Isolation, Characterization and Topographical Relationships of Pigment-protein Complexes from Membranes of *Rhodomicrobium vannielli*

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Pigment–protein complexes from *Rhodomicrobium vannielli* were prepared by detergent solubilization of intra-cytoplasmic membranes followed by gel electrophoresis or sucrose gradient centrifugation. These procedures gave rise to two native pigmented complexes. The major one (designated B800-865) was associated with two polypeptides of $M_r$ 11 000 and 13 000 and was identified with the 'accessory' light-harvesting complex II found in other members of the *Rhodospirillaceae*. The minor complex (designated B885-RC) contained both reaction centre and light-harvesting bacteriochlorophyll. Detergent fractionation and reversible chemical cross-linking, followed by two-dimensional polyacrylamide gel electrophoresis, indicated a specific relationship between a membrane-bound cytochrome c-553 and the $M_r$ 31 000 subunit of the reaction centre.

INTRODUCTION

The photosynthetic apparatus in members of the *Rhodospirillaceae* consists of a series of pigment–protein complexes which are localized in either the cytoplasmic membrane or a system of intra-cytoplasmic membranes (ICM) derived from it (Drews & Oelze, 1981). Information regarding the nature of these complexes has come largely from work with *Rhodopseudomonas sphaeroides* and *Rhodopseudomonas capsulata* (Kaplan, 1981). These microbes contain a vesicular ICM system in which the photosynthetic unit is comprised of two types of light-harvesting (LH) antenna complex (B870 or LHI and B800-850 or LHII, according to their *in vivo* absorption maxima) combined with the photochemical reaction centre (Lien et al., 1973; Sauer & Austin, 1978).

Although a considerable amount of data is now available on the structure, composition and development of the photosynthetic apparatus in *R. sphaeroides* and *R. capsulata* (Aagaard & Sistrom, 1972; Drews & Oelze, 1981; Kaplan, 1981; Kaplan et al., 1983), less is known about these aspects in other members of the *Rhodospirillaceae*, particularly those with lamellate ICM systems. Within this group, pigment–protein complexes have been isolated from *Rhodopseudomonas palustris* (Firsova & Drews, 1977; Hayashi et al., 1982a, b; Varga & Staehelin, 1985), *Rhodopseudomonas acidiphila* (Cogley et al., 1983) and the bacteriochlorophyll b containing *Rhodopseudomonas viridis* (Jay et al., 1984; Peters et al., 1984). Such studies have revealed marked differences in the composition of these complexes, both between these species and in comparison with those of *R. sphaeroides* and *R. capsulata*.

In this paper we report on the characterization of pigment–protein complexes from *Rhodomicrobium vannielli*, a member of the *Rhodospirillaceae* which exhibits extensive cellular differentiation (Whittenbury & Dow, 1977; Dow et al., 1983; Kelly & Dow, 1984), and which has a lamellate ICM system (Conti & Hirsch, 1965). Our data indicate that, despite similarities in the cell cycles and physiology of the prosthecate and budding *Rhodospirillaceae*, *Rm. vannielli* displays several unique properties in its photosynthetic apparatus, some of which are also distinct from the *R. sphaeroides* and *R. capsulata* group.

**Abbreviations:** Bchl, bacteriochlorophyll; DTSP, dithiobis(succinimidyl propionate); ICM, intra-cytoplasmic membranes; LH, light-harvesting; RC, reaction centre; TMBZ, 3,3',5,5'-tetramethylbenzidine.
METHODS

Organism and growth conditions. Rhodomicrobium vannieli strain Rm5 from the University of Warwick culture collection was used throughout. Bacteria were grown in PM medium (Whittenbury & Dow, 1977) containing (g l\(^{-1}\)); NH\(_4\)Cl, 0.5; MgSO\(_4\), 7H\(_2\)O, 0.4; NaCl, 0.4; CaCl\(_2\), 2H\(_2\)O, 0.05; sodium pyruvate, 1.5; sodium hydrogen malate, 1.5. The pH was adjusted to 6.8 before autoclaving and K\(_2\)HPO\(_4\)/KH\(_2\)PO\(_4\) buffer was added aseptically to a final concentration of 5 mm. Quickfit flasks containing 5 l PM medium were capped with Suba-seals (William Freeman & Co., Barnsley, UK), the headspace gassed with O\(_2\)-free N\(_2\) (15 min), inoculated (0.2\%, v/v) and incubated with stirring at 30 °C under an incident light intensity of 1000 lx from tungsten lamps. Cultures were grown to the late exponential phase (OD\(_{730}\) 1.0-2.0), and harvested by centrifugation (20000 g, 4 °C for 20 min). Cell pellets were stored at -20 °C.

Isolation of ICM. Cells were resuspended in 10 mM-Tris/HCl buffer pH 7.4 and broken by two passages through a French press (137 MPa). Unbroken cells and debris were removed by centrifugation (30000 g, 4 °C for 30 min) and the supernatant (3 ml) was layered onto a 3 ml cushion of 25% (w/v) sucrose in 10 mM-Tris/HCl pH 7.4. After centrifugation (100000 g, 4 °C for 5 h) in the SW28 rotor of a Beckman L8 ultracentrifuge, the membrane pellet was resuspended in 10 mM-Tris/HCl pH 7.4, or 50 mM-triethanolamine buffer pH 8.3 (for cross-linking studies) to a protein concentration of 15-20 mg ml\(^{-1}\) and samples were stored at -20 °C.

Isolation of pigment–protein complexes. The method of Hayashi et al. (1982a) was modified as follows. An equal volume of 68 mM-Tris/HCl pH 6.8 containing 1.6% (w/v) SDS and 1.6% (w/v) Triton X-100 was added to a membrane suspension containing 15-20 mg protein ml\(^{-1}\). After 5 min incubation at room temperature, the mixture was microfuged for 5 min and the supernatant retained. To study the effects of different detergents, the same solubilization procedure was followed, except that either SDS or Triton X-100 alone (0.8% w/v, final concentration) was used. For electrophoresis, 40 μl of the supernatant plus 10 μl 75% (v/v) glycerol was applied to a 10% (w/v) polyacrylamide slab gel with a 3% (w/v) polyacrylamide stacker (Laemmli, 1970). Both gels and the running buffer contained either 0.1% (w/v) SDS or 0.05% (w/v) each of SDS and Triton X-100. Samples were electrophoresed (25 mA for 6 h at 4-10 °C), and the pigmented bands cut out and homogenized in cold 10 mM-Tris/HCl pH 7.4. Larger amounts of the complexes were prepared by layering 300 μl ICM solubilized with SDS plus Triton X-100 onto a 10-40% (w/v) sucrose step gradient [1.1 ml each of 10, 20, 30 and 40% (w/v) sucrose in 10 mM-Tris/HCl pH 7.4 plus 0.05% (w/v) each of SDS and Triton X-100] followed by centrifugation (100000 g for 16 h at 4 °C) in the SW50.1 rotor of a Beckman L8 ultracentrifuge. Gradients were fractionated (150 μl samples) from the top, sucrose concentrations were determined by refractometry and absorbance values determined on an LKB Ultraspec spectrophotometer. For cross-linking studies, complexes prepared on sucrose gradients were dialysed against 50 mM-triethanolamine buffer pH 8.3 containing 0.05% (w/v) each of SDS and Triton X-100.

Spectrophotometry. Absorption spectra were measured on a Pye-Unicam SP8-200 double beam spectrophotometer in 1 cm light path cuvettes. To measure absorption changes due to chemical oxidation of the isolated complexes (Picorel et al., 1984), 1 mM Fe(CN)\(_6\) in 10 mM-Tris/HCl buffer pH 7.4 was added to the sample cuvette to a final concentration of 50 mM and absorption spectra were recorded at intervals. Cytochrome difference spectra were recorded with a few crystals of sodium dithionite as reductant.

Analytical gel electrophoresis. Proteins were analysed on 10-30% (w/v) exponential gradient polyacrylamide gels (Hames & Rickwood, 1981). Samples were denatured in 62.5 mM-Tris/HCl pH 6.8, 2% (w/v) SDS, 5% (w/v) 2-mercaptoethanol, 10% (w/v) glycerol and 0.01% (w/v) bromophenol blue (Laemmli sample buffer; Laemmli, 1970) by heating at 75 °C for 2 min. Gels were run at 20 mA constant current overnight in the cold, fixed in 50% (v/v) methanol and silver-stained according to Wray et al. (1981). For two-dimensional analysis of pigment–protein complexes, 45 μl ICM solubilized with SDS plus Triton X-100 was electrophoresed (10 mA for 2 h at 4 °C) on a 6% (w/v) polyacrylamide tube gel (10 cm long, 2 mm diameter) with a 3% (w/v) polyacrylamide stacking gel, each gel containing 0.05% (w/v) each of SDS and Triton X-100. After soaking in Laemmli sample buffer at 60 °C for 5 min the tube gel was sealed to the stacker of a 10-30% (w/v) polyacrylamide gradient slab gel with 1% (w/v) agarose dissolved in sample buffer. Electrophoresis in the second dimension was at 14 mA for 16 h at room temperature.

Reversible chemical cross-linking. The method of Peters & Drews (1983) was modified for use with first dimension polyacrylamide tube gels as follows. Membranes resuspended in 50 mM-triethanolamine buffer pH 8.3 (200 μg protein in 100 μl) were cross-linked with dithiobis(succinimidyl propionate) (DTSSP; 5 mM final concentration) dissolved in dimethyl sulphoxide (DMSO). After 1 h at room temperature, 33 μl stop buffer [5 mM Tris/HCl pH 6.8, 8% (w/v) SDS, 5% (w/v) glycerol, 0.01% (w/v) bromophenol blue] was added, the mixture was heated at 75 °C for 2 min and 50 μl samples were electrophoresed (6 mA for 2 h at 4 °C) on 10% (w/v) polyacrylamide tube gels (10 cm long, 2 mm diameter) with a 3% (w/v) polyacrylamide stacking gel. Cross-links were cleaved in the presence of 2-mercaptoethanol by equilibration (30 min) in Laemmli sample buffer. After sealing the tube gel to the stacker of a 10-30% (w/v) polyacrylamide gradient gel, as above, electrophoresis in the second dimension was done at 14 mA for 16 h at room temperature. The B885-RC complex was cross-linked in the same manner except that the DTSSP concentration was 1 mM and the reaction time 10 min. The final DMSO
Fig. 1. Isolation of pigment–protein complexes by PAGE (a) with both SDS and Triton X-100 present and (b) with only SDS in the gel and running buffer. Tracks 1 and 4, ICM solubilized with 0.8% (w/v) each of SDS and Triton X-100; track 2, ICM solubilized with 0.8% (w/v) Triton X-100; track 3, ICM solubilized with 0.8% (w/v) SDS. The gel was photographed unstained.

concentration in both cases was 5% (v/v). These conditions of cross-linking did not result in any change in the absorption spectra of membranes or complexes.

**Analytical methods.** Polyacrylamide gels were stained for haem-associated peroxidase activity according to the method of Thomas et al. (1976). Fractions from sucrose gradients were assayed in a reaction mixture containing 0.98 ml 1.9 m-3,3',5,5'-tetramethylbenzidine (TMBZ) in 30% (v/v) methanol/0.175 M-sodium acetate buffer pH 5.0, 10 μl of the appropriate fraction and 10 μl 30% (v/v) H₂O₂. The A₆₉₀ was measured immediately against a blank containing only TMBZ and H₂O₂.

Protein was determined using the Folin phenol reagent according to the method of Lowry.

**Chemicals.** SDS was from Bio-Rad, Triton X-100 from BDH, DTSP from Pierce Eurochemie, TMBZ from Sigma and acrylamide from Eastman-Kodak. All other chemicals were of the highest purity available. Unstained molecular weight standards were obtained from Pharmacia and were used to calibrate pre-stained standards from BRL as described by Tsang et al. (1984).

**RESULTS**

**Isolation of pigment–protein complexes.** Two native pigmented bands were resolved upon low temperature PAGE of *Rm. vannielii* ICM treated with a mixture of SDS and Triton X-100, provided both detergents were present in the gel and the running buffer (Fig. 1 a). Similar results were obtained in this gel system when SDS or Triton X-100 alone were used for solubilization, although the resolution with Triton X-100 was less satisfactory (Fig. 1 a). However, if the gel and running buffer contained only SDS, a number of other, more diffuse pigmented bands were observed (Fig. 1 b). As these results indicated that Triton X-100 was required to maintain the stability of the complexes, the latter were characterized further using solubilization and PAGE in the presence of both detergents.

**Absorption spectra.** In order to identify the spectral forms of bacteriochlorophyll (Bchl) contained within the complexes, they were eluted from the gel by homogenization in Tris/HCl buffer and room temperature absorption spectra were obtained (Fig. 2). The upper pigmented band, which migrated with an apparent Mᵦ of 150000 in the SDS plus Triton X-100 gel system, exhibited three peaks in the IR region of the spectrum (760, 800 and 885 nm). As in other members of the *Rhodospirillaceae* (Drews & Oelze, 1981), these could be ascribed to reaction centre (RC) Bchl (800 nm), RC bacteriopheophytin (760 nm) and a larger concentration of 'B885' light-harvesting Bchl (A₈₈₅ : A₈₀₀ = 3–6).
Fig. 2. Room temperature absorption spectra of (a) ICM, (b) the lower pigmented band and (c) the upper pigmented band from ICM solubilized with SDS plus Triton X-100 followed by gel electrophoresis with both detergents.

The lower pigmented band migrated with an apparent $M_r$ of 90,000 and exhibited two peaks in the IR region at 800 nm and 865 nm ($A_{865}:A_{800} = 1.5-3.0$). This complex was accordingly designated B800–865. Both complexes also exhibited carotenoid absorption maxima at wavelengths (460, 490 and 525 nm) that were identical to those of the intact membrane (Fig. 2).

Larger quantities of these pigment–protein complexes were prepared by subjecting ICM solubilized with SDS plus Triton X-100 to sucrose gradient centrifugation instead of PAGE. This procedure resulted in the formation of two pigmented bands, spectrally identical to the complexes B800–865 (density 1.07 g cm$^{-3}$) and B885-RC (density 1.10 g cm$^{-3}$). Such material was used in all further experiments.

**Ferricyanide induced absorption changes.** Chemical oxidation of Bchl $a$ in isolated pigment–protein complexes has been used as a probe in characterization (Picorel et al., 1984). The ferricyanide induced changes in the absorption spectra of the B885-RC and B800–865 complexes from *Rm. vannielii* were quite different (Fig. 3). Oxidation of the former complex resulted in a rapid decline of the 885 nm peak coupled with a blue shift of 10 nm while both the 760 nm and 800 nm peaks remained unaltered. In contrast, the 800 nm band of the B800–865 complex was very sensitive to oxidation while the 865 peak was only moderately sensitive and was blue-shifted by only 2–3 nm.

**Polypeptide composition.** The polypeptide compositions of the isolated complexes are compared with those of the intact ICM in Fig. 4. The B800–865 complex contained only two polypeptides of $M_r$ 11,000 and 13,000, while the B885-RC complex contained six proteins. Five of these could be attributed to the RC triplet ($M_r$ 26,000, 28,000 and 31,000) and the B885 complex itself ($M_r$ 12,000 and 14,000), as has been found in other members of the *Rhodospirillaceae* (Drews & Oelze, 1981). However, a striking difference was observed in the ease with which these pigment–protein complexes were denatured. While incubation at room temperature in Laemmli sample buffer was sufficient to effect denaturation of the B885-RC complex, the B800–865 complex required heating at 75 °C or above for full denaturation. These differences were also evident in the polypeptide profiles of ICM treated in a similar manner (Fig. 4).
Fig. 3. Chemical oxidation of the isolated pigment–protein complexes with 50 mM-potassium ferricyanide for (a) the B885-RC complex and (b) the B800–865 complex. The numbers with arrows refer to the time (min) after the addition of ferricyanide.

Fig. 4. Polypeptide composition of the B885-RC complex (tracks 1 and 2), ICM (tracks 3 and 4) and the B800–865 complex (tracks 5 and 6). In tracks 1, 3 and 6, the samples were not heated in the Laemmli sample buffer before application to the gel. In tracks 2, 4 and 5, the samples were heated at 75 °C for 2 min to effect complete denaturation.

Association of cytochrome with the B885-RC complex. The B885-RC complex also contained a polypeptide of $M_r$ 38 000 which, apart from the light-harvesting polypeptides, appeared to be the most abundant membrane protein (Fig. 4). This protein was identified as a cytochrome by staining gels for haem-associated peroxidase activity (Fig. 5). In membrane preparations, three bands of $M_r$ 38 000, 32 000 and 28 000 reacted positively, while in the B885-RC complex only the polypeptide of $M_r$ 38 000 reacted. Although the presence of mercaptoethanol is often inhibitory to the detection of cytochrome by the method of Thomas et al. (1976), we found no increase in
sensitivity in its absence, but instead observed a decreased resolution of some protein bands, especially that of the 38000 \( M_r \) cytochrome (Fig. 5). Dithionite-reduced minus air-oxidized difference spectra showed the presence of a \( c \)-type cytochrome in the B885-RC complex, with an \( \alpha \)-peak at 553 nm. Several lines of evidence suggested that this cytochrome was an integral part of the isolated complex and not merely a contaminant. Firstly, a peak of haem-associated peroxidase activity was observed when ICM were solubilized with SDS and Triton X-100 and layered on a 10−40% (w/v) sucrose gradient. This peak corresponded with the B885-RC complex. Similarly, a modification of the Triton X-100 plus SDS PAGE system to include a second dimension separation under denaturing conditions (Fig. 6) did not reveal any complex containing this cytochrome other than that which also contained the RC and B885 polypeptides. In addition, chemical cross-linking studies (see below) indicated a specific relationship between the cytochrome \( c \)-553 and the RC.

Chemical cross-linking studies. Reversible chemical cross-linking procedures are an accepted method of probing topographical relationships within photosynthetic membranes and complexes (Peters et al., 1983). We used the hydrophobic reagent DTSP combined with the two-dimensional diagonal mapping technique described by Peters & Drews (1983) to study such relationships in membranes and the isolated B885-RC complex of \( Rm. \) vannielii (Fig. 7).

At pH 8.3, homo-oligomers of the LH polypeptides in the B885-RC complex were undetectable above the presumed dimer (Fig. 7b) and we observed specific vertical relationships between the smaller B885 polypeptide \( (M_r, 12000) \) and the 28000 \( M_r \) RC subunit in both membranes (Fig. 7a) and the B885-RC complex itself (Fig. 7b). A relationship between the RC subunits of \( M_r, 26000 \) and 28000 was also evident in both cases. Most interesting, however, was the degree of cross-linking observed between the 31000 \( M_r \) RC subunit and the 38000 \( M_r \) cytochrome \( c \)-553. The cross-linked product had an \( M_r \) or approximately 68000 from which a
Pigment-protein complexes of *Rm. vannielii*

Fig. 6. Two-dimensional gel analysis of pigment–protein complexes. Membranes solubilized with SDS plus Triton X-100 were electrophoresed in the first dimension on a 6% (w/v) tube gel at 4 °C. The gel was photographed unstained and then heated for 5 min in Laemmli sample buffer (60 °C) before electrophoresis in the second dimension, followed by silver staining.

stoichiometry of 1:1 may be deduced (Fig. 7b). With the intact complex, the yield of this cross-linked product was quite high, judging from the amount of the two polypeptides left on the diagonal. The relationship between the 38 000 *M*, cytochrome and the 31 000 *M*, RC subunit was also observed with intact membranes cross-linked with 5 mM-DTSP (Fig. 7a). Variation of the DTSP concentration between 1 and 10 mM gave the same cross-linking patterns with membranes, and control experiments in the absence of DTSP showed no spots below the diagonal (data not shown).
Fig. 7. Reversible chemical cross-linking patterns of (a) ICM and (b) the B865-RC complex. Cross-links in the first dimension were cleaved by 2-mercaptoethanol (2-Me) in the second dimension and the products analysed by silver staining. Vertical arrows indicate cross-linked polypeptides.
Pigment–protein complexes of \textit{Rm. vannielii}

\textbf{DISCUSSION}

The fact that the 870 nm IR peak of the \textit{Rm. vannielii} ICM lies between the 865 and 885 nm peaks of the complexes is suggestive of the preservation of pigment–protein interactions during their isolation. Interestingly, the carotenoid absorption maxima of both membranes and isolated complexes were identical, unlike \textit{R. sphaeroides}, in which RC and B875 associated carotenoids are blue-shifted by 7–10 nm (Broglie \textit{et al.}, 1980).

The greater number of pigmented bands observed during low temperature PAGE of solubilized membranes in the presence of SDS alone is in accord with the results of Broglie \textit{et al.} (1980) for \textit{R. sphaeroides}, where the bands consisted largely of mixtures of the different complexes in various proportions. Our data, and those of others (Helenius \& Simons, 1975; Hayashi \textit{et al.}, 1982\textit{a}), indicate a stabilizing effect of Triton X-100, and it is clear that conclusions about the in \textit{vivo} organization of the photosynthetic apparatus based on such gel patterns must take account of the possibility of micellar artefacts. Accepting this caveat, the dual detergent method used here for the isolation of pigment–protein complexes from \textit{Rm. vannielii} suggests that the membrane bound photopigments are organized into two major complexes, one of which contains the RC. Previous studies of the general structure of the photosynthetic apparatus in members of the \textit{Rhodospirillaceae} (Monger \& Parson, 1977; Firson \& Drews, 1977; Feick \textit{et al.}, 1980; Drews \& Oelze, 1981) have revealed the existence of both reaction centre associated (LHI) and accessory (LHII) antenna complexes with which the B885 and B800–865 components of \textit{Rm. vannielii} may be identified. However, it is now known that several subclasses of LHI and LHII complexes exist (Thornber \textit{et al.}, 1983). Amongst the LHI group both B890- and B875-protein complexes have been identified while two types of 'B800–850' complex, differing in the ratios of their near IR peak heights, characterize the LHII group. The B800–820 complex of \textit{Chromatium} and \textit{R. acidophila} also belong to the latter group (Thornber \textit{et al.}, 1983; Cogdell \textit{et al.}, 1983). From the near IR absorption spectrum, the \textit{Rm. vannielii} LHI complex most closely resembles the B890 class, while a type I 'B800–850' (LHII) complex is also present, with the long wavelength band shifted to 865 nm. This combination is unusual amongst the prosthecate and budding members of the \textit{Rhodospirillaceae}. The physiologically similar \textit{R. palustris} has a B875 LHI complex, associated with one (Firson \& Drews, 1977) or possibly two (Varga \& Staehelin, 1985) polypeptides and the 800 and 850 nm LHII peak heights do not exhibit a constant ratio to each other, as in the archetypal type I LHII complex from \textit{R. sphaeroides} (Hayashi \textit{et al.}, 1982\textit{a, b}; Varga \& Staehelin, 1983). In addition, the B890 complex of \textit{R. acidophila} is not associated with a type I B800–850 accessory LHII complex alone (Cogdell \textit{et al.}, 1983).

The redox properties of the pigment–protein complexes from \textit{Rm. vannielii} were not, however, particularly unusual. The relative susceptibility of the IR peaks of the isolated pigment–protein complexes to chemical oxidation is in part a reflection of their monomeric or dimeric bacteriochlorophyll structure (Picorel \textit{et al.}, 1984). The ease of oxidation of the 800 nm band of the LHII complex of \textit{Rm. vannielii} suggests it to be monomeric while the 865 nm peak is probably dimeric, as in other LHII complexes (Sauer \& Austin, 1978; Picorel \textit{et al.}, 1984). Similarly, the 760 and 800 nm peaks of the LHII–RC complex are resistant to oxidation, consistent with a dimeric structure for these RC bacteriopheophytin and Bchl peaks (Feher \& Okamura, 1978). However, more detailed studies involving circular dichroism spectra will be needed to confirm these data and to resolve the structure of the 885 nm LHI peak.

In all species of the \textit{Rhodospirillaceae} so far examined, the light-harvesting photopigments are bound to very low molecular weight polypeptides (Drews \& Oelze, 1981). Consistent with this, the four light-harvesting proteins from \textit{Rm. vannielii} have \textit{M}*, values of 11–14,000, but these values are probably over-estimates, as it is known that such proteins exhibit anomalous migration in SDS polyacrylamide gels (Tadros \textit{et al.}, 1983). The \textit{M}*, values of the RC triplet (26,000, 28,000 and 31,000) are subject to the same constraints, but are comparable to those of other members of the \textit{Rhodospirillaceae} (Feher \& Okamura, 1978) and most probably correspond to the L, M and H subunits of the archetypal \textit{R. sphaeroides} RC.

We have not attempted to isolate the RC and B885 complexes separately in this study. Instead we wished to use the complementary approaches of detergent fractionation and reversible
chemical cross-linking to probe relationships between these components and the membrane bound c-type cytochrome associated with them. The cytochrome composition of *Rm. vannielli* has been studied (Morita & Conti, 1963; Bartsch, 1978) and a loosely bound or soluble c2 type cytochrome (c-550) is present in addition to the membrane bound c-553. We have shown that the cytochrome c-553 is a component of the B885-RC complex. Interestingly, however, RC or RC-LHI complexes from other Bchl a containing members of the *Rhodospirillaceae* do not appear to contain cytochrome (Feher & Okamura, 1978; Gingras, 1978; Firsow & Drews, 1977; Hayashi et al., 1982b) but such preparations from the Bchl b containing *R. viridis* (Pucheu et al., 1976; Jay et al., 1984), *Thiocapsa pfennigii* (Sefor & Thornber, 1984) and several other Bchl a containing members of the *Chromatiaceae* (Lin & Thornber, 1975; Bartsch, 1978; Lefebvre et al., 1984) do contain multi-haem cytochrome c-552–558 bound to a polypeptide of M, 35–45,000. However, only in *R. viridis* have cross-linking studies been done to investigate the spatial relationships between the RC and this c-type cytochrome (Peters et al., 1984) and these have indicated binding to the 27,000 M, (M) RC subunit. In *Rm. vannielli* cytochrome c-553 is bound to the 31,000 M, RC subunit which most probably corresponds to the H subunit of the *R. capsulata*/R. viridis RC. In addition, the smaller LHI polypeptide (M, 12,000) of *Rm. vannielli* cross-links with the 28,000 M, (M) RC subunit, while in both *R. capsulata* and *R. viridis* cross-linking to the H subunit was observed (Peters et al., 1984). These data indicate that in *Rm. vannielli* interactions between LHI and RC Bchl are mediated by the 28,000 M, (M) RC subunit while the 31,000 M, (H) RC subunit could co-ordinate cytochrome–RC interactions. That these relationships were observed in both membranes and the isolated complex lends further credence to this view and it may not be incompatible with previously suggested roles for the H subunit in *R. sphaeroides* (Feher & Okamura, 1978) and *R. capsulata* (Peters et al., 1983) in binding proteins associated with the electron transport chain.

The photosynthetic apparatus of *Rm. vannielli* has a unique set of properties, some of which are more akin to those of the Bchl b containing *R. viridis* than to other Bchl a containing members of the *Rhodospirillaceae*. In this respect the phylogenetic relationship between these two microbes may be of importance (Gibson et al., 1979). It is interesting that, despite fundamental similarities in the cell cycles and physiology of many members of the *Rhodospirillaceae* (Whittenbury & Dow, 1977; Kelly & Dow, 1984), individuality is expressed in the composition and interactions in so conserved a structure as the photosynthetic apparatus. The ease of preparation of the pigment–protein complexes from *Rm. vannielli* in potentially large quantities by sucrose gradient centrifugation may be useful in further studies on their biosynthesis or structure. Most importantly, as it is possible to obtain large synchronous populations of *Rm. vannielli* swarmer cells (Whittenbury & Dow, 1977), there is the possibility of studying the development of the photosynthetic apparatus in selection synchronized cultures. We believe that the photosynthetic prosthecate and budding bacteria possess distinct advantages for such studies and that they will assume increasing importance in the future as alternatives to *R. sphaeroides* and *R. capsulata* where culture heterogeneity may be problematic (Gest et al., 1983; Pellerin & Gest, 1983; Kelly & Dow, 1984).

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