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ANALYSIS OF THE PIGMENT CONTENT OF AN ANTENNA PIGMENT-PROTEIN COMPLEX FROM THREE STRAINS OF *RHODOPSEUDOMONAS SPHAEROIDES*

RICHARD J. COGDELL [a,*] and ANTONY R. CROFTS [b]

[a] *Department of Botany, University of Glasgow, Glasgow G12 8QQ, and* [b] *Department of Biochemistry, Medical School, University of Bristol, Bristol BS8 ITD (U.K.)*

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Summary

The pigment content of a B800-850 light-harvesting pigment-protein complex isolated from three different stains of *Rhodopseudomonas sphaeroides* has been determined. In each case the ratio of carotenoid to bacteriochlorophyll present is very nearly 1 : 3 an no specificity with regard to carotenoid type was observed.

The fourth derivative of the infra-red absorption bands of the complex was determined and it is concluded that the minimal functional unit of B800-850 complex consists of 1 carotenoid molecule and three bacteriochlorophyll molecules. The data presented here, together with the previous study of Austin, (Austin, L.A. (1976) Ph.D. Thesis, University of California at Berkeley, Lawrence Berkeley Laboratory Report No. LBL 5512) suggest that the 800 nm absorption band represents one of these bacteriochlorophyll molecules while the remaining two bacteriochlorophylls are responsible for the 850 nm band.

The absorption spectra and circular dichroism spectra of the complexes suggests that their structure has not been greatly altered during the purification.

Introduction

There is a division of labour within the photosynthetic unit. Most of the bacteriochlorophylls and carotenoids constitute the light-harvesting antenna and funnel the absorbed radiant energy to the other component, the specialised chlorophylls in the reaction centre, where that energy is "trapped".

* To whom requests for reprints should be addressed.
Abbreviation: SDS, sodium dodecyl sulphate.

Reaction centres have been isolated and purified from a variety of strains of *Rhodopseudomonas sphaeroides* [1—4] and have been well characterised both with regard to their content of pigments and proteins [3—6] and to their function [7].

The antenna pigments also occur as pigment-protein complexes. In carotenoid-containing strains of *Rps. sphaeroides* there are probably two types of antenna complexes B (Bulk) 800-850 and B870 (where the numbers refer to the absorption maxima in nm of the complexes in the near infrared) [8]. The B800-850 complex has been isolated from wild-type *Rps. sphaeroides* and its protein moiety has been studied [9—11]. The complex probably exists in vivo as aggregates of a polypeptide whose molecular weigth is somewhere between 8000 and 14 000.

Recently, functional studies with this complex have begun (for example see ref. 12); however, before further work is undertaken it is important to define the pigment content of this complex, and such an analysis is presented below.

Experimental

Cells of *Rps. sphaeroides* strains 2.4.1 (wild type), GlC and Ga (two green mutants) were grown photosynthetically, with succinate as the sole carbon source. The cells were harvested, disrupted by shaking with glass beads (Ballotoni No. 10) in a Braun Cell homogeniser (Type 53030, B. Braun, Melsungen, G.F.R.) and chromatophores isolated by differential centrifugation.

Reaction centers were removed from the chromatophores following treatment with 0.25% lauryldimethyl-amine-*N*-oxide in 0.1 M phosphate buffer, pH 7.5, at 26°C, after the method of Jolchine and Reiss-Husson [3]. The B800-850 antenna complex was then isolated and purified from the residue of the chromatophores by the method of Clayton and Clayton [9]. In most cases the B800-850 complexes obtained in this way were further purified by ammonium sulphate precipitation and a second sucrose gradient centrifugation.

The absorption spectra were recorded upon a Pye-Unicam SP800 spectrophotometer and the circular dichroism spectra upon a Cary 60 spectrophotometer. The room temperature and low temperature absorption spectra of the complexes in the infrared, together with their respective fourth derivative spectra were measured in a computer-linked spectrophotometer as previously described [13].

The bacteriochlorophyll concentration was measured following extraction with a 7 : 2 (v/v) mixture of acetone and methanol, using the extinction coefficient of 75 $mM^{-1} \cdot cm^{-1}$ at 772 nm [14].

The carotenoid content and concentration was determined as described in ref. 4 except that in this case commercial silica gel thin layer chromatography plates (silica gel 60 precoated plates, Merck, G.F.R.) were used.

The protein concentration was determined by the Lowry method [16] and the homogeneity of the complexes was analysed by sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis after the method of Laemmli [15] as described in ref. 4.

Results and Discussion

The absorption spectra of the three B800-850 complexes were recorded (Fig. 1), then the concentration of bacteriochlorophyll and carotenoid was determined for each one. The results are presented in Table I.

In each case, the ratio of bacteriochlorophyll to carotenoid present was very nearly 3 : 1. A similar quantitative analysis of the bacteriochlorophyll/carotenoid ratio was carried out upon the intact chromatophores from which the B800-850 complexes were derived (Table II). The bacteriochlorophyll/carotenoid ratio in the chromatophores is lower than in the purified complexes and is more variable from one batch of cells to another. This ratio in chromatophores has been shown previously to be quite variable and dependent upon the growth conditions [17]. The values presented here fall within the range of previous studies (for example see ref. 17). *Rps. sphaeroides* reaction centres prepared from carotenoid-containing strains show a remarkable degree of specificity with regard to which types of carotenoid are bound to the reaction centre [4]. The B800-850 complexes, however, do not exhibit such specificity.

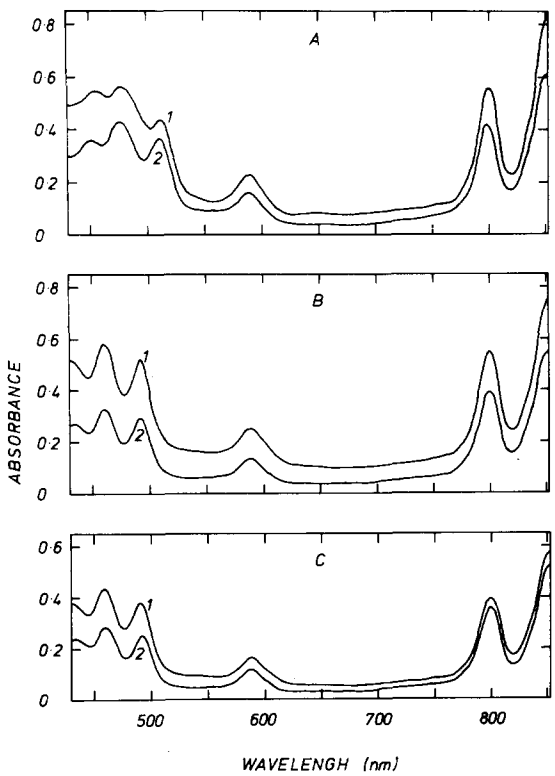

Fig. 1. The absorption spectra of the B800-850 light-harvesting complexes and their parent chromatophores. In each case the complexes and chromatophores were suspended in 50 mM Tris · HCl pH 8.0 (and 1% lauryldimethyl-amine-*N*-oxide with the complexes). A: Strain 2.4.1; (1), B800-850 complex; (2) chromatophores. B: Strain G.1C.; (1) chromatophores; (2), B800-850 complex. C: Strain Ga; (1), chromatophores; (2), B800-850 complex.

TABLE I

THE RELATIVE CONCENTRATIONS OF BACTERIOCHLOROPHYLL AND CAROTENOID IN THE B800-850 ANTENNA COMPLEXES

It should be noted that the concentration of carotenoid is only probably accurate to 5—10% since there is uncertainty in their extinction coefficients.

Sample	A_{850} (cm^{-1})	[Bacteriochlorophyll] * (μm)	[Carotenoid] * (μm)	Bacteriochlorophyll/carotenoid ratio
2.4.1.	0.84	7.2	2.46	2.92 : 1
Ga	5.75	47.2	15.35	3.07 : 1
G1C	4.72	33.4	11.5	2.91 : 1

* Average of two determinations.

TABLE II

THE RELATIVE CONCENTRATIONS OF BACTERIOCHLOROPHYLL AND CAROTENOID IN THE CHROMATOPHORES FROM WHICH THE B800-850 ANTENNA COMPLEXES WERE DERIVED

Sample	A_{850} (cm^{-1})	[Bacteriochlorophyll] * (μm)	[Carotenoid] * (μm)	Bacteriochlorophyll/carotenoid ratio
2.4.1.	63	540	319	1.69 : 1
Ga	26.5	246	98.5	2.52 : 1
G1C	85	772	296	2.6 : 1

* Average of two determinations.

TABLE III

THE CAROTENOID COMPOSITION OF THE B800-850 ANTENNA COMPLEXES TOGETHER WITH THAT OF THEIR PARENT CHROMATOPHORES

Sample	R_f of major bands *	Composition (%)	Identity of the carotenoid
2.4.1 chromatophores	0.72	0.06	neurosporene
	0.50	92.00	spheroidene
	0.42	7.94	spheroidenone
2.4.1 B800-850 complex	0.73	0.06	neurosporene
	0.54	91.00	spheroidene
	0.46	8.94	spheroidenone
Ga chromatophores	0.72	60.10	neurosporene
	0.50	13.40	chloroxanthin
	0.20	26.50	unknown
Ga B800-850 complex	0.72	60.00	neurosporene
	0.49	13.50	chloroxanthin
	0.20	26.50	unknown
G1C chromatophores	0.72	100%	neurosporene
G1C B800-850 complex	0.72	100%	neurosporene

* With strain 2.4.1 several minor bands with lower R_f values were also present but only in such small amounts that their characterisation was not practical.

Table III compares the carotenoid composition of the B800-850 complexes with that of their parent chromatophores. As noted by previous workers, chromatophores from strain 2.4.1 contain mainly spheroidene and spheroidenone [18], those from strain Ga mainly neurosporene, chloroxanthin and an unidentified carotenoid with the same absorption spectrum as neurosporene [19], those from strain GlC only neurosporene [13]. The carotenoid composition of the B800-850 complexes apparently mirrors that of the parent chromatophores.

An important question is how many bacteriochlorophyll molecules there are in the smallest functional unit of the B800-850 complex. But in order to answer this not only is a pure B800-850 complex required but also an accurate knowledge of its molecular weight. Clayton and Clayton [9] estimated that their preparation contained about 16% bacteriochlorophyll, which, if their molecular weight is taken as 9000, is equivalent to just less than 1.5 mol bacteriochlorophyll per mol protein. The B800-850 complexes used in this study also contain 14—16% bacteriochlorophyll and like Clayton and Clayton's [9] preparation SDS polyacrylamide gel electrophoresis reveals contamination with a protein of 45 000—50 000 molecular weight as well as the major protein band with a molecular weight of 9000. So far attempts to remove this with chromatography on DEAE-cellulose or by ammonium sulphate precipitation have failed. However, this is being actively pursued in an attempt to get an accurate bacteriochlorophyll content.

Recently, Austin [20], using a combination of two detergents (Triton X-100 and SDS), has succeeded in obtaining very pure preparations of the B855 light-harvesting complex from *Rps. sphaeroides* R26 (carotenoidless strain) and the B800-850 light-harvesting complex from *Rps. sphaeroides* strain 2.4.1. His careful analysis of the percent of bacteriochlorophyll of the purified complexes and the molecular weigths of their polypeptides led him to conclude that the B855 complex contained one baceteriochlorophyll per 9000 dalton polypeptide while the B800-850 complex contained 1.5 bacteriochlorophylls per 9000 dalton polypeptide. Circular dichroism (CD) analysis of the complexes in the infrared region reveals that in each type the absorption band around 850 nm consists of two exciton coupled bacteriochlorophylls. This led him to conclude that the minimal unit of each complex is a dimer of two 9000 dalton polypeptides.

Butler and Hopkins [21] have shown that under optimal conditions the fourth derivative of an absorption band can provide information about the number of absorbing species which contribute to that band. Fig. 2 shows the fourth derivative curves obtained from the absorption spectra at 293 K and 77 K of the B800-850 complex from strain 2.4.1. Very similar spectra were obtained with the Ga and GlC complexes. At room temperature the simplest interpretation of the fourth derivative curve is that the absorption band at 800 nm contains a single transition, while the absorption band centred at 850 nm contains two transitions. At 77 K, where the absorbtion bands are sharper and better separated, the 800 nm band still shows a single transition; however, the two transitions seen in the 850 nm band at room temperature are now indistinguishable. It appears that at 77 K the two transitions which make up the 850 nm have shifted their relative orientations so that they now are indistinguishable.

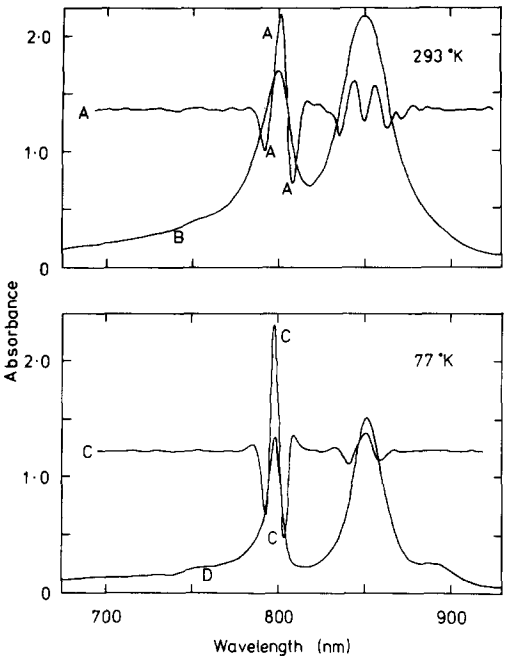

Fig. 2. The infrared absorption spectra of the B800-850 complex from strain 2.4.1. and their fourth derivatives. Trace A is the fourth derivative of the room temperature absorption spectrum of the B800-850 complex from strain 2.4.1. The complex was suspended in 50 mM Tris · HCl, pH 8.0, 1% lauryldimethyl-amine-*N*-oxide in a 1 cm pathlength cuvette. For both trace A and trace C the derivative intervals were 4.0, 3.5, 3.0 and 2.5 nm. Trace C is the fourth derivative of the 77 K absorption spectrum. The complex was suspended in 60% glycerol/40% 50 mm Tris · HCl, pH 8.0/1% lauryldimethyl-amine-*N*-oxide in a 1 mm cuvette in a homemade Dewar. The vertical scale for the fourth derivative curves is arbitrary, but the same in each case. The size of the peak in the fourth derivative depends upon the width of the absorption band, the narrower the band the larger and sharper the fourth derivative. Thus, the peak in the 800 nm band of trace C is larger than that in trace A.

Austin [20] was able to selectively remove the 800 nm component of the B800 complex by a combination of freezing and thawing and exposure to light in the presence of 0.5% Triton X-100. The remaining 850 nm band still contained a pair of exciton-coupled bacteriochlorophylls. This evidence, taken with the fourth derivative spectra suggests that the 800 nm band represents one bacteriochlorophyll molecule while the 850 nm band represents the other two bacteriochlorophylls. It seems reasonable, therefore, to assume that the minimal functional unit of the B800-850 complex contains 3 molecules of bacteriochlorophyll and 1 molecule of carotenoid.

If these B800-850 complexes are to be used in functional studies it must be asked how much their structure has been deranged by extraction from the chromatophore membrane into detergent solution. Fig. 1 compares the absorption spectra of these complexes to those of the parent chromatophores. When allowance is made for the different carotenoid content it is clear that the solubilised complexes show absorption spectra which are very similar to those of the intact chromatophores. This suggests little alteration in their basic structure has occurred during their preparation.

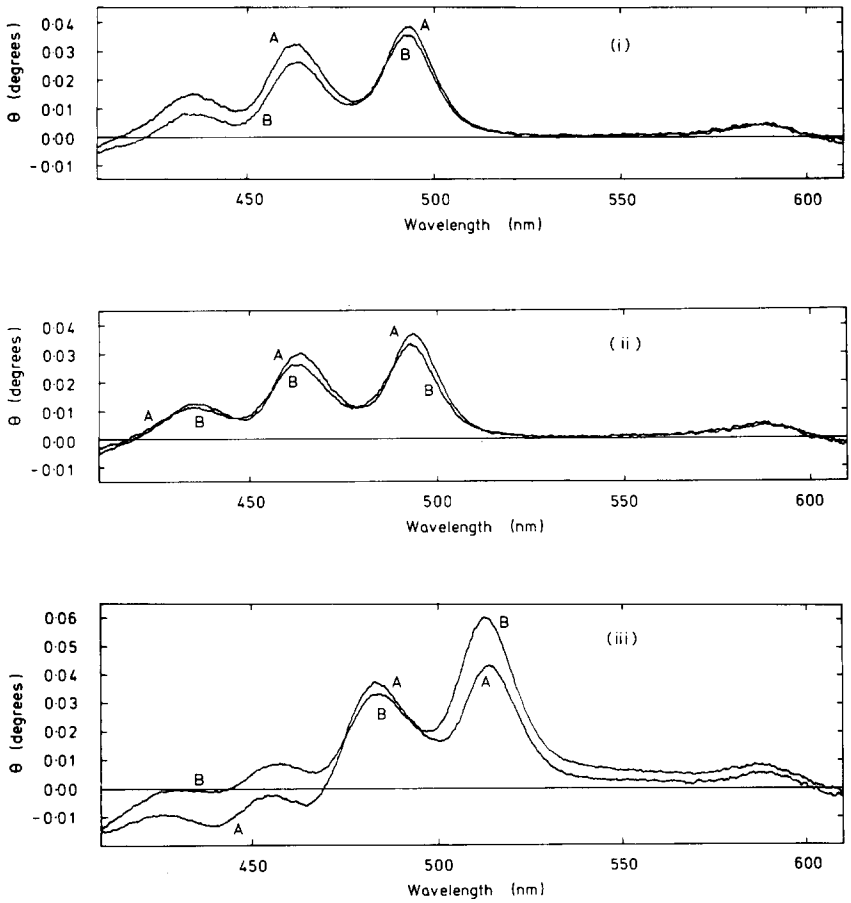

Fig. 3. The CD spectra of the B800—850 complexes and their parent chromatophores. The B800-850 complexes were resuspended in 50 mM Tris · HCl, pH 8.0/1% lauryldimethyl-amine-*N*-oxide, the chromatophores just in 50 mM Tris · HCl, pH 8.0. The concentration of the B800-850 complexes and the chromatophores were adjusted so that they had an absorbance of approx. 1.0 at 500 nm. i: Strain Ga; trace A, B800-850 complex; trace B, chromatophores. ii: Strain GlC; trace A, B800-850 complex, trace B, chromatophores. iii: Strain 2.4.1; trace A, B800-850 complex; trace B, chromatophores.

This conclusion is reinforced when the CD spectra of the complexes are compared with the CD spectra of intact parent chromatophores (Fig. 3). The CD spectra of the complexes and the parent chromatophores are very similar, indeed in strains Ga and GlC they are virtually superimposable. The difference seen in Fig. 3 between the 2.4.1. complex and the 2.4.1. chromatophores probably just reflects the difference in the amount of carotenoid in the two cases (see Tables I and II).

This similarity of the CD spectra is particularly significant in the 420—530 nm region where the changes are due to the carotenoids. It has been shown previously that the carotenoids present in these B800-850 complexes, when extracted into organic solvent, exhibit no CD spectra [4]. This means that the circular dichroism seen in the intact complexes must be due to either the assymetric binding of the carotenoid to the protein or to exciton coupling

with other pigments. In either case the CD spectrum would be expected to be a rather sensitive probe of the structure of the complex. Therefore, the fact that the CD spectrum is not significantly altered when the B800-850 complex is extracted means that these complexes are probably ideal for functional studies.

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