

A further characterisation of the B890 light-harvesting pigment–protein complex from *Rhodospirillum rubrum* strain S1

Richard J. Cogdell, J. Gordon Lindsay⁺, Jane Valentine and Irene Durant

Department of Botany and ⁺Department of Biochemistry The University of Glasgow, Glasgow GL2 8QQ, Scotland

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An intact B890 light-harvesting pigment–protein complex has been obtained from *Rhodospirillum rubrum* strain S1. We show that this complex contains two types of low- M_r polypeptide. Both these polypeptides are present in the intact chromatophore membrane. Analysis of the pigment content of this complex suggests that per pair of polypeptides the complex contains 2 molecules of bacteriochlorophyll and one molecule of carotenoid.

Photosynthetic bacteria *R. rubrum* Light-harvesting complex

1. INTRODUCTION

The major antenna complex from *Rhodospirillum rubrum* is the B890 pigment–protein complex [1–4]. This antenna complex has been isolated and studied in detail by several groups [1,2,5]. However, in most cases its isolation has only been achieved upon denaturing the complex.

Here, we describe the isolation of a 'native' spectrally-intact, preparation of the B890 complex, and because of this, are able to determine its pigment and polypeptide content. Contrary to [1,2,5], we show below that the B890 complex contains two low- M_r polypeptides, and demonstrate why the second polypeptide was missed in the previous reports.

2. MATERIALS AND METHODS

Cells of *R. rubrum* strain S1 were grown anaerobically in the light with succinate as the sole carbon source. The cells were harvested, washed in 100 mM sodium phosphate (pH 7.5) 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

[6] and resuspended in 100 mM sodium phosphate (pH 7.5).

The chromatophores were initially depleted of reaction centres by treating with 0.25% LDAO as in [7]. The depleted chromatophores were resuspended in 50 mM Tris–HCl (pH 8.0) to give an A_{890} of 100 cm⁻¹. LDAO was then added dropwise, while stirring, to give 1% (v/v) final conc. The solubilised, depleted chromatophores were immediately diluted 3-fold in 50 mM Tris–HCl (pH 8.0) and loaded onto a DE52 column. The column had been pre-washed with 50 mM Tris–HCl (pH 8.0) and was usually loaded to ~1/3rd of its capacity. The solubilised material was washed onto the column with 50 mM Tris–HCl (pH 8.0), 0.2% LDAO (v/v). The column was developed in this buffer by stepwise addition of NaCl. At 50 mM NaCl the free pigment was eluted, while the purified B890 was usually eluted between 100–150 mM NaCl.

The integrity of the isolated B890 complex was checked by recording its absorption spectrum (400–850 nm in a Pye-Unicam SP8000 and from 850–950 nm in a Pye-Unicam SP500). Good samples retained their characteristic single strong absorption maximum in the near-infrared at 885 nm and, by eye, looked a pleasing pink-red colour. Denatured samples absorbed at 775 nm (in-

Abbreviation: LDAO, lauryldimethylamine-*N*-oxide

dicative of 'free' bacteriochlorophyll) and looked a dirty, green colour. The best preparations we obtained when the chromatography was run on small columns (e.g., 10 cm long \times 2.5 cm diam.), so that the time for this stage can be kept to a minimum. Good preparations (as judged by the absorption spectrum) can also be obtained by column chromatography on hydroxylapatite.

The concentration of bacteriochlorophyll was determined by extraction with acetone/methanol (7:2, v/v), using the extinction coefficient of $76 \text{ mM}^{-1} \cdot \text{cm}^{-1}$ at 772 nm [8]. [Protein] was determined as in [9]. The carotenoid composition and content of the isolated B890 complex was determined as in [10,11]. The quantitation of the amount of carotenoid present was also checked by an alternative method. A given extract of B890 (containing both bacteriochlorophyll and carotenoid) was put into the measuring cuvette of the spectrophotometer, while the reference cuvette was filled with a purified sample of bacteriochlorophyll (purified from the carotenoidless mutant of *R. rubrum*, G9). The absorbance of the reference cuvette was adjusted so that it equalled that of the measuring cuvette at 772 nm. This procedure effectively removes the contribution of the bacteriochlorophyll and allows the absorption spectrum of the carotenoid (and therefore its concentration) to be directly determined, without the need for any further purification. Both methods gave identical results (see below).

The polypeptide composition of the whole chromatophores and the isolated, purified B890 pigment-protein complex was determined by electrophoresis on SDS gradient polyacrylamide gels (11.5–16.5% acrylamide) as in [12,13].

3. RESULTS AND DISCUSSION

Properties of reaction centre-depleted membranes of *R. rubrum* were described in [14]. During this analysis, evidence was presented that the light-harvesting bacteriochlorophyll appeared to be associated with two low- M_r polypeptides. However, when the presumed B890 antenna complex was isolated [1–3,5] only a single polypeptide was found.

Usually these isolation procedures (for the B890 complex) involved extraction into chloroform and methanol and this of course produced a denatured

complex. This organic extraction method has, however, proved useful in allowing enough of the single polypeptide to be purified to allow it to be sequenced [5]. The organic, soluble polypeptide consists of 52 amino acids and has M_r 6106 [5]. Unfortunately for this method, the bacteriochlorophyll and carotenoid are noncovalently bound to the B890 complex. Because of this, once the complex has been denatured, it is difficult to be sure that the correct light-harvesting polypeptides have indeed been obtained.

Therefore, we have adopted an alternative approach. We have sought to begin by isolating and purifying an intact antenna complex and, only when we have achieved this, to take it apart and determine its composition.

Fig. 1 shows the absorption spectrum of the isolated, purified B890 complex. Unlike [1–3], it is essentially free of reaction centres and is still in its native, 885 nm absorbing form.

The polypeptide composition of the isolated B890 complex is depicted in fig. 2. The complex contains two rather low- M_r polypeptides, both of which are also present in the whole chromatophore membrane (as in [14]).

To reconcile our data with that of the previous studies we have applied the organic solvent extraction procedure to our isolated B890 preparation. Some B890 complex was freeze-dried and then extracted with 1:1 (v/v) chloroform-methanol, as in [1]. In fig. 3 we have compared the polypeptide composition of the unextracted B890 complex,

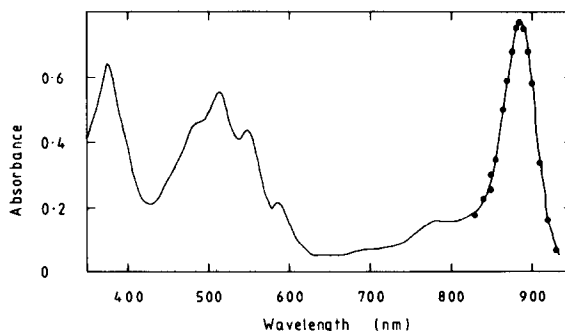


Fig. 1. The absorption spectrum of the isolated B890 light-harvesting pigment-protein complex from *R. rubrum* strain S1: (—) recorded on the Pye-Unicam SP8000; (—●—) recorded on the Pye-Unicam SP500.

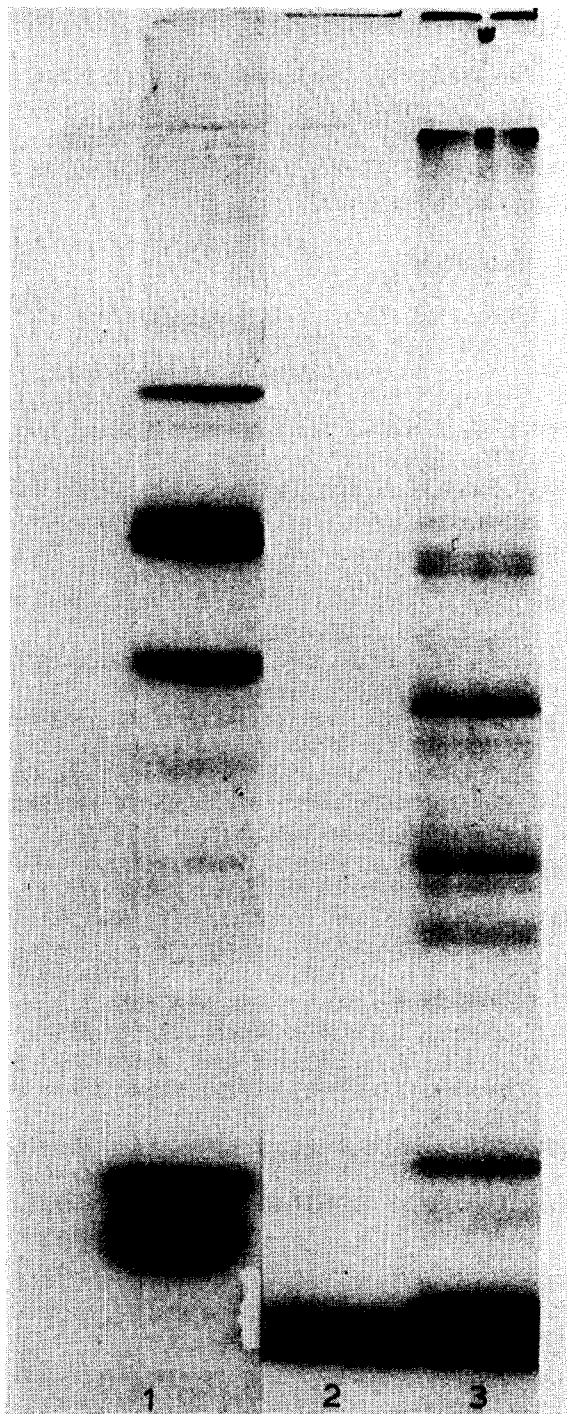


Fig. 2. A comparison of the polypeptide composition of the isolated B890 complex with that of the whole chromatophore membrane of *R. rubrum* strain S1 on an SDS-polyacrylamide gradient gel (11.5–16.5% acrylamide: (1) standard proteins (M_r) bovine serum albumin (68 000), alcohol dehydrogenase (41 000), myoglobin (17 200) cytochrome *c*, (12 200); (2) the isolated, purified B890 complex; (3) chromatophores from *R. rubrum* S1. All these tracks were taken from a single slab gel, but for convenience only 3 tracks have been presented.

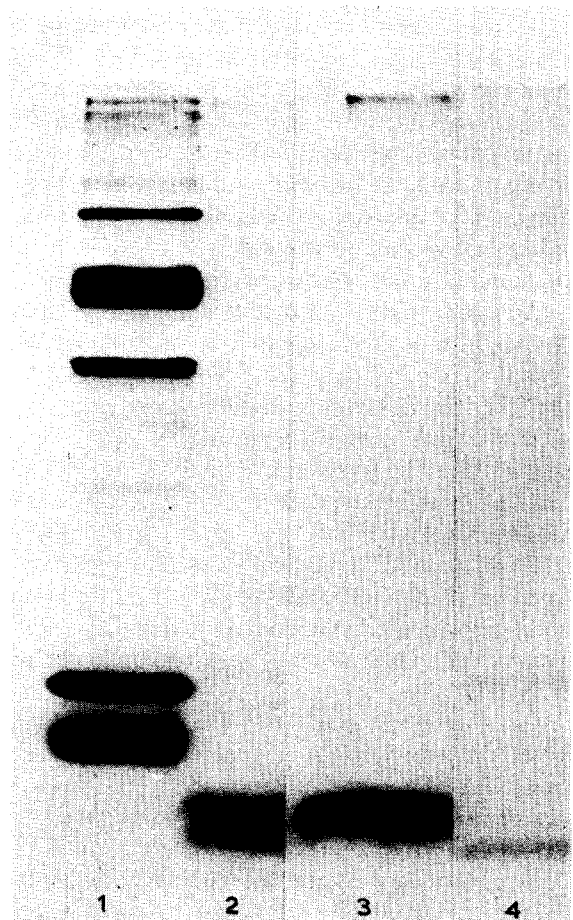


Fig. 3. The effect of chloroform:methanol (1:1, v/v) extraction upon the polypeptide composition of the isolated B890 antenna complex from *R. rubrum* S1 analysed by SDS-polyacrylamide gradient gel electrophoresis: (1) standard polypeptides as in fig. 2; (2) the unextracted B890 complex; (3) the organic soluble fraction of the B890 complex; (4) the organic insoluble fraction of the B890 complex. All these tracks were taken from a single slab gel, but for convenience only 4 tracks have been presented here.

with the organic-soluble and organic-insoluble material. Clearly, mainly the upper of the two light-harvesting polypeptides is removed by the organic solvent, while most of the lower band remains in the organic-insoluble material. Therefore, it seems that the previous studies, where only a single light-harvesting polypeptide was found, missed the second polypeptide because of its differing solubility in chloroform-methanol.

Analysis of the carotenoid composition shows that >99% of the carotenoid in our isolated B890 complex is spirilloxanthin. This assignment was verified by absorption spectroscopy, thin-layer chromatography on silica gel and by mass spectroscopy [15]. The quantitative analysis of the carotenoid present in the B890 complex yielded a carotenoid:bacteriochlorophyll ratio (mol:mol) of 1:1.94 (the total analytical method, average of 4 separate determinations) and of 1:1.92 (the quick spectrophotometric method, av. 6 determinations), that is very nearly 1:2. These results agree well with [11].

The determination of the bacteriochlorophyll:protein ratio in several different preparations yielded average values of $16\% \pm 1.5\%$ (w/w) (av. 8 determinations). If it can be assumed that the second polypeptide described here has a similar M_r -value to the polypeptide previously sequenced, then these ratios are equivalent to two bacteriochlorophyll molecules and one carotenoid molecule per pair of polypeptides.

Since this study was completed, we have discovered that the groups of Professor Zuber and Professor Batchofen from Zurich have also recognised the presence of the second polypeptide from the B890 complex, and will soon present its complete primary structure. They confirm that it was originally missed because of its non-solubility in 1:1 (v/v) chloroform-methanol.

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