Biochimica et Biophysica Acta, 766 (1984) 259–262
Elsevier

BBA Report

BBA 40038

STRUCTURAL CHARACTERIZATION AND COMPARISON OF ANTENNA COMPLEXES OF R26 AND R26.1 MUTANTS OF *RHODOPSEUDOMONAS SPHAEROIDES*

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Key words: Bacterial photosynthesis; Light-harvesting complex; Resonance Raman spectroscopy; Bacteriochlorophyll; (Rps. sphaeroides)

A reverted R26.1 strain of *Rps. sphaeroides* contains two types of light-harvesting complexes, absorbing near 880 and 850 nm. Resonance Raman spectroscopy provides evidence that the compensating mutation which, in R26.1, restored an 850-nm-absorbing antenna population, results in a local structure around the bacteriochlorophylls *a* that is the same as in the B850 (800-depleted) complexes from *Rps. sphaeroides* 2.4.1 (wild strain). Yet, this compensation is accompanied by a marked change in the control mechanisms of the synthesis of the antenna complexes.

The intracytoplasmic membranes of several species of the *Rhodospirillaceae* family appear to contain two distinct types of antenna pigment-protein complexes, generally named B880 and B850–800 [1]. The *Rhodopseudomonas sphaeroides* species contains these two types of complexes. The *Rps. sphaeroides* R26, carotenoidless mutant exhibits a single electronic band in the 800–900 nm region. When originally isolated, this mutant had an absorption peak near to 870 nm [2]. This, and developmental studies suggested that this strain contained a single B880 type of antenna [2,3,4]. However, recent observations showed that in most laboratories, this band now occurs at shorter wavelengths, around 855–860 nm and that it splits at low temperature into two components. On this basis, Davidson and Cogdell [5] considered that the R26 strain had mutated further, and proposed to rename this new strain R26.1. Before this observation, pigment-protein complexes were extracted from the R 26.1 strain. These complexes generally presented an absorption at 850 nm and were either considered as a modified B850–800 type complex, lacking B800 [6,7]] or as modified B880 [8]. Davidson and Cogdell favored the latter hypothesis, but later showed that the polypeptide composition of the complex extracted from the R26.1 strain had to be close to that of a B850–800 complex [9].

Resonance Raman spectroscopy probes the binding sites of the BChl *a* molecules within intracytoplasmic membranes or antenna complexes of purple bacteria. It provides information about the local, ground-state environmental interactions assumed by these molecules [10,11], among which the bonding of their conjugated C=O groups can be very precisely observed. In particular, we recently used this method to characterize B880-, B850–800-, B850 (800 depleted)- and B820–800-type complexes from various *Rhodospirillaceae* and *Chromatiaceae* [12]. It was shown that the electronically interacting BChl pairs present in B880-type and in B850 (800-depleted)-type complexes from *Rhodospirillaceae* assumed slightly but definitely different ground-state interactions [12,13]. In addition, if B880 complexes appeared to assume a single structure according to these criteria, the B850–800 complexes presented a larger variability among the *Rhodospirillaceae* [12].

It thus appeared that resonance Raman spec-

Abbreviation: BChl, bacteriochlorophyll *a*.

troscopy should be helpful in further characterizing the antenna complexes present in R26 and R26.1 strains of *Rhodopseudomonas sphaeroides*.

Cells of the 2.4.1. wild type, R26 and R26.1 strains of *Rps. sphaeroides* were grown anaerobically in the light. The R26 strain was a kind gift from Dr. R.J. Cogdell. Chromatophores were prepared as in Ref. 14. B880 and B850–800 complexes were isolated from LDAO-treated 2.4.1. chromatophores by ultracentrifugation on sucrose gradient [13]. A sample of a B850 (800-depleted) preparation of *Rps. sphaeroides* 2.4.1. was kindly provided by Dr. R.K. Clayton [15]. No attempt was made to extract antenna complexes from R26 and R26.1 chromatophores, which were directly examined using Raman resonance spectroscopy.

Resonance Raman spectra were obtained at 30 K, using a 363.8 nm excitation wavelength. The experimental set-up and recording procedure have been described elsewhere [10].

Fig. 1 compares the 650–900 nm regions of electronic absorption spectra of R26 and R26.1 chromatophores obtained at 10 K on a Cary 17 spectrometer. While the Q_y band of R26 chromatophores appears to be single component, with a maximum at 880 nm, that of R26.1 chromatophores has two components at 880 and 863 nm, as previously observed [16].

Fig. 1 also indicates that in the R26.1 strain, the biosynthesis of the revertant B850 complex may well occur at the expense of the B880 complex. Indeed, the overall integrated intensities of the Q_y bands of antenna BChl, normalized to those of the reaction center bands at 800 or 760 nm, are closely the same for R26 and R26.1. These values are, for example, 23 and 19, taking the 800 nm bands as

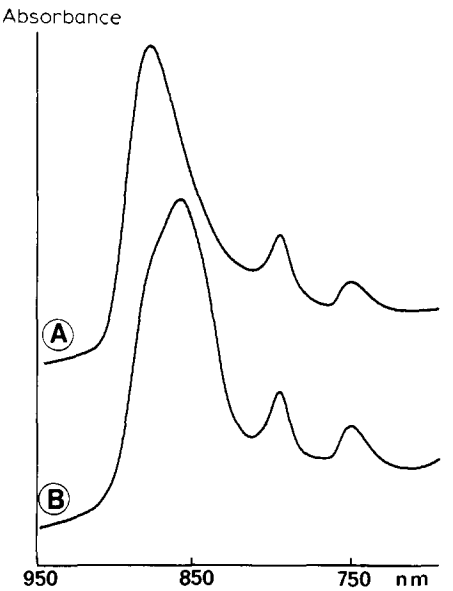

Fig. 1. Low-temperature absorption spectra (10 K) of chromatophores from carotenoidless strains of *Rps. sphaeroides*; (A), R26 strain; (B), R26.1 strain.

references, in spectra of R26.1 and R26 of Fig. 1, respectively. In the R26.1 spectrum, the B880 complex accounts for less than 25% of the overall intensity of the antenna Q_y band, that is, the molecular ratio of the B880 complexes to the reaction centers is lower in R26.1 (4–7 : 1) than in R26, for which it is 20–25 : 1 [3]. In addition, contrarily to what observed for *Rps. sphaeroides* 2.4.1 and many other *Rhodospirillales*, neither culture age nor illumination levels during growth significantly affected the B880 : B850 molecular ratios significantly in R26.1 (not shown). This also indicates that, in R26.1, the synthesis of B880 and B850 are not independent.

TABLE I

INTENSITY RATIOS FOR SKELETAL RAMAN BANDS OF CHROMATOPHORES (R26 AND R26.1 STRAINS) AND OF B880 AND B850–800 PURIFIED COMPLEXES (WILD-TYPE) FROM *RPS. SPHAEROIDES*.

For each ratio indicated, the intensities of the reference bands constitute the denominator. Excitation wavelength, 363.8 nm; $T = 30$ K.

	I_{360}/I_{380}	I_{579}/I_{590}	I_{767}/I_{700}	I_{950}/I_{900}	I_{1067}/I_{1615}
R26	0.65	0.60	1.15	0.65	0.50
R26.1	1.0	0.85	0.85	0.55	0.35
B880	0.65	0.55	1.20	0.65	0.45
B850–800	1.15	1.0	0.55	0.45	0.20

Thus, the R26.1 strain used in the present experiments behaves very differently from the wild type of *Rps. sphaeroides* and from many other bacteria, departing from Sistrom's model [3] on two essential points, namely (i) the B880/reaction center molecular ratio, (ii) the independence of the biosynthesis controls of B880 and of B850. It appears that, in R26.1, the control mechanisms for the synthesis of the antenna complexes are modified, being largely common to B880 and B850.

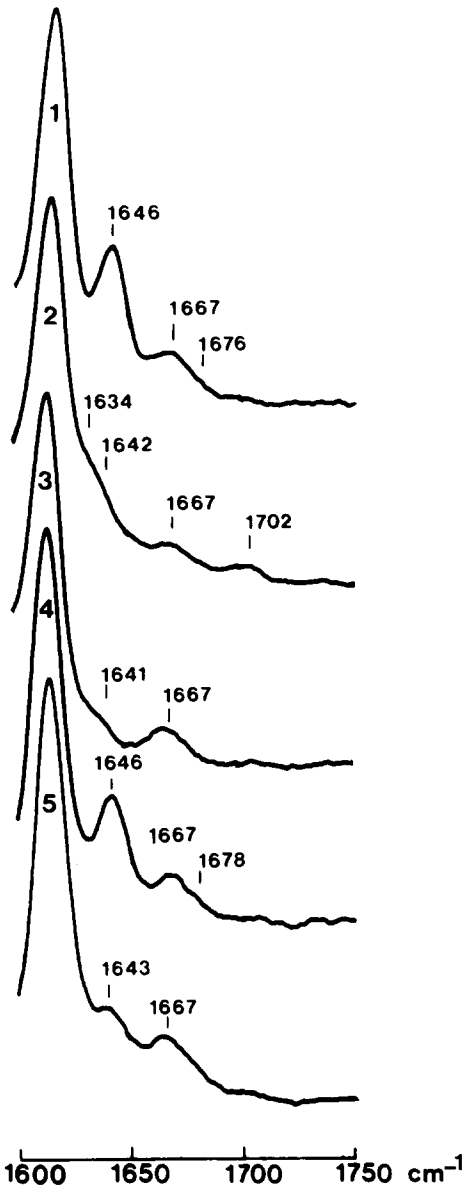

Fig. 2. Resonance Raman spectra (carbonyl stretching region) of: (1), B880 complexes from *Rps. sphaeroides* 2.4.1; (2), B850–800 complexes from *Rps. sphaeroides* 2.4.1; (3), B850 complexes from *Rps. sphaeroides* 2.4.1; (4), chromatophores from *Rps. sphaeroides* R26; (5), chromatophores from *Rps. sphaeroides* R26.1. Excitation wavelength, 363.8 nm; $T = 30$ K.

These observations constituted an additional motivation for undertaking a detailed structural comparison of the antenna complexes of R26.1 with those of the other *Rps. sphaeroides* strains.

Resonance Raman spectra of the BChl a molecules contained in R26 and R26.1 chromatophores at 30 K exhibit large differences, particularly in the 360–380, 570–590, 700–800 900–950 cm^{-1} regions, and at the level of the 1068 cm^{-1} band [17]. Most of these differences concern alterations in relative band intensities and are related to complex vibrational modes of the dihydrophorbin skeleton [10]. Table I indicates the major intensity discrepancies observed between R26 and R26.1 spectra along with the differences seen between the B880 and B850–800 complexes isolated from the wild-type strain. Interestingly, the differences between the R26 and R26.1 spectra involved the same bands seen to differ between the B880 and B850–800 complexes isolated from *Rhodospirillales*. Moreover, Table I shows that the intensity ratios found for R26 are very close to those measured for the B880 complex of 2.4.1. On the other hand, the values found for R26.1 fall somewhere in between those measured for B880 and B850–800 complexes of 2.4.1.

Similar observations can be made in the 1620–1710 cm^{-1} regions of the spectra, where bands arise from the stretching modes of the conjugated carbonyls of the BChl molecules. Here both frequency and apparent relative intensity differences occur between R26 and R26.1 (Fig. 2). All of these necessarily arise from the presence of additional, different BChl populations, with specific bonding of their conjugated carbonyls, in one type of chromatophores and not in the other [10]. More precisely, the R26 spectrum in this region is identical to that of the B880 complex from 2.4.1. (Fig. 2) and, thus shares the intensity pattern which has recently been shown to be characteristic of B880 complexes of several *Rhodospirillales* [12]. On the contrary, the 1620–1710 cm^{-1} region of the R26.1 spectrum differs from those of both the B880 and B850–800 complexes of 2.4.1, but can be simulated with great accuracy

by adding a B850 (800 depleted) spectrum to a B880 spectrum, with proper relative weights – here 4 : 1 – with normalization done on the 1615 cm^{-1} bands (Fig. 3).

Taking those results together, it can be concluded that the B880 complexes present in both the R26.1 and the original R26 strains are structurally identical to those of the wild type of *Rps. sphaeroides*, inasmuch as their two unequivalent BChl molecules share the typical local environments which are common to B880-type complexes of *Rhodospirillales* [12]. On the other hand, the B850 complex of the R26.1 strains appears identical (in terms of pigment environments) to the B850 (800-depleted) complex of *Rps. sphaeroides* 2.4.1. In particular, its resonance Raman spectrum contains a 1640 cm^{-1} band arising from stretching of intermolecularly-bound acetyl carbonyls (Fig. 2). This band is characteristic of B850 (800-depleted) from 2.4.1, occuring, e.g., in *Rps. palustris*, at 1633 cm^{-1} [12]. No satisfactory simulation of the 1620–1710 cm^{-1} region of R26.1 chromatophores could indeed be obtained using a resonance Raman spectrum of B850 (800 depleted) from *Rps. palustris*, in place of that from *sphaeroides* 2.4.1. It thus appears that the compensating mutation which converts the R26 strain into the R26.1 one, constitutes a partial suppression of the original mutation, this latter strain again synthesizing a BChl-*a*-protein complex produced by the wild-type. However, this partial suppression is obtained through a marked change in the control mechanism of the biosynthesis of the antenna complexes.

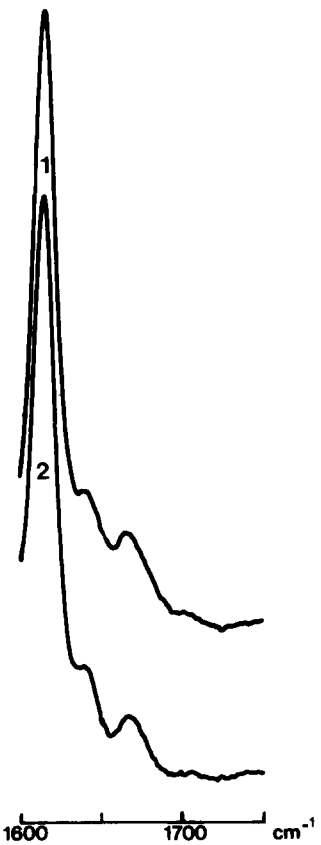

Fig. 3. (1), Resonance Raman spectrum (carbonyl stretching region) of chromatophores from the R26.1 strain of *Rps. sphaeroides*; (2), simulated spectrum obtained by adding resonance Raman spectra of B880 and of B850 complexes from *Rps. sphaeroides* 2.4.1 (see text).

We wish to thank Dr. Richard J. Cogdell for the gift of the R26 strain as well as Dr. R.K. Clayton for a sample of the B850 (800-depleted) complex.

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