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Development and Growth of Photosynthetic Membranes of *Rhodospirillum rubrum*

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In cell-free extracts from low-aeration suspensions of *Rhodospirillum rubrum* strain G-9, bacteriochlorophyll *a* was distributed in two bands after rate-zone sedimentation in sucrose density gradients. From the physicochemical properties of these fractions, it was concluded that the upper band consisted of small membrane fragments, whereas the major band was composed of fragmented vesicular intracytoplasmic membrane (chromatophores). After a pulse with L-[^{35}S]methionine, apparent polypeptide subunits of the reaction center and light-harvesting complexes within the upper pigmented fraction were labeled more rapidly than those of chromatophores; after a chase with excess unlabeled L-methionine, radioactivity from these components within the upper band appeared to be chased into the corresponding polypeptides of chromatophores. These labeling patterns are interpreted to reflect growth initiation and maturation of the photosynthetic apparatus and may, in part, represent a general mechanism for the development of vesicular intracytoplasmic membranes.

It is generally accepted that the intracytoplasmic photosynthetic membrane (ICM) of facultatively photoheterotrophic *Rhodospirillaceae* arises initially at points of invagination of preexisting cytoplasmic membrane (CM) (14, 24). Biochemical evidence in support of this possibility in *Rhodospirillum rubrum* has been provided by pulse-chase experiments, which demonstrated that, at least during the initial stages of ICM development, the CM provides material for ICM growth (23). In recent pulse-chase studies of membrane development in phototrophically growing *R. sphaeroides* (20, 22), radioactivity destined eventually for the ICM appeared first in a distinct pigmented membrane fraction; this rapidly labeled material formed an upper pigmented band that sedimented more slowly than the fragmented ICM (chromatophore) band. The upper band was found to be enriched in newly synthesized bacteriochlorophyll *a* (Bchl)-protein complexes, and these pulse-chase results, together with fluorescence yield (13) and photochemical (12) evidence, all suggested that this fraction is derived largely from specific sites at which CM invagination is initiated.

In this communication, these observations have been extended to *R. rubrum*, which also possesses a vesicular ICM that has been suggested to be continuous with the CM (11). The carotenoidless mutant G-9 was chosen because, unlike the wild type, French pressure cell extracts of this strain yielded readily separable pigmented fractions after rate-zone sedimentation. For this study, Bchl formation was induced gratuitously in concentrated cell suspensions at low aeration in the dark. Under such circumstances, ICM development was essentially uncoupled from cell division and the synthesis of cell envelope layers. The repigmenting cells were subjected to pulse-labeling and pulse-chase analyses, and the distribution of radioactivity from L-[^{35}S]methionine into Bchl-associated polypeptides within the pigmented membrane fractions was examined.

MATERIALS AND METHODS

Induction of photosynthetic membrane development. *R. rubrum* carotenoidless mutant G-9 (kindly supplied by Judy Wall, Department of Biochemistry, University of Missouri, Columbia) was grown aerobically at 30°C in the dark in 2.8-liter Fernbach flasks on a defined medium (28) containing 0.125% ammonium sulfate and 0.1% yeast extract except where noted. Cells were harvested aseptically in the exponential phase, suspended to an optical density at 680 nm of 2.3 to 2.6 (Beckman DU spectrophotometer, 1-cm light path) (equivalent to 1.1×10^9 to 1.3×10^9 cells per ml) in fresh medium and incubated in the dark under low aeration in Erlenmeyer flasks, as described elsewhere (21), to induce ICM formation.

Membrane isolation and characterization. French pressure cell extracts were prepared as described (21) and centrifuged at 10,000 × *g* for 10 min, and the supernatant was subjected directly to rate-zone sedimentation on sucrose density gradients (22). The gradients consisted of 5 to 40% (wt/wt) sucrose with a 4-ml cushion of 60% (wt/wt) sucrose prepared in 1.0 mM Tris-hydrochloride buffer (pH 7.5). They were centri-

fuged at 27,000 rpm (96,000 × g_{avg}) for 160 min in a Beckman SW27 rotor at 4°C. The upper pigmented band was sedimented at 368,000 × g_{av} for 2 h and was purified further on a 20 to 55% (wt/wt) sucrose density gradient prepared in 10 mM Tris-hydrochloride buffer containing 20 mM EDTA (pH 7.5) and centrifuged at 200,000 × g_{avg} for 6.25 h in a Beckman SW40 Ti rotor at 4°C. Succinate dehydrogenase (succinate: phenazine methosulfate oxidoreductase [EC 1.3.99.1]) activity of gradient fractions, and the Bchl and protein contents of the isolated membranes were determined as described elsewhere (18). Lipid was obtained from trichloroacetic acid-precipitated gradient fractions by two chloroform-methanol (2:1, vol/vol) extractions, and phosphate was determined on combined lipid extracts and purified membrane fractions as described previously (2). Results were averaged from gradients and the membrane fractions derived from them in two different experiments.

Radiolabeling procedures. One liter of low-aeration cell suspension in which ICM development had been induced for 10.5 h was divided into five 200-ml samples that were pulse-labeled with 200 μCi of L-[^{35}S]methionine (1,270 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) for 30 s, 1.0, 2.0, 3.0, and 4.0 min, respectively, on a shaker at 200 rpm and 25°C. Protein synthesis was terminated by pouring the cultures over crushed ice in the presence of chloramphenicol (22), and the pigmented membranes were isolated from each sample as described above. For pulse-chase studies, 800 ml of low-aeration cell suspension induced for 11 h was pulse-labeled with 800 μCi of L-[^{35}S] methionine (980 to 1,050 Ci/mmol) for 5 min and chased with 2.5 mM L-methionine at 30°C. The incorporation of radioactivity was linear over this time period, and controls with trichloroacetic acid-precipitated whole cells showed that labeling was effectively terminated by the chase. Samples were removed at the beginning of the chase and at several intervals thereafter; protein synthesis was terminated, and the pigmented membrane fractions were isolated. For direct radioactivity measurements, membrane fractions were applied to filter paper disks, treated as described elsewhere (22), suspended in scintillation fluid (1), and counted in a Beckman LS-3150T liquid scintillation spectrometer, using the ^{14}C fixed-window iso-set module.

Electrophoresis. The upper pigmented and chromatophore fractions isolated from the radiolabeled cells were solubilized by boiling for 45 s in a buffered 2% sodium dodecyl sulfate (SDS) solution (1) and subjected to SDS-polyacrylamide gel electrophoresis (1) on gel slabs formed with a gradient of 10 to 15% acrylamide. Stained and dried slab gels were exposed directly to X-ray film (Du Pont Cronex-2DC; E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) at −80°C, and the developed autoradiograms were scanned in an Ortec model 4310 scanning densitometer (Ortec Inc., Oak Ridge, Tenn.). For the pulse-labeling experiment, radioactivity levels of individual polypeptide bands were integrated by gravimetric analysis of peak areas cut out from triplicate photocopies of the densitometric scans. The response of the film was found to follow an essentially linear relation with radioactivity in dried gels.

Specific radioactivity estimates. In the pulse-chase analysis of protein insertion into growing photosynthetic membranes of *R. sphaeroides* (22), specific radioactivities were determined from ^{3}H/^{14}C ratios in a double-labeling procedure. Before the preparation of cell-free extracts, fixed amounts of steady-state ^{14}C-labeled cells were added to ^{3}H-pulsed cell samples removed during a chase with the respective unlabeled amino acid. The ^{14}C radioactivity thus provided a measure of protein concentrations. Because it has been suggested that the ICM grows by both the formation of new invaginations and the lengthening of existing ones (9), it is necessary during the extended chase to compensate for the dilution of pulsed radioactivity in the ICM arising from the completely unlabeled invaginations which are newly formed at the CM during this period. The use of constant levels of the ^{14}C-labeled cells in the previous study (22) obviated this problem. Adjustment of radioactivities in this manner effectively confined the analysis to the dilution of only the original ^{3}H-pulse-labeled membrane protein increment in the existing invaginations by protein newly synthesized during the chase period. In this manner, it is possible in the ICM to follow the elongation of these existing invaginations in the absence of the ambiguity introduced by the formation of new ones. Furthermore, this analysis can be applied not only to maturing ICM with the isolation characteristics of chromatophores, but also to putative transient invagination sites isolated in this and in previous studies (20, 22) that represent an intermediate stage in ICM development. When radioactivities were balanced in the above procedure, the process during the chase of only the transient invagination sites existing at the end of the pulse was followed; the increment of new and entirely unlabeled invaginations formed in excess of these was thereby eliminated.

Although the radioautography procedure used in the present [^{35}S]methionine-labeling studies in *R. rubrum* afforded improved sensitivity and resolution in the examination of individual polypeptide components separated in SDS-polyacrylamide gel electrophoresis, it was necessary to employ different methods to estimate specific radioactivities and compensate appropriately for the formation of new membrane invagination sites during the 4-h chase. For this purpose, the full-scale deflections of densitometric scans of the autoradiograms were normalized to the cellular Bchl content at the end of the pulse. This was possible because the analysis was confined to polypeptide components of the Bchl complexes in which pigment is thought to be associated in fixed stoichiometries (4, 27, 31). Since the membrane preparations used in the electrophoretic analyses were always equalized on a protein basis, no further adjustments were necessary to obtain estimates of relative specific radioactivities of the resolved chromatophore polypeptides. For the membrane fraction containing putative transient invagination sites, however, it was also necessary to correct for Bchl complexes resident in the CM (10, 24) that were present in these preparations and from which radioactivity had apparently not been chased. For this purpose, the specific Bchl content of this material, which increased from 7.8 to 11.3 μg of Bchl per mg of protein during the chase, was equalized to that at the end of the pulse. In the short-term pulse-labeling study, the full-scale deflections of the scans were equalized on the basis of Bchl levels in isolated membrane preparations, as will be explained below.

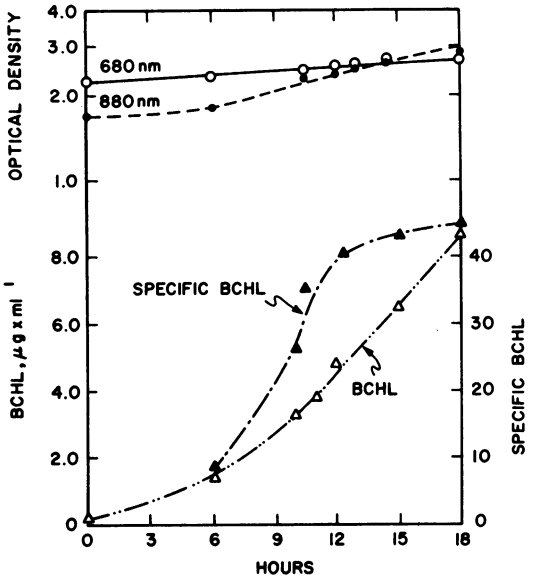

FIG. 1. The kinetics of ICM induction and low-aeration cell suspensions. Specific Bchl values (micrograms of Bchl per milligram of protein) were determined for chromatophores purified as described in the text. Culture density was monitored from the optical density at 680 nm, and initial estimates of Bchl levels were monitored from 880-nm readings (in vivo absorption band of light-harvesting complex). Bchl levels of cell suspension were determined as described in the text.

RESULTS

In the low-aeration *R. rubrum* G-9 suspensions, Bchl synthesis was initiated without a significant lag, and an essentially exponential rise in the cellular Bchl content from approximately 0.2 to 9.0 μg/ml of culture was observed over a period of 18 h (Fig. 1). In contrast, the specific Bchl content of chromatophores purified between 6 and 18 h followed a hyperbolic course; it increased initially at a rate greater than that of the cellular Bchl content and began to approach a steady state at 12 h, reaching a value near 45 μg of Bchl per mg of protein at 18 h. The optical density at 680 nm of the culture increased only slightly, consistent with limited cell division expected for concentrated cell suspensions (21).

Development of the ICM was also followed in electron micrographs of thin cell sections performed as described previously (2). In the aerobically grown cells, the CM appeared in close apposition to the cell wall, and no ICM was observed, whereas in the induced cells some vesicular ICM was detected near the cell periphery after 6 h, which became more evident at 12 h (data not shown). Unlike the ultrastructure of *R. sphaeroides* carotenoidless mutant R-26 (17), no lamellar ICM was observed in *R. rubrum* G-9 cells.

Although chromatophores formed the major pigmented fraction after rate-zone sedimentation of cell-free extracts from the induced *R. rubrum* G-9 cultures (Fig. 2A), an upper band that contained significant levels of Bchl *a* was also observed. Although an upper fraction was observed in the wild type as well, the two bands were not as widely separated, probably as a result of their greater diffusion within the gradients. The upper pigmented band from the G-9 mutant was separated from ribosomal material by a 2-h sedimentation followed by isopycnic centrifugation through a second sucrose density gradient (Fig. 2B). The specific Bchl values for the purified upper fractions ranged from 7.8 to 11.3 μg/mg of protein, which were one fourth to one half those of chromatophores. The sedimentation properties, the succinate dehydrogenase activity profile (Fig. 2A), and the appearance in electron micrographs of thin sections (20) all suggested that the upper pigmented band consisted mainly of small membrane fragments. Furthermore, in cells induced 11 to 12 h, the upper pigmented fractions, as they were isolated together with the CM, contained 52% of the lipid phosphorus after rate-zone sedimentation compared with 25% in the chromatophore fractions. Overall, the purified upper band had a 0.54 (wt/wt) ratio of phospholipid to protein, whereas for chromatophores, this value was 0.21.

A reconstruction experiment was performed to test the extent to which the upper pigmented fraction was contaminated by chromatophores. L-[^{3}H]leucine-labeled chromatophores from the experiment described in the legend to Fig. 5 were combined with unlabeled induced cells, and the mixture was subjected to French pressure cell treatment and rate-zone sedimentation. Virtually all of the radioactivity remained with the chromatophore fraction, and less than 4% was found in the upper pigmented band. By comparison, the upper fraction contained 28% of the total Bchl distributed in the two pigmented fractions.

In addition to numerous unidentified polypeptide bands, SDS-polyacrylamide gel electrophoresis suggested that the upper pigmented fraction contained apparent polypeptides of the Bchl-protein complexes observed in chromatophores (Fig. 3). These consisted of the three polypeptide subunits H, L, and M (27, 31) of the photochemical reaction center (apparent M_r 30,500, 24,500, and 21,000, respectively) and one or two polypeptides with M_r near 9,000 to 11,000, which form part of the light-harvesting complex (4, 25, 30, 31). Figure 3 also shows that after a pulse with L-[^{35}S]methionine, SDS-poly-

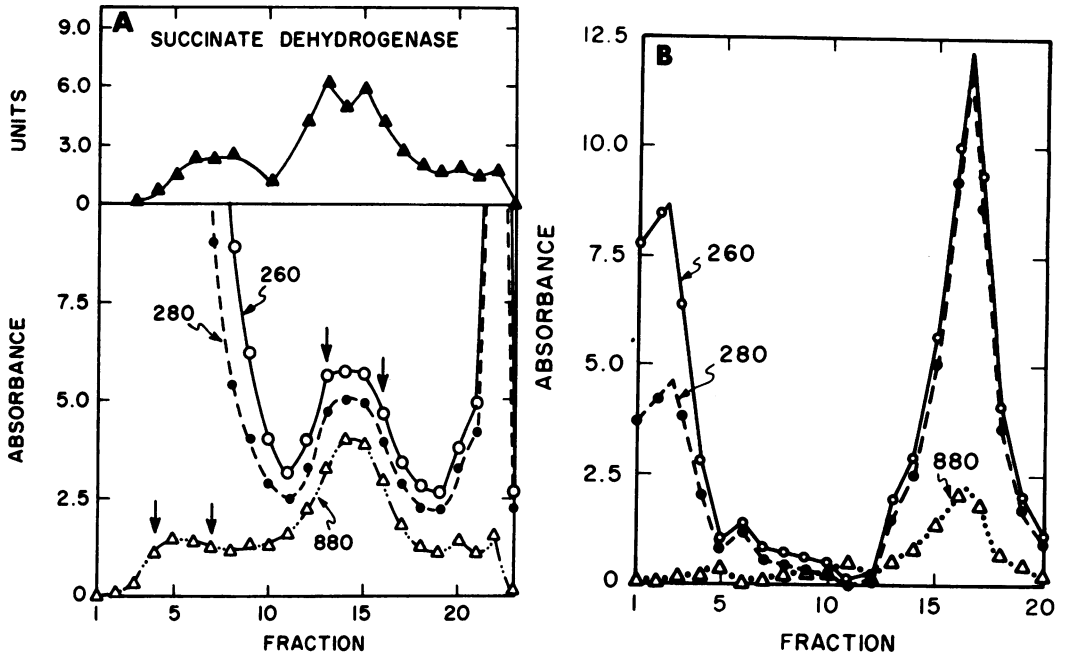

FIG. 2. Isolation of pigmented membrane fractions. ICM formation was induced for 11 h at low aeration. (A) Cell-free extract was subjected to rate-zone sedimentation on a sucrose density gradient as described in the text. Bchl was localized from 880-nm absorbance. UV absorbance was measured at 260 and 280 nm. Upper pigmented band, fractions, 4 through 7; chromatophores, 13 through 16; cell wall fraction, 21 through 23. (B) Further purification of the sedimented upper pigmented band by isopycnic centrifugation, as described in the text. Purified upper band, fractions 14 through 18. In both gradients, sedimentation is to the right.

acrylamide gel electrophoresis, and subsequent radioautography, radiolabeling of the upper fraction was confined mainly to those polypeptide components that comigrated with their apparent Bchl-associated counterparts in the chromatophore membrane.

Since such polypeptide components were labeled preferentially in an analogous upper fraction isolated from phototrophically grown *R. sphaeroides* (22), their kinetics of biosynthesis and assembly were examined in the upper pigmented and chromatophore fractions of *R. rubrum* G-9, undergoing ICM induction at low aeration. A comparison of the kinetics of L-[^{35}S]methionine incorporation into each of these two membrane fractions suggested that the upper pigmented band is also labeled preferentially in *R. rubrum* (Fig. 4A). For the overall membrane fractions, the upper pigmented band was labeled nearly three to six times more rapidly than chromatophores when they were equalized on protein and Bchl bases, respectively (Table 1). By subjecting the isolated membrane preparations to SDS-polyacrylamide gel electrophoresis, it was possible to assess for the first time the relative rates of biosynthesis and insertion of polypeptides in the various cellular membrane regions from which upper pigmented and chromatophore fractions are derived (Fig. 4B and C). When compared on protein and Bchl bases, the putative polypeptide components of Bchl-protein complexes within the upper band were labeled 1.1 to 3.5 and 3 to 10 times faster, respectively, than their chromatophore counterparts (Table 1). These data generally corroborate the results obtained with the overall fractions. Since under the induction conditions used here, the majority of the pulse-labeling in both the upper pigmented and chromatophore fractions occurred in the reaction center and light-harvesting protein (Fig. 3), a more valid comparison between these two membrane preparations for kinetic studies was obtained when they were normalized on the basis of Bchl content. Furthermore, the apparent invagination sites within the upper pigmented band are contaminated by CM, as suggested in SDS-polyacrylamide gels stained with Coomassie brilliant blue (data not shown) and by the lower specific Bchl and higher phospholipid content of this fraction in comparison to chromatophores. Thus, equalization on a protein basis includes a substantial quantity of unpigmented CM protein that is not relevant to this analysis.

Pulse-chase procedures were applied to the induced cell suspensions to critically assess the

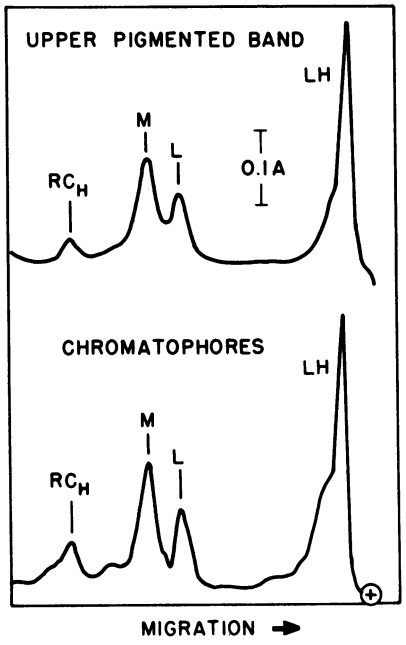

FIG. 3. Densitometric scans of autoradiograms obtained after SDS-polyacrylamide gel electrophoresis of membrane fractions isolated from cells subjected to a pulse-chase procedure. A low-aeration cell suspension in which ICM development was induced for 10 h was pulsed for 9 min with 1.0 μCi of L-[^{35}S]methionine per ml of culture and chased as described in the text. To facilitate maximal labeling, the upper pigmented fraction was isolated from cells at the end of the pulse, whereas the chromatophores were obtained 120 min after the start of the chase. In each case, 100 μg of sample protein was applied to the gel. Abbreviations: RC$_H$, M, and L, polypeptide subunits H, M, and L of photochemical reaction center; LH, protein associated with light-harvesting Bchl.

role of the upper band in the development of the *R. rubrum* ICM. In two separate experiments, cell suspensions were labeled with L-[^{35}S]methionine for 4 and 9 min, respectively, and further uptake was halted by a chase with excess unlabeled L-methionine. As expected, at the end of the pulse, the apparent polypeptide components of the Bchl complexes within the upper fraction were each more highly labeled than those of chromatophores (Fig. 5). Thereafter, radioactivity appeared to be chased into Bchl-associated polypeptides of the ICM, as indicated by the distinct decrease in their labeling within the upper fraction concomitant with an increase in their radioactivity observed in chromatophores. However, the unidentified band that migrated between the reaction center and light-harvesting polypeptides in chromatophores did not show increased radioactivity. When the upper pigmented band was only corrected for the increase in total cellular Bchl during the chase, no consistent removal of radioactivity from this fraction could be observed; however, because this fraction contains CM, which has been suggested to contain a significant quantity of Bchl complexes (10, 24), from which pulsed radioactivity would not be expected to be chased, this increment of Bchl was compensated for as described above (Fig. 5). With the exception of reaction center subunit H, this resulted in decreases in radioactivity during the chase period that ranged from approximately 1.2- to 1.7-fold for other polypeptides of the upper fraction, with the greatest decrease in the light-harvesting protein. In contrast, these components in the chromatophores showed approximately 1.3- to 4.2-fold increases over this period, with reaction center subunit H showing the most dramatic increase. These overall results are consistent with the possibility that some of the radioactivity which eventually accumulated within the ICM Bchl-protein complexes appeared initially in the upper band.

DISCUSSION

The results of this study further support the hypothesis that the *R. rubrum* ICM originates at invaginations of the peripheral CM (24). The

TABLE 1. Pulse-labeling rates of membrane polypeptides

Fraction	Radioactivity (10^3 cpm)		Relative rate	
	Per mg of protein per min[a]	Per μg of Bchl per min	Protein basis	Bchl basis
Upper pigmented	82.4[b]	8.6	1.00	1.00
Chromatophore	40.5	1.5	0.39	0.17
Upper pigmented				
LH[c]	681.1[d]	72.1	1.00	1.00
RC$_M$	492.4	51.4	0.72	0.72
RC$_L$	411.0	42.9	0.60	0.60
RC$_H$	81.4	8.5	0.12	0.12
Chromatophore				
LH	623.7	23.1	0.92	0.32
RC$_M$	232.2	8.6	0.34	0.12
RC$_L$	113.4	4.2	0.17	0.06
RC$_H$	64.8	2.4	0.10	0.03

[a] From slopes computed by linear regression analysis on plots of radioactivity versus minutes shown in part in Fig. 4. Correlation coefficients ranged between −0.91 and −0.99.

[b] Radioactivity expressed in 10^3 counts per minute. Values represent duplicate analyses at each time point.

[c] Abbreviations are defined in the legend to Fig. 3.

[d] Radioactivity integrated from autoradiograms of SDS-polyacrylamide gel slabs.

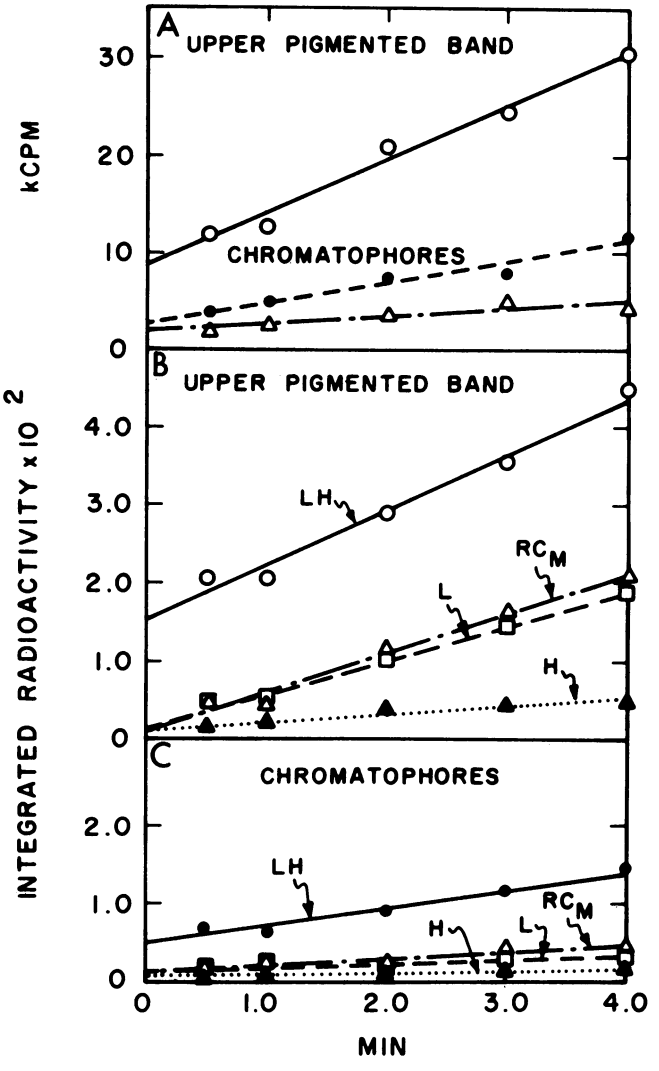

FIG. 4. Pulse-labeling of membrane polypeptides. Low-aeration cell suspensions in which ICM formation was induced for 10.5 h were pulse-labeled with L-[^{35}S]methionine for 4 min. (A) Upper pigmented and chromatophore fractions were purified at the indicated times, and radioactivity was determined as described in the text; symbols: ●, chromatophores equalized to upper fraction on a protein basis; △, chromatophores equalized to upper band on a Bchl basis. (B and C) Radioactivity of resolved polypeptide components integrated from autoradiograms of SDS-polyacrylamide gel slabs of the upper pigmented and chromatophore fractions, respectively. Integration is in arbitrary units as described in the text. Points were averaged from duplicate electrophoretic analyses at each time interval, and the Bchl levels of the upper pigmented and chromatophore fractions were equalized throughout since the analysis was confined to polypeptide components of the Bchl complexes. Lines through the points were calculated by linear regression analysis. Only the resolved polypeptide components of the pigment-protein complexes were analyzed. Abbreviations are defined in the legend to Fig. 3. Only the most highly labeled peak in the light-harvesting region is shown.

physicochemical and pulse-labeling properties of the upper pigmented band isolated here suggest that it contains material derived from membrane domains at which newly synthesized Bchl-protein complexes appear preferentially and that this fraction could include the numerous newly formed invagination sites observed in electron micrographs (9). Although the pulse-labeling of these complexes was more limited in chromatophores, it appears that much of their newly synthesized apoprotein is also inserted directly into the preexisting ICM (6, 16). The observed

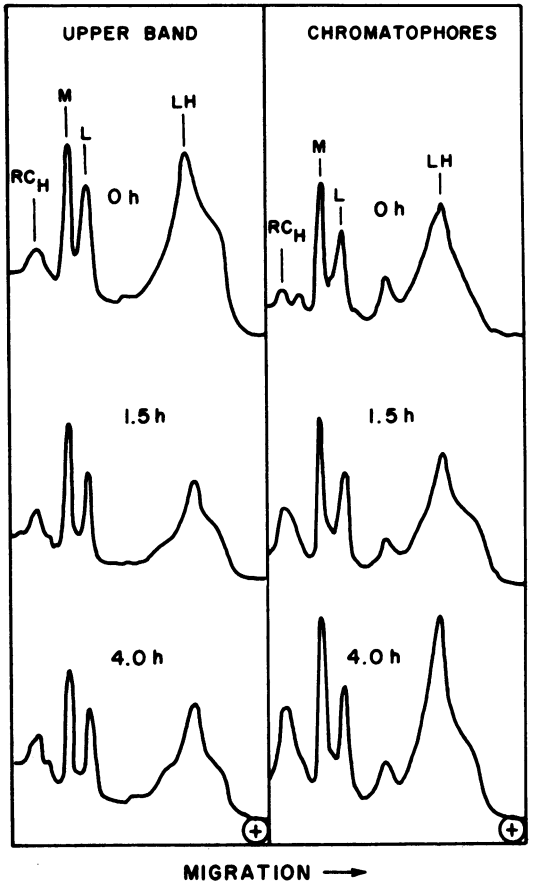

FIG. 5. Pulse-chase relations in polypeptide components of upper pigmented and chromatophore fractions. A low-aeration cell suspension was pulsed with L-[^{35}S]methionine for 5 min and chased with unlabeled amino acid for 4 h, as described in the text. Densitometric scans are presented for autoradiograms derived after SDS-polyacrylamide electrophoresis of the pigmented membranes isolated during the chase. Each lane of the gels contained 100 μg of protein. The height of the reaction center subunit M peaks in the chromatophores at 4.0 h and the upper band at 0 h have been equalized. The actual differences in relative radioactivities between polypeptide components of the upper and chromatophore fractions after a pulse are presented in Fig. 4. The chase effects appeared to be delayed for approximately 30 min. The scans of the membranes from the chased cells have been corrected for the formation of a new increment of membrane invaginations, as described in the text. The upper band from the chased cells has also been corrected for increased specific Bchl content during this period to compensate in part for the Bchl-associated polypeptides not chased from this fraction (10, 24). Essentially the same results were observed after a 9-min pulse with L-[^{35}S]methionine and when a low-aeration cell suspension induced for 10 h in the absence of yeast extract was pulse-labeled for 9 min with 1.0 μCi of L-[3,4,5-^{3}H]leucine (135 Ci/mmol; New England Nuclear Corp.) per ml of culture followed by a chase with 5.0 mM L-leucine. For the latter experiment, the gels were sliced into 1-mm segments, and radioactivity was determined by liquid scintillation counting (2). The decreases in radioactivity in the upper fraction over a 3-h chase ranged from 1.1- to 1.8-fold for polypeptide components of the reaction center and light-harvesting complexes. With chromatophores, 1.2- to 1.4-fold increases in these components were observed.

chase kinetics, although not as striking as those seen with *R. sphaeroides* (20, 22), may in part reflect the growth of the newly invaginated membrane regions to form more fully developed ICM with the isolation characteristics of chromatophores. The current study extends the observations made for phototrophically growing *R. sphaeroides* to essentially nondividing *R. rubrum* suspensions undergoing gratuitous induction of ICM formation. In comparison to the *R. sphaeroides* system, however, the pulse-chase procedures in these partially induced *R. rubrum* cells were complicated somewhat by the more limited amounts of proliferating ICM observed in electron micrographs and consequently reflected in the lower yields of pigmented membranes relative to the cell wall fraction (cf. reference 3).

An additional consideration in the present study is the apparent difference in the structure of the CM in *R. sphaeroides* and *R. rubrum* (10, 19, 24, 29). An essentially unpigmented CM fraction has been isolated from phototrophically grown *R. sphaeroides* and was thought to be derived from peripheral membrane regions conserved from chemotrophic cells (19, 29). In contrast, some incorporation of Bchl-protein complexes directly into the peripheral CM has been proposed for phototrophically growing *R. rubrum* (24), and in a recent freeze-fracture analysis (10), a significant increase in the number of intramembrane particles was observed on the plasmic fracture face of this structure in comparison to that from chemotrophically grown cells. This was largely attributed to an increase in particles of the size class predominant on the plasmic face of the ICM (10). No such difference was observed between the CM of phototrophically and chemotrophically grown *R. sphaeroides* (10), in agreement with the identical polypeptide composition of the isolated fractions (29). In this connection, it is also noteworthy that in a recent ultrastructural study of 3,3'-diaminobenzidine photooxidation in *R. rubrum*, this cytochemical reagent was observed to accumulate in both the CM and ICM regions of the cell (J. S. Gierczak, H. S. Pankratz, and R. L. Uffen, Abstr. Annu. Meet. Am. Soc.

Microbiol. 1980, J18, p. 83). This suggests further that in *R. rubrum*, components involved in photochemical reactions are distributed throughout the membrane continuum.

These apparent differences in CM architecture are also supported in the present study by the increasing specific Bchl content of the upper pigmented band during *R. rubrum* ICM development, together with the presence in these preparations of CM polypeptides and substantial quantities of phospholipid. Thus, in addition to transient membrane invagination sites, this fraction also appears to contain Bchl-protein complexes of the CM that show product rather than precursor behavior in the pulse-chase study. As a consequence, it was only possible to demonstrate precursor behavior for the apparent transient invagination sites by correcting for the presence of these CM Bchl complexes within the upper pigmented fraction. As pointed out in a recent review (15), precursor-product relationships between such invagination sites of upper pigmented fractions and isolated chromatophores apparently reflect their original cellular localizations. Thus, the pulsed radioactivity is ultimately displaced from the former to the latter by the continued membane growth during the chase.

Although results of pulse-labeling with [^{35}S]methionine suggest that the upper pigmented fraction is derived, in part, from membrane regions in which newly synthesized reaction center and light-harvesting proteins appear preferentially, it is not yet possible to distinguish whether this results from accelerated rates for translation of their mRNAs or for their insertion into the membrane. Nevertheless, it can be concluded that in these membrane regions and in the ICM, the light-harvesting polypeptides are pulse-labeled more rapidly than those of the reaction center. This is consistent with the differential labeling observed in a total membrane fraction isolated from *R. rubrum* during the initial 3 h of adaptive formation of these polypeptides at low aeration (26). In the present study, the most marked differences in the rates of pulse-labeling were observed between the reaction center L and M subunits of the upper pigmented and chromatophore fractions, respectively. Their pulse-labeling rates suggest that they appear coordinately in the membrane regions that give rise to the upper band; in contrast, the H subunit of the reaction center is labeled at less than one fifth this rate despite having the same number of methionine residues as the L subunit (31). For chromatophores, the increase in radioactivity during the chase was most marked with the H subunit, whereas that of the other polypeptides of the pigment complexes increased essentially in parallel. Furthermore, radioactivity pulsed into this subunit within the upper fraction was not removed during the chase. Thus, the H subunit appears to be directly assembled into the ICM independently of the rest of the reaction center. The possibility must also be considered, however, that reaction center subunit H has comigrated in the gel with another rapidly labeled polypeptide.

From the distribution of oxidoreductase and photophosphorylation activities in pigmented membrane fractions isolated during adaptation to phototrophic growth, a differential incorporation of functional components into discrete membrane domains has also been suggested for *R. capsulata* (7). Furthermore, the upper membrane fraction of *R. capsulata* has recently been shown (8) to share fluorescence yield and spectral properties reported previously for *R. sphaeroides* (12, 13) that are also consistent with the origin of this material from a membrane site into which some pigment-protein complexes are inserted preferentially. This was supported further by the differential pulse-labeling of the two pigmented membrane fractions (5); however, unlike results obtained with *R. sphaeroides* (20, 22) and with *R. rubrum* in the present study, no chase of radioactivity from the upper to the lower fraction could be demonstrated with *R. capsulata* (5). This may be explained in part by the differences in membrane architecture in the latter organism in which tubular structures apparently differentiate into a vesicular ICM (5). Aside from the differences in the apparent membrane distribution of pigment-protein complexes between *R. sphaeroides* and *R. rubrum* discussed above, the architecture of their photosynthetic apparatuses is similar, and mainly vesicular membrane invaginations are present. Thus, the labeling patterns observed in these two organisms appear in part to represent a common mechanism for de novo growth initiation and maturation of a vesicular ICM.

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