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A COMPARISON OF THE CONSTITUENT POLYPEPTIDES OF THE *B*-800—850 LIGHT-HARVESTING PIGMENT-PROTEIN COMPLEX FROM *RHODOPSEUDOMONAS SPHAEROIDES*

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Summary

The polypeptide composition of the *B*-800—850 light-harvesting pigment-protein complex from *Rhodopseudomonas sphaeroides* has been determined. The complex consists of equimolar amounts of two small polypeptides. The two polypeptides have very similar molecular weights and amino acid composition but are clearly separable by either SDS polyacrylamide gradient gel electrophoresis or isoelectric focussing.

Introduction

The past five years has seen a rapid increase in our understanding of the mechanisms of the primary reactions in bacterial photosynthesis [1]. These advances are entirely due to the availability of well-characterised, isolated reaction centres, which are devoid of antenna pigments. In comparison the light-harvesting pigment-protein complexes have received little attention. However procedures for their purification are now beginning to be described for a variety of antenna complexes and their detailed characterisation is now under way in several laboratories [2—5].

The photosynthetic unit of *Rhodopseudomonas sphaeroides* strain 2.4.1 is constructed out of three components: the photochemical reaction centres and two types of light-harvesting complexes (*B*-870 and *B*-800—850 [6]). The *B*-800—850 complex is easier to isolate and purify [2] and is probably the best characterised of all the antenna complexes so far studied.

Recent studies by Sauer and Austin [7] and Cogdell and Crofts [8] suggest

that the *B*-800—850 complex exists in vivo as aggregates of a basic 'minimal unit'. This unit consists of two polypeptides (with an apparent molecular weight of 9000—10 000), three molecules of bacteriochlorophyll and one molecule of carotenoid. The 850 nm absorption band arises from two of the bacteriochlorophylls while the 800 nm absorption band represents the other. The two bacteriochlorophylls in the 850 nm band are exciton coupled.

Similar *B*-800—850 light-harvesting complexes have been isolated from *Rhodopseudomonas capsulata* [5] and *Rhodopseudomonas palustris* [9]. In both of these cases analysis of the constituent polypeptides of the *B*-800—850 complexes by SDS polyacrylamide gradient gel electrophoresis has revealed at least two types of polypeptides to be present in the 8000—12 000 mol. wt. region. This contrasts with previous studies, with 10% acrylamide SDS gels, upon the *B*-800—850 complex from *Rps. sphaeroides* where only a single polypeptide has been resolved [2, 3 and 10].

In the present study, using a combination of SDS polyacrylamide gradient gel electrophoresis and isoelectric focussing, we have been able to resolve the *B*-800—850 complex from *Rps. sphaeroides* into two component polypeptides, having very similar molecular weights. These two polypeptides have then been partially characterised. A preliminary report of some of this work has appeared elsewhere [11].

Materials and Methods

(a) The preparation of the B-800—850 complexes from Rps. sphaeroides and Rps. capsulata

Cells of *Rps. sphaeroides* strain 2.4.1 and *Rps. capsulata* strain Z1 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 then disrupted by passage through a French Pressure cell at 10 tons per square inch. Chromatophores were then isolated from the broken cells by differential centrifugation [12] and resuspended in 20 mM Tris-HCl, pH 8.0.

The *B*-800—850 light-harvesting complex from *Rps. sphaeroides* was prepared, following treatment of reaction centre depleted chromatophores with lauryl-dimethylamine-*N*-oxide by the method of Clayton and Clayton [2] as described in Cogdell and Crofts [8].

The *B*-800—850 antenna complex from *Rps. capsulata* was prepared as described by Feick and Drews [9] except that the sucrose gradient centrifugation step was omitted. This modification gave better yields with no detectable loss of purity.

The protein concentration was determined by the method of Mejbaum-Katzenellenbogen and Drobryszycka [13]. The concentration of bacteriochlorophyll was determined by extraction with acetone/methanol (7 : 2, v/v), using a mM extinction coefficient of 75 cm^{-1} at 772 nm [14]. The integrity of the complexes was confirmed by checking their absorption spectra with Unicam SP500 and SP800 spectrophotometers (Fig. 1). The two spectra shown compare very well with those previously reported [2, 8 and 9].

(b) Analysis of the polypeptide composition of the B-800—850 complexes

SDS polyacrylamide gel electrophoresis was carried out on the purified

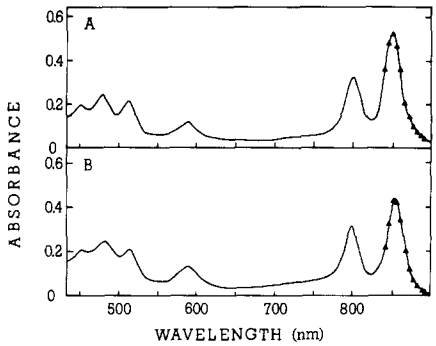

Fig. 1. The absorption spectra of the *B*-800—850 antenna pigment-protein complexes from *Rps. sphaeroides* and *Rps. capsulata*. A. The absorption spectrum of the *B*-800—850 antenna complex from *Rps. sphaeroides* strain 2.4.1. suspended in 20 mM Tris-HCl, pH 8.0. From 430 to 840 nm the spectrum was recorded on the SP8000 and from 840 to 900 nm on the SP500 (▲———▲). B. The absorption spectrum of the *B*-800—850 complex from *Rps. capsulata* strain Z1. The conditions were the same as in A.

complex as described by Laemmli [15]. Gradient gels (usually 11.5—16.5% acrylamide) were poured by stabilising the gradient with sucrose (15% sucrose with the 11.5% acrylamide and 70% sucrose with the 16.5% acrylamide). This procedure was carried out on ice, which retarded the polymerisation of the gels long enought to allow the construction of a smooth gradient. The gels were then run at room temperature.

Isoelectric focussing was carried out in the presence of 9.6 M urea and the non-ionic detergent, Nonidet NP-40, as described by O'Farrell [16], using several of the modifications described by Ames and Nikaido [17]. After isoelectric focussing the gels were fixed in 10% (v/v) trichloroacetic acid (72 h) and washed with water (>2 h) to remove all the ampholines. The protein bands were then visualized by staining with Coomassie Brilliant Blue R-250 [18].

The pH gradient of the gels was measured directly with an antimony microelectrode as described by Beeley et al. [19].

(c) Amino acid analysis and N-terminal analysis of the constituent polypeptides of the B-800—850 complex from Rps. sphaeroides

Samples of the *B*-800—850 antenna complex from *Rps. sphaeroides* and its individual constituent polypeptides were hydrolysed and analysed by the method of Spackman et al. [20]. Some samples of the intact *B*-800—850 complex were hydrolysed in 6 N HCl for 24, 48 and 72 h. The analyses were then extrapolated to zero time to check for any non specific degradation of the amino acids during the hydrolysis. However very little degradation was found. No alkaline hydrolyses were carried out so that tryptophan was not determined. Norleucine was used as an internal standard in all the analyses.

The analysis of the intact *B*-800—850 complex was either performed upon the complex directly after its purification or following an extra electrophoretic purification on 10% SDS polyacrylamide gels. In the latter case the complex was electrophoresed under non-denaturing conditions. The pigmented band was cut out of the gel and the complex eluted from it by boiling the mascerated gel slices in a small volume of 1% (v/v) SDS. Similar results were obtained with either method.

The amino acid analysis of the two constituent polypeptides of the *B*-800—850 complex was performed after they had been separated by isoelectric focussing. A set of identical isoelectric gels of the complex were run. The gels were then fixed in 10% (v/v) trichloroacetic acid and washed with water to remove the ampholines and most of the urea. One sample gel was then stained for protein with Coomassie Blue. This sample gel was then used to indicate the position of the two polypeptides in the other isoelectric gels. The two bands were cut out of the gels and eluted from the gel slices as described above for the SDS polyacrylamide gels. The remaining traces of ampholines and urea were removed by dialysis.

These samples were then concentrated by precipitating the protein with 80% acetone at 4°C and used for the amino acid analysis.

The N-terminal analyses were performed by dansylation procedures as described by Hartley [21] and Gray [22]. The determinations were carried out only on the intact *B*-800—850 complex.

The standard proteins were obtained from Sigma Chemical Co., Ltd.

Results and Discussion

Most previous studies on the polypeptide composition of the *B*-800—850 antenna pigment-protein complex from *Rps. sphaeroides* have used SDS polyacrylamide gel electrophoresis [2,7,8 and 11]. Usually 10% acrylamide gels were run [2,7 and 8] and only a single polypeptide band was resolved, with an apparent molecular weight of approx. 9000.

However Takemoto and Lascelles [23] were able to resolve two components in the region thought to be due to the *B*-800—850 complex, running SDS-urea polyacrylamide gels on whole membranes of *Rps. sphaeroides*. It has also been reported that SDS polyacrylamide gel electrophoresis with the isolated complex using a Tris-phosphate buffer system will resolve two polypeptides, one of approx. 12 000 and one of approx. 9000 [24]. We have not been able to repeat this latter method and consistently obtain two polypeptides. The exception to this (described below) is with 'old' samples of the complex where an artefactual second band appears.

The *B*-800—850 complex is reproducibly resolved into two components on 11.5—16.5% SDS polyacrylamide gradient gels. We have compared the electrophoretic behaviour of the *B*-800—850 complex from *Rps. sphaeroides* with that of the analogous complex from *Rps. capsulata* (Fig. 2). As described by Feick and Drews [9] the *B*-800—850 complex from *Rps. capsulata* has three polypeptide components (I, II and III). When the complex is run under non-denaturing conditions two bands are seen on the gel (Fig. 2A). The upper band is pigmented and contains the two polypeptides II and III while I is dissociated from the complex and runs with an apparent molecular weight of 14 000. As the complex is progressively more denatured prior to electrophoresis more of the pigmented band is dissociated into the individual II and III polypeptides, which run with apparent molecular weights of 10 000 and 8000 respectively. The *B*-800—850 complex from *Rps. sphaeroides* shows a similar electrophoretic behaviour (Fig. 2B).

When undenatured the complex mainly runs as a single pigmented band. No

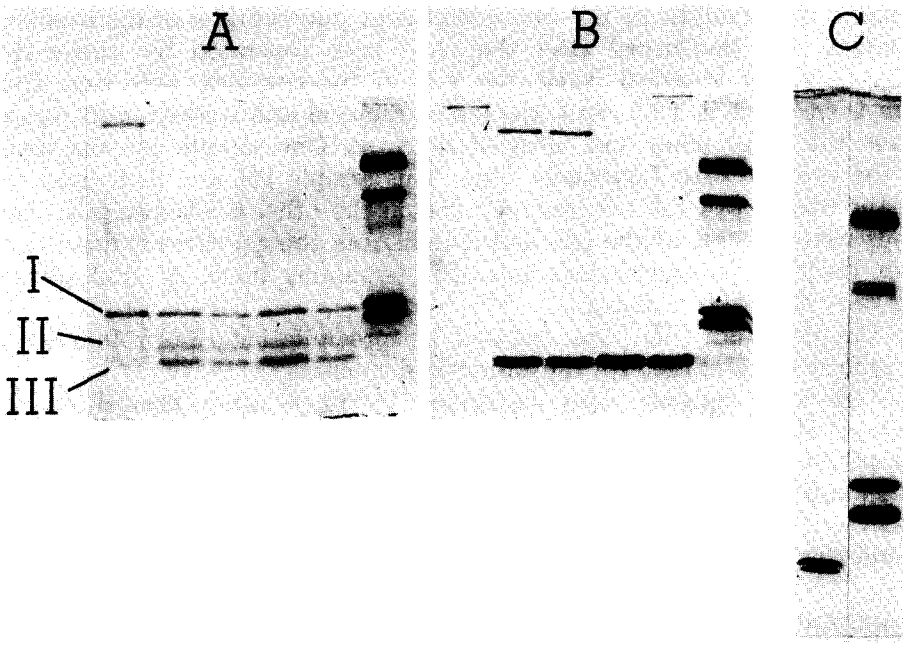

Fig. 2. SDS gradient polyacrylamide gels of the *B*-800—850 antenna pigment-protein complexes from *Rps. sphaeroides* and *Rps. capsulata*. A. An 8 cm slab gel of the *B*-800—850 antenna complex from *Rps. capsulata*. The right most track contained four standard proteins, bovine serum albumin (68 000), alcohol dehydrogenase (41 000), myoglobin (17 200), and equine cytochrome *c* (11 700). Samples were mixed with an equal volume of denaturing solution (50 mM Tris-HCl, pH 8.0, 2% (w/v) SDS, 10% glycerol, 2% β-mercaptoethanol and 0.1% (w/v) Bromophenol Blue) except the sample in the left hand track which was mixed with an equal volume of Tris-glycine, 0.1% SDS and 0.1% Bromophenol Blue, pH 8.3. Samples from left to right were treated as follows: no treatment, 15 min at 60°C, 30 min at 60°C, 1 min at 100°C and 3 min at 100°C. 12.5 μg protein was applied to each track. The left hand track shows a pigmented complex (95 000) and band I (14 000). All other tracks show bands I, II (10 000) and III (8000) of the complex. B. An 8 cm slab gel of the *B*-800—850 antenna complex from *Rps. sphaeroides*. Tracks contain samples denatured as described in A. 12.5 μg of protein were applied to each track. The left hand track shows a pigmented band (115 000). The next two tracks show a pigmented band (90 000) and two low molecular weight polypeptides (approx. 9000). C. A 14 cm slab gel of the *B*-800—850 antenna complex from *Rps. sphaeroides*. The left hand track contains 10 μg of *B*-800—850 complex after boiling in denaturing solution for 1 min. The two polypeptides have apparent molecular weights of approx. 8000 and 9000. The right hand track shows standard proteins as described in A.

polypeptide equivalent to the I polypeptide of the *Rps. capsulata* complex is detectable. Electrophoresis of the denatured complex from *Rps. sphaeroides* resolves two polypeptides, which appear to be present in equal amounts (Fig. 2B). The resolution is not very good as the two polypeptides have very similar molecular weights of approx. 9000. The two bands are not easily apparent if the gels are overloaded. However, improved resolution can be achieved by analysis on longer gradient gels (Fig. 2C).

Since the separation of the two polypeptides on SDS polyacrylamide gels was insufficient to allow us to isolate them for routine analysis, we have turned instead to isoelectric focussing.

Fig. 3 shows a typical gel of a denatured sample of the *B*-800—850 complex from *Rps. sphaeroides* after isoelectric focussing. Two bands, present in

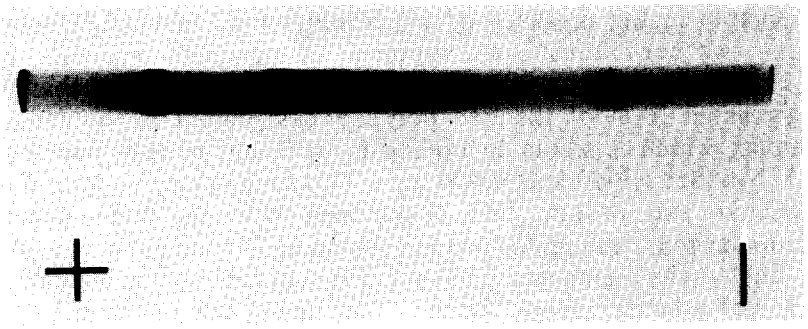

Fig. 3. An isoelectric focusing gel of the *B*-800—850 antenna pigment-protein complex from *Rps. sphaeroides*. A sample of the *B*-800—850 antenna complex was denatured and the isoelectric focusing was carried out with 2% (v/v) ampholines, pH range 3.5—10.0.

approximately equal amounts as judged by the intensity of staining, are very clearly resolved. Their isoelectric points are pH 7.2 ± 0.25 and pH 7.8 ± 0.2 (the average of five separate determinations). These are probably only apparent values, though, since they vary somewhat depending on how much of the ionic detergents (lauryl-dimethylamine-*N*-oxide and SDS) are carried over with the sample prior to electrophoresis.

It is clear from Figs. 2 and 3 that, as it is prepared, the *B*-800—850 complex from *Rps. sphaeroides* contains two types of polypeptide. However it is not trivial to try and show that they both belong to the native, functional antenna complex and that one of them is not just a contaminant, comparable to the I polypeptide in the *Rps. capsulata* *B*-800—850 complex.

To test this point more thoroughly, a sample of the undenatured *B*-800—850 complex from *Rps. sphaeroides* was electrophoresed on a 10% SDS polyacrylamide gel. The pigmented band (cf. Fig. 2B) was cut off the gel, the complex eluted, denatured and then subjected to isoelectric focussing. Again the same two isoelectric bands were detected, and again they were present in approximately equal amounts. Since it is unlikely that a contaminant would have remained stoicheiometrically bound to the native complex after electrophoresis in the presence of SDS, the best interpretation of this experiment is that the native complex consists of equal amounts of the two different polypeptides. This interpretation is strengthened by the result of SDS polyacrylamide gel electrophoresis on the native *B*-800—850 complex from *Rps. capsulata*. With this species the 'I' polypeptide copurifies with the native complex, but is clearly removed by the electrophoresis in SDS (Fig. 2A).

The loss of the 'I' polypeptide also seems to leave the *B*-800—850 complex from *Rps. capsulata* functionally unaffected, since its absence does not disrupt the delicate pigment-protein interactions of the native complex [9].

On occasions when 'old' samples of the *B*-800—850 complex from *Rps. sphaeroides* (which had been subjected to repeated freezing and thawing) were analysed on 10% SDS polyacrylamide gels two clearly resolved components were observed: one with the usual apparent molecular weight of approx. 9000 and another with a larger apparent molecular weight of approx. 12 000—13 000. The 12 000 band was eluted and isoelectric focussing per-

formed on this sample in the presence of 1% Nonidet P-40 and 9.6 M urea. In addition to the two normal bands a component of intermediate p*I* is now apparent. This 12 000 band is presumably an artefact in the sense that it represents an aggregated form of the two polypeptides present in the native *B*-800—850 complex which is stable to boiling in SDS and is only partially dissociated in 1% Nonidet NP-40 and 9.6 M urea. This finding may explain the results of Moskalenko and Erokin [24]. Sometimes when the undenatured complex is electrophoresed, staining for protein reveals an extra band on the gel just below that due to the pigmented band (cf. Fig. 1B depicted in Ref. 26). This, however, is not a contaminant. When it was eluted out of the gel and re-run on isoelectric focusing gels it yields the same two polypeptides as the native *B*-800—850 complex. This suggests that the second band represents a partially denatured state of the complex where the pigments have been stripped off but the two component polypeptides are still associated.

It is thus important to be aware of these unusual electrophoretic properties of the *B*-800—850 complex otherwise a whole series of artefactual and confusing bands can be obtained. Multiple bands upon isoelectric gels are often obtained from single homogenous polypeptides due to de-amidation or insolubility problems during the process of electrophoresis [25]. However, in this case, the possibilities seem untenable, since the constituent polypeptides are also separable on SDS gradient gels indicating that they have similar but differing molecular weights.

We have been able to confirm these observations by determination of the amino acid composition of the purified *B*-800—850 complex and also of its individual polypeptides isolated from isoelectric focusing gels. The data are presented in Table I.

The general features of the analysis indicate the *B*-800—850 complex from *Rps. sphaeroides* has a similar overall composition to those reported for light-harvesting protein-pigment complexes from related photosynthetic bacteria [3,5,7 and 10]. It is characterised by containing no cysteine residues and having a high glycine, alanine and proline content. As expected, the protein is hydrophobic in nature being rich in hydrophobic and neutral amino acids. The content of hydrophobic amino acids is 64.5%.

Analysis of the 2 constituent polypeptides demonstrates that they are closely related with similar amino acid compositions. However, the upper band (more alkaline p*I*) from isoelectric focussing gels apparently contains a number of additional residues, the 2 chains contain 62—63 and 54—56 amino acids, respectively (tryptophan not included). These values would suggest molecular weights of approx. 6400 and 5700 in reasonable agreement with the 9000, originally reported on 10% polyacrylamide gels [2]. From these figures it is clear why the 2 components are not readily separable in standard SDS-polyacrylamide gel systems.

As seen in Table I, there is good agreement between the value for total number of residues of each amino acid present in the native complex and the sum of the determinations from the constituent polypeptides. This is strong evidence for the occurrence of the subunits in a 1 : 1 relationship in the native light-harvesting complex.

In one or two instances, notably valine and tyrosine, it has not been possible

TABLE I

AMINO ACID COMPOSITION OF THE NATIVE *B* 800—850 LIGHT-HARVESTING COMPLEX AND ITS CONSTITUENT POLYPEPTIDES FROM *RPS. SPHAEROIDES* 2.4.1

The results presented in this table have been corrected for non-specific degradation by comparison of the analyses of the intact complex after 24, 48 and 72 h of hydrolysis. The number of residues in each individual polypeptide has been calculated from the mol % by dividing by half the phenylalanine value. This is because both the His and Arg mol % values are half the phenylalanine value and therefore there must be more than one phenylalanine per molecule. For the intact complex the number of residues has been doubled since we know there are 2 types of polypeptide chain present. The minimal molecular weights of the individual chains are approx. 5700 and 6400 (Trp not determined) and this gives the complex a minimal molecular weight of approx. 12 800.

Amino acid	Native *B* 800—850 complex		Lower isoelectric focusing		Upper isoelectric focusing	
	mol%	No. of residues (2 chains)	mol%	No. of residues (nearest whole number)	mol%	No. of residues
Lys	3.84	4.84 (5)	3.85	2.08 (2)	5.03	3.14 (3)
His	1.54	1.94 (2)	2.13	1.15 (1)	2.01	1.26 (1)
Arg	1.49	1.88 (2)	2.05	1.11 (1)	1.85	1.16 (1)
Asp	6.04	7.60 (8)	7.01	3.79 (4)	5.93	3.70 (4)
Thr	8.21	10.32 (10)	8.32	4.50 (4—5)	6.92	4.32 (4—5)
Ser	4.77	6.01 (6)	5.57	3.01 (3)	5.11	3.19 (3)
Glu	9.09	11.44 (11—12)	8.36	4.52 (4—5)	9.49	5.93 (6)
Pro	6.51	8.18 (8)	5.55	3.00 (3)	8.08	5.05 (5)
Gly	9.01	11.34 (11—12)	10.58	5.72 (6)	9.39	5.87 (6)
Ala	17.41	21.90 (22)	18.20	9.84 (10)	19.44	12.15 (12)
Cys	0	0	0	0	0	0
Val	9.47	11.92 (12)	7.10	3.84 (4)	6.23	3.89 (4)
Met	2.76	3.48 (3—4)	2.81	1.52 (2)	2.78	1.74 (2)
Ile	3.73	4.70 (4—5)	3.61	1.95 (2)	3.39	2.12 (2)
Leu	9.20	11.60 (11—12)	9.36	5.06 (5)	9.44	5.90 (6)
Tyr	3.74	4.71 (4—5)	1.79	0.97 (1)	1.71	1.07 (1)
Phe	3.18	4.00 (4)	3.70	2.00 (2)	3.19	2.00 (2)
Total residues		123—129		54—56		62—63

to account for all the residues present in the intact *B*-800—850 complex. These discrepancies may result from the technical difficulties involved in the analysis of the small amounts of polypeptide isolated from isoelectric focussing gels. However, they may also represent the genuine loss of a small hydrophobic fragment from the complex during the isoelectric focussing purification procedure. It will be particularly interesting to compare these data with the amino acid composition of the polypeptide(s) of *B*-870 complex from the same organism to see if they are related.

Careful analysis of the native *B*-800—850 complex reveals the presence of only one N-terminal amino acid, namely methionine. This strongly suggests that both polypeptides have identical N-termini (or one N-terminus is blocked). It is not yet clear whether the additional residues present in one polypeptide are present in a single extra sequence although it appears a strong possibility. Peptide mapping of the purified polypeptides is currently being undertaken to determine whether the two polypeptides are indeed closely related.

Our amino acid composition data is only qualitatively similar to an amino acid composition reported previously by Fraker and Kaplan [10]. In particular, these authors found histidine to be absent from their organic-soluble poly-

peptide although in other respects e,g, lack of cysteine and high alanine and proline content, some general areas of similarity can be observed with our own analysis. There is also agreement on methionine as the N-terminal amino acid. The reasons for these discrepancies are not clear.

Acknowledgements

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