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Effects of Incident Light Levels on Photosynthetic Membrane Polypeptide Composition and Assembly in *Rhodopseudomonas sphaeroides*

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Cells of *Rhodopseudomonas sphaeroides* were grown anaerobically with incident light levels ranging between 4,500 and 400 footcandles (ca. 48,420 and 4,304 lux). Cells grown with the higher light levels had lower contents of total bacteriochlorophyll and incorporated L-[U-^{14}C]leucine into membrane protein at higher rates than cells grown with lower light levels. The former cells also contained relatively lower amounts of light-harvesting membrane polypeptides as compared with the latter cells. In contrast, the relative amounts of reaction center membrane polypeptides were approximately the same with varying incident light levels. The relative amounts of these membrane polypeptides were correlated with differences in rates of synthesis and assembly of the polypeptides into membrane by measuring the rates of incorporation of L-[U-^{14}C]leucine into the membrane-bound polypeptides. No significant differences in rates of turnover of these polypeptides were detected under the varying incident light levels as measured in pulse-chase radioactive labeling experiments.

Cells of the photosynthetic non-sulfur bacterium *Rhodopseudomonas sphaeroides*, grown anaerobically in light or with low aeration in the dark, contain an extensive vesicular intracellular membrane system that houses the photosynthetic apparatus (13, 17). Under these growth conditions, specific membrane proteins occur apparently in association with the intracellular membrane system (3, 7, 8, 25). Among the most prominent of these proteins are three polypeptides with molecular weights of 21,000, 24,000 and 28,000, which are involved with reaction center function, and one or two polypeptides with molecular weights between 9,000 and 11,000, which are believed to be involved with light-harvesting function (2, 25). All of these polypeptides occur in association with bacteriochlorophyll *a*. The intracellular membrane system and these specific polypeptides are not detectable in cells grown with high aeration (24).

Sistrom (21) and Cohen-Bazire and Sistrom (4) showed that variations in light intensity during anaerobic growth of *R. sphaeroides* affect the growth rate, the extent of photosynthetic membrane development as revealed by electron microscopy, and the cellular pigment content. Recently, Aagard and Sistrom (1) demonstrated that the relative amounts of the B850 form of light-harvesting bacteriochlorophyll and reaction center bacteriochlorophyll were a function of incident light levels. More specifically, cells grown under low light levels contained large ratios of B850 light-harvesting bacteriochlorophyll to reaction center bacteriochlorophyll, and cells grown under high light levels contained smaller ratios. In contrast, the amounts of the B875 form of bacteriochlorophyll, also believed to have light-harvesting function, occurred in fixed stoichiometry to the amounts of reaction center bacteriochlorophyll.

In view of the relationships between incident light levels and ratios of light-harvesting to reaction center bacteriochlorophyll, it was of interest to consider how the specific polypeptides associated with these forms of bacteriochlorophyll varied in amounts and rates of assembly in the photosynthetic membrane. The present report describes the relative amounts and rates of assembly of the reaction center and light-harvesting membrane polypeptides in cells of *R. sphaeroides* grown anaerobically with different light levels.

MATERIALS AND METHODS

Organism and growth conditions. Cells of *R. sphaeroides* NCIB 8253 were grown anaerobically in MG medium (12) in 125-ml prescription bottles filled to capacity and capped. The bottles were incubated at 30°C with no agitation and grown to an optical density of 1.0 to 1.5 measured at 680 nm. Incandescent light was supplied by 25-, 60-, 75-, or 150-W household light bulbs (General Electric) to provide

400, 1,800, 2,200, and 4,500 footcandles (ca. 4,304, 19,368, 23,672, and 48,420 lux), respectively, measured with a Weston model 756 light meter.

Preparation of membrane fractions. Cells were harvested by centrifugation at 5,000 × *g* for 10 min and washed once with 10 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.5). The washed cells were suspended in the same buffer to an optical density of 40 measured at 680 nm. The suspended cells were disrupted by two passages through a French pressure cell (Aminco) at 16,000 to 18,000 lb/in^2. Approximately 200 μg of deoxyribonuclease I per ml was added, and the crude extracts were incubated at room temperature for 20 min. The crude extracts were centrifuged at 15,000 × *g* for 20 min at 8°C. The supernatants were recovered and centrifuged at 144,000 × *g* for 90 min at 8°C. The resultant pellets were suspended in 10 mM Tris-hydrochloride (pH 7.5) and centrifuged again at 144,000 × *g* for 90 min at 8°C. The washed pellets were suspended in the same buffer (15 mg of protein per ml) (designated membrane fractions) and stored at 5°C.

Acetone-methanol extraction of membrane fractions. Bacteriochlorophyll, carotenoid pigments, and lipids were extracted from membrane fractions by adding 10 volumes of acetone-methanol (7:2, vol/vol) to membrane preparations, shaking the mixtures, and centrifuging the mixtures at 3,000 × *g* for 10 min. The supernatants were removed, and the pellets were extracted a second time. The extracted pellets were dissolved in 0.1 N NaOH (15 mg of protein per ml) (designated the extracted membrane fractions) and stored at 5°C.

Pulse-labeling and pulse-chase-labeling experiments. For radioactive pulse-labeling of membrane polypeptides, 25 μCi of L-[U-^{14}C]leucine (312 mCi/mmol) was added to 125 ml of anaerobically growing cells with a culture optical density of approximately 1.0 measured at 680 nm. After 15 min, chloramphenicol (0.7 μg/ml) was added, the cells were harvested, and membrane fractions were prepared as described previously. In the pulse-chase experiments, 800 μmol of L-leucine was added to the culture after the above-mentioned labeling period, and the cells were incubated for an additional 15 min. Chloramphenicol was then added, the cells were harvested, and membrane fractions were prepared as described above.

SDS-polyacrylamide gel electrophoresis and autoradiography. Membrane fractions were mixed with equal volumes of a solution containing 40 mM Tris-hydrochloride (pH 8.0), 2% (wt/vol) sodium dodecyl sulfate (SDS), 2% (vol/vol) 2-mercaptoethanol, and 20% (vol/vol) glycerol. The mixture was heated at 100°C for 1 min. Approximately 20 μg of membrane fraction protein or membrane fraction containing 50,000 cpm of radioactivity was subjected to electrophoresis in each column. SDS-polyacrylamide gel electrophoresis procedures were essentially those of Laemmli (11), except that acrylamide and *N,N'*-bis-methyleneacrylamide concentrations were 12% (wt/vol) and 3.14% (wt/vol), respectively, in the separating gel and 4.5% (wt/vol) and 1.13% (wt/vol), respectively, in the stacking gel. Electrophoresis was performed in a slab gel apparatus (Hoefer) with 1.5-mm spacers. The slab gels were stained with Coomassie brilliant blue by the procedures of Fairbanks et al. (6). Gel autoradiographic procedures were done as described previously (24), except that the gels were dried on a slab gel dryer (Bio-Rad Laboratories) prior to exposure to X-ray film. Stained gels and autoradiograms were scanned, and the peaks were integrated on a Gelman Digiscreen scanner.

Other procedures. Protein was determined by the method of Lowry et al. (14) with bovine serum albumin as the standard.

Bacteriochlorophyll *a* levels were determined in acetone-methanol (7:2, vol/vol) extracts of whole-cell suspensions, using a millimolar extinction value of 76 at 770 nm (5).

Chemicals. L-[U-^{14}C]leucine (312 mCi/mmol) was purchased from Schwarz/Mann. Electrophoresis-grade SDS and acrylamide were obtained from Bio-Rad Laboratories. All other biochemicals were obtained from Sigma Chemical Co.

RESULTS

Bacteriochlorophyll content and membrane protein synthesis. The cellular content of bacteriochlorophyll varied inversely with incident light intensity during anaerobic growth (Table 1). Over a 11.3-fold range of light intensities there was an approximately fivefold difference in bacteriochlorophyll content, as observed previously (1, 4). In contrast, the rate of incorporation of radioactive leucine into total membrane protein varied proportionately with light intensity (Table 1). This parameter varied approximately 2-fold over the 11.3-fold range of light intensities. This relationship may reflect the higher growth rates (and thus higher rates of protein synthesis) observed for cells growing with higher light intensities as compared with the lower growth rates for cells growing with lower light intensities (4).

Membrane polypeptide composition. The

TABLE 1. *Bacteriochlorophyll content and rate of radioactive L-[U-^{14}C]leucine incorporation into total membrane protein of R. sphaeroides growing with different light levels*

Light level (footcandles)	Bacteriochlorophyll content (nmol/mg [dry wt] of cells)	Radioactive amino acid incorporation into total membrane protein (cpm/mg of membrane protein)
4,500 (48,420)[a]	3.8	409,393
2,200 (23,672)	8.0	361,704
1,800 (19,368)	8.1	309,850
400 (4,304)	20.0	239,827

[a] Numbers in parentheses indicate lux equivalents.

membrane polypeptide composition of cells grown with different light intensities was revealed by SDS-polyacrylamide gel electrophoresis and staining with Coomassie brilliant blue (Fig. 1). Polypeptide bands 9, 10, and 11 are associated with reaction center function. The light-harvesting polypeptides were clearly resolved into two bands, 15A and 15B, in the SDS-gel electrophoresis system as described previously (25). The occurrence of two bands suggests two distinct polypeptides, but other possibilities explaining this observation exist (see Discussion). Densitometric scans of these stained gels are given in Fig. 2. At higher light levels the relative staining intensities of the light-harvesting polypeptides were less than the staining intensities of these polypeptides of cells grown with lower light levels. In contrast, the relative staining intensities of the reaction center polypeptides remained essentially constant over the light level range. Determinations of the relative areas under the stained peaks in the scans are shown in Table 2. The area corresponding to polypeptide peak 10 was arbitrarily taken as equal to 1.00 since the staining intensity of this polypeptide was constant over the varying light intensities. The data indicate that the relative staining intensities of the peaks corresponding to the light-harvesting polypeptides 15A and 15B increased 2.84- and 7.3-fold, respectively, from the highest to the lowest light levels. In contrast, the relative staining intensities of the peaks corresponding to reaction center polypeptides did not vary significantly.

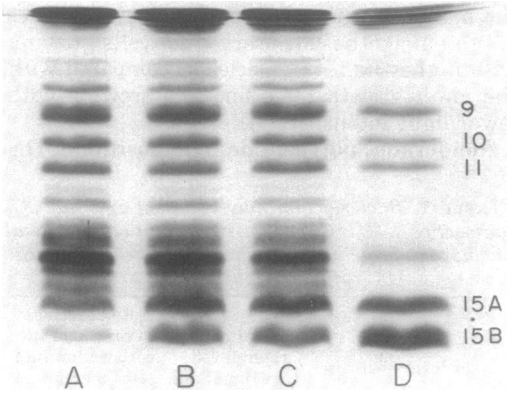

FIG. 1. *SDS-polyacrylamide slab gel of unextracted membrane fractions isolated from cells grown with (A) 4,500, (B) 2,200, (C) 1,800, and (D) 400 footcandles (for lux equivalent, see Table 1) of incident light. The gel was stained with Coomassie brilliant blue. The numbered bands correspond to photosynthetic membrane polypeptides as described in the text.*

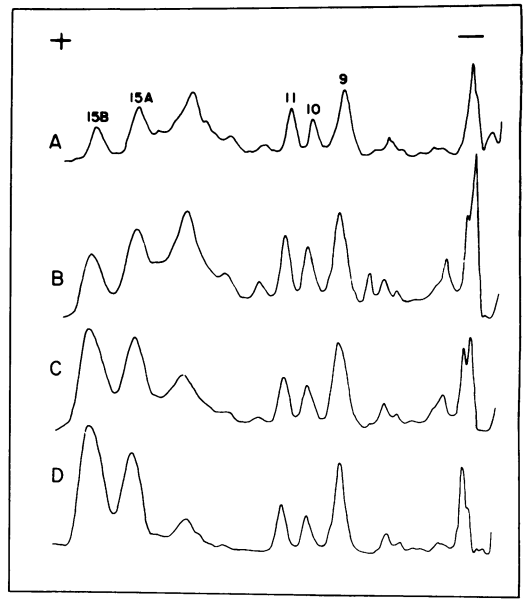

FIG. 2. *Densitometric scans of Coomassie brilliant blue-stained SDS-polyacrylamide slab gels of unextracted membrane fractions (Fig. 1). Membrane fractions were isolated from cells growing anaerobically with (A) 4,500, (B) 2,200, (C) 1,800, and (D) 400 footcandles (for lux equivalents, see Table 1) of incident light.*

TABLE 2. *Relative staining intensities of photosynthetic membrane polypeptides of unextracted membranes of R. sphaeroides growing with different light levels*

Light level[a] (footcandles)	Relative staining intensity[b] of polypeptide:				
	9	10	11	15A	15B
4,500	2.34	1.00	1.24	1.76	1.06
2,200	1.93	1.00	1.11	2.18	1.45
1,800	2.27	1.00	1.14	3.61	4.30
400	2.86	1.00	1.26	5.00	7.74

[a] For lux equivalents, see Table 1.

[b] Calculated by designating the area under stained polypeptide peak 10 equal to 1.00.

The area under polypeptide peak 9 was about twice that under polypeptide peak 10. Because the molar stoichiometry of the three reaction center polypeptides has been shown to be 1:1:1 (20), the larger area under polypeptide peak 9 is probably due to other protein which comigrated with polypeptide 9.

Because pigments and/or lipid may also stain with Coomassie brilliant blue, SDS-polyacrylamide gel electrophoresis was performed with

membrane fractions extracted with acetone-methanol. Densitometric scans of these stained gels are shown in Fig. 3, and determinations of relative peak areas are given in Table 3. In contrast to gels of unextracted membrane fractions, gels of extracted membrane fractions show that the light-harvesting polypeptide peaks 15A and 15B had relative increases of 1.8- and 3.2-fold, respectively, from the highest to lowest light levels. This represents 1.5- and 2.3-fold differences in staining intensities of peaks 15A and 15B, respectively, when comparing extracted and unextracted membranes.

These differences are most likely due to anomalous staining of pigment and/or lipids that comigrated with polypeptide bands 15A and 15B in SDS-polyacrylamide gels. The differences are probably not due to protein that was soluble in acetone-methanol. The acetone-methanol extracts contained protein ranging between 18 and 20% of the total membrane protein. However, polypeptide peaks 15A and 15B represent between 15 and 30% of the total stainable material as determined from densitometric scans of stained gels of unextracted membranes (Fig. 2). Hence, the small differences in the amounts of extracted soluble protein do not account for the large differences in the staining intensities between extracted and unextracted membranes.

Rates of membrane polypeptide assembly. To measure the rates of assembly of individual membrane polypeptides, cells growing with various incident light levels were pulse-labeled with L-[U-^{14}C]leucine. In these experiments, more than 90% of the radioactivity was incorporated into protein (data not presented). Densitometer scans of SDS-polyacrylamide gel autoradiograms of unextracted membrane fractions from these labeled cells are shown in Fig. 4. As in the case of the stained gels, the rate of labeling of polypeptide 10 was constant under the

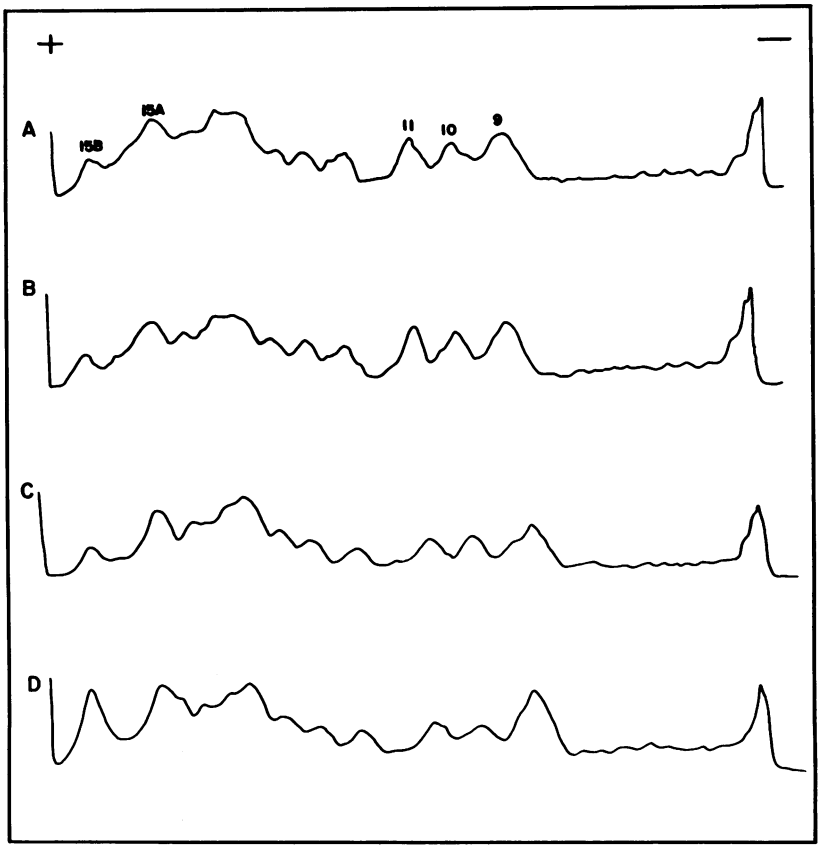

FIG. 3. *Densitometric scans of Coomassie brilliant blue-stained SDS-polyacrylamide slab gels of acetone-methanol-extracted membrane fractions. The membrane fractions were isolated from cells growing anaerobically with (A) 4,500, (B) 2,200, (C) 1,800, and (D) 400 footcandles (for lux equivalents, see Table 1) of incident light.*

TABLE 3. *Relative staining intensities of photosynthetic membrane polypeptides of acetone-methanol-extracted membranes of R. sphaeroides growing with different light levels*

Light level[a] (footcandles)	Relative staining intensity[b] of polypeptide:				
	9	10	11	15A	15B
4,500	1.60	1.00	0.94	1.60	0.58
2,200	1.80	1.00	1.05	1.78	0.60
1,800	1.24	1.00	1.02	2.01	0.91
400	2.04	1.00	1.02	2.85	1.83

[a] For lux equivalents, see Table 1.

[b] Calculated by designating the area under stained polypeptide peak 10 equal to 1.00.

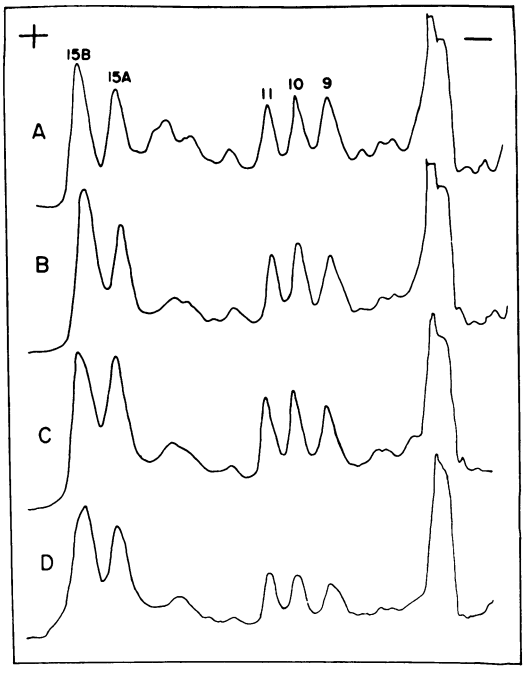

FIG. 4. *Densitometric scans of SDS-polyacrylamide gel autoradiograms of unextracted membrane fractions. The membrane fractions were isolated from cells growing anaerobically with (A) 4,500, (B) 2,200, (C) 1,800, and (D) 400 footcandles (for lux equivalents, see Table 1) of incident light. The cells were pulse-labeled with L-[U-^{14}C]leucine prior to cell harvest as described in the text.*

varying light intensities. Determinations of relative peak areas corresponding to the reaction center and light-harvesting polypeptides are given in Table 4. The area under polypeptide 10 was arbitrarily set equal to 1.00. The data show relative increases of 1.9- and 2.5-fold, respectively, of polypeptide peaks 15A and 15B over the 11.3-fold range of decreasing light intensities.

The amount of radioactivity in the peak corresponding to polypeptide 15B, when compared with that of polypeptide peak 10, significantly exceeds the relative staining intensity of polypeptide peak 15B in comparison to that of polypeptide 10 in stained gels of extracted membranes (cf. Tables 3 and 4, e.g., 1.29 and 0.58 for peak 15B at 4,500 footcandles [ca. 48,420 lux]). This may be largely attributed to the incorporation of radioactive label into protein that comigrated with polypeptide 15B and was extracted by acetone-methanol. Approximately the same amount of radioactivity was incorporated into the extractable protein in cells growing under the various light levels.

Turnover of membrane polypeptides. As shown above, the relative increases of radioactivity incorporation rates into polypeptides 15A and 15B quantitatively resemble the relative increases in the staining intensities of these polypeptides in SDS-polyacrylamide gels of extracted membranes with decreasing incident light intensities. This observation implies little difference in the rates of turnover of reaction center and light-harvesting polypeptides in cells growing with different incident light intensities. To confirm this observation, pulse-chase-labeling experiments were performed with cells growing under the various light intensities. SDS-polyacrylamide gel autoradiograms of pulse-chased membrane fractions were scanned densitometrically (Table 5). The relative amounts of radioactivity incorporated in polypeptides 9, 10, and 11 remained constant, and the relative amounts of radioactivity in polypeptide peaks 15A and 15B increased 1.8- and 2.9-fold, respectively, with decreasing incident light levels. These rates of incorporation quantitatively resemble those observed with pulse-labeled polypeptides. Therefore, the reaction center and light-harvesting polypeptides turnover at approximately equal rates or, alternatively, do not degrade appreciably after as-

TABLE 4. *Relative rates of L-[U-^{14}C]leucine incorporation into photosynthetic membrane polypeptides of R. sphaeroides growing with different light levels*

Light intensity[a] (footcandles)	Relative amt of radioactivity[b] in polypeptide:				
	9	10	11	15A	15B
4,500	1.21	1.00	0.826	1.23	1.29
2,200	1.10	1.00	0.847	1.41	2.00
1,800	1.09	1.00	0.941	1.91	1.89
400	1.07	1.00	0.975	2.31	3.12

[a] For lux equivalents, see Table 1.

[b] Calculated by designating the area under the autoradiogram polypeptide peak 10 equal to 1.00.

TABLE 5. *Relative amounts of L-[U-^{14}C]leucine incorporated into photosynthetic membrane polypeptide after pulse-chase labeling of R. sphaeroides growing with different light levels*

Light level[a] (foot-candle)	Relative amt of radioactivity[b] in polypeptide:				
	9	10	11	15A	15B
4,500	1.17	1.00	0.87	1.19	0.96
2,200	1.22	1.00	0.96	1.53	1.81
1,800	0.92	1.00	0.88	1.57	2.71
400	0.92	1.00	0.90	2.22	2.83

[a] For lux equivalents, see Table 1.

[b] Calculated by designating the area under the autoradiogram polypeptide peak 10 equal to 1.00.

sembly into membrane in cells growing anaerobically with different incident light levels.

DISCUSSION

The results presented indicate that the relative amounts of light-harvesting polypeptides in the photosynthetic membranes of *R. sphaeroides* vary inversely with the level of incident light. The relative amounts of reaction center polypeptides remain constant with varying incident light levels. The relative amounts of light-harvesting and reaction center polypeptides occurring in the membrane are correlated with the rates of synthesis and assembly of these polypeptides into membranes and are apparently not due to differences in turnover or degradation rates in the membranes.

A distinction between rates of polypeptide synthesis and subsequent assembly into the membrane cannot be made solely on the basis of the presented data. However, the close correlation between relative rates of incorporation of radioactive L-leucine into the membrane-bound polypeptides and the relative amounts of membrane polypeptides observed in stained polyacrylamide gels implies that assembly occurs soon after, or concomitantly with, synthesis. Previous attempts to find membrane polypeptide precursors not yet incorporated into membranes and possibly existing in the soluble, nonmembranous fractions of *R. sphaeroides* cells have failed (9, 23).

In a related study, Irschik and Oelze (10) reported a preferential decrease in the synthesis of a photosynthetic membrane protein fraction in cells of *Rhodospirillum rubrum* (zone G proteins identified in phenol-urea-acetate-containing polyacrylamide gels) adapting from growth under low light levels to growth under high light levels. This protein fraction was previously associated with the synthesis of bacteriochlorophyll *a* (19) and is possibly analogous to the light-harvesting polypeptides of *R. sphaeroides*. Of considerable interest in the case of *R. rubrum* is that the ratio of light-harvesting to reaction center bacteriochlorophyll remains invariant over a range of incident light levels (1) despite the apparent change in the amounts of associated zone G proteins.

The occurrence of two polypeptide bands, 15A and 15B, in the presently used SDS-polyacrylamide gel electrophoresis system suggests that the light-harvesting-associated protein is composed of two polypeptides as discussed previously (25). Clayton and Clayton (2) reported a single light-harvesting polypeptide in the same region on SDS-polyacrylamide gels. The apparent coordinate rates of assembly of polypeptides of bands 15A and 15B in membranes of cells growing with different light intensities and in adapting cells with differentiating membranes (23) suggest that polypeptides in both bands are associated with the light-harvesting complex. The relationship between the two polypeptide bands, however, is unclear. They may, in fact, represent two distinct polypeptides or, alternatively, may represent different conformations of the same polypeptide or different degrees of SDS binding to one kind of polypeptide. Clearly, the polypeptide composition of the light-harvesting complex requires further investigation.

The relative amounts and rates of assembly of polypeptides 15A and 15B reported here correlate with the relative amounts of the B850 forms of light-harvesting bacteriochlorophyll synthesized during anaerobic growth under varying incident light levels (1). This suggests a possible physical association of this form of bacteriochlorophyll to polypeptides 15A and 15B. The relative amounts of total cellular bacteriochlorophyll (Table 1) and relative amounts of polypeptides 15A and 15B (Table 3) are not directly proportional over the various incident light levels. Since the bulk of the total cell bacteriochlorophyll is B850 light-harvesting bacteriochlorophyll, the data indicate the absence of a fixed stoichiometry between these polypeptides and this form of bacteriochlorophyll with growth under various light levels.

In contrast, the amounts of the B875 form of light-harvesting bacteriochlorophyll remain stoichiometrically fixed to the amounts of reaction center bacteriochlorophyll (1), suggesting at least no fixed stoichiometry and possibly no association between this form of light-harvesting bacteriochlorophyll and polypeptides 15A and 15B, and perhaps instead an association with the reaction center polypeptides. In view of these suggestions, however, it is of interest to consider that a carotenoidless mutant of *R.*

sphaeroides, strain R-26, which lacks B850 but has B875, was reported to contain a low-molecular-weight membrane polypeptide with an electrophoretic mobility similar to those of polypeptides 15A and 15B in SDS-polyacrylamide gels (2, 3). We have confirmed and extended this observation by detecting polypeptides 15A and 15B in membrane preparations of strain R-26 cells, albeit in significantly lesser amounts than observed in membranes of photosynthetically grown wild-type *R. sphaeroides* cells (unpublished data). In addition, cells of *R. sphaeroides* mutant strain PM-8 contain B875 but lack the three reaction center polypeptides (3, 22). Thus, it seems conceivable that a small fraction of the light-harvesting polypeptides 15A and 15B is complexed with B875 bacteriochlorophyll and together are associated with reaction center components in a stoichiometrically fixed relationship. The possible association of the B875 form of bacteriochlorophyll with these polypeptides requires further investigation.

The constancy in relative amounts of reaction center polypeptides and contrasting variability in relative amounts of light-harvesting polypeptides in cells grown in varying light levels suggests separate regulatory systems for the synthesis of these polypeptides, as discussed previously (1, 15). Measurements of the kinetics of synthesis and assembly of these polypeptides during the biogenesis of the photosynthetic membrane system in *R. sphaeroides*, *R. capsulata*, and *R. rubrum* show that the reaction center polypeptides are coordinately synthesized and assembled with overall kinetics that differ from that of the synthesis and assembly of light-harvesting polypeptides (16, 18, 23). These observations likewise suggest a separate biosynthetic regulatory system for these polypeptides. The elucidation of the mechanisms by which these regulatory systems are coordinated through the interactions of light energy and/or oxygen levels remains an open and intriguing problem.

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