A comparison of the primary structures of the two B800–850-apoproteins from wild-type *Rhodopseudomonas sphaeroides* strain 2.4.1 and a carotenoidless mutant strain R26.1

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The α- and β-apoproteins from the B800–850-complex of *Rhodopseudomonas sphaeroides* strain 2.4.1 have been sequenced. These results have been compared with those previously obtained with the analogous antenna apoproteins from the carotenoidless mutant R26.1 [9]. The α-apoproteins differ at position 24, where a valine residue present in the wild-type sequence is replaced by a phenylalanine residue in the mutant. The β-apoproteins differ at the N-terminus. In the wild-type β-apoprotein some chains have methionine at the N-terminus and some have an N-terminal threonine, while in the mutant the N-terminus is threonine.

Photosynthetic bacteria Rps. sphaeroides *Sequence comparison* *Light-harvesting complex*

1. INTRODUCTION

The photosynthetic unit in wild-type cells of *Rhodopseudomonas sphaeroides* consists of photochemical reaction centres and two types of light-harvesting pigment–protein complexes [1] (the B875-complexes and the B800–850-complexes). When the well-known carotenoidless mutant of *Rps. sphaeroides*, R26, was first described [2], as well as having no coloured carotenoids, it was also shown to lack the B800–850-complex. Since this time R26 has become very widely used, especially as a convenient source of reaction centres [3]. However over these intervening years, since its original isolation, the status of the 'R26' in current use in most laboratories became unclear [4–6].

The long wavelength bacteriochlorophyll absorption band in the original R26 isolate was at 870 nm, while in the 'R26' now in common use this absorption maximum has 'slipped' between 5 and 10 nm to the blue (see fig.1 below). Authors in [7] were able to show that the changed 'R26' was a partial revertant of the original R26, having regained the B800–850-apoproteins. The revertant was renamed R26.1 [7] and this nomenclature will be used in the present study.

In vivo, the B800–850-complex from *Rps. sphaeroides* 2.4.1 most probably exists as an aggregate of a 'minimal compositional unit' [1]. This minimal unit consists of 3 molecules of bacteriochlorophyll *a*, one molecule of carotenoid and one each of two, low molecular mass, hydrophobic polypeptides (called the B800–850-α-apoprotein and the B800–850-β-apoprotein [8]). In R26.1 the analogous antenna complex (the B850-complex), although similar in polypeptide composition to the wild-type B800–850-complex, lacks both carotenoids and the 800 nm absorbing bacteriochlorophyll molecule [4].

We have recently described the primary structures of the α- and β-apoproteins of the B850-complex from the carotenoidless mutant [9]. Here, we set out to determine the primary structures of the two wild-type B800–850-apoproteins

Abbreviation: NIR, near infrared

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and then to compare them with those of the mutant apoproteins. It was hoped that this might allow us to suggest reasons why the 800 nm absorbing bacteriochlorophyll molecule is absent in R26.1.

2. MATERIALS AND METHODS

Cells of *Rps. sphaeroides* strains 2.4.1 (wild-type), R26 (the original carotenoidless mutant) and R26.1 (the partially reverted carotenoidless mutant) were grown anaerobically in the light as previously described [10]. The cells were harvested, washed in 20 mM Tris–HCl (pH 8.0) and disrupted by passage through a French pressure cell at 10 ton·in^{-2}. Chromatophores were then isolated from the broken cell suspension by differential centrifugation [11]. Large quantities of chromatophores from strain 2.4.1 were very kindly provided in Zürich by Dr Vreni Wiemken.

The NIR absorption spectra of the chromatophores were recorded with a Pye-Unicam SP8-500 spectrophotometer.

The light-harvesting polypeptides from strain 2.4.1 were isolated from freeze-dried chromatophores by extraction with 1:1 (v/v) chloroform and methanol containing 100 mM ammonium acetate [9]. The individual α- and β-apoproteins from the B800–850-complex were then purified and sequenced as previously described for strain R26.1 [9]. The sequence data were also checked by amino acid analysis of the purified individual apoproteins [9].

3. RESULTS AND DISCUSSION

The NIR absorption spectra of chromatophores from the 3 strains of *Rps. sphaeroides* used in the present study are shown in fig.1. A comparison of the absorption spectrum of strain 2.4.1 with that of the mutant R26 shows the absence of the B800–850-complex in the mutant, while a similar comparison of the absorption spectra of the two carotenoidless mutants illustrates the blue-shift of the bacteriochlorophyll absorption band associated with the change of R26 to R26.1. An absorption difference spectrum of chromatophores from R26.1 minus chromatophores of R26 (fig.2) shows clearly that the difference between the two

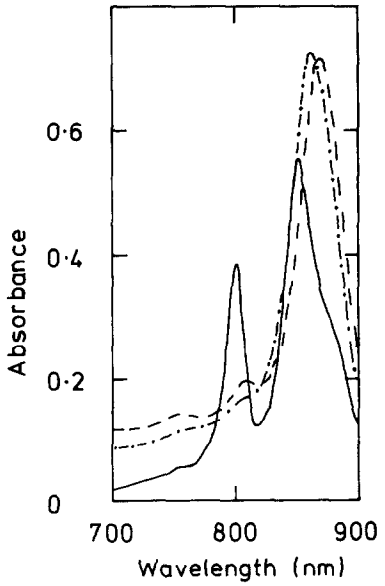

Fig.1. The NIR absorption spectra of chromatophores from 3 strains of *Rps. sphaeroides*. The chromatophores were suspended in 20 mM Tris–HCl (pH 8.0) in a 1 cm path length cuvette and their absorption spectra recorded in a PYE-UNICAM SP 8-500 spectrophotometer. (——) wild-type strain 2.4.1; (– – –) R26; (-·-·-) R26.1.

mutants is due to the presence of the B850-complex in R26.1.

The amino acid sequence of the B800–850-α-apoprotein from *Rps. sphaeroides* strain 2.4.1 is presented in fig.3, together with the previously determined sequence of the analogous α-apoprotein from the B850-complex from R26.1 [9]. The two α-apoproteins both contain 54 amino acids and only differ in a single position. At position 24 there is a valine in the wild-type apoprotein which is replaced by a phenylalanine in the mutant. We have been able to confirm this result by comparing the amino acid composition of the two α-apoproteins (table 1). The change from valine to phenylalanine reflects a single point mutation.

The sequence of the wild-type β-apoprotein is presented in fig.4 and compared there with the corresponding amino acid sequence of the β-apoprotein from the mutant [9]. The two polypeptides are identical except at the N-terminus. In the R26.1 β-apoprotein the N-terminal residue is threonine and the polypeptide contains 50 amino acids. However, in the wild-type sequence there is

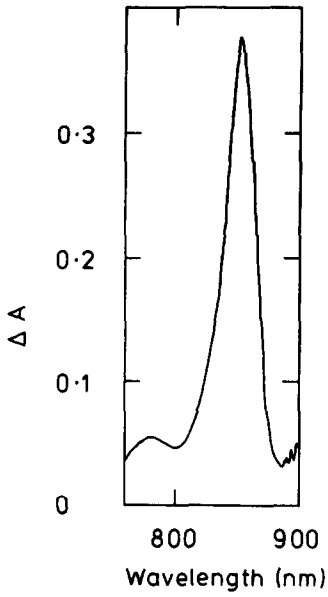

Fig.2. The NIR difference spectrum of chromatophores from *Rps. sphaeroides* R26.1 – chromatophores from R26. The chromatophores were suspended in 20 mM Tris–HCl (pH 8.0) in a 1 cm path length cuvette and their concentrations adjusted so that they had equal absorbances at 900 nm. The difference between R26.1 – R26 was then recorded.

a degree of heterogeneity at the N-terminus, with some chains being identical with the β-apoprotein from R26.1 and others having an additional methionine residue at the N-terminal position. Those chains with the extra methionine therefore contained 51 amino acids. In general the amino acid analyses of the two β-apoproteins confirmed the sequence data (table 2), however the values for methionine and histidine in the wild-type apoprotein were too low. These discrepancies probably

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A: B (800-850) - α (2.4.1)

B: B 850 - α       (R-26.1)
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                                5                  10                  15
A:  H-Met-Thr-Asn-Gly-Lys-Ile-Trp-Leu-Val-Val-Lys-Pro-Thr-Val-Gly-

B:  H-Met-Thr-Asn-Gly-Lys-Ile-Trp-Leu-Val-Val-Lys-Pro-Thr-Val-Gly-

                        20                  25                  30
A:    Val-Pro-Leu-Phe-Leu-Ser-Ala-Ala-Val-Ile-Ala-Ser-Val-Val-Ile-

B:    Val-Pro-Leu-Phe-Leu-Ser-Ala-Ala-Phe-Ile-Ala-Ser-Val-Val-Ile-

                        35                  40                  45
A:    His-Ala-Ala-Val-Leu-Thr-Thr-Thr-Thr-Trp-Leu-Pro-Ala-Tyr-Tyr-

B:    His-Ala-Ala-Val-Leu-Thr-Thr-Thr-Thr-Trp-Leu-Pro-Ala-Tyr-Tyr-

                        50
A:    Gln-Gly-Ser-Ala-Ala-Val-Ala-Ala-Glu-OH

B:    Gln-Gly-Ser-Ala-Ala-Val-Ala-Ala-Glu-OH
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Fig.3. The amino acid sequence of the B800–850-α-apoprotein from *Rps. sphaeroides* strain 2.4.1 and its comparison with the previously determined sequence of the B850-α-apoprotein from R26.1 [9].

Table 1

Amino acid composition of B(800–850) – α

	B(800–850) – α (2.4.1)			B850 – α (R-26.1)[g]		
	Residues/ molecule[a]	Nearest integer	Composition[b]	Residues/ molecule[a]	Nearest integer	Composition[b]
Asp	1.3	(1)	0	1.2	(1)	0
Asn			1			1
Thr[c]	5.7	(6)	6	5.5	(6)	6
Ser[c]	3.0	(3)	3	2.9	(3)	3
Glu	2.3	(2)	1	2.3	(2)	1
Gln			1			1
Pro	3.0	(3)	3	3.0	(3)	3
Gly	3.4	(3)	3	3.1	(3)	3
Ala[d]	9.9	(10)	10	9.5	(10)	10
Val[d]	8.6	(9)	9	7.6	(8)	8
Met[d]	0.7[e]	(1)	1	1.2	(1)	1
Ile[d]	2.8	(3)	3	3.0	(3)	3
Leu[d]	5.0	(5)	5	5.0	(5)	5
Tyr	2.0	(2)	2	2.2	(2)	2
Phe[d]	1.1	(1)	1	2.1	(2)	2
Lys	2.0	(2)	2	2.0	(2)	2
His[d]	0.6[e]	(1)	1	0.9	(1)	1
Arg	–	(0)	0	0.2	(0)	0
Trp[f]	2.2	(2)	2	2.0	(2)	2

[a] As calculated on the basis of two lysine residues per polypeptide. The values represent the averages of 3 analyses each obtained after 24, 48 and 72 h of hydrolysis
[b] As derived from the sequence
[c] Uncorrected values after 24 h hydrolysis
[d] Values after 72 h hydrolysis
[e] Partially destroyed
[f] Determined after 24 h hydrolysis in methanesulfonic acid
[g] Data taken from [9]

reflect partial destruction of these two amino acids during the hydrolysis. The difference between the two β-apoproteins indicates a change in the degree of post-translational processing in the two strains.

There are two possible simple explanations for the lack of the 800 nm absorbing bacteriochlorophyll molecule in the B850-complex from R26.1. The 800 nm absorbing bacteriochlorophyll could require the presence of carotenoids for binding, or, on the other hand, the two changes in the primary structure of the antenna apoproteins described above may have effectively removed its binding site.

Authors in [10,12] have shown that it is possible to functionally reconstitute carotenoids into the B850-complex from R26.1. However, they completely failed to restore the 800 nm bacteriochlorophyll absorption band irrespective of whether bacteriochlorophyll was added back in the presence or the absence of carotenoids [10,12]. This result may mean that in the mutant R26.1 the binding site for the 800 nm bacteriochlorophyll is absent. However there is always a problem of knowing how to interpret negative results. It could also be argued that these authors failed to reconstitute the 800 nm absorption band just because the wrong conditions had been employed.

A comparison of the available primary structures of bacterial antenna apoproteins has revealed the presence of strongly conserved histidine

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A:  B (800-850) - β  (2.4.1)

B:  B 850 - β        (R-26.1)


                                5                  10                  15
A:  (Met)-Thr-Asp-Asp-Leu-Asn-Lys-Val-Trp-Pro-Ser-Gly-Leu-Thr-Val-Ala-

B:       Thr-Asp-Asp-Leu-Asn-Lys-Val-Trp-Pro-Ser-Gly-Leu-Thr-Val-Ala-


                           20                  25                  30
A:       Glu-Ala-Glu-Glu-Val-His-Lys-Gln-Leu-Ile-Leu-Gly-Thr-Arg-Val-

B:       Glu-Ala-Glu-Glu-Val-His-Lys-Gln-Leu-Ile-Leu-Gly-Thr-Arg-Val-


                           35                  40                  45
A:       Phe-Gly-Gly-Met-Ala-Leu-Ile-Ala-His-Phe-Leu-Ala-Ala-Ala-Ala-

B:       Phe-Gly-Gly-Met-Ala-Leu-Ile-Ala-His-Phe-Leu-Ala-Ala-Ala-Ala-


                           50
A:       Thr-Pro-Trp-Leu-Gly-OH

B:       Thr-Pro-Trp-Leu-Gly-OH
```

Fig.4. The amino acid sequence of the B800–850-β-apoprotein from *Rps. sphaeroides* strain 2.4.1 and its comparison with the previously determined sequence of the B850-β-apoprotein from R26.1 [9].

residues [13], one in the α-apoproteins and two in the β-apoproteins. This has led to the suggestion that these histidine residues are directly liganded to the magnesium atoms at the centre of the bound bacteriochlorophyll molecules [13]. It was, therefore, somewhat disappointing that the changes in the primary structures of the B800–850-apoproteins between the wild-type and the mutant could not be interpreted in a straightforward way as they appear to involve residues that are probably not in direct contact with the bound bacteriochlorophyll molecules. It is still possible, however, that these changes in primary structure are sufficient to significantly alter the conformation of the apoprotein and in this way prevent the binding of the 800 nm bacteriochlorophyll molecule.

It will obviously require further work to show which of the reasons discussed above is indeed responsible for the loss of the 800 nm bacteriochlorophyll.

The carotenoid in the B800–850-complex is responsible for the electrochromic carotenoid band shift [14–16], and it has been proposed [17] that this carotenoid is polarised by a strong fixed charge. It was suggested from the sequence of the R26.1 β-apoprotein that arginine-29, could fulfil this role [9] and it is worthwhile pointing out that this residue is indeed retained in the wild-type, carotenoid-containing complex.

Table 2

Amino acid composition of B(800–850) – β

	B(800–850) – β (2.4.1)			B850 – β (R-26.1)[g]		
	Residue/ molecule[a]	Nearest integer	Composition[b]	Residue/ molecule[a]	Nearest integer	Composition[b]
Asp	3.1	(3)	2	3.2	(3)	2
Asn			1			1
Thr[c]	3.9	(4)	4	3.9	(4)	4
Ser[c]	1.0	(1)	1	0.9	(1)	1
Glu	4.1	(4)	3	4.2	(4)	3
Gln			1			1
Pro	2.0	(2)	2	1.9	(2)	2
Gly	5.2	(5)	5	4.9	(5)	5
Ala[d]	8.0	(8)	8	8.0	(8)	8
Val[d]	4.1	(4)	4	4.0	(4)	4
*Met	(1.1)[e]	(1)	1–2	0.9	(1)	1
Ile[d]	2.0	(2)	2	2.0	(2)	2
Leu[d]	7.0	(7)	7	6.8	(7)	7
Tyr	–	(0)	0	0.1	(0)	0
Phe[d]	2.0	(2)	2	2.0	(2)	2
Lys	2.0	(2)	2	2.0	(2)	2
*His[d]	(1.4)[e]	(2)	2	1.7	(2)	2
Arg	0.9	(1)	1	1.0	(1)	1
Trp[f]	2.0	(2)	2	1.8	(2)	2

* These values appear to be too low and are probably due to partial destruction during the hydrolysis

For explanation of the footnotes please refer to table 1

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