-Met-Ile-Phe-Asp-Pro-Arg-Arg-
B: (HCO)-Met-Ser-Lys-Phe-Tyr-Lys-Ile-Trp-Met-Ile-Phe-Asp-Pro-Arg-Arg-

                    ↓        20                  25                  30
A:                  Val-Phe-Val-Ala-Gln-Gly-Val-Phe-Leu-Phe-Leu-Leu-Ala-Val-Met-
B:                  Val-Phe-Val-Ala-Gln-Gly-Val-Phe-Leu-Phe-Leu-Leu-Ala-Val-Met-

                              35                 ↓40                 45
A:                  Ile-His-Leu-Ile-Leu-Leu-Ser-Thr-Pro-Ser-Tyr-Asn-Trp-Leu-Glu-
B:                  Ile-His-Leu-Ile-Leu-Leu-Ser-Thr-Pro-Ser-Tyr-Asn-Trp-Leu-Glu-

                              50                  55
A:                  Ile-Ser-Ala-Ala-Lys-Tyr-Asn-Arg-Val-Ala-Val-Ala-Glu-OH
B:                  Ile-Ser-Ala-Ala-Lys-Tyr-Asn-Arg-Val-Ala-Val-Ala-Glu-OH
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Fig.1. Amino acid sequence determined for the α-polypeptide of the B875 complex from *R. sphaeroides* 2.4.1 (A), and comparison with sequence of B870-α-polypeptide from R26.1 (B), reported in [9]. Arrows show position of central hydrophobic domain believed to span the membrane [9,17].

C-terminal regions and contains the single conserved histidine residue at position 32, characteristic of polypeptides of the α-group, that is believed to be involved in BChl interactions [9, 14–19]. The amino acid composition of the B875-α subunit of the 2.4.1 strain (table 1) both confirms the sequence analysis and establishes that this polypeptide is identical to that of R26.1 in all respects.

The sequence determined for the B875-β chain from the wild-type strain (fig.2) consists of 48 amino acid residues with a molecular mass of 5457 Da and contains two histidines at positions characteristic of light-harvesting polypeptides of the β-group [9,17,18]. It is identical to the corresponding β-polypeptide of R26.1 with the exception that the leucine at position 29 in 2.4.1 is replaced by a proline in the mutant strain. Both this amino acid exchange and the sequence of the wild type β-subunit were verified by amino acid analysis (table 1). The proposed central hydrophobic domain includes residues 22–42 and thus, the amino acid exchange in R26.1 is located within this segment of the β-chain.

It can be anticipated that the substitution of a leucine residue by a proline will have a dramatic effect on the conformation of this hydrophobic seg-

Table 1

Amino acid composition of B875-α- and -β-polypeptides of *R. sphaeroides* 2.4.1

	B875-α			B875-β		
	Residues/mol[a]	Nearest integer[b]	Composition[b,c]	Residues/mol[a]	Nearest integer	Composition[c]
Asp	3.1	(3)	1	3.1	(3)	3
Asn			2			0
Thr[d]	1.2	(1)	1	1.9	(2)	2
Ser[d]	3.9	(4)	4	3.6	(4)	4
Glu	3.3	(3)	2	4.2	(4)	2
Gln			1			2
Pro	2.3	(2)	2	1.1	(1)	1 [2][h]
Gly	1.3	(1)	1	3.1	(3)	3
Ala	6.1[g]	(6)	6	5.9	(6)	6
Val	6.2[g]	(6)	6	3.9[g]	(4)	4
Met[e]	2.8[g]	(3)	3	0.8	(1)	1
Ile	5.0[g]	(5)	5	1.9[g]	(2)	2
Leu	7.1[g]	(7)	7	5.9[g]	(6)	6 [5][h]
Tyr	3.0	(3)	3	2.8	(3)	3
Phe	5.1[g]	(5)	5	2.1[g]	(2)	2
Lys	3.0	(3)	3	1.0	(1)	1
His[e]	0.7[g]	(1)	1	1.5[g]	(2)	2
Arg	3.0	(3)	3	0.9	(1)	1
Trp[f]	2.3	(2)	2	3.0	(3)	3

[a] Calculated on basis of 3 and 1 lysine residues per α- and β-polypeptide, respectively. Values represent averages of 3 analyses each obtained after 24, 48 and 72 h hydrolyses
[b] Identical to values reported for B870-α of R26.1 [9]
[c] Derived from sequence
[d] Uncorrected values after 24 h hydrolysis
[e] Partially destroyed
[f] Determined after 24 h hydrolysis in methanesulfonic acid
[g] Values after 72 h hydrolysis
[h] Values reported for R26.1 B870-β [9]; composition otherwise identical

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                    5                   10                  15
A:  H-Ala-Asp-Lys-Ser-Asp-Leu-Gly-Tyr-Thr-Gly-Leu-Thr-Asp-Glu-Gln-
B:  H-Ala-Asp-Lys-Ser-Asp-Leu-Gly-Tyr-Thr-Gly-Leu-Thr-Asp-Glu-Gln-

                   20                  25                  30
A:    Ala-Gln-Glu-Leu-His-Ser-Val-Tyr-Met-Ser-Gly-Leu-Trp-Leu-Phe-
B:    Ala-Gln-Glu-Leu-His-Ser-Val-Tyr-Met-Ser-Gly-Leu-Trp-Pro-Phe-

                   35                  40                  45
A:    Ser-Ala-Val-Ala-Ile-Val-Ala-His-Leu-Ala-Val-Tyr-Ile-Trp-Arg-
B:    Ser-Ala-Val-Ala-Ile-Val-Ala-His-Leu-Ala-Val-Tyr-Ile-Trp-Arg-

A:    Pro-Trp-Phe-OH
B:    Pro-Trp-Phe-OH
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Fig.2. Amino acid sequence determined for the β-polypeptide of the B875 complex from *R. sphaeroides* 2.4.1 (A), and comparison with sequence of B870-β-polypeptide from R26.1 (B), reported in [9]. The box shows the position of an amino acid substitution in R26.1 and arrows designate position of hydrophobic domain [9,17].

ment and therefore on protein-protein interactions within the B870-α/β heterodimer. Whereas it is probable that the heterodimer of the wild-type B875 complex can form two regular, transmembrane α-helical rods such as those proposed for the α- and β-subunits of the B800-850 protein [19], in the case of the B870-β subunit of R26.1, the helical structure would be disrupted and a bend of ~20° would occur at the position of the proline residue. As a result, one carbonyl group of the backbone would become exposed which would be energetically unfavorable in the hydrocarbon phase of the membrane. These effects would be expected to result in a weakening of the B870 heterodimer structure.

This structural alteration in the β-polypeptide may be responsible for the reduced stability of the R26.1 heterodimer in detergent solutions since the B870 complex could not be isolated even under mild conditions in the presence of LDS in which the corresponding wild-type complex is stable (fig.3). Only the B850 complex lacking the 800-nm band of the wild-type was isolated from R26.1, although it is suggested from both the position and width of the near-IR absorption band that the B870 complex was also present in these R26.1 chromatophores. The R26.1 strain is a B850-containing revertant [20] of the original R26-carotenoidless mutant [21] and apparently retains the B870 complex of R26 in which the near-IR absorption band is shifted ~5 nm to shorter wavelengths in comparison to the wild-type B875 complex [13]. The B870 of R26 chromatophores

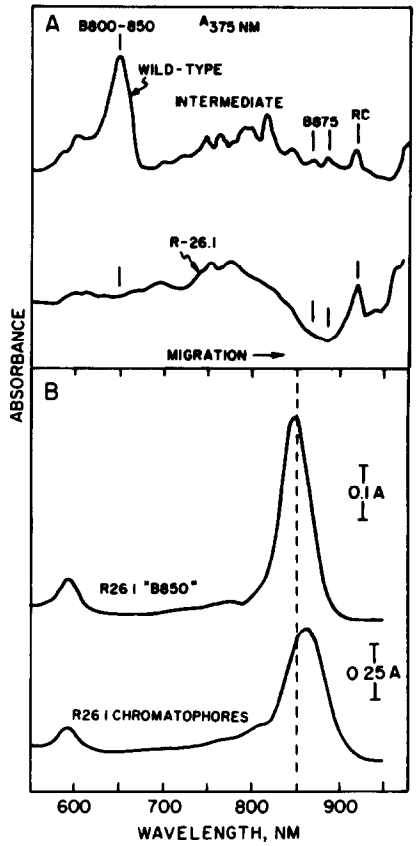

Fig.3. Stability of BChl-protein associations of antenna complexes of *R. sphaeroides* R26.1. (A) Chromatophores of strain R26.1 and wild-type strain NCIB8253 were subjected to LDS-polyacrylamide gel electrophoresis at 4°C as in [6] and scanned at 375 nm in an Ortec model 4310 scanning densitometer. The positions of pigment-protein complexes are indicated. Note absence of B875 complex and enrichment of reaction center (RC) particle consisting of L- and M-subunits [6] in R26.1. Although the B850 complex of R26.1 banded mainly in the position of the wild-type intermediate complexes containing B800-850 and B875 in various associations, it only gave rise to apparent B800-850 polypeptide subunits in SDS-polyacrylamide gel electrophoresis (not shown); this implies that the B870-α- and -β-polypeptides of R26.1 bind BChl in a weak, LDS-unstable association. (B) Near-IR absorption spectra of R26.1 chromatophores and B850 complex derived from them. Spectra were obtained on a Johnson Research Foundation DBS-3 spectrophotometer.

also retains low-temperature fluorescence polarization and CD spectra similar to those of the wild-type [13], but complete optical data are not yet available for R26.1 chromatophores nor has the sequence been determined for the R26 B870 protein. Nevertheless, any stable BChl-protein associations within the in situ B870 complex were disrupted upon removal from the membrane and although both the binding [12] and orientation [13] of the B870 BChls may be similar to that of the BChls in the wild-type B875, the results in fig.3 suggest that an intact α-helix in at least the B875-β-polypeptide is essential for preservation of pigment-protein interactions and stability within the isolated complex. In addition, these results demonstrate that a single amino acid exchange can have important structural consequences for a chlorophyll-protein complex.

With regard to the basis for the mutational alteration determined here in the R26.1 mutant strain, the leucine-to-proline exchange was likely effected by a single point mutation (T→C) in a leucine codon. The exchange of a proline for a leucine has also been reported in the sequence of the N-terminal signal peptide of the *Escherichia coli* maltose binding protein [22]; this also had important structural consequences and prevented export of the protein possibly through alteration of an α-helical conformation in the signal sequence. Other mutational alterations in the *R. sphaeroides* blue-green mutant strains result in a failure to produce colored carotenoids [21], the exchange of a phenylalanine for a valine in the B850-α-polypeptide as determined for R26.1 [14] and greatly reduced levels of the B800-850 polypeptides which we have observed in the original R26 strain. The reversion to R26.1 is characterized by the restoration of high levels of the B850 polypeptides and may provide a basis for the increased growth rate of this strain. This apparent selective advantage may mimic the possible evolutionary origin of B800-850 through duplication of B875 genes as proposed in [23].

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