THE POLYPEPTIDE COMPOSITION OF THE B850 LIGHT-HARVESTING PIGMENT—PROTEIN COMPLEX FROM *RHODOPSEUDOMONAS SPHAEROIDES*, R26.1

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1. Introduction

In most species of purple photosynthetic bacteria the light-harvesting pigments (bacteriochlorophyll *a* and carotenoids) are organised into two broad classes of antenna pigment—protein complexes [1—5]. The first class only show a single strong absorption band in the near infrared (NIR), while the second show two strong absorption bands in the NIR. The light-harvesting apparatus of wild-type cells of *Rhodopseudomonas sphaeroides* is now well characterised and contains both of these types of antenna complexes, called B875 and B800—850 (after the respective maxima of their major NIR bacteriochlorophyll absorption bands) [1,2,4]. However, in contrast, the nature of the major light-harvesting pigment- protein complex from the well known carotenoidless-mutant of *Rps. sphaeroides* R26, has been a matter for speculation [6—8].

When R26 was first isolated its long wavelength absorption maximum was at ~870 nm (see, e.g., fig.2 of [9]). It was clear then from both the spectral evidence [9] and developmental studies [10] that this represented a carotenoidless B875-type complex. More recently, a number of carotenoidless mutants have been isolated from the closely related species *Rps. capsulata* [11,12], and again these only contain the B875-type of antenna complex. However, since R26 was first isolated, the strain of R26 used by most laboratories has subtly changed and its long wavelength absorption maximum has moved down to ~855—860 nm (see, e.g., fig.1 of [13]). We have been very fortunate in this study to have been given a vintage culture of the original R26 by Professor W. R. Sistrom, which still absorbs maximally at 870 nm. To distinguish between these two strains, we have decided to designate the 'changed' strain as R26.1

and reserve the term R26 for the original 870 nm absorbing strain. In [14] it was shown that at 4 K the long wavelength absorption band of the bacteriochlorophyll in whole cells of *Rps. sphaeroides* R26.1 split into two components. They suggested that R26.1 does in fact contain 2 types of antenna complex.

A purified, detergent solubilised antenna complex (B850) has been isolated from *Rps. sphaeroides* R26.1 [6- 8] and is being used in a variety of functional studies. It is therefore important to determine which types of antenna complexes are present in membranes from R26.1, and to try and decide which type of antenna complex the isolated B850 represents.

Here we have compared the polypeptide composition of chromatophores from R26, R26.1 and wild-type cells. We show below that R26.1 membranes contain the polypeptides from both the B875, and B800—850-types of antenna complex, and that the B850 pigment—protein complex represents an altered B800—850-type of antenna complex.

In [8] we had considered that the B850 complex was an altered B875 antenna type. This, however, was incorrect and we now agree with [6,7,15] that the B850 from R26.1 is a B800—850 antenna-type.

2. Materials and methods

Cells of *Rps. sphaeroides* strains 2:4:1 (wild-type), R26 (obtained from Professor W. R. Sistrom) and R26.1 were grown anaerobically in the light with succinate as the sole carbon source. The cells were harvested, washed in 20 mM Tris—HCl (pH 8.0) and disrupted by passage through a French pressure cell at 10 tons . in^{-2}. Chromatophores were then isolated from the broken cells by differential centrifugation [16].

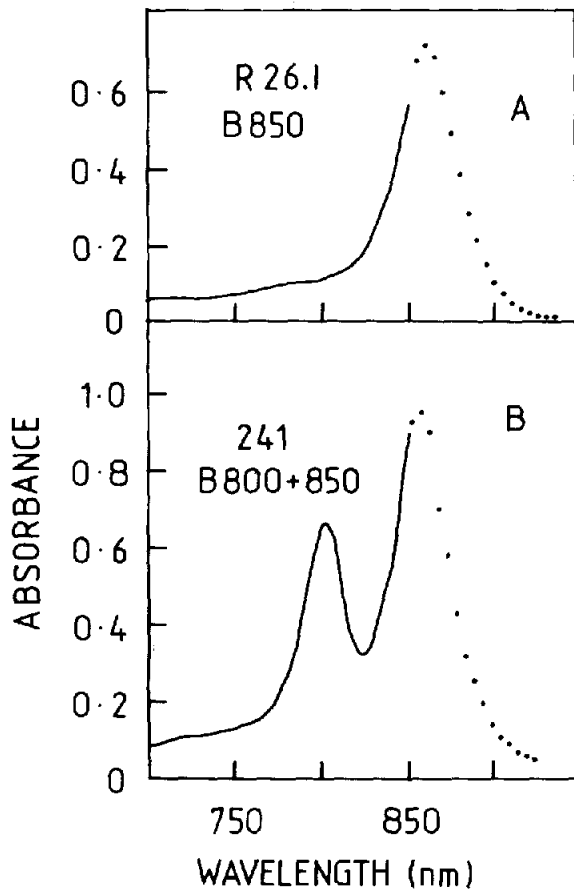The B800—850 light-harvesting pigment—protein

Fig.1. Near infrared absorption spectra of (A) B850 light-harvesting complex from *Rps. sphaeroides* R26.1 and (B) B800–850 light-harvesting complex from *Rps. sphaeroides* 2.4.1. Complexes in 20 mM Tris–HCl (pH 8.0). Spectra below 850 nm were recorded on an Unicam SP8000 scanning spectrophotometer (—). Spectra above 850 nm were recorded on an Unicam SP500 manual spectrophotometer (● ● ●).

complex from *Rps. sphaeroides* 2.4.1 was prepared by the method in [2] as modified [17]. The B850 antenna complex from *Rps. sphaeroides* R26.1 was prepared as in [8].

The integrity of the isolated complexes was checked by recording their absorption spectra with Unicam SP500 and SP8000 spectrophotometers (fig.1). The protein concentration was determined as in [18].

SDS–Polyacrylamide gel electrophoresis was done as in [19]. Gradient slab-gels (usually 11.5–16.5% acrylamide) were poured and run as in [3]. Isoelectric focusing was carried out on the purified complexes in the presence of 8 M urea and the non-ionic detergent,

Nonidet NP-40, as in [20], using the modifications in [21]. After the isoelectric focusing the gels were fixed and stained as in [3]. Individual tracks from the slab gels were cut out, after staining, and scanned at 550 nm using a Gilford 240 spectrophotometer equipped with a linear-transport device.

3. Results and discussion

The polypeptides from the light-harvesting pigment–protein complexes are present in such high concentrations within the chromatophore membrane that they are easily distinguished on SDS–polyacrylamide gradient gels of the whole membrane [4,22]. The light-harvesting polypeptides are a group of hydrophobic, low molecular mass polypeptides, and in chromatophores from *Rps. sphaeroides* 2.4.1 they are resolved into 3 major components (track D, fig.2). B875 is composed of band 1 and part of band 3 [4], while B800–850 is made up of band 2 and the remainder of band 3 [3,4].

It is clear from fig.2 that, even though R26.1 chromatophores lack both carotenoids and the major bacteriochlorophyll absorption band at 800 nm, they
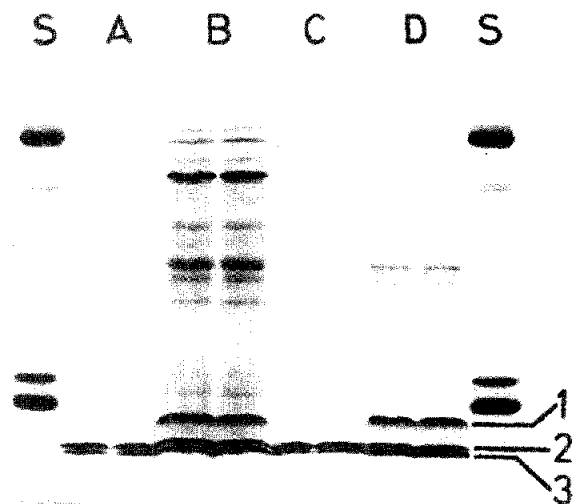

Fig.2. Comparison of polypeptides from *Rps. sphaeroides* 2.4.1 and R26.1 on an SDS–polyacrylamide gradient gel (11.5–16.5% acrylamide): (A) B850 complex from R26.1; (B) R26.1 chromatophores; (C) B800–850 complex from 2.4.1; (D) 2.4.1 chromatophores; (S) standard proteins: bovine serum albumin 68 000 M_r; alcohol dehydrogenase 41 000 M_r; myoglobin 17 200 M_r; cytochrome *c* 12 200 M_r. Bands 1–3 represent polypeptides from the light-harvesting complexes.

contain all 3 of the light-harvesting polypeptide bands, just as in wild-type membranes. This suggests that R26.1 contains 2 types of light-harvesting complex.

In fig.2 we have also compared the polypeptide composition of the isolated B800—850 wild-type antenna complex (track C) with that of the B850 complex from R26.1 (track A). Wild-type B-800—850 had been shown to contain only bands 2 and 3 [3]. Band 1, observed in wild-type chromatophores, is not present since it is a component of the B875 complex [4] which is removed during purification of B800—850. The isolated B850 complex from R26.1 seems to have the same polypeptide composition as the wild-type B800—850 complex. That is, it lacks band 1 (typical of B875), but clearly shows the presence of bands 2 and 3. We therefore conclude that the B850 antenna complex from R26.1 represents a B800—850-type of complex, albeit in a spectrally altered form.

When the region of the light-harvesting polypeptides from the chromatophores is scanned (fig.3), band 3 is usually more intense than band 2. However, this situation is reversed when the gel tracks from the isolated pigment—protein complexes are scanned. Isolation of the B850 from R26.1 therefore removes band 1 and part of band 3 which are present in the R26.1 chromatophores. In [4], it was shown that the B875 antenna complex of wild-type *Rps. sphaeroides*

consisted of both band 1 and part of band 3. This suggests that R26.1 contains the polypeptides of a B875-type of complex in addition to the B850 complex. This B875-type is lost, probably denatured, when the B850 complex is isolated.

The two polypeptides from the B800—850 antenna complex are also well resolved by isoelectric focussing. We have compared the wild-type B800—850 and the B850 from R26.1 by isoelectric focussing to see whether they differ in net charge and whether this might provide an explanation for the lack of the 800 nm bacteriochlorophyll in the B850 complex. The individual complexes were run either separately or together as a mixture to allow a direct comparison within a single gel. Both complexes only gave 2 major bands and these were indistinguishable even when they were co-focused. This suggests that if the differences in the composition of the wild-type B800—850 antenna complex and the B850 complex from R26.1 are due to altered polypeptides, then the changes must be in the content of neutral and hydrophobic amino acids.

In fig.4 we have compared the polypeptide composition of chromatophores from strains 2.4.1, R26 and R26.1. As we had expected R26 only shows light-harvesting bands 1 and 3, consistent with the presence of only B875. The other 2 strains show a full complement of antenna polypeptide types.
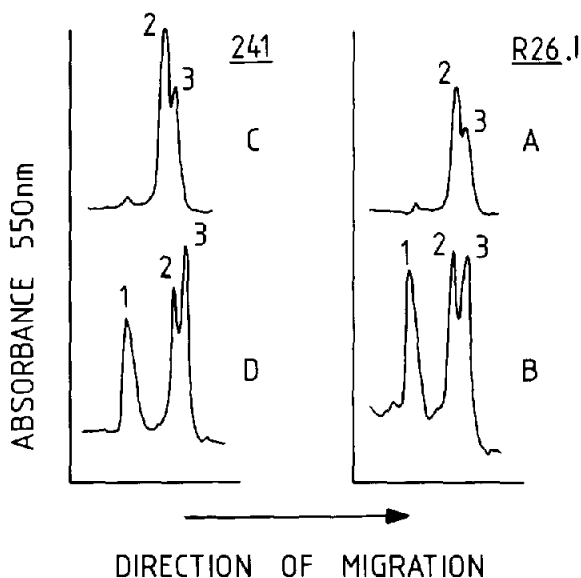

Fig.3. Scans of low molecular mass region of gel shown in fig.2: (A) B850 light-harvesting complex from R26.1; (B) R26.1 chromatophores; (C) B800—850 complex from 2.4.1; (D) 2.4.1 chromatophores.
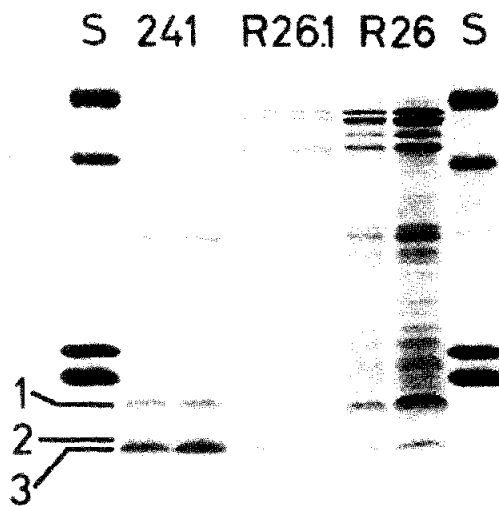

Fig.4. Comparison of polypeptides from chromatophores of strains of *Rps. sphaeroides* on an SDS—polyacrylamide gradient gel (11.5—16.5% polyacrylamide) standards as in fig.2. Bands 1 – 3 represent polypeptides from the light-harvesting complexes.

Acknowledgements

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