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Physiological and Structural Analysis of Light-Harvesting Mutants of *Rhodobacter sphaeroides*

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Two mutants of *Rhodobacter sphaeroides* defective in formation of light-harvesting spectral complexes were examined in detail. Mutant RS103 lacked the B875 spectral complex despite the fact that substantial levels of the B875-α polypeptide (and presumably the β polypeptide) were present. The B800-850 spectral complex was derepressed in RS103, even at high light intensities, and the growth rate was near normal at high light intensity but decreased relative to the wild type as the light intensity used for growth decreased. Mutant RS104 lacked colored carotenoids and the B800-850 spectral complex, as well as the cognate apoproteins. This strain grew normally at high light intensity and, as with RS103, the growth rate decreased as the light intensity used for growth decreased. At very low light intensities, however, RS104 would grow, whereas RS103 would not. Structural analysis of these mutants as well as others revealed that the morphology of the intracytoplasmic membrane invaginations is associated with the presence or absence of the B800-850 complex as well as of carotenoids. A low-molecular-weight intracytoplasmic membrane polypeptide, which may play a role in B800-850 complex formation, is described, as is a 62,000-dalton polypeptide whose abundance is directly related to light intensity as well as the absence of either of the light-harvesting spectral complexes. These data, obtained from studies of mutant strains and the wild type, are discussed in light of photosynthetic membrane formation and the abundance of spectral complexes per unit area of membrane. Finally, a method for the bulk preparation of the B875 complex from wild-type strain 2.4.1 is reported.

The B875 and B800-850 light-harvesting (LH) antenna bacteriochlorophyll *a* (Bchl)-protein complexes are the major pigment-protein complexes (8) located in the inducible photosynthetic intracytoplasmic membrane (ICM) system of *Rhodobacter sphaeroides* (recently renamed from *Rhodopseudomonas sphaeroides* [23]). The LH complexes absorb photons and direct light energy to the photochemical reaction center (RC) complex (8, 18). The combination of these three Bchl-protein complexes has been designated the photosynthetic unit (PU) (25). Synthesis of the ICM and the associated Bchl-protein complexes is induced by low oxygen partial pressures, whereas the amount and composition of the ICM are controlled by incident light intensities (11, 25). In steady-state photosynthetically grown *R. sphaeroides*, the number of PUs per unit area of ICM appears to be constant and is independent of the light intensity of growth (46). Within the PU, the ratio of the B875 to RC complexes remains constant (10 to 15:1) and comprises what has been designated the fixed component of the PU (1, 8, 25). In contrast, the amount of the B800-850 complex within the PU is inversely related to the incident light intensity of growth and has, therefore, been designated the variable component of the PU (1, 6, 8, 25).

The ability to purify both LH complexes as spectrally active entities has been central to determining their composition and to understanding structure-function relationships. The purified B875 complex consists of equimolar amounts of two polypeptides, B875-α and B875-β, two Bchl molecules, and two carotenoids (3). The B875-α and B875-β polypeptides have been sequenced, and their molecular weights are 6,809 and 5,457, respectively (41). Detergent-purified B800-850 complexes (7) contain two polypeptide subunits, B800-850-α and B800-850-β, which by amino acid sequence are 5,599 and 5,448 daltons, respectively (43). There are 6 molecules of Bchl per two pairs of polypeptide subunits while the Bchl/carotenoid ratio is 3:1 (50).

Although substantial spectroscopic and biochemical data have been derived from analysis of detergent-solubilized pigment-protein complexes in vitro, it has been more difficult to describe their interactions in vivo. Through the isolation and analysis of *R. sphaeroides* mutants altered in these spectral complexes, we hope to determine what factors and genetic regions are necessary for synthesis of functional spectral complexes in vivo. Since the structural genes for the B875, B800-850, and RC polypeptides from *R. sphaeroides* have been cloned and sequenced, and their operon structure has been determined (16, 26, 27, 44, 45, 47, 48), such an approach provides the foundation for further studies to understand the genetic and molecular mechanisms regulating the synthesis and stoichiometry of individual LH complexes in vivo.

In this paper, we describe a physiological and ultrastructural analysis of two *R. sphaeroides* mutants: RS103, which lacks the B875 complex; and RS104, which lacks both B800-850 complexes and colored carotenoids. We have previously characterized these mutant strains with regard to their efficiency of light energy transfer to the RC (32). This study reports on the effect of these mutations on the synthesis of Bchl-protein complexes, as determined by spectral analysis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and Western blot (immunoblot) analysis with specific antibodies against previously characterized

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LH polypeptides. The data are discussed with regard to the role of individual LH complexes in photosynthetic growth at different light intensities, the regulation of synthesis of individual Bchl-protein complexes, and the effects of the loss of individual LH complexes on structure and assembly of the ICM.

MATERIALS AND METHODS

Bacterial strains. *R. sphaeroides* RS2, RS103, and RS104 were obtained by S. Harayama as previously described (32). Strain RS104, a blue-green mutant, and RS103, a pale reddish brown mutant, were obtained by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine mutagenesis of strain RS2. *R. sphaeroides* 2.4.1 is a wild-type strain isolated by Van Niel and obtained from W. R. Sistrom.

Growth of bacteria. For preparation of pigment-protein complexes, *R. sphaeroides* 2.4.1 was grown photoheterotrophically at 32°C in Sistrom medium A (30) supplemented with 0.2% Casamino Acids (Difco Laboratories, Detroit, Mich.) in completely filled sealed carboys with continuous stirring, surrounded by a bank of incandescent lamps. Cells were harvested by continuous centrifugation of late-logarithmic-phase cultures (approximately 150 Klett units with a no. 66 filter; 1 Klett unit = 10^7 cells per ml). The cell pellet was frozen and stored at −70°C prior to use. Parent and mutant strains, to be used for chromatophore, thin-section electron microscopy, or spectral analysis, were grown in Sistrom medium A (30) at 32°C by initially sparging with an atmosphere of 25% O_2–1% CO_2–74% N_2. At a culture density of 1.5×10^8 cells per ml, the gas mixture was changed to 10% O_2, 2.5% CO_2, and 87.5% N_2 for 30 min, then to 5% O_2, 2.5% CO_2, and 92.5% N_2 for 60 min, to 2.5% O_2, 2.5% CO_2, and 95% N_2 for 30 min, and finally to 2.5% CO_2 and 97.5% N_2 for the remainder of the experiment. Cultures were illuminated at the light intensities indicated (measured as previously described [6]), 15 min after shifting to 2.5% CO_2–97.5% N_2.

Thin-section electron microscopy. Photosynthetically grown cells (10 W/m^2) were fixed with 0.5% glutaraldehyde in the growth medium for 1 h at room temperature (RT). Cells were washed and postfixed in 1% OsO_4 (aqueous) and then washed and stained en bloc with 0.25% uranyl acetate (aqueous) overnight at 4°C. The fixed cells were dehydrated in acetone and embedded in Spurrs resin. Silver sections were stained with lead salts (36) and visualized in a Hitachi H600 microscope at 75 kV.

Preparation of chromatophores. Chromatophores (vesicularized ICM) were prepared through sucrose gradient centrifugation as previously described (20, 30).

Isolation of pigment-protein complexes. Approximately 50 g of frozen *R. sphaeroides* 2.4.1 was disrupted and fractionated to obtain crude membranes as previously described (40). The membrane pellet was suspended by homogenization in 0.1 M sodium phosphate buffer, pH 7.5, and the volume was adjusted such that the A_{850} was 50. The membrane suspension was brought to RT, and lauryldimethylamine-*N*-oxide (LDAO) (30%) was added dropwise to a final concentration of 0.25%, as previously described (7). The suspension was stirred slowly for 10 min at RT and then centrifuged for 10 min at 15,000 × *g* (4°C). The supernatant was removed and centrifuged at 186,000 × *g* for 2 h at 4°C. The particulate fraction, which contained the 0.25% LDAO-extracted membranes, was suspended in 10 mM Tris hydrochloride, pH 7.5, by homogenization, and the volume was adjusted such that the solution had an A_{850} of 100, and the ratio of A_{800}/A_{875} was 1.6.

For purification of B875 complexes from the wild-type organism, the optimum concentration of the 0.25% LDAO-extracted membranes was specifically determined for each preparation of membranes. The suspension of 0.25% LDAO-extracted membranes was diluted 1:1, 1:2, and 1:3 (vol/vol) with cold 10 mM Tris hydrochloride, pH 7.5, and placed at RT, when LDAO (30%) was added steadily with stirring to a final concentration of 0.5% and stirred for 10 min at RT. A 3-ml portion of this suspension was immediately layered onto 20%/40% (wt/vol) sucrose (in 10 mM Tris hydrochloride, pH 7.5) step gradients and centrifuged for 14 h at 27,000 rpm in a Beckman SW28 rotor at 4°C. Two bands were visible at the 20%/40% sucrose interface: a brown-green fraction which contained most of the membranes and an orange band immediately above it. The orange band, which contained the B875 complex, was removed from the side and analyzed for spectral activity and by SDS-PAGE. The dilution factor used for processing the remainder of the 0.25% LDAO-extracted membrane suspension was determined from the gradient which contained the highest yield and spectral purity of the B875 complex. The B875 preparation was concentrated by pooling several fractions, adjusting the solution with cold 10 mM Tris hydrochloride pH 7.5, such that the refractive index was equivalent to 20% sucrose, and using the resulting solution as the upper layer in a second 20%/40% sucrose step gradient, using the same centrifugation conditions as before. The orange fraction, which did not enter the 40% sucrose solution, was removed. On occasion, the B875 preparation contained detectable amounts of the B800-850 complex, which could be removed by including either *N*-octyl-β-D-glucopyranoside or lithium dodecyl sulfate at a final concentration of 0.1% in the 20% sucrose solution during the second centrifugation step.

The B800-850 complex was purified by the LDAO detergent solubilization method of Cogdell and Crofts (7), with several modifications. To enhance the separation of the B800-850 complex from other pigmented material, the sucrose step gradient centrifugation time was lengthened from 2 to 20 h. The pigmented material obtained from the 20%/40% interface was pelleted at 69,000 rpm for 5 h in a Beckman 70Ti rotor at 4°C, suspended in 10 mM Tris hydrochloride, pH 7.5–1.0% LDAO, layered onto a second 20%/40% sucrose step gradient, and centrifuged as before.

SDS-PAGE and Western blotting. Electrophoresis was carried out with 11.5 to 18.0% linear acrylamide gradient SDS-polyacrylamide gels with 0.5% NaCl in the resolving gel to increase the resolution of low-molecular-weight polypeptides (14). Transfer of proteins from polyacrylamide gels to 0.2-μm nitrocellulose was accomplished by electrophoresis at 40 V for 2.5 h in 25 mM Tris, pH 8.3–192 mM glycine–0.1% SDS–20% (vol/vol) methanol. Filters were treated with specific antibody as previously described (13).

Preparation of antisera. Use of antibodies to the purified ICM polypeptides 15A, 15B, and 15C has been previously described (5). These three low-molecular-weight polypeptides were suggested to be components of the LH machinery due to their size and abundance in purified chromatophores (9, 10). The polypeptide subunits of the B875 complex were separated by preparative SDS-PAGE, using the electrophoresis conditions described above, visualized with anilinonaphthalene sulfonate (22), excised from the gel, and electroeluted from the gel slices into <3,000-molecular-weight cutoff dialysis tubing as previously described (37). Since the low-molecular-weight Bchl-binding polypeptides were previously observed to be relatively poor immunogens (L. Cohen and S. Kaplan, unpublished observations), the polypeptides

purified by preparative SDS-PAGE were cross-linked to keyhole limpet hemocyanin by treatment with ethyl-dimethylaminopropylcarbodiimide at a 50-fold excess of hemocyanin relative to the LH polypeptides (21). New Zealand White rabbits were injected with approximately 200 μg of isolated protein emulsified with complete Freund adjuvant for the primary inoculation and with incomplete Freund adjuvant for monthly boosters (100 μg of protein). Immunoglobulin fractions were prepared from sera as previously described (13).

Biochemical techniques. Bchl and carotenoids were extracted in acetone-methanol (7:2) and estimated by the extinction coefficients E_{775} = 75 mM^{-1} (for Bchl) and E_{484} = 128 mM^{-1} (for carotenoids) (11). Absorption spectra were obtained on either a Cary 14 or a Varian 2390 scanning spectrophotometer. Protein was determined by a modified Lowry assay (31).

Reagents. Nitrocellulose, 0.2 μm, was purchased from Schleicher & Schuell, Keene, N.H. LDAO was the gift of Onyx Chemical Co., Jersey City, N.J. ^{14}C-labeled amino acid mixture was obtained from New England Nuclear Corp., Boston, Mass., and contained 1.25 mCi/ml. All other chemicals were of reagent grade.

RESULTS

Analysis of purified LH spectral complexes. To biochemically characterize the LH mutants derived from RS2, it was essential to purify the LH spectral complexes from the wild-type strain for comparison. *R. sphaeroides* 2.4.1 was used for purification of the LH complexes since this strain has been used previously (3, 7). By spectroscopic measurement, SDS-PAGE profiles, and cross-reactivity to various ICM-specific antibodies, RS2 is indistinguishable from 2.4.1 (data not shown).

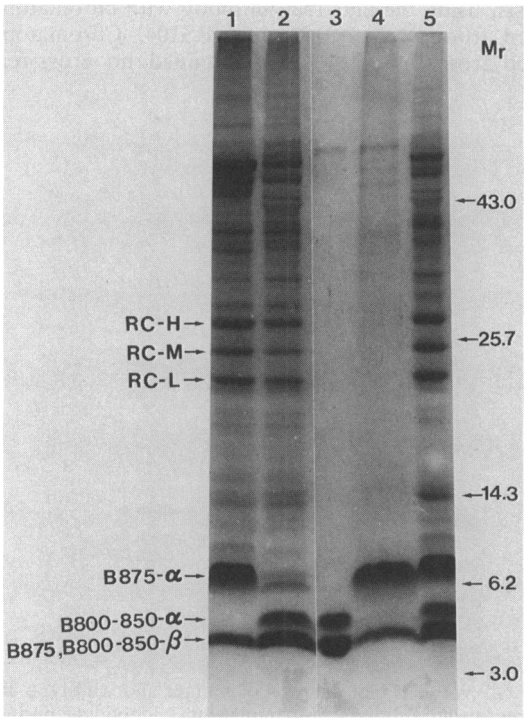

FIG. 1. Analysis of chromatophores from RS104, RS103, and RS2 and purified LH complexes by SDS-PAGE. Lanes 1, and 2 contain 40 μg of chromatophore protein from RS104 and RS103, respectively; lane 3, 15 μg of B800-850 complex; lane 4, 10 μg of B875 complex; lane 5, 40 μg of chromatophore protein from RS2. Samples were prepared for electrophoresis as in the legend to Fig. 7, except the reductant was 2-mercaptoethanol (5.0%).

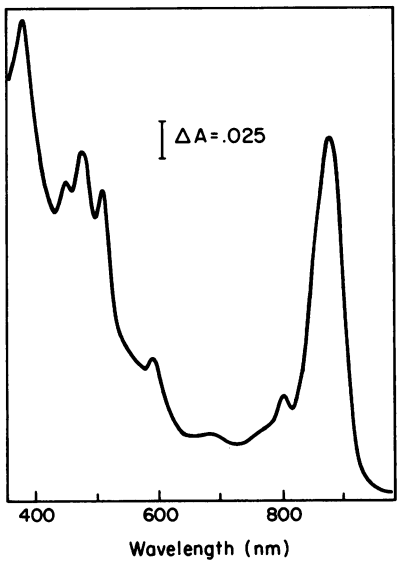

FIG. 2. Analysis of spectral complexes. Shown is the absorption spectrum of 46 μg of B875 complex.

The purified B800-850 complex had characteristic absorption maxima in the near infrared, the typical spectrum of carotenoids (data not shown), and a ratio of Bchl/carotenoids similar to those of other isolated *R. sphaeroides* B800-850 complexes (50; R. Cogdell, personal communication). Analysis of the complex by SDS-PAGE showed the presence of two polypeptides of apparent molecular weights of 5,200 and 4,300 (Fig. 1, lane 3), corresponding to the B800-850-α and the B800-850-β subunits, respectively.

The B875 complex was purified from wild-type 2.4.1 by modifying the method used for isolating the B800-850 complex (7). The B875 complex could be differentially solubilized from the 0.25% LDAO-extracted membranes with 0.5 rather than 1.0% LDAO, which was necessary for solubilizing the B800-850 complex. LDAO concentrations of >0.5% resulted in a loss of the A_{875} due to selective denaturation of the B875 complex, whereas concentrations of <0.5% resulted in most of the A_{875} remaining in the membrane fraction. In addition, treatment of the membrane suspension obtained after the 0.25% LDAO extraction with 1% *N*-octyl-β-D-glucopyranoside for 10 min at RT yielded a fraction that contained almost exclusively B875 and RC-H, RC-M, and RC-L polypeptides (data not shown). Further optimization of this procedure may lead to the purification of an intact active RC-875 complex which would be useful for future structure-function studies.

Analysis of the absorption spectrum of the purified B875 complex showed one major near-infrared peak at 875 nm as well as the characteristic carotenoid absorption spectrum (Fig. 2). Some minor A_{800} indicated the presence of small amounts of B800-850 complex, and some Bchl breakdown was occasionally observed as monitored by the A_{690}. Analysis of the purified B875 complex by SDS-PAGE revealed two major polypeptide subunits, one corresponding to B875-α (apparent molecular weight, 6,700) and the second corresponding to B875-β (apparent molecular weight, 4,300) (Fig. 1, lane 4). Note that on this gel system, the B800-850-β

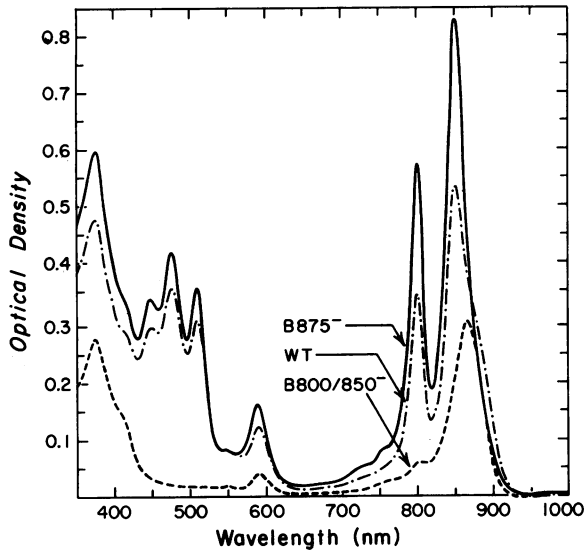

FIG. 3. Absorption spectra of cell extracts of mutant and wild-type (WT) strains grown as described in Materials and Methods at 10-W/m^2 light intensity. Strain RS103 is B875$^-$, strain RS104 is B800-850$^-$ Car$^-$, and strain RS2 is wild type. Each sample was scanned at a protein concentration of 520 μg/ml.

polypeptide comigrates with the B875-β polypeptide. This method was an improvement over lithium dodecyl sulfate preparative electrophoresis (3), which gave variable results in our hands while only allowing solubilization of limited amounts of membrane at a time, and offered the advantage of preparing the complexes from wild-type cells, unlike other published procedures which used spectral mutants (2, 50).

Spectral analysis of mutants. Figure 3 shows the absorption spectra obtained with equivalent amounts of crude cell protein from two mutants (RS103 and RS104) and the wild-type parental strain (RS2) all grown at a light intensity of 10 W/m$_2$, as described in Materials and Methods. Mutant RS103 lacks the characteristic absorption maximum at 875 nm, while mutant RS104 lacks both colored carotenoids and the 800- and 850-nm absorption peaks. These spectra were characteristic of other B800-850- and B875-deficient mutants derived from *R. sphaeroides* 2.4.1 by Nano and Kaplan (34).

Comparison of chromatophore polypeptide profiles with purified B875 and B800-850 complexes. Chromatophores were prepared from mutant and wild-type strains and analyzed together with purified LH complexes by SDS-PAGE to relate the loss of spectral activity to the absence of specific chromatophore polypeptides. The chromatophore polypeptide profile of RS2 showed the expected RC polypeptides, RC-H, RC-M, and RC-L (18), and the LH polypeptides B875-α, B800-850-α, B800-850-β, and B875-β (both β polypeptides comigrate on SDS-PAGE) (Fig. 1, lanes 3 to 5). The chromatophore polypeptide profile of RS103 (Fig. 1, lane 2) contained the normal complement of RC and B800-850 polypeptides but showed apparently reduced amounts of B875-α polypeptide relative to the wild-type strain (see below). There was an apparent alteration in the electrophoretic mobility of the putative B875-α polypeptide such that it ran slightly ahead of the B875-α polypeptide from RS2; this may reflect the amount of B875-α polypeptide analyzed per lane (see Western blot analysis). Since B800-850-β comigrates with B875-β on SDS-PAGE, it could not be determined whether B875-β was present in RS103 by Coomassie blue staining of SDS-PAGE gels. Chromatophores from RS104 (Fig. 1, lane 1) lacked the B800-850-α polypeptide and presumably the B800-850-β polypeptide since there was a decrease in Coomassie blue staining in the RS104 sample in the region where the B800-850-β and B875-β polypeptides comigrate in the wild-type strain. The relative ratio of B875-α/B875-β in RS104 was similar to that observed in the purified B875 complex, as judged by Coomassie blue staining, suggesting that the B800-850-β polypeptide is absent or greatly reduced in RS104 (see below).

Western blot analysis. Verification of the loss of or reduction in LH polypeptides was obtained by Western blot analyses. ^{14}C-amino acid-labeled chromatophores were always included in the SDS-PAGE gel and subsequent transfer to nitrocellulose to compare the relative mobility of the cross-reacting material with the known LH polypeptides (Fig. 4A, lane 1, and B, lane 5). The antibody raised against polypeptides 15B and 15C reacted specifically with the B800-850-β polypeptide from purified B800-850 complexes (Fig. 4B, lane 4; data not shown), and therefore this antibody was used to assay for B800-850-β polypeptide in chromatophores. There was no detectable cross-reacting material present in the chromatophores derived from RS104 (Fig. 4B, lane 1) compared with RS2 (Fig. 4B, lane 3) when the antibody directed against polypeptide 15 B was used. Like the wild-type RS2 (Fig. 4B, lane 3), chromatophores derived from RS103 and purified B800-850 complexes (Fig. 4B, lanes 2 and 4, respectively) contained cross-reacting material to anti-15B antibody. Similar results were observed with the antibody against polypeptide 15C (data not shown).

Antibody directed against the 15A polypeptide did not cross-react with purified B875 or B800-850 complexes (data not shown). Figure 4A shows the results of a Western blot analysis, using the anti-15A antibody with chromatophores derived from RS2, RS103, and RS104. Chromatophores derived from mutant RS104 contained no cross-reacting

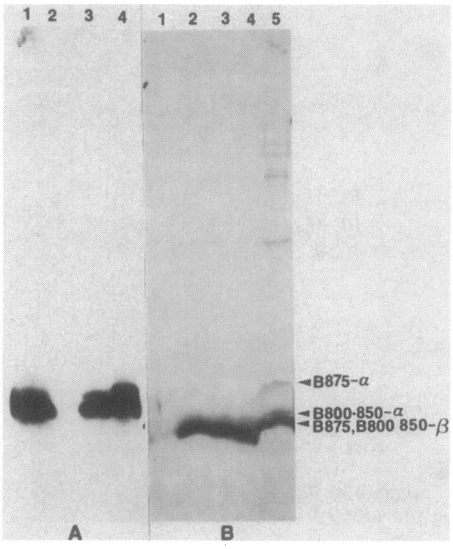

FIG. 4. Western blot analysis of mutant and wild-type chromatophores, using antibody prepared against individual purified LH polypeptides 15A and 15B: autoradiograph of nitrocellulose replicas treated with individual LH antibodies to 15A (A) and 15B (B). Panels A, lanes 2 to 4, and B, lanes 1 to 3, contain 40 μg of chromatophore protein prepared from strains RS104, RS103, and RS2 respectively. Panel B, lane 4, contains 10 μg of B800-850 complex, and panels A, lane 1, and B, lane 5, contain 50 μg of ^{14}C-amino acid-labeled RS2 chromatophores.

material to anti-15A antibody (Fig. 4A, lane 2), but cross-reacting material was present in chromatophores derived from RS103 and RS2 (Fig. 4A, lanes 3 and 4, respectively). The cross-reacting material always appeared as a doublet in this analysis, the origin of which is unknown at this time. In L37, another B800-850-deficient mutant (34) derived from strain 2.4.1, the amount of 15A was reduced significantly relative to wild type (data not shown). L37, unlike RS104, contains carotenoids.

The presence of reduced amounts of B875-α polypeptide in chromatophores derived from RS103 was confirmed by Western blot analysis, using anti-B875-α antibody (Fig. 5). There was approximately 25% of the cross-reacting B875-α polypeptide in chromatophores derived from RS103 than was found in the equivalent amount of chromatophore protein derived from RS2 (as determined by densitometer scans of X-ray films well within linear response of film exposure). The relative mobility of B875-α from both RS2 and RS103 appeared to be the same in this analysis, although in Fig. 3 we had observed that the putative B875-α polypeptide in RS103 detected by Coomassie blue staining appeared to run ahead of the B875-α polypeptide in membrane samples derived from the wild type. As suggested earlier, this apparent difference in migration could be due to either the smaller chromatophore load or the reduced amount of B875-α protein within the RS103 chromatophores present in the samples used in this Western blot analysis. Smaller chromatophore loads were used to ensure that binding of this protein to nitrocellulose and its detectability by antibody was within the linear range of the assay. The patterns of cross-reactivity of mutant (RS103 and RS104) and wild-type (RS2 and 2.4.1) chromatophores, as well as purified B800-850 and B875 complexes with antibody against polypeptides 15A, 15B, 15C, and B875-α, are summarized in Table 1.

Growth of RS103, RS104, and RS2 under photosynthetic conditions. Early in these studies we observed that direct placement of chemoheterotrophic grown cultures of RS103 and RS104 under strict anaerobic photosynthetic conditions (a nongratuitous shift) resulted in a high proportion of cells with an apparent wild-type phenotype (i.e., revertants) appearing in the population. The reversion frequency for each of these mutants to a wild-type-like phenotype was determined by plating chemoheterotrophically grown cells in anaerobic jars in the light and scoring the dark-pigmented, faster-growing colonies as presumptive wild type. The absorption spectra from a number of the revertants were examined, and they were indistinguishable from the wild-type spectrum. We determined that the reversion frequency to apparent wild type was 4.5×10^{-4} for RS103 and 1.4×10^{-6} for RS104. Because of this relatively high reversion frequency to a wild-type phenotype, we developed growth conditions that minimized the selective advantage the wild type would have in outgrowing the mutants in the population. This was accomplished by shifting cells at a low culture density through a series of decreasing oxygen tensions prior to reaching anaerobic photoheterotrophic conditions. The use of the low-oxygen gratuitous shift conditions (described in Materials and Methods) was sufficient to allow the induction of photosynthetic membranes to occur prior to the imposition of stringent nongratuitous conditions of anaerobic photosynthetic growth. Under these growth conditions no significant level of wild-type-like revertants in the population could be detected by analysis of the absorption spectra.

Regulation of spectral complex formation in RS103, RS104, and RS2 by incident light intensity. Figure 6 shows the

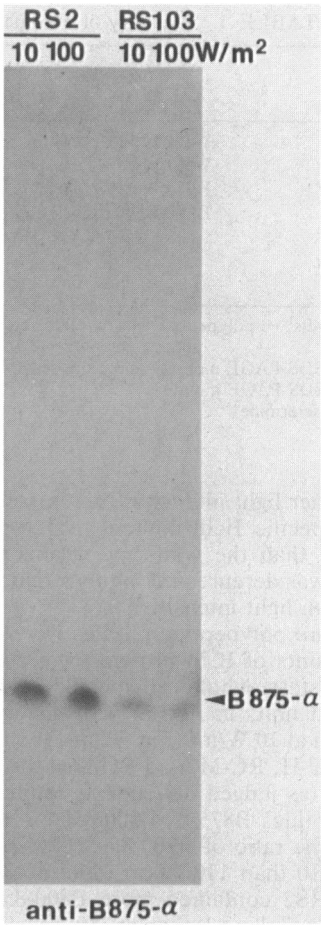

FIG. 5. Western blot analysis with antibody raised against B875-α polypeptide, using ^{125}I-labeled protein A for detection. Chromatophore polypeptides were separated by 11.5 to 18% SDS-PAGE, transferred to nitrocellulose, and probed with antibody to B875-α. Each lane contains 2.5 μg of chromatophore protein.

kinetics of growth of RS103, RS104, and RS2 following illumination at 10 and 110 W/m^2 (Fig. 6A and B, respectively). Both wild-type and mutant strains showed similar lag periods and doubling times at 110 W/m^2. However, when shifted to photosynthetic conditions at 10 W/m^2, the mutants exhibited a longer lag phase before reaching exponential growth, when their generation times were approximately twice as long as that of the wild type. This is presumably due to the defect in the PU.

From Fig. 6, it was determined that the amount of Bchl per milligram of cell protein achieved a steady-state value at mid-logarithmic phase for all cultures grown at either light intensity. Furthermore, the whole-cell-specific Bchl content at mid-logarithmic phase for RS2 grown at 10 W/m^2, using the shift regimen described, was equivalent to that measured when this strain was grown under steady-state photosynthetic conditions at the same light intensity (data not shown). This suggested that photosynthetic membrane synthesis was fully induced with the low-oxygen shift regimen at least in wild-type cells. Therefore, we can directly compare the steady-state, whole-cell-specific Bchl contents of the mutant strains to the wild-type as a function of incident light intensity (Table 2). Comparison of the data from Fig. 6 showed that the whole-cell-specific Bchl content of strain RS104 was two- to threefold less than that determined for the

TABLE 1. Summary of chromatophore polypeptide composition of LH mutants and wild-type strains

Strain/fraction	Phenotype	Detection of given LH polypeptide[a]				
		B875-α[b]	15A[b]	B800-850-α[c]	B800-850-β[b]	B875-β[c]
RS2	Wild type	+	+	+	+	+
2.4.1	Wild type	+	+	+	+	+
RS103	B875$^-$	+/−	+	+	+	ND[d]
RS104	B800-850$^-$, Car$^-$	+	−	−	−	+
B800-850 complex		−[c]	−	+	+	ND
B875 complex		+	−	−	−[c]	+

[a] +, Wild-type levels; −, polypeptide was not detected by Western blot or SDS-PAGE analysis; +/−, substantially reduced amounts relative to the wild-type level.
[b] Determined by SDS-PAGE mobility and cross-reactivity to specific antisera in a Western blot analysis.
[c] Determined by SDS-PAGE mobility.
[d] ND, Could not determine.

wild type at either light intensity. In contrast, strain RS103 had a higher specific Bchl content at 110 (2-fold) and 10 (1.3-fold) W/m^2 than the wild-type strain, suggesting that Bchl synthesis was derepressed in this strain relative to wild type even at high light intensities.

Chromatophore polypeptide profiles were examined with equivalent amounts of ICM protein from cultures that had reached steady-state, whole-cell-specific Bchl levels (i.e., at 100 to 150 Klett units in Fig. 6). Chromatophores derived from RS2 grown at 10 W/m^2 (Fig. 7 lane 3) contained the RC polypeptides RC-H, RC-M, and RC-L in the expected equimolar amounts (as judged by Coomassie blue staining) and the LH polypeptides B875-α, B800-850-α, B800-850-β, and B875-β. Since the ratio of B800-850/B875 spectral complex was greater at 10 than 110 W/m^2 (data not shown), it was expected that RS2 contained more B800-850-α relative to B875-α at 10 (Fig. 7, lane 3) than at 110 (Fig. 7, lane 4) W/m^2. Also, the amount and the ratio of B875 and RC polypeptides appeared constant in chromatophores from RS2 grown at either 10 or 110 W/m^2. This was expected since the number of PU per unit area of chromatophore membrane (46) as well as the ratio of B875/RC complexes do not change as a function of incident light intensity (1). Therefore, the higher whole-cell-specific Bchl content (Table 2; Fig. 6) observed in RS2 grown at 10 W/m^2 versus that observed at 110 W/m^2 was due to an increase in the relative amount of the B800-850 complex per chromatophore (which is also reflected in the chromatophore-specific Bchl content; Table 2) and an increase in the total amount of ICM per cell.

By Coomassie blue staining, the level of RC and B875 polypeptides in chromatophores derived from cells grown at 10 W/m^2 was equivalent in RS104 and RS2. The ratios of B875/RC polypeptides in chromatophores prepared from RS104 grown at 10 and 110 W/m^2 appeared similar. However, RS104 appeared to have less of the B875 and RC polypeptides per equivalent amount of chromatophore protein from cells grown at 110 W/m^2 than from those grown at 10 W/m^2 (Fig. 7, lanes 5 and 6). The decrease in B875 and RC polypeptides in chromatophores from RS104 grown at 110 W/m^2 was consistent with its lower specific Bchl content (Table 2). It is not possible to distinguish at this time whether the increase in whole-cell-specific Bchl content at 10 versus 110 W/m^2 in RS104 was due solely to the increase in the number of photosynthetic units per unit chromatophore membrane or also to an increase in the amount of ICM per cell. The decrease of RC and LH polypeptides in chromatophores derived from RS104 grown at 110 W/m^2 appeared to be correlated with an increase in the amount of an apparent 62,000-molecular-weight polypeptide. The 62,000-dalton polypeptide was most abundant in chromatophores derived from RS104 grown at 110 W/m^2. Moreover, the amount of this polypeptide was always greater in chromatophores isolated from all strains grown at 110 W/m^2 (high light) than from those grown at 10 W/m^2. Although synthesis or accumulation or both of the 62,000-dalton polypeptide may be associated with decreased amounts of Bchl-protein complexes, there was more 62,000-dalton polypeptide in the mutants than in the wild type at all light intensities. The physiological function of this polypeptide is unknown, but we cannot exclude the possibility that the relative abundance of the 62,000-dalton polypeptide is a consequence of either cytoplasmic or outer membrane contamination of these chromatophores. However, SDS-PAGE profiles of cytoplasmic and outer membrane fractions from the wild type do not show the presence of an abundant 62,000-dalton polypeptide (38).

There was more B800-850-α relative to RC polypeptides in RS103 chromatophores from cells grown at 10 W/m^2 than from those grown at 110 W/m^2 (Fig. 7, lanes 1, 2), although the magnitude of the increase in this ratio was much less in RS103 than in the wild type. The apparent ratio of B800-850-α/RC polypeptides in cells grown at 110 W/m^2 is significantly higher in RS103 than in RS2; this is consistent with the higher specific Bchl content of RS103 chromatophores (Table 2) and suggests that at high light (110 W/m^2) synthesis of the B800-850 complex is derepressed in RS103 relative to wild type. Since the amount of RC in RS103 chromatophores appears to be less than in RS2, there could also be fewer photosynthetic units per chromatophore (similar to RS104), although the size of the photosynthetic unit in RS103 would be much larger than in RS104.

Thin-section electron microscopy. *R. sphaeroides* RS103 was essentially identical in membrane morphology to its parent strain RS2 (Fig. 8). There appeared to be a slightly greater number of chromatophores per cell in RS103 than in RS2 grown at 10-W/m^2 light intensity, which, in addition to the derepression of B800-850 complexes, can account for the increased whole-cell-specific Bchl levels relative to wild type.

In contrast, RS104 showed greatly altered morphology (Fig. 8). Large irregularly shaped membranes, presumably containing RC and B875 complexes, were attached to the cytoplasmic membrane (Fig. 8). Long tubes of membrane were seen in many cells, running the entire length of the cell. These membrane tubes appeared to interfere with cell division by preventing complete cell separation, resulting in the

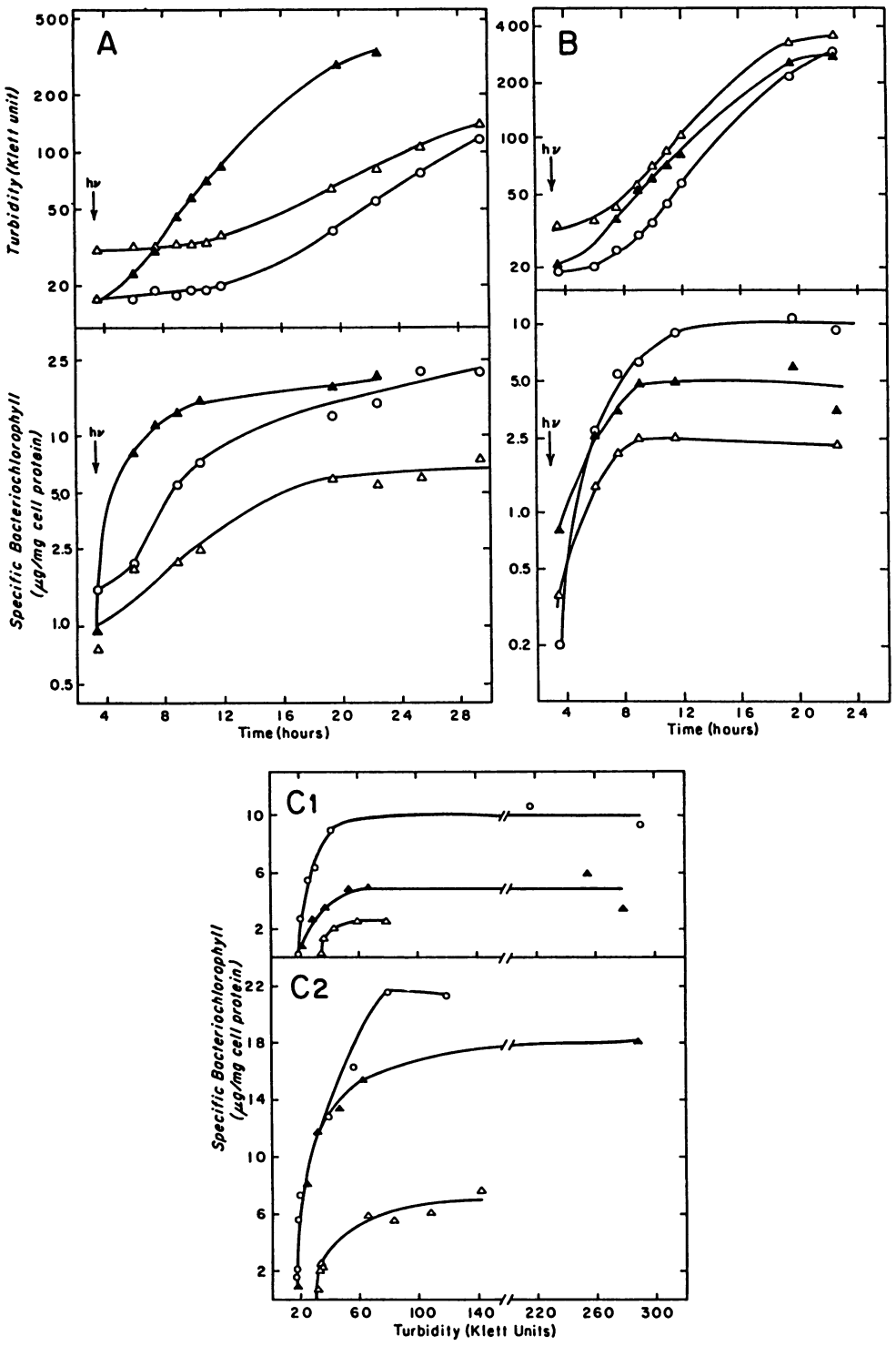

FIG. 6. Effect of incident light intensity on cell growth and specific Bchl content of RS2, RS103, and RS104. Cells were grown as described in Materials and Methods. Cultures of RS103 (○), RS104 (△), and RS2 (▲) were illuminated 15 min after shifting to no O_2 as indicated by arrows. Incident light intensity was 10 (A) or 110 (B) W/m^2. In panel C, the data from panels A and B have been replotted to show the change in specific Bchl *a* content as a function of the increase in culture turbidity (Klett units). Cells were grown at 110 (C1) and 10 (C2) W/m^2.

formation of long chains of cells (Fig. 8). It is not clear if the long tubules arise from the irregularly shaped membrane or if the composition of the two membrane types is homogeneous throughout any given cell. The cells observed were grown at 10 W/m^2; it is possible that at high light intensities (110 W/m^2) there are different ratios of the long tubules when compared with the cytoplasmic membrane-attached irregular vesicles which may give rise to an altered fractionation of

TABLE 2. Effect of incident light intensity on whole cells and chromatophores from mutant and wild-type *R. sphaeroides*

Strain	Incident light intensity (W/m^2)	Specific Bchl content (μg/mg of protein, mean ± SD)	
		Whole cell	Chromatophore
RS2	110	4.7 ± 0.8	25.7 ± 4.9
	10	18.2 ± 1.7	53.1 ± 8.9
RS103	110	9.9 ± 1.9	36.2 ± 5.6
	10	24.2 ± 6.8	65.3 ± 15.5
RS104	110	3.8 ± 0.7	13.7 ± 3.0
	10	8.8 ± 1.6	28.0 ± 2.4

such membranes when cells are broken. These differences may contribute to the differences seen in SDS-PAGE analyses shown in Fig. 7.

R. sphaeroides L37 (34), a mutant which lacks B800-850 spectral complexes but contains carotenoids, was also examined. These cells showed aberrant membrane morphology (Fig. 9). There were large sacklike membranes attached to the cytoplasmic membrane (Fig. 9), and many cells showed what seemed to be multiple membrane sacks almost exclusively at the poles of the cells. In contrast to RS104, there was no evidence of the long membrane tubules in L37 and the cells appeared to be of normal size. SDS-PAGE profiles of L37-derived chromatophores were similar to those of RS104 except that the previously mentioned 15A polypeptide is present, although in greatly reduced amounts in these chromatophores (data not shown), whereas it is absent altogether in RS104 (see above). Finally, although only a limited number of cells were examined, there appeared to be an unmistakable correlation between the appearance of "new" membrane sacs localized at the poles and the equatorial constriction of the cells during cell division.

Examination of aerobic chemoheterotrophically grown strain 2.4.1 for B875-α polypeptide. The above analysis demonstrated that Bchl-binding polypeptides can accumulate in RS103 chromatophores even if they are not assembled into a functional complex. We are currently determining whether the reduced levels of B875-α in RS103 chromatophores is due to decreased synthesis of this protein or to an increased rate of turnover due to the lack of functional B875 complexes or both. Since it has been shown previously that mRNAs specific for the B875 polypeptides are present in chemoheterotrophic cells (47, 48), we assayed soluble and particulate fractions derived from chemoheterotrophic and photosynthetic cells in a Western blot analysis, using the B875-α antiserum (Fig. 10). No B875-α was detected in either soluble or particulate fractions from aerobically grown cells. As expected, the majority of B875-α in photosynthetic cells was found in the membrane fraction. The low level of B875-α found in the soluble extracts was due to the slight contamination of this fraction with photosynthetic membranes, since the soluble fraction was derived following a 1-h 150,000 × *g* centrifugation. Also, note that the protein load was considerably less in the photosynthetic membrane sample. These data suggest a different mechanism for the control of synthesis or membrane association of B875-α under chemoheterotrophic versus photosynthetic conditions.

DISCUSSION

In this study, we have characterized two LH mutants (RS103 and RS104) by their in vivo absorption spectra, photosynthetic growth characteristics, and chromatophore composition. The energy transfer capabilities of RS103 and RS104 have been previously characterized (32). RS104 transferred light energy to the RC with a wild-type efficiency even though the Bchl/RC ratio (i.e., PU size) was much lower (32). However, in RS103, the efficiency of energy transfer was reduced to 24% of that measured for RS2 even when measured at the same Bchl/RC ratio (32). Since only RS103 was affected in energy transfer to the RC, these data support the model in which light energy absorbed by the B800-850 complex is transferred to the RC in an obligatory fashion through the B875 complex (33). Recently, the absorption spectrum of RS103 was reexamined at 77 K (24) to confirm the absence of B875 complexes.

Our characterization of the LH mutants was aided by purification and analysis of LH spectral complexes. Purification of the LH spectral complexes also allowed us to address the function of the three major ICM polypeptides (15A, -B, and -C) previously purified by Cohen and Kaplan (9, 10). These three low-molecular-weight polypeptides were proposed to be part of the LH apparatus based upon their

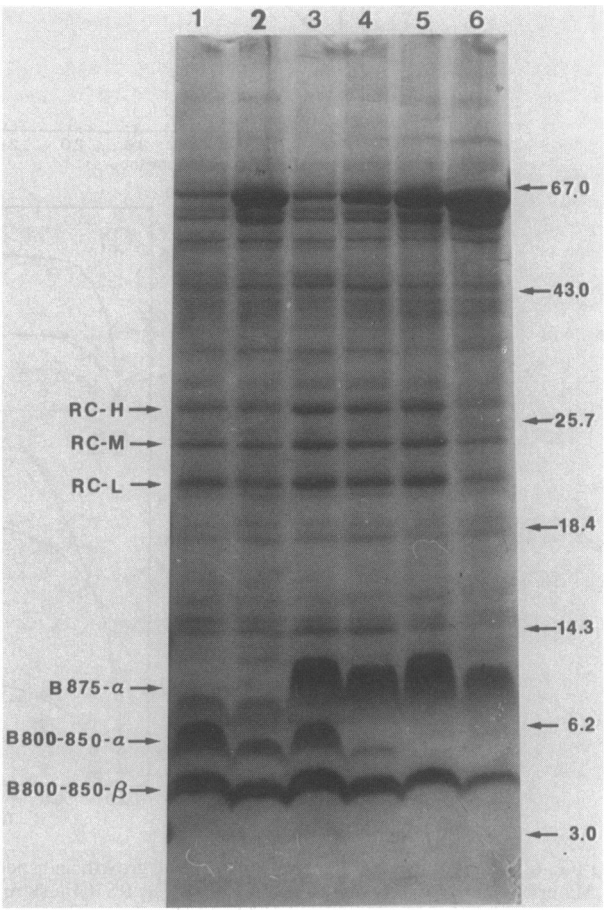

FIG. 7. Analysis by SDS-PAGE of chromatophores prepared from RS103, RS2, and RS104 grown at 10 (lanes 1, 3, and 5) and 110 (lanes 2, 4, and 6) W/m^2. Each lane contains 40 μg of chromatophore protein as follows: RS103, lanes 1 and 2; RS2, lanes 3 and 4; RS104, lanes 5 and 6. Samples were prepared for electrophoresis by solubilization at 75°C for 10 min in 62.5 mM Tris hydrochloride, pH 6.8–10% glycerol–2% SDS–5 mM dithiothreitol–0.01% bromophenol blue at a protein concentration of 2 mg/ml.

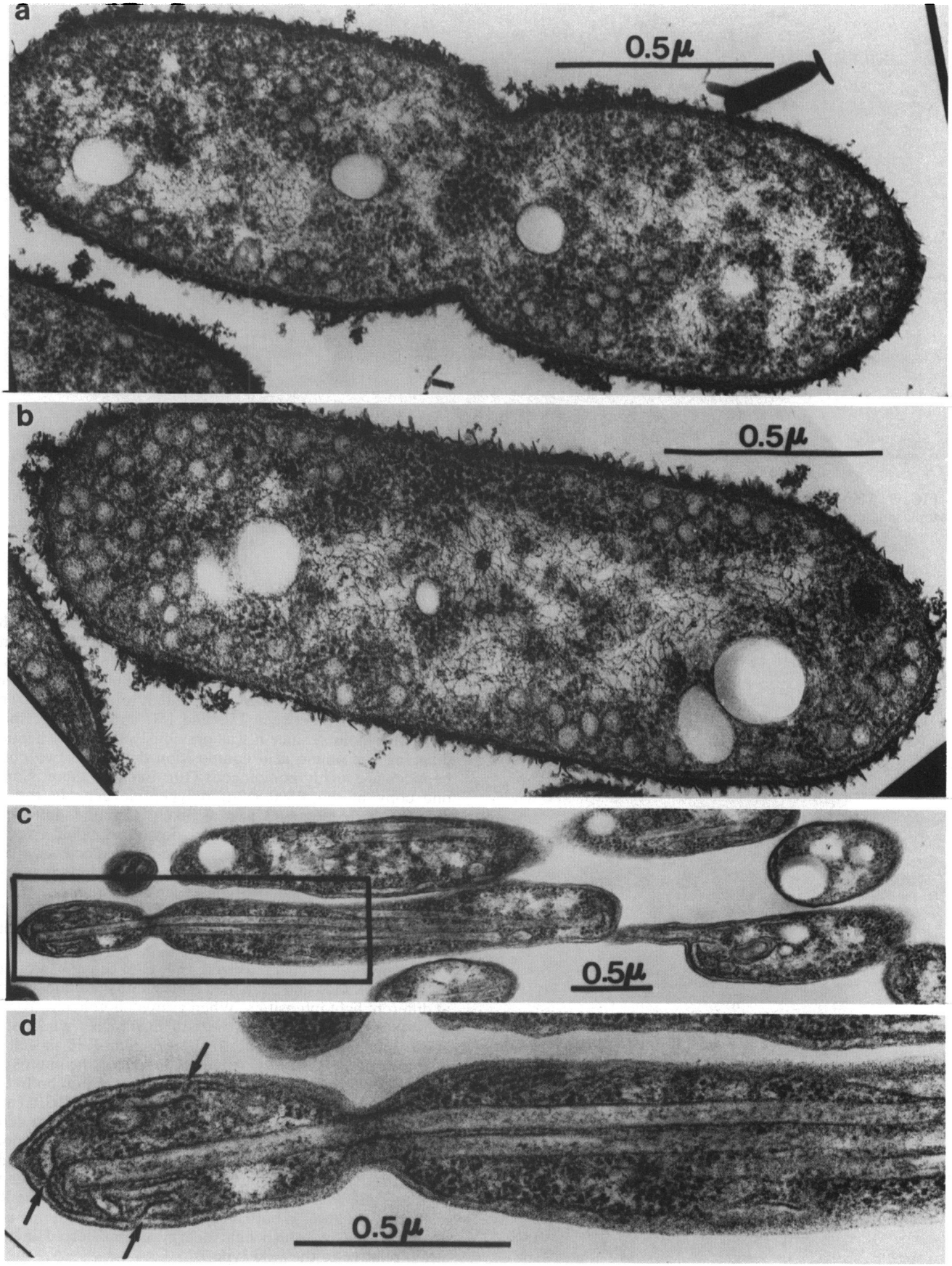

FIG. 8. Thin-section electron micrographs of wild-type and mutant strains of photosynthetically grown *R. sphaeroides*. (a) *R. sphaeroides* RS2 grown at 10-W/m^2 incident light intensity. Many approximately 50-nm ICM vesicles can be seen as well as large nonstaining β-polyhydroxybutyrate granules. (b) *R. sphaeroides* RS103 grown at 10-W/m^2 incident light intensity. This mutant is morphologically identical to the wild-type strain (RS2). (c) *R. sphaeroides* RS104 grown at 10-W/m^2 incident light intensity. Note the irregularly shaped ICM at the cell periphery and the long tubes of membrane running the entire length of the cell and continuing into adjacent cells. Boxed area is shown in detail in panel d. (d) Enlargement of boxed area of panel c. Arrows indicate clear connections of the peripheral ICM to the cytoplasmic membrane.

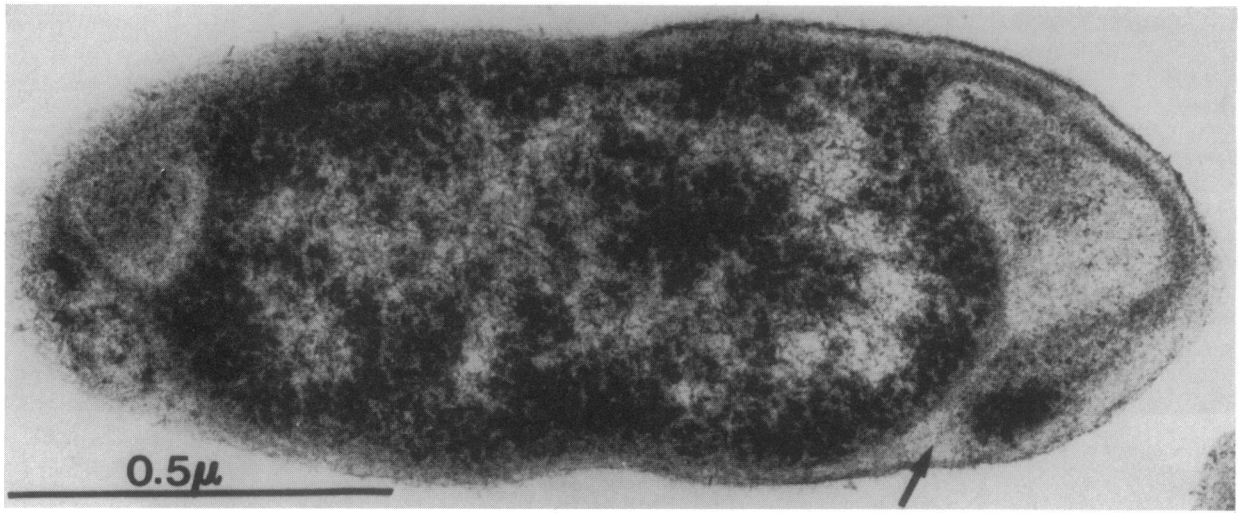

FIG. 9. Thin-section electron micrograph of *R. sphaeroides* L37 (B800-850$^-$). Arrow indicates connection of large ICM vesicle to cytoplasmic membrane near the cell pole; an additional large ICM vesicle can be seen at the opposite cell pole.

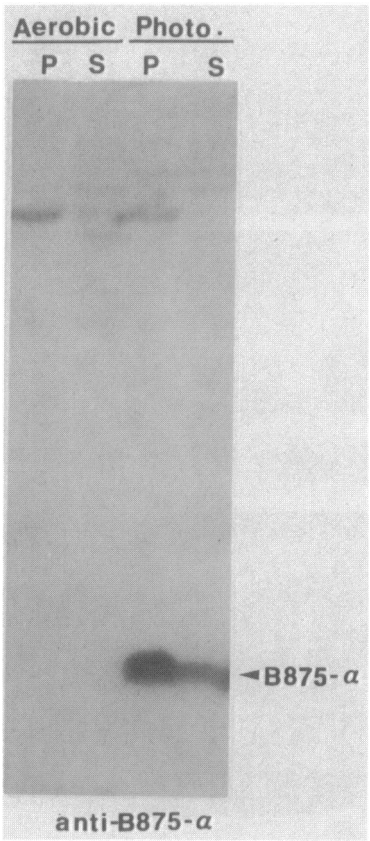

FIG. 10. Western blot analysis with antibody raised against B875-α polypeptide, using ^{125}I-labeled protein A for detection. A 20-μg portion each of particulate (P) and soluble (S) fractions (17) were analyzed from aerobic chemoheterotrophically grown strain 2.4.1. From photosynthetic cells, 7.5 μg of the particulate and 20 μg of the soluble extract were analyzed.

abundance in purified chromatophores. Comparison of the isoelectric points and amino acid compositions of 15B and 15C (9, 10) with those deduced from the amino acid sequences for the B800-850-α and B800-850-β polypeptides (43) demonstrated that 15B is equivalent to B800-850-β. The major difference between 15B and 15C appears to be their isoelectric points, since it was previously shown by peptide mapping and amino acid composition that these two polypeptides are highly homologous (10). Because there is only one copy of the B800-850-β polypeptide gene in the *R. sphaeroides* genome (27), it is likely that the difference between 15B and 15C is due to posttranslational events. Theiler et al. (42, 43) previously demonstrated heterogeneity in the amino termini of B800-850-β polypeptide; some contained amino-terminal methionine and the remainder had blocked amino-terminal threonine residues. This would be consistent with posttranslational modification of the β subunit.

We have also characterized the ICM composition of RS103 and RS104 and examined their photosynthetic growth at different light intensities. When grown photoheterotrophically at either 110 or 10 W/m^2, RS103 had higher whole-cell- and chromatophore-specific Bchl levels than RS2 as well as an increased B800-850/RC polypeptide ratio in the photosynthetic membrane. This appears to result from both the increased B800-850/RC spectral complex ratio in RS103 and an increase in ICM per cell. The previously observed derepression of B800-850 complex synthesis in RS103 cannot compensate for the documented inefficient transfer of light energy to the RC (32). Thus, it appears that the presence of increased B800-850 in the absence of B875 "blinds" the RC in RS103, resulting in both a decrease in the growth rate and the efficiency by which light energy is transferred in this strain relative to the wild type.

RS104 lacked B800-850 spectral complexes, apoproteins, and colored carotenoids. Interestingly, the 15A polypeptide of Cohen and Kaplan (9, 10) was also lacking in RS104 chromatophores. Chory et al. (5) have previously shown that the 15A polypeptide is not present in cells grown under

high-oxygen (25%) chemoheterotrophic conditions and that the 15A polypeptide accumulated in membranes subsequent to B800-850 spectral complexes or 15B and 15C polypeptides when chemoheterotrophically grown *R. sphaeroides* 2.4.1 was shifted to strict anaerobic photosynthetic conditions (nongratuitous shift) (5). The physiological function of 15A remains to be determined, but the lack of this polypeptide in RS104 or B800-850 complexes combined with the kinetics of its appearance following a nongratuitous shift suggests that it is not required in stoichiometric amounts for B800-850 spectral activity. In *Rhodobacter capsulatus*, a relatively abundant 14,000-dalton chromatophore-polypeptide has been shown to be associated with the B800-850 complex, although it is not required for spectral activity (19). Moreover, some blue-green mutants in *R. capsulatus* are deficient in this 14,000-dalton polypeptide but, unlike those in *R. sphaeroides*, they still contain the B800-850-α and -β subunits (49). Whether 15A is equivalent to the *R. capsulatus* 14,000-dalton polypeptide is unclear, especially since 15A does not appear to be present in stoichiometric amounts with other LH polypeptides in chromatophores. Analysis of the *puc* operon indicates that the coding region for polypeptide 15A is not linked to the B800-850 structural genes (27). Moreover, in *R. capsulatus*, mutations leading to the blue-green phenotype have been mapped to the carotenoid biosynthetic genes, which are not closely linked to the *puc* operon (49).

The characterization of *R. sphaeroides* mutants deficient in LH complexes has also been useful in addressing questions of ICM assembly and its regulation. For example, the B875/RC complex ratio in chromatophores remained constant in RS104, providing genetic evidence that regulation of the B875/RC complex ratio is fixed at the molecular level due to the differential transcription of the *puf* operon (48). Since the LH complexes constitute the major ICM polypeptides, it was interesting that RS104 formed long tubes rather than vesicular invaginations. The work presented here implies that the B800-850 complex may be important for attaining normal photosynthetic membrane morphology in *R. sphaeroides*. Mutants lacking a functional B800-850 complex (RS104, L37, and R26 [29]) all exhibit grossly altered membrane morphology. Interestingly, the SDS-PAGE profile of chromatophores derived from R26 (12) is similar to that from both RS104 and L37. At this time we cannot determine whether it is the loss of the B800-850 polypeptides, the functional pigment-protein complex, or some additional non-pigmented component which leads to the overall effect on membrane structure. The genetic defect in RS104 and L37 is currently unknown, but either could be in synthesis of the B800-850 polypeptides or in a component critical for synthesis, assembly, or insertion of the B800-850 complex, which is, in turn, necessary for normal ICM structure. Moreover, the (approximately) 62,000-dalton polypeptide which accumulated in the ICM at high light intensities may function to maintain the normal protein/phospholipid ratio, which might be altered upon the loss of these major polypeptide species in the mutants. It has recently been suggested that carotenoids have an effect on the conformation of the B890 apoproteins from *Rhodospirillum rubrum* (4), suggesting that further studies are required to determine the basis for the pleiotropic nature of B800-850-deficient mutants in the formation of the photosynthetic membranes. The availability of the structural genes for the B800-850 complex (27) and the recent identification and isolation of the *R. sphaeroides* genes for carotenoid biosynthesis (35, 39) will allow such mutants to be characterized at the molecular level.

The study of RS103 has been equally enlightening. Using antibody to the B875-α polypeptide, we have been able to detect significant levels of this polypeptide in RS103 chromatophores, although there is no B875 spectral complex present. This suggests that the B875-α polypeptide can be inserted into and accumulate in chromatophores in the absence of functional B875 complexes. We know that the mutation in RS103 does not affect transcription of this operon (J. Davis and S. Kaplan, unpublished results) so the fact that less than wild-type levels of this protein are found does not preclude the previously suggested stabilizing effect of Bchl on polypeptide insertion (8, 28). The observation that the B875-α polypeptide can be inserted into the ICM in RS103 leads us to conclude that the lack of detectable B875-α polypeptide in chemoheterotrophically grown wild-type cells cannot be simply due to the requirement for association with Bchl for insertion or stability of this polypeptide. Moreover, the properties of RS103 raise interesting questions about how Bchl synthesis is coupled to synthesis of the individual LH complexes. For example, the steady-state level of Bchl and B800-850 complex synthesized in RS103 was higher than in the wild type, particularly when high light intensities were used for growth. In contrast, synthesis of B875 complexes is not derepressed in RS104 relative to the wild type at any light intensity. These observations suggest that synthesis of the B800-850 complex is more tightly coupled to the levels of Bchl than is synthesis of the B875 complex. Whether coupling of synthesis of individual LH complexes to Bchl levels occurs at the transcriptional or the translational level remains to be determined, but we anticipate that further analysis of the synthesis of such complexes in mutants such as these will be useful.

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