Energetic and Topographic Properties of a *Rhodopseudomonas capsulata* Mutant Deficient in the B870 Complex

W. J. Jackson,* R. C. Prince, G. J. Stewart,‡ and B. L. Marrs§
Biological Sciences Laboratory, Exxon Research and Engineering Company, Annandale, New Jersey 08801
Received May 2, 1986; Revised Manuscript Received August 6, 1986

ABSTRACT: The energetic and topographic properties of a photosynthesis-deficient mutant of *Rhodopseudomonas capsulata*, PBS108, were examined by a variety of methods. Spectral and physical analyses indicate that chromatophores obtained from PBS108 contain no B870 complex. Kinetic absorption spectra reveal that reaction centers in PBS108, although functional, are not coupled energetically either to cytochrome c_2 and the cytochrome bc_1 oxidoreductase, which are its normal reaction partners, or to the B800–850 complex. Whereas photooxidized reaction centers in the wild type are rereduced by cytochrome c_2 associated with the periplasmic membrane surface, approximately 80% of the reaction centers in the mutant are accessible to cytochrome *c* from the cytoplasmic side. Although the carotenoids of the B800–850 complex respond to transmembrane potentials with their usual electrochromic shift, there is no light-induced response, indicating that electron transfer in the reaction center does not contribute to a transmembrane potential in PBS108. Protease sensitivity experiments using purified chromatophores suggest a change in the membrane topography of reaction centers. These data suggest that reaction centers are not properly assembled into the photosynthetic membrane in the absence of B870.

Photosynthesis in the purple non-sulfur photosynthetic bacteria depends upon two distinct types of pigment–protein complexes: reaction centers (RCs)[1] and antenna complexes [for reviews, see Drews (1985), Okamura et al. (1982), and Parson & Ke (1982)]. The antennae or light-harvesting (LH) complexes direct absorbed light energy to the reaction center where primary photochemistry occurs (Knox, 1975). Excitation energy reaching "open" reaction centers is quickly trapped and oxidizes a special bacteriochlorophyll (BChl) dimer (Clayton, 1978). The ejected electron temporarily resides on an intermediate acceptor believed to be a molecule of bacteriopheophytin (Tiede et al., 1976). This charge separation, the primary photochemical event, is stabilized by the rapid transfer of the electron to the primary acceptor, an iron–quinone complex, and the rereduction of the oxidized BChl dimer by cytochrome c_2 (Dutton et al., 1975; Kirmaier et al., 1985). Cyclic electron flow is completed by the cytochrome bc_1 complex, and the free energy initially present in the absorbed photon is conserved in the form of a transmembrane proton gradient (Dutton & Prince, 1978). A proton-driven ATPase couples the dissipation of this electrochemical gradient to the synthesis of ATP.

In *Rhodopseudomonas capsulata*, both RC and LH complexes contain BChl and carotenoid. These chromophores are bound noncovalently to specific hydrophobic polypeptides embedded in the photosynthetic membrane. Each RC contains three different polypeptide subunits in a stiochiometry of 1:1:1. These subunits, designated H, M, L on the basis of their relative mobility in denaturing gels (28, 26, and 21 kDa, respectively), have been extensively characterized and sequenced at both the protein and DNA levels (Sutton et al., 1982; Williams et al., 1983; Youvan et al., 1984). Together, the L and M subunits bind all the pigments and cofactors found in the RC. Purified L/M complexes have spectral characteristics similar to those of the native RC and perform primary photochemistry in the absence of H (Okamura et al., 1974; Feher & Okamura, 1978). The function of the H subunit is not completely understood. H does form contacts with certain LH polypeptides and may also interact with the bc_1 oxidoreductase and ATPase (Peters et al., 1983; Reed et al., 1975; Valkirs, 1976).

While photosynthetic bacteria contain only a single type of RC, the LH complexes can be subdivided into two groups on the basis of their near-infrared absorption spectrum and relative abundance (Belasco et al., 1985; Cogdell & Thornber, 1980). The B870 complex has a single BChl absorption maxima around 870 nm and is present in a fixed stoichiometry of approximately 20 B870 BChl molecules per RC (Kaufman et al., 1982). In contrast, the amount of B800–850, which has two absorption maxima (800 and 850 nm), varies inversely with the incident light intensity (Golecki, 1980). Both LH complexes consist of two homologous but distinct pigment-binding polypeptides, the α and β subunits, having molecular masses ranging from 6 to 12 kDa in denaturing gels (Peters & Drews, 1983b; Tadros et al., 1982). All four LH subunits have one central hydrophobic region that probably forms a transmembrane α-helix. One (α) or two (β) conserved histidine residues are present in these segments and are thought to bind and orient BChl in the protein (Eccles & Honig, 1983; Tadros et al., 1983). The B800–850 complex also contains a third, larger (14 kDa), polypeptide of unknown function (Feick & Drews, 1979). Analysis of isolated LH complexes indicates that the α and β subunits are present in equimolar amounts and bind two to three molecules of BChl and one to two molecules of carotenoid per $\alpha\beta$ pair (Shiozawa et al., 1982).

‡Present address: Department of Biology, University of South Florida, Tampa, FL 33620.
§Present address: Central Research and Development Department, Agricultural Research Station, E. I. du Pont de Nemours and Co., Wilmington, DE 19898.

[1] Abbreviations: RC, reaction center; *Rps.*, *Rhodopseudomonas*; LH, light harvesting; BChl, bacteriochlorophyll; EDTA, ethylenediaminetetraacetic acid; SDS, sodium dodecyl sulfate; PS^-, photosynthesis deficient; kDa, kilodalton; PMSF, phenylmethanesulfonyl fluoride; GTA, gene transfer agent; MOPS, 3-(*N*-morpholino)propanesulfonate; PAGE, polyacrylamide gel electrophoresis.

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Although the composition of the RC and both LH complexes has been determined, much less is known about energy transfer between components or their overall membrane organization. Kinetic fluorescent emission studies suggest that excitation energy is channeled from the LH complexes to the RC via the long-wavelength antenna, i.e., from the B800–850 complex to the B870 complex and then to the RC (Monger & Parson, 1977; van Grondelle et al., 1983). Similarly, chemical cross-linking, immunofractionation, and nearest-neighbor analysis indicate that RCs are surrounded and interconnected by oligomers of the B870 complex (Peters & Drews, 1983a; Valkirs & Feher, 1982). Fluorescence polarization data indicate that only a few of these B870 complexes (15%) are in direct contact with the RC (Kramer et al., 1984). The concentric B870–reaction center assemblies are themselves surrounded and interconnected by oligomers of the B800–850 complex to form a large photosynthetic array. From these studies, it appears that B870 functions as an antenna complex and an intermediate in energy flow from the B800–850 apparatus to the RC. To further investigate the role of B870 in energy transfer and assess its contribution to the overall topography of the photosynthetic apparatus, we have examined a mutant of *Rps. capsulata* which lacks the B870 complex.

Materials and Methods

Bacterial Strains and Growth Conditions. All strains used in this study are derivatives of *Rps. capsulata* [*Rhodobacter capsulatus* in the taxonomy of Imhoff et al. (1984)] B10. The photosynthetic mutant PAS108 (*rxc*A-108, *hsd*-1, StrR) was derived from PAS100 (*hsd*-1, StrR) by tetracycline suicide (Taylor et al., 1983). The "green" derivative PBS108 (*crt*D-121, *rxc*A-108, *hsd*-1, StrR) was generated by transferring the *crt*D mutation from strain R121 to PAS108 via the gene transfer agent (GTA) (Yen et al., 1979). The *crt*D mutation, which blocks normal carotenoid biosynthesis and gives a green color to the colony, was used to simplify quantitation of the carotenoid electrochronic response (Scolnik et al., 1980) and measurements of cytochromes by spectroscopy. PJS108 (*crt*D-121, *hsd*-1, StrR), an isogenic photosynthetic competent strain, was constructed by treating PBS108 with GTA obtained from the wild-type overproducing strain Y262 and selecting for photosynthetic growth. RCV malate-minimal salts media supplemented with 0.1% yeast extract (Difco) and 0.1% casamino acids (Difco) were used to grow all strains (Weaver et al., 1975). Oxygen-limiting growth was achieved by incubating cultures (1.5 L in a 2-L conical flask) at 35 °C for 2 days with gentle shaking (80 rpm) in a New Brunswick G25 gyrorotary incubator. Ambient fluorescent light conditions existed throughout cultivation. Photosynthetic growth rates were measured at 35 °C with cultures (approximately 1×10^8 cells/mL) grown aerobically (20% O_2, 75% N_2, and 5% CO_2) for several generations and then made anaerobic through a sequential decrease in the dissolved O_2 concentration (10% O_2, 85% N_2, and 5% CO_2 for 45 min; 5% O_2, 90% N_2, and 5% CO_2 for 30 min; 2% O_2, 93% N_2, and 5% CO_2 for 30 min; 0% O_2, 95% N_2, and 5% CO_2 for 30 min) before being illuminated with light of varying intensity. Light intensity (in watts per meter squared) was measured through a Schott RG-9 colored glass filter (700-1150-nm transmittance) with a Yellow Springs–Kettering Model 65A radiometer. Cell growth was measured with a Klett-Summerson colorimeter using a number 66 filter with 1 Klett unit $\simeq 10^7$ cells/mL.

Vesicle Preparation. Chromatophores were prepared at 4 °C in 20 mM 3-(*N*-morpholino)propanesulfonate (MOPS), 100 mM KCl, and 1 mM $MgCl_2$ for spectral measurments or in 50 mM $NaHPO_4$ (pH 7.4), 1 mM EDTA, and 0.05% NaN_3 (SEA buffer) for gel electrophoresis. Cells were grown to late logarithmic phase (300–400 Klett units), washed, and then disrupted after resuspension in 40 mL of buffer by two consecutive passages through an Aminco French pressure cell. Disruption occurred at 15 000 lb/in.^{2} in the presence of approximately 5 μg of deoxyribonuclease and 5 μg of ribonuclease per milliliter. Unbroken cells and lysis debris were removed by centrifugation at 20000*g* for 15 min in a Ti70 rotor (Beckman Instruments). Crude chromatophores were pelleted at 150000*g* for 1.5 h by using the Ti70 rotor and resuspended in a minimal volume of appropriate buffer. Prior to gel electrophoresis, chromatophores (approximately 150–200 μg of BChl) were further purified by sedimentation in a 36-mL 20–35% (w/v) linear sucrose density gradient formed in SEA buffer. Centrifugation was carried out at 25000*g* for 14–16 h at 20 °C in an SW28 rotor (Beckman Instruments). Individual gradients were fractionated (1.2-mL fractions at 2 mL/min) by using an Isco model 640 density gradient fractionator fitted with an optical flow cell. The absorbance at 436 nm was continuously monitored by an Isco type 9 optical unit equipped with a mercury vapor lamp, and plotted on an UA5 absorbance monitor. Fractions containing chromatophores were pooled and dialyzed 6–10 h against two 500-mL changes of SEA buffer. Purified chromatophores were pelleted as above and then resuspended by homogenization in a minimal volume of buffer. Chromatophores for salt-induced carotenoid band shifts were prepared in a metal ion free buffer, where all monovalent cations were replaced by choline or tetramethylammonium cations (Jackson et al., 1968).

Determination of Buoyant Density. The refractive index (η) of every other fraction was determined with an AbbE-3L refractometer (Bausch and Lomb). Buoyant density was calculated by first determining the weight percent sucrose of the fraction then using this value to obtain the density. All density determinations have been corrected to 20 °C.

Electrophoresis. SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was performed on a 10 × 14 × 0.15 cm 10–20% (w/v) linear gradient gel (Youvan et al., 1983). Samples were prepared by adding an equal volume of 2× sample buffer to the aqueous chromatophore suspension and heating at 65 °C for 1 min immediately before loading. The gel was run at a constant power setting of 3 W until the pigment front reached the bottom of the gel. Prestained protein molecular mass standards were obtained from Bethesda Research Laboratories with the following molecular masses: ovalbumin, 43 kDa; α-chymotrypsinogen, 25 kDa; β-lactoglobulin, 18 kDa; lysozyme, 14 kDa; bovine trypsin inhibitor, 6 kDa; and insulin (α and β chains), 3 kDa. Gels were stained with 0.1% Coomassie Brilliant Blue R-250 [in methanol/acetic acid/water (3:1:6)] for 2–4 h and then destained with repeated changes of the same solvent.

Protease Treatment. Digestion of purified chromatophores with proteinase K was performed at 30 °C essentially as described (Peters & Drews, 1984). Digestion was terminated by adding cold phenylmethanesulfonyl fluoride (PMSF) to a final concentration of 5 mM. Protease-treated samples were prepared for electrophoresis as described above and immediately analyzed on SDS gels.

Spectrophotometric Analysis. Visible absorption spectra were recorded at ambient temperature (25 °C) on a DU7 spectrophotometer (Beckman Instruments) equipped with a high-resolution graphics system. Low-temperature spectra were recorded at −196 °C by using a Hitachi Model 557 dual-beam spectrophotometer in the dual-beam mode. Samples (~200 μM BChl) were mixed with an equal volume of

glycerol to prevent amorphous crystallization and then frozen in a mylar cuvette having a 1-mm path length. Flash-induced optical absorbance changes were measured with a rapidly responding double-beam spectrometer constructed by the Bio-Instrumentation Group at the University of Pennsylvania, interfaced via a Nicolet 3091 oscilloscope to an IBM personal computer. Actinic flashes were provided by a xenon flash lamp (full width at half-height about 12 μs), filtered through a Schott RG780 filter.

Assays. BChl content was estimated from the absorption spectrum by using an extinction coefficient of 100 mM^{-1}/cm^{-1} at 850 nm. Protein concentration was determined according to the method of Lowry et al. (1951).

Chemicals. All biochemicals, buffers, and reagents were of analytical grade. Culture media were purchased from Difco Laboratories. Acrylamide, *N,N'*-methylenebis(acrylamide), sucrose, SDS, and PMSF were obtained from Bio-Rad Laboratories. Horse heart cytochrome *c* was purchased from Sigma Chemical Co. Proteinase K was obtained from Bethesda Research Laboratories.

RESULTS

Phenotypic Characterization. Preliminary genetic analysis has shown that PBS108 carries a mutation within the *puf* operon (previously *rxcA*) (Taylor et al., 1983): the region of the *Rps. capsulata* genome encoding the B870 α and β subunits and two (L and M) of the three RC subunits (Marrs, 1981; Youvan et al., 1984). PBS108 reverts spontaneously to a wild-type photosynthetic phenotype and is complemented in trans by segments of DNA encoding the B870 genes (unpublished observations). Genetic mapping places the lesion in PBS108 within the promoter proximal region of the B870 structural genes (unpublished observations). Under aerobic conditions, PBS108 grows at wild-type rates with doubling times (T_2) of 2.2 h. Although PBS108 is unable to grow under normal photoheterotropic conditions (T_2 of >18 h as compared to 1.9 h for the control under anaerobic conditions and 20–30 W/m^2 of light), this impediment can be partially overcome by high-intensity illumination (T_2 of 6.7 h at light intensities >120 W/m^2). When sedimented in a linear sucrose density gradient, chromatophores prepared from PBS108 band at a lower density than those from the control (1.0872 and 1.0978 g/cm^3, respectively). Since membrane sedimentation can be related to the overall lipid/protein composition (Osborn et al., 1972), this finding suggests that chromatophores from PBS108 are missing a major protein component of the photosynthetic apparatus.

Absorption Spectra. In the near-infrared region, both mutant and control have two absorption peaks around 800 and 850 nm that are characteristic of the B800–850 complex (Figure 1). The absorption maximum of the shorter wavelength B800–850 component is the same for both strains, 801 nm, while the longer wavelength component of PBS108 is shifted 2 nm toward shorter wavelengths (849 nm) with respect to the control (851 nm). Close inspection of the 850–900-nm region reveals a slight "broadening" of this region in PJS108 relative to the mutant. We attribute this broadening to low levels of the B870 complex.

In the 400–500-nm region where carotenoids absorb, the major peaks of PJS108 have maxima of 429, 457, and 489 nm, whereas the carotenoid peaks of PBS108 are shifted 1–3 nm toward longer wavelengths and have maxima of 430, 460, and 491 nm. Both the B800–850 blue shift and the carotenoid red shift seen with PBS108 have been previously observed in strains of *Rps. sphaeroides* and *Rps. capsulata* deficient in the B870 complex (Meinhardt et al., 1985; Scolnik et al., 1980).

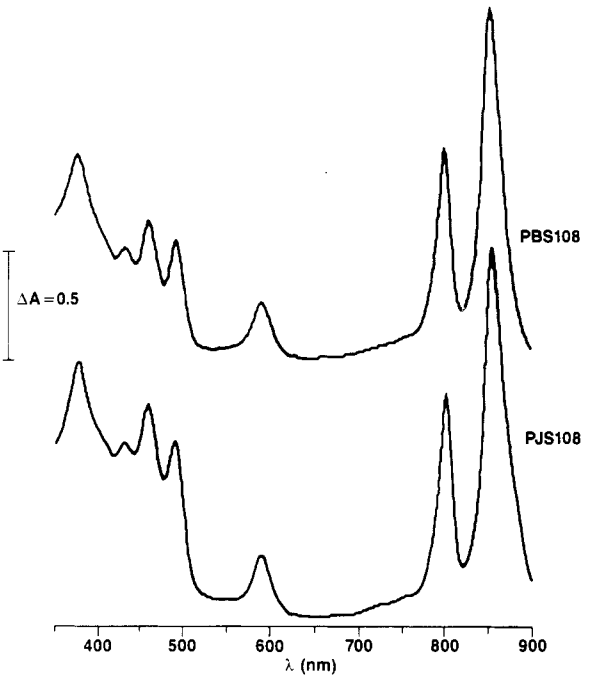

FIGURE 1: Visible absorption spectra of chromatophores prepared from PJS108 and PBS108. Chromatophores were isolated from strains cultured in a supplemented minimal salts media under oxygen-limiting conditions and examined at 25 °C as detailed under Materials and Methods.

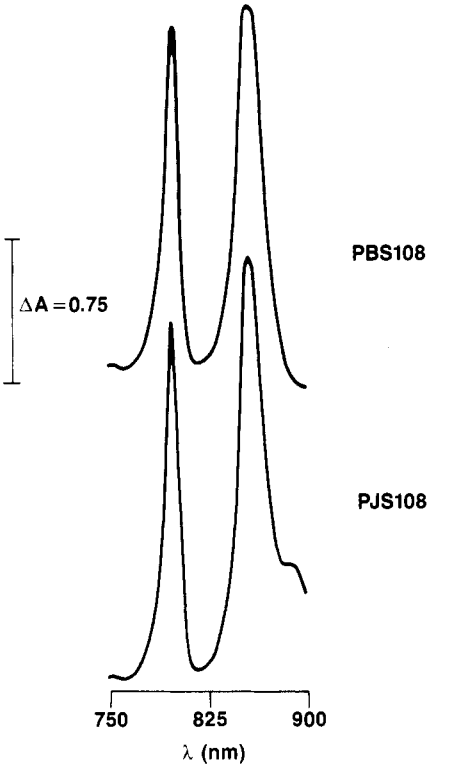

FIGURE 2: Low-temperature near-infrared absorption spectra of chromatophores. Chromatophores were prepared as in Figure 2, and approximately 200 μM BChl was diluted with an equal volume of glycerol before freezing at −196 °C.

To resolve B870 from B800–850, low-temperature absorption spectra were obtained (Figure 2). At −196 °C, the 850-nm peak in PJS108 is sharpened, and a small, though detectable, B870 peak is shifted to 890 nm. In contrast, the overall spectrum of PBS108 remained unchanged. This demonstrates that the photosynthetic membranes from the

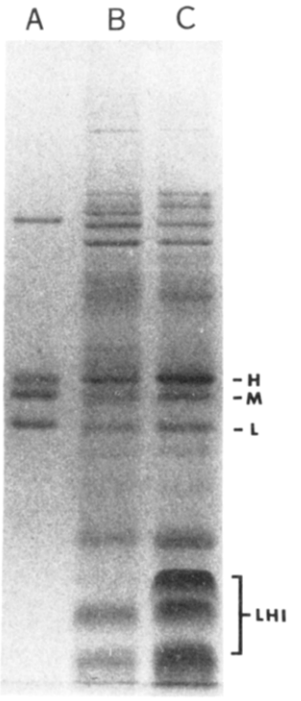

FIGURE 3: SDS–PAGE of purified chromatophores from PJS108 and PBS108. After centrifugation in a sucrose gradient, chromatophores were electrophoresed in a 10–20% polyacrylamide gradient gel as described under Materials and Methods and stained with Coomassie blue. Bands marked H, M, and L designate the 28-, 26-, and 21-kDa RC subunits. The 12- and 6.5-kDa B870 polypeptides are indicated. Lane A, purified RCs from *Rps. sphaeroides* R-26; lane B, PBS108; lane C, PJS108.

mutant contain no B870 complex. It is not surprising that only a small amount of B870 is present in PJS108 since strains were grown under conditions that enhance expression of B800–850 relative to B870, i.e., dark microaerophyllic growth.

Polypeptide Analysis. Since the visible absorption peaks of the LH complexes arise solely from the BChl present in these complexes, we examined chromatophores for the presence of the LH α and β subunits by SDS–PAGE. Consistent with spectral data, the B800–850 subunits (14, 10, and 6 kDa) were present in both mutant and control. Unlike PJS108, chromatophores from PBS108 did not contain either the 12- or the 6.5-kDa B870 subunits (Figure 3). (The broad band present in the 6–7-kDa region of the control is a doublet which consists of the B870 β and the B800–850 β subunits; due to their similar molecular masses, these proteins are not easily resolved.) Polypeptides with molecular masses expected of the RC subunits (28, 26, and 21 kDa) were also observed in both strains. Densitometry of the gel showed that PBS108 contains equivalent concentrations of RC subunits, relative to the B800–850 bands, as the photosynthetic control (unpublished observations). These results confirm the absence of the B870 complex in PBS108 and support the hypothesis that the strict photosynthesis-deficient phenotype results from this deficiency.

Spectral Analysis. Recently, a strain of *Rps. sphaeroides*, RS103, has been described which lacks the B870 complex yet grows photoheterotropically at wild-type rates when exposed to high light intensities (>100 W/m^2) (Meinhardt et al., 1985). Since this is not the case with PBS108, we hypothesized that in addition to the absence of B870, our mutant may not incorporate functional RCs into the photosynthetic membrane. To test for photochemical activity, we measured the reversible light-induced bleaching of the RC absorption peak at 605 nm (Figure 4). While both PJS108 and PBS108 contain equivalent concentrations of photoactive RCs, those in the

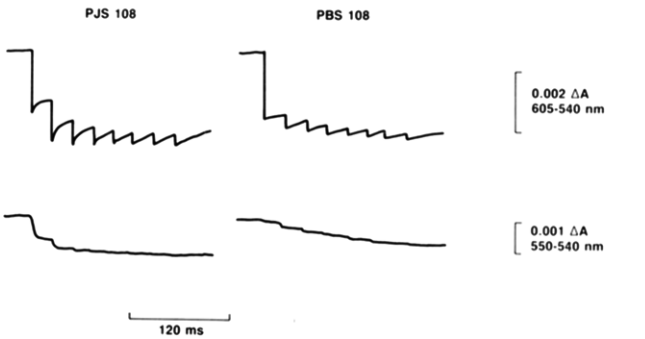

FIGURE 4: Oxidation/reduction kinetics of RCs and cytochrome c_2 in PJS108 and PBS108. The oxidation, seen as a downward deflection of the trace, and rereduction, seen as an upward deflection of the trace, of RCs (top) and cytochrome c_2 (bottom) were monitored at 605 and 550 nm, respectively, related to an isosbestic point at 540 nm, during a series of eight actinic flashes (16 ms between flashes). Chromatophores were suspended to a concentration of approximately 19 μM BChl (~100 nM RCs) in 50 mM MOPS (pH 7.0) and 200 mM KCl, and the E_h was adjusted to ≃200 mV by the addition of sodium ascorbate. Antimycin (2 μM) was present to prevent rereduction of cytochrome c_2, and thus RC turnover, by the bc_1 complex. The total amount of light-induced RC oxidation was measured by the differences from the base line after the fourth flash.

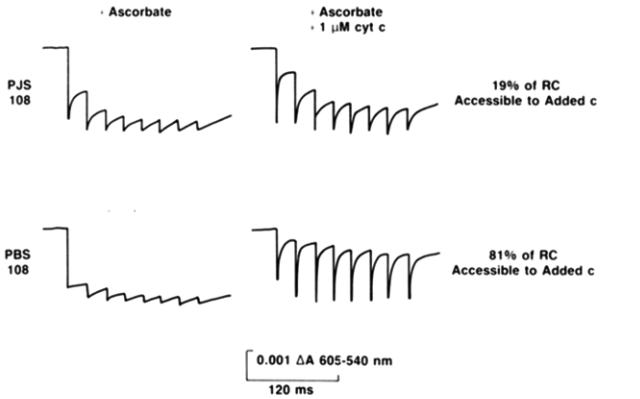

FIGURE 5: Effect of exogenously added cytochrome *c* on the kinetics of RC rereduction in PJS108 and PBS108. Assay conditions were as described in Figure 4 except ascorbate was added to ensure all cytochromes were reduced and horse heart cytochrome *c* (1 μM) was present where indicated. The percent of RCs accessible to exogenous cytochrome *c* was derived from the increase in rereduction produced by the added cytochrome after the first flash.

mutant are not rereduced following the first flash. Consistent with this observation is the low level of light-induced cytochrome c_2 oxidation, as measured by the photobleaching of the cytochrome *c* absorption peak at 550 nm. Difference spectra obtained over the region where cytochromes absorb, 500–600 nm, showed that chromatophores from both strains contain similar amounts of both *b*- and *c*-type cytochromes (unpublished observations). Thus, the absence of RC rereduction in the mutant is not due to a cytochrome deficiency.

In the wild type, RCs are asymmetrically oriented across the membrane such that the photooxidized BChl dimer is rereduced by cytochrome c_2 associated with the periplasmic membrane surface (Prince et al., 1975). To determine if the RC orientation is perturbed in PBS108, we measured the effect of adding exogenous cytochrome *c* on RC bleaching. As shown in Figure 5, more than 80% of the photoactive RCs in PBS108 are rereduced by the added cytochrome, compared to less than 20% in the control. Although consistent with RCs being improperly inserted into the membrane, this result is not definitive. An alternative explanation is that the "chromatophore" fraction obtained from the mutant contains only a few intact membrane vesicles. In this case, the external

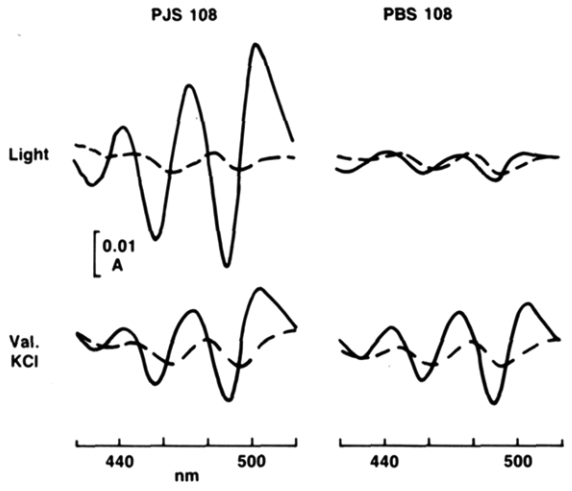

FIGURE 6: Difference spectra of the carotenoid band shifts in PJS108 and PBS108. Light minus dark (top) or valinomycin–KCl minus KCl (bottom) difference spectra were obtained over the 420–520-nm region where carotenoids absorb. Chromatophores were prepared and suspended to a final concentration of approximately 20 μM BChl in 50 mM MOPS (pH 7.0) and 100 mM choline chloride. Salt-induced band shifts (solid line) were produced by the addition of 0.1 μM valinomycin and 12 mM KCl. Changes due to identical additions of water are shown by dotted lines. Light-induced changes (solid line) were produced with saturating continuous illumination. The dashed lines denote the final spectra 1 min after the actinic light had been turned off.

cytochrome would have access to the periplasmic side of the membrane and could reduce RCs normally. To test the integrity of vesicles obtained from PBS108, we assayed chromatophores for their ability to generate a transmembrane potential.

Carotenoid Band Shift. The carotenoids associated with the B800–850 complex undergo an electrochromic red shift in response to a transmembrane potential (Scolnik et al., 1980). Transmembrane potentials can be generated in intact chromatophores either by the electron translocations associated with photosynthesis or by treatment with potassium and the ionophore valinomycin (Jackson et al., 1968). In either case, a carotenoid band shift was observed with PJS108 (Figure 6). Unlike the control, or the *Rps. sphaeroides* B870 mutant RS103 (unpublished observations), there was no light-induced response with PBS108. A carotenoid red shift, having a magnitude equal to that of the control, was produced when the mutant was treated with valinomycin–KCl. This salt-induced response demonstrates the integrity of chromatophores and shows that the B800–850 carotenoids in PBS108 respond to membrane potentials in a normal fashion, yet do not respond to the light-induced electron translocations occurring in the reaction center.

Protease Digestion. The RC subunits of *Rps. capsulata* have been reported to exhibit a hierarchy of protease sensitivity when chromatophores are treated with a nonspecific protease (Peters & Drews, 1984). In these experiments, the H and M subunits were completely degraded before the L subunit was cleaved. If RCs in PBS108 have a membrane orientation different from those in the wild type, we might expect this difference to alter the proteolytic sensitivity of the RC subunits. To investigate the membrane topography of RCs in PBS108, chromatophores were subjected to partial proteolysis with proteinase K and the digestion products examined on SDS gels. In contrast to the previous report, our experiments failed to show a major difference in subunit sensitivity when digestion was performed as described (unpublished observations). Even when the protease:BChl ratio was decreased, the L subunit

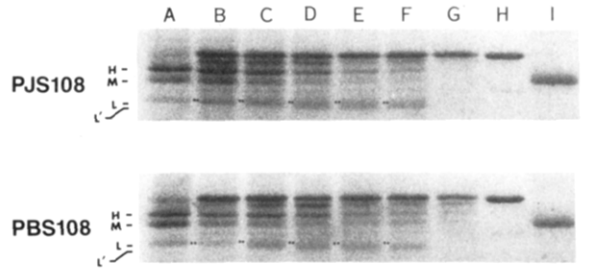

FIGURE 7: Proteinase K digestion of RC subunits. Digestion was performed by using equal concentrations (micrograms per milliliter) of BChl and protease as described under Materials and Methods. Samples were electrophoresed in a 10–20% SDS–polyacrylamide gel until the pigment band reached the bottom of the gel and then stained with Coomassie blue. Only the region of the gel containing the native RC subunits is shown ($R_f \sim 0.4$). Bands marked H, M, L, and L′ refer to the native RC subunits of approximately 28, 26, and 21 kDa and the 20.5-kDa primary degradation product of L, L′ (Peters & Drews, 1983a). Lane A, chromatophores without protease; lanes B–G, chromatophores incubated with protease for 5, 15, 30, 60, 90, and 120 min, respectively; lane H, proteinase K; lane I, 25-kDa marker. Dots designate the location of the L subunit relative to L′.

was only slightly more resistant to degradation than either H or M, with approximately half of the L subunit being cleaved to the more stable L′ form (approximately 20.5 kDa) within 30 min (top panel of Figure 7, lane E). These discrepancies with the previous study may result from a difference in the activities of the protease preparations used in the two studies; however, different protease preparations (both lot and manufacturer) yielded similar results (unpublished observations). Nevertheless, relative to the control, a change in subunit sensitivity was observed with PBS108. Essentially all of the L subunit was cleaved to the L′ form within 15 min for PBS108, whereas more than 60 min was required to achieve similar digestion of L in PJS108 (top panel of Figure 7, lane D). Close inspection of the gel suggests that the H and M subunits are slightly more resistant to cleavage in the mutant (compare lanes F of top and bottom panels of Figure 7).

Energy Transfer. Due to the absence of the B870 complex and a change in RC orientation, we tested the ability of the B800–850 apparatus to transfer absorbed energy directly to the RC in PBS108. This was done by measuring RC activity under conditions where photooxidation of the BChl dimer depends primarily upon energy transferred from the LH apparatus, i.e., at subsaturating flash intensities. As shown in Figure 8, saturating flashes produce the same level of photoactivity in both PJS108 and PBS108; however, fewer RCs are photooxidized in the mutant as the flash intensity decreases. This demonstrates that the B800–850 complexes in the mutant do not transfer energy directly to the RC. Subsaturating flashes also produce more photooxidation in an equivalent concentration of purified RCs, where photooxidation results entirely from the direct absorption of excitation energy rather than the energy transferred from the LH complexes. This finding suggests that the B800–850 complexes in PBS108 are acting as "traps" that shield neighboring RCs from illumination.

Discussion

In this report, we have described the energetic and topographic properties of a photosynthetic mutant of *Rps. capsulata*, PBS108. We have demonstrated, using several different methods, that the photosynthetic membranes of this strain contain no B870 complex. A rigorous approach was necessary to show this deficiency because of the low levels of B870 expressed when strains are grown under dark, oxygen-limiting conditions, i.e., the conditions required to induce

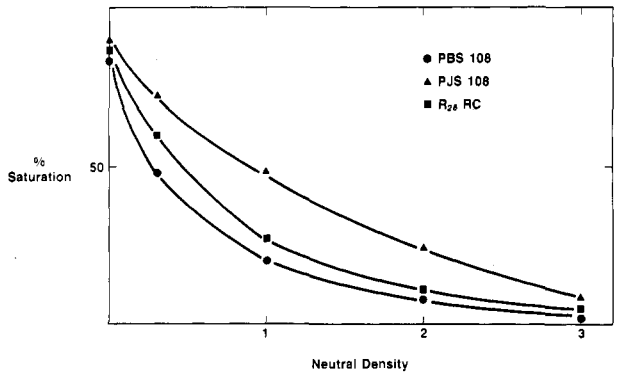

FIGURE 8: Photooxidation of RCs as a function of flash intensity. The intensity of actinic light was varied by the use of neutral density (ND) filters; ND1 is approximately equivalent to 10% T, ND2 $\simeq$ 1% T, and ND3 $\simeq$ 0.1% T. RC oxidation was monitored as in Figure 4. The percent of total RC oxidation was determined by comparing the RC activity produced at each intensity to that obtained with the maximal lamp intensity after the first flash. The failure to saturate RCs on the first flash reflected the age of the xenon flash tube used in these experiments. Samples contained approximately 20 μM BChl and 5 μM diaminodurene (DAD); the ambient potential was poised at 425 $\pm$ 5 mV to prevent RC rereduction by cytochrome c_2. (▲) PJS108; (●) PBS108; (■) purified RCs from *Rps. sphaeroides* R-26.

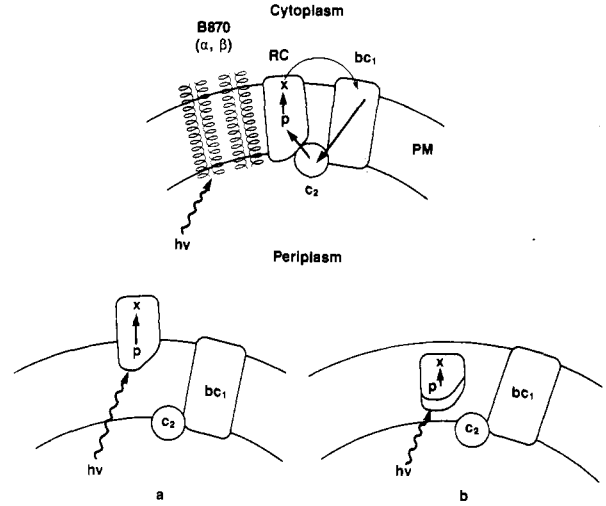

FIGURE 9: Models for the membrane topography of RCs in PBS108. (Top) Native orientation. (Bottom) Peripheral (a) and misaligned models (b) for the RC orientation in PBS108. bc_1, cytochrome bc_1 oxidoreductase; c_2, cytochrome c_2; B870, B870 antenna complex; PM, photosynthetic membrane; P, photoactive BChl dimer; X, primary acceptor. Heavy arrows denote electron translocations believed to induce an electrochromic response from the B800–850 carotenoids (Jackson & Dutton, 1973).

expression of the photosynthetic apparatus in a photosynthetic mutant.

The most important results from this study, however, indicate that in the absence of the B870 complex, RCs are not properly inserted into the membrane. This imprecise insertion energetically uncouples the RC from both the cytochrome bc_1 complex and cytochrome c_2. This effect was clearly demonstrated by the almost complete absence of a light-induced carotenoid band shift with PBS108. Kinetic absorption spectroscopy has resolved this electrochromic response into three separate phases, each of which accompanies a particular electron translocation that occurs during photosynthesis and contributes to the overall membrane potential. Phases II and III of this response are the contributions from the rereduction of the oxidized BChl dimer by cytochrome c_2 and the electron flow through the bc_1 complex, respectively (Jackson & Dutton, 1973). The absence of a phase I band shift, the component due to electron translocation from the primary donor to the primary acceptor, shows that the RCs in PBS108 are oriented such that this translocation no longer produces an electrical gradient across the membrane. These findings can be interpreted in terms of a model where, in the absence of B870, the reaction centers either are misaligned or are not fully embedded in the membrane (Figure 9). It is important to note that the differences observed with protease digestion and the failure to selectively extract RCs from the PBS108 membrane with low concentrations of detergent (unpublished observations) are more consistent with RCs being intrinsic membrane complexes that are improperly oriented rather than being weakly associated with the membrane surface. That RCs are randomly oriented in the membrane seems unlikely since no additional carotenoid band shift was seen in the presence of added cytochrome *c* and a reproducible hierarchy of subunit digestion was observed.

Fluorescence polarization data indicate that the B870 apparatus is not a homogeneous system (Kramer et al., 1984). A minor fraction (15%) of the B870 complexes in *Rps. sphaeroides* was found to have an absorption maxima at a longer wavelength (896 nm) than the majority of complexes, suggesting that only a few B870 complexes are in direct contact with the RC. If a similar organization is present in *Rps. capsulata*, then it is likely that this minor B896 component is involved in orienting the RC.

A strain of *Rps. sphaeroides*, RS103, impaired in its ability to grow photosynthetically and missing the LHI apparatus, has been described (Meinhardt et al., 1985). However, the phenotypic and energetic properties of this mutant are strikingly different from those of PBS108. For example, RS103 has a T_2 of approximately 6 h when grown under standard photoheterotropic conditions (see above) but grows at wild-type rates when exposed to high light intensities. Unlike PBS108, the RCs in the *Rps. sphaeroides* mutant remain coupled to both cytochrome c_2 and the cytochrome bc_1 complex. These properties were attributed to the loss of an intermediate, i.e., B870, necessary for energy transfer between the B800–850 complex and the RC. The observed dissimilarities between PBS108 and RS103 may reflect a dependence on the B870 complex for proper RC orientation in *Rps. capsulata* but not in *Rps. sphaeroides*. Alternatively, since the B870 deficiencies in the *Rps. sphaeroides* mutant were based on ambient spectral data, these differences may reflect the presence of low but undetected levels of B870 in the membrane of RS103.

Experiments designed to identify the mutation in PBS108 and assess its effect on the B870 subunits as well as to determine the number of B870 complexes required for proper RC insertion are currently in progress.

ACKNOWLEDGMENTS

We thank Doug Youvan and Ed Bylina for helpful discussions and communicating results prior to publication and Copper Haith for cheerful and competent technical assistance. We also thank Meg Landmesser and Marianne Kane for typing the manuscript.

Registry No. Bacteriochlorophyll P870, 54577-63-8; cytochrome c_2, 9035-43-2.

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