JOURNAL OF BACTERIOLOGY, July 1986, p. 89–95
0021-9193/86/070089-07$02.00/0

Vol. 167, No. 1

Immunocytochemical Ultrastructural Analysis of Chromatophore Membrane Formation in *Rhodospirillum rubrum*

SUZANNE M. CROOK, SUZANNE B. TREML, AND MARY LYNNE PERILLE COLLINS*

Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin 53201

Received 23 December 1985/Accepted 31 March 1986

An immunocytochemical ultrastructural study of *Rhodospirillum rubrum* cultured under semiaerobic conditions was conducted to correlate the localization of functional components with membrane formation. *R. rubrum* is a facultatively phototrophic organism. Under reduced oxygen, this bacterium forms an intracytoplasmic chromatophore membrane that is the site of the photosynthetic apparatus. Immunogold techniques were used to localize intracellular protein antigens associated with the photosynthetic apparatus. Antibody, demonstrated by immunoblotting to be specific for the reaction center and light-harvesting photochemical components, was conjugated to colloidal gold particles and used for direct immunolabeling of fixed, sectioned specimens. Membrane invaginations appeared by 4 h after transition to induction conditions, and mature chromatophore membrane was abundant by 22 h. The occurrence of chromatophore membrane was correlated with bacteriochlorophyll *a* content and the density of the immunolabel. In uninduced (aerobic) cells and those obtained from cultures 0.5 h posttransition, the immunogold preferentially labeled the peripheral area of the cell. In contrast, in cells obtained after 22 h of induction, the central region of the cell was preferentially immunolabeled. These findings provided immunocytochemical evidence supporting the hypothesis that the chromatophore membrane is formed by invagination of the cytoplasmic membrane.

Rhodospirillum rubrum is a facultatively photosynthetic bacterium that has been used as a model in studies of membrane structure and formation (22, 36). Under aerobic conditions nonpigmented cells undergo chemotrophic metabolism. Under anaerobic conditions in the light, *R. rubrum* grows phototrophically. Bacteriochlorophyll *a* (BCHL) and carotenoids and associated proteins are localized in the intracytoplasmic chromatophore membrane. Ultrastructural and physical evidence has suggested that this chromatophore membrane is continuous with the cytoplasmic membrane (for a review, see reference 39). Moreover, studies revealing the opposite asymmetry of the chromatophore and cytoplasmic membranes in *R. rubrum* (34, 42) and other photosynthetic bacteria (8, 11, 25) imply the existence of a membrane continuum because the cytoplasmic face of the chromatophore membrane forms the outer surface of isolated chromatophores.

The apparent continuity between the chromatophore and cytoplasmic membrane has led to the hypothesis that the chromatophore membrane is formed by invagination of the cytoplasmic membrane (35). Evidence to support this hypothesis is provided by the freeze-etching studies of Golecki and Oelze (13). These investigators transferred chemotrophic cells to anaerobic, light conditions and observed indentations and protuberances on the convex and concave fracture faces, respectively, that were interpreted to be invaginations of the cytoplasmic membrane. The number of these invaginations was correlated with the increase in BCHL content of the cells.

Niederman and co-workers (32) isolated a fraction from phototrophically growing *Rhodopseudomonas sphaeroides* called upper pigmented band (UPB) because of its sedimentation properties. On the basis of kinetics in pulse-chase experiments, these investigators suggested that the UPB may be the site of membrane growth. These workers have further proposed that the UPB arises from the periphery of the cell and may represent membrane invagination sites (20). A similar UPB fraction was isolated from *R. rubrum* G9 that had been induced to form chromatophore membrane by incubation at low aeration (19).

Evidence consistent with the hypothesis that chromatophore membrane is formed by invagination of the cytoplasmic membrane is provided by morphological studies and by studies involving isolation of cell fractions and analysis of their functional constituents. The present communication reports immunocytochemical analysis of chromatophore membrane formation in *R. rubrum*. Immunocytochemistry permits functional analysis of ultrastructure by providing the means to correlate cellular structures with their biological role. Colloidal gold is a useful marker because it is electron dense, can be conjugated to immunoglobulins and other ligands, and can be quantified (14, 17). Only recently has colloidal gold labeling been applied to microorganisms. In most cases, surface antigens (1, 9, 43, 50) or nonprotein antigens (2, 23, 44) have been detected. Immunocytochemical localization of intracellular components requires that specimens first be fixed by freezing or chemical treatment and sectioned to expose the antigens; the conditions of this treatment must not destroy antigenic structure. Several intracellular protein antigens have been localized with indirect techniques (21, 41, 45, 46) in which a secondary electron-dense ligand is used to localize the site of binding of the primary ligand. Indirect immunolabeling provides greater sensitivity because the density of labeling is amplified (16). However, indirect methods result in higher levels of background labeling (16). Direct immunogold labeling of frozen thin sections has been reported (15, 48). We previously reported a protocol for specimen preparation and direct immunogold labeling that resulted in adequate preservation of ultrastructure and antigenicity and highly specific labeling (29; C. R. Myers, M. L. P. Collins, M. Agresti, and G. Bergtrom, J. Insect Physiol., in press). By using immunogold labeling in the present study, the localization of

* Corresponding author.

photochemical components was correlated with membrane biogenesis.

MATERIALS AND METHODS

Cultivation of bacteria and membrane preparation. *R. rubrum* inoculum prepared from exponential-phase cultures was stored in 10% glycerol at −75°C. Cultures were inoculated with a 3% volume and grown in the medium described by Ormerod et al. (37) with 9.5 mM $(NH_4)_2SO_4$ substituted for glutamate and supplemented with 0.1% yeast extract (Difco Laboratories, Detroit, Mich.). The pH of the medium was 6.8. Aerobic cultures of *R. rubrum* S1 were grown in 2.8-liter Fernbach flasks containing 500 ml of media, incubated at 30°C, and aerated by shaking at 300 rpm in a controlled-environment incubator shaker (New Brunswick Scientific Co., Inc., Edison, N.J.). For phototrophic growth, *R. rubrum* G9 was incubated at 30°C with illumination provided by 2 25-W standard incandescent light bulbs (approximately 2,000 lx). Aerobic and phototrophic cells were obtained from exponentially growing cultures that had reached an optical density at 680 nm (OD_{680}) of 0.4 to 0.8 and 1.5, respectively, determined on a Beckman DU spectrophotometer (Beckman Instruments, Inc., Palo Alto, Calif.; modified by Update Instruments, Madison, Wis.). Cells were harvested and washed, and membranes were prepared as described previously (28).

Induction of chromatophore membrane. Induction of chromatophore membrane was performed essentially as with cultures of *R. sphaeroides* (9). Aerobically grown *R. rubrum* cultures were harvested in early log phase (0.4 to 0.5 OD_{680}) by centrifugation at 4°C in sterile bottles, aseptically pooled, and suspended in fresh media to achieve a final OD_{680} of 3.5. A portion of the cell suspension was retained for the time zero control, and the remaining cell suspension was dispensed in portions in sterile 125-ml Erlenmeyer flasks filled to 80% capacity. The flasks were incubated under semiaerobic conditions (31) by shaking at 200 rpm at 30°C. Samples were removed at 0.5, 1, 2, 4, 7, and 22 h after initiation of induction. Portions of cells obtained at these time intervals were used for ultrastructural analysis and immunocytochemistry. The remainder of the samples were washed, suspended in double-distilled H_2O, and used for determination of A_{680} and A_{880} and for measurement of BCHL content. The remaining sample was disrupted by sonication (Sonic 300 Dismembrator; Artek Systems Co., Farmingdale, N.Y.). These samples were held in an ice bath and treated with 3 30-s bursts of ultrasound at 30% efficiency setting with a relative output of 0.5. Sonicated samples were used for protein determination.

Electron microscopy. Cells were harvested, washed, and suspended in a minimal amount of 10 mM potassium phosphate buffer (pH 7.0) and held at 0°C until cell blocks were prepared with 2% agarose (Seakem HGT; FMC Corp., Marine Colloids Div., Rockland, Maine). Double-distilled water used in all reagents was boiled for 10 min and filter sterilized. For cell block preparation, cell suspensions were brought to room temperature and mixed with an equal volume of agarose on glass microscope slides. Cell blocks of 1-mm cubes were cut from the solidified droplet and were fixed in 0.5% glutaraldehyde (8% electron microscopy-grade glutaraldehyde; Ted Pella Inc., Tustin, Calif.) buffered with 10 mM potassium phosphate (pH 7.0) for 1 h at 0°C. For samples intended for ultrastructural but not immunocytochemical analysis, washed glutaraldehyde blocks were postfixed in 1% OsO_4 (4% OsO_4; Lanum Co., Homewood, Ill.) buffered with 10 mM potassium phosphate (pH 7.0) for 1 h at 0°C. Specimen blocks were washed after fixation with 10 mM potassium phosphate (pH 7.0) and dehydrated through a graded ethanol series followed by two changes of 100% propylene oxide. After drawing off the propylene oxide, the specimen blocks were treated with a mixture of equal volumes of propylene oxide and Poly-Bed 812 epoxy resin (Polysciences, Inc.), and mixed by gentle hand rotation. The resin formula was prepared by the procedure of Luft for Epon 812 (27). Continuous infiltration was performed by overnight rotation on a rotary shaker (Elenco Model, Northbrook Ill.). The cell blocks were transferred to new vials of Poly-Bed 812 for final infiltration. Specimens were polymerized by slow curing at temperature stages of 35, 45, and 60°C each for a period of 24 h. Ultrathin sections of silver interference color (60 to 90 nm) were cut with a diamond knife on a Porter-Blum MT-II Ultramicrotome (Ivan Sorvall, Inc., Norwalk, Conn.) and collected on copper grids (200 mesh; Lanum Co.). Before examination in the electron microscope, sections were poststained with 2% ethanolic uranyl acetate and lead citrate (40).

Antibody preparation. Immunogen was prepared from a lauryl dimethyl amine oxide extract of membranes (detergent/protein ratio of 1) of phototrophic cells. This extract was partially purified by DEAE-cellulose chromatography, precipitation with $(NH_4)_2SO_4$ at 45% saturation, and chromatography on Sephacryl 400 (Pharmacia Fine Chemicals, Piscataway, N.J.). New Zealand White female rabbits were immunized by subcutaneous injection with immunogen (5 μg of protein) in 50% Freund incomplete adjuvant (Difco) by the immunization protocol reported previously (10). The immunoglobulin fraction was purified from crude antiserum and concentrated by $(NH_4)_2SO_4$ precipitation and dialysis (10). To obtain antibody specific for components of phototrophic cells, this antibody preparation was adsorbed with membranes prepared from aerobically grown cells (20 mg of membrane protein per ml of purified antibody).

Preparation of colloidal gold conjugate and immunogold labeling. All glassware was cleaned in nitric acid, and filtered double-distilled water was used in reagent preparation. Colloidal gold (cAu) was prepared by the method of Frens (12). To 25 ml of double distilled H_2O, 0.25 ml of 1% chloroauric acid and 0.125 ml of 1% sodium citrate were added, and the mixture was refluxed for 30 min and cooled in a water bath. This resulted in gold particles approximately 35 nm in diameter. The ratio of antibody to gold sol was optimized as described by Horisberger and Rosset (18). Twice the protein sufficient to prevent flocculation was used for conjugation. Normal (nonimmune) rabbit serum (lot 27385; Polysciences) was purified as described above and coupled to colloidal gold (control cAu) in the above manner to be used as a control of immunocytochemical labeling. Gold conjugates were stored at 4°C in 0.15 M NaCl in 50 mM Tris hydrochloride (pH 7.4) containing 1% polyethylene glycol. Before use, the suspension was vortexed and then centrifuged for 10 min at 2,500 rpm (750 × *g*) to remove aggregates. Ultrathin sections were labeled with immunogold (immune cAu) by the procedure that we described previously (29) modified by a final step in which the grids were treated with 0.05% glutaraldehyde.

Electrophoresis and immunoblot analysis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of phototrophically grown *R. rubrum* chromatophore membranes purified by density centrifugation (30) was done by using vertical slab gels (Protean II Vertical Electrophoresis system; Bio-Rad Laboratories, Richmond, Calif.) modified (30) from the method of Laemmli (24). Molecular weight markers

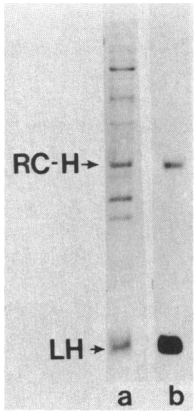

FIG. 1. Immunoblot analysis of antibody specificity. (a) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis separation of chromatophore polypeptides. Arrows, LH and heavy subunit of RC designated H. (b) Autoradiograph of immunoblot prepared with specific antibody. Bands corresponding to RC-H and LH were detected by binding of radiolabeled protein A to primary antibody that was bound to polypeptides transferred to nitrocellulose.

(Dalton VI; Sigma Chemical Co., St. Louis, Mo.) were coelectrophoresed in a separate lane. Gels were removed, and a portion of the gel not to be electroblotted was excised, fixed for 4 to 18 h in dye solvent (methanol-water-glacial acetic acid; 227:227:46 [vol/vol/vol]), stained with 0.25% R250 Coomassie brilliant blue in dye solvent for 4 h, and destained in several changes of dye solvent for 24 to 48 h. Electrophoretically resolved proteins on the remainder of the gel were transferred to nitrocellulose sheets (Schleicher & Schuell, Inc., Keene, N.H.) at 40 V (approximately 1 to 2 mA) at 4°C for 18 h by using an electrophoretic Transblot apparatus (Bio-Rad). After transfer, the nitrocellulose sheet was cut into strips corresponding to lanes of the electrophoresis gel. Strips were washed with gentle rotation in a blocking solution (29) for 1 h with two changes. This procedure was necessary to saturate nonspecific binding sites and remove adhering polyacrylamide gel particles and residual sodium dodecyl sulfate. The same reagent used for blocking served as diluent for primary antibody and for subsequent washes to remove unbound reagents. The strips were incubated with gentle agitation. The final antibody concentration used corresponded to approximately a 1:7,000 dilution with respect to original serum concentration. After incubation for 18 h, the strips were washed in blocking reagent six times for 30 min and then further washed with five changes of Tris-buffered saline (pH 7.5) (0.9% NaCl in 19 mM Tris hydrochloride [pH 7.5]). The nitrocellulose strips were then incubated with 5×10^5 cpm of ^{125}I-labeled protein A per ml for 4 h with gentle agitation. This was followed by five 10-min washes in Tris-buffered saline (pH 7.5). The radiolabeled nitrocellulose strips were dried on filter paper for 20 min, wrapped in Saran Wrap, and exposed on Kodak XS-5 X-ray film for 15 to 30 min at room temperature with an intensifying screen. Radioiodinated protein A was prepared by the lactoperoxidase method (47).

Analytical procedures. Protein content was determined by the method of Lowry et al. (26), modified as described previously (8). Bovine serum albumin (Miles Laboratories, Inc., Elkhart, Ind.) was used as the standard. BCHL content was calculated from the A_{770} of acetone-methanol (7:2 [vol/vol]) extracts (7) by using the extinction coefficient of Clayton (4).

Localization of immunogold was assessed by examining cross sections of 300 cells at each time point. Particles bound to the outer one-sixth of the cell (i.e., one-sixth of the diameter of the cross section), including the cytoplasmic membrane, were scored as peripheral; gold particles associated with the center of the cell were scored as cytoplasmic. Only particles bound to cross sections of cells were included in this analysis. Statistical comparisons of the data were made by using a standardized formula for comparison of sample means for $n > 30$:

$$Z = \frac{(x_1 - x_2)}{\sqrt{(s_1^2/n_1 + s_2^2/n_2)}}$$

where x_1 and x_2 represent the means of the two sample sets being compared; s_1 and s_2 represent the sample standard deviations, and n_1 and n_2 represent the sample sizes. The values of z obtained were compared to standardized t-table values for $n > 30$.

RESULTS

Immunocytochemistry requires antibody of defined specificity. The specificity of the antibody conjugated to colloidal gold for this investigation was determined by immunoblot analysis. Chromatophore polypeptides were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. (Fig. 1a). Antibody, detected by labeling with radioiodinated protein A, bound to bands (Fig. 1b) identified (5, 33, 38, 49, 51) as the H subunit of the photochemical reaction center (RC) and the light-harvesting antenna (LH).

Incubation of aerobically grown *R. rubrum* under semiaerobic conditions resulted in BCHL synthesis. An increase in the ratio of BCHL content to cell mass, measured by OD_{680}, was observed (Fig. 2a). The specific BCHL content of aerobic cells (time zero posttransition) was negligible (0.363 μg of BCHL per mg of protein); a 15-fold

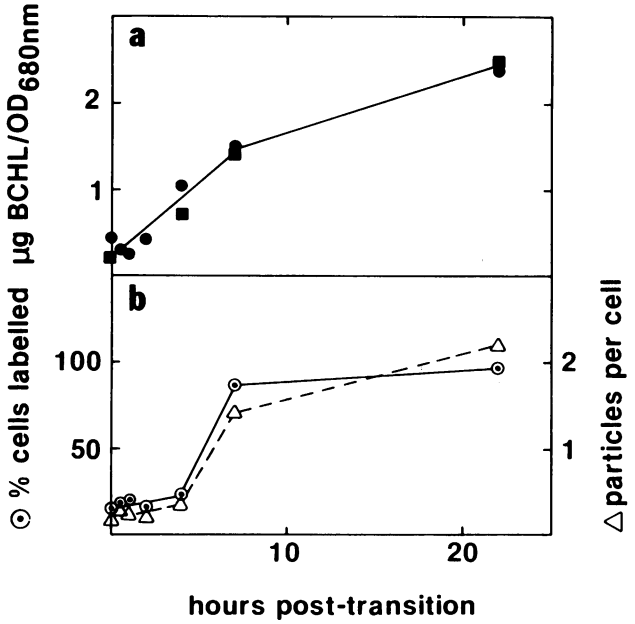

FIG. 2. Increase in BCHL and RC or LH antigens in *R. rubrum* induced by incubation at low aeration. (a) Increase in BCHL at OD_{680}. ●, Experiment 1; ■, experiment 2. (b) Increase in percentage of cells (viewed in cross section) labeled and number of immunogold particles per cross section of cell.

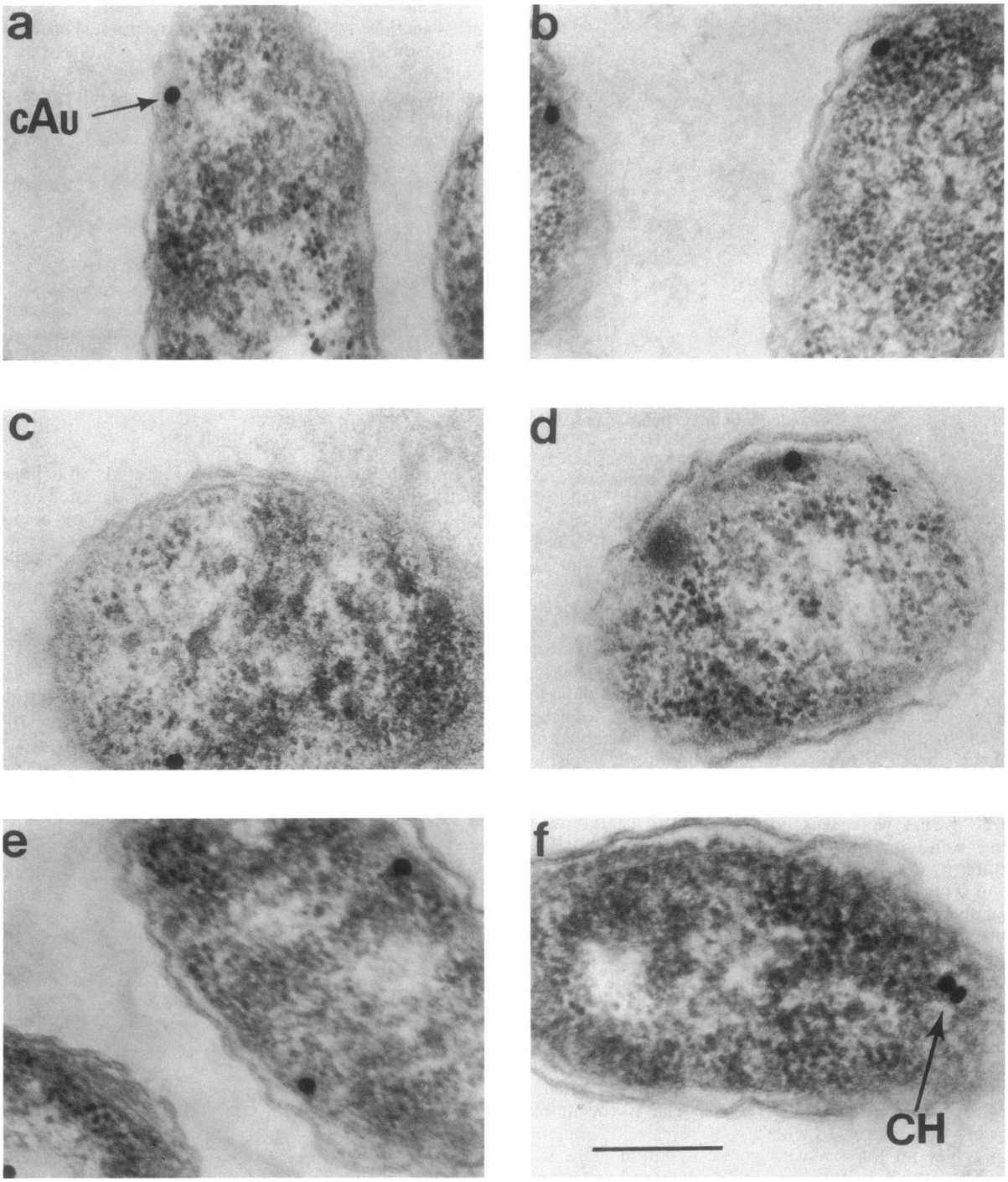

FIG. 3. Electron micrographs of immunogold-labeled specimens induced by incubation at low aeration for the following time periods in hours: 0 (a), 1 (b), 2 (c), 4 (d), 7 (e), 22 (f). cAu, Immunogold particle; CH, chromatophore membrane. Although longitudinal and cross sections are shown, only cross sections were used for statistical analysis. Bar = 0.25 nm.

increase (5.35 μg of BCHL per mg of protein) occurred during 22 h of incubation.

Immunogold specific for the RC and LH antigens was used to localize these components in induced *R. rubrum*. The site of immunogold binding is illustrated by micrographs (Fig. 3). At the early time points in the induction (0 and 1 h; Fig. 3a and b), the immunogold was peripheral and associated with the cytoplasmic membrane. By 22 h (Fig. 3f), chromatophore membrane was visible and was labeled by the immunogold. Quantitation of bound gold particles showed that the increase in immunolabeling corresponded to the increase in BCHL content (Fig. 2). The percentage of cells labeled and the number of particles per cell increased in parallel (Fig. 2b). No increase (>95% confidence level) occurred between 0 and 2 h; the cells sampled at 22 h posttransition exhibited a 12-fold increase in density of labeling with the

immunogold reagent in comparison with the time zero sample (Fig. 2b; Table 1). The specificity of the labeling was demonstrated by comparison with sections labeled with control cAu. A significant (>99% confidence level) difference in the density of the labeling of the immune cAu-labeled and control cAu-labeled specimens was observed at all time points (Table 1).

A difference in the intracellular distribution of the immunogold was detected at different points during the course of the induction (Fig. 4). The periphery, including the cytoplasmic membrane, was preferentially (>95% confidence level) labeled in aerobic cells and in cells 0.5 h posttransition. In contrast, the cytoplasmic area, including the intracytoplasmic chromatophore membrane, was preferentially labeled in cells 22 h posttransition (>99% confidence level).

The preservation of membrane ultrastructure in the nonosmicated sections used for immunogold labeling, although superior to frozen sections, was not optimal. Therefore, specimens postfixed in OsO_4 were examined. Cells harvested at 4 and 7 h after transition to low aeration contained invaginations at the periphery of the cell (data not shown). Cells from cultures 22 h posttransition showed abundant intracytoplasmic chromatophore membrane (data not shown). In contrast, no intracytoplasmic membrane was observed in time zero cells or in cells obtained from cultures 2 h posttransition (data not shown).

DISCUSSION

Direct immunogold labeling was used to analyze chromatophore membrane formation. The antigens labeled by the immunogold reagent were identified as LH or RC on the basis of the determination of antibody specificity toward these chromatophore membrane components (Fig. 1). The sites labeled must be considered minima, since a cross section represented <3% of a complete cell and only those antigenic determinations exposed at the surface of the section would be expected to bind antibody (3). Further underestimation of the number of antigenic sites is due to the possibility that not all of the antigenic determinants remained after glutaraldehyde fixation and embedment in resin. The extent of labeling of RC or LH was clearly correlated with the BCHL content (Fig. 2). The labeling was highly specific, as was demonstrated by the labeling with control cAu compared with that of immune cAu (Table 1) and by the lack of immunogold binding to the resin.

Immunogold particles appeared to be associated with chromatophore membrane in induced cells (Fig. 3f). However, this membrane was poorly resolved in specimens prepared without osmium tetroxide. We previously observed incomplete fixation of membrane in nonosmicated specimens (29). In this study, it was necessary to use samples postfixed in OsO_4 to achieve optimal preservation of membrane ultrastructure. In samples treated in this way, mature chromatophore membrane was observed in cells obtained 22 h posttransition, and invaginations at the cell periphery were detected at 4 and 7 h. This is consistent with the results of Cohen-Bazire and Kunisawa (6) and is correlated with the localization of immunogold in the present study. The preferential localization of immunogold at the cell periphery early in transition and in the cytoplasm at 22 h suggests that the photochemical components are inserted into the cytoplasmic membrane which then invaginates to form intracytoplasmic chromatophore membrane. This interpretation is consistent with the studies of Niederman and co-workers (19, 20, 32), since the invagination sites may correspond to the fraction isolated in the UPB. Furthermore, this study extends the findings of Golecki and Oelze (13) by localizing functional components in the invaginating chromatophore membrane.

TABLE 1. Density of cAu particles per cell cross section

Posttransition time (h) to low aeration	Density[a] (mean ± SD) of cAu/cell for the following cAu types:	
	Immune	Control
0	0.180 ± 0.485	0.043 ± 0.274
0.5	0.257 ± 0.599	0.063 ± 0.355
1	0.233 ± 0.560	0.037 ± 0.205
2	0.217 ± 0.551	0.063 ± 0.336
4	0.347 ± 0.713	0.037 ± 0.221
7	1.383 ± 0.906	0.037 ± 0.221
22	2.233 ± 1.696	0.070 ± 0.335

[a] Density of labeling was evaluated by scoring cross sections of 300 cells viewed at each time interval.

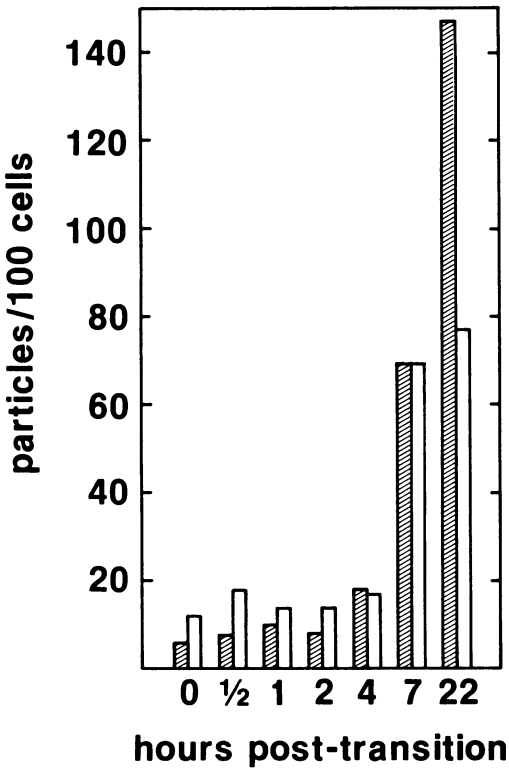

FIG. 4. Relationship between immunogold distribution and progress of induction. ▭, Density of immunogold binding at cell periphery; ▨, density of immunogold binding to cytoplasmic area.

ACKNOWLEDGMENTS

This investigation was supported by grants from the National Science Foundation (DCB-8309063) and from the Graduate School of the University of Wisconsin-Milwaukee through the Shaw Discretionary Funds.

We are grateful to C. F. Shaw for preparation of chloroauric acid, to J. Faragher for antibody preparation, to A. Durski for preparation of chromatophores, to C. R. Myers for preparation of nonimmune immunoglobulin G and many helpful discussions, and to C. C. Remsen and M. Schaller for advice throughout this investigation.

LITERATURE CITED

1. **Bayer, E. A., E. Setter, and R. Lamed.** 1985. Organization and distribution of the cellulosome in *Clostridium thermocellum*. J. Bacteriol. **163**:552–559.
2. **Bayer, M. E., E. Carlemalm, and E. Kellenberger.** 1985. Capsule of *Escherichia coli* K29: ultrastructural preservation and immunoelectron microscopy. J. Bacteriol. **162**:985–991.
3. **Bendayan, M., and H. Stephens.** 1984. Double labelling cytochemistry applying the protein A-gold technique, p. 143–154. *In* J. M. Polak and I. M. Varndell (ed.), Immunolabelling for electron microscopy. Elsevier Science Publishing, Inc., New York.
4. **Clayton, R. K.** 1963. Toward the isolation of a photochemical reaction center in *Rhodopseudomonas spheroides*. Biochim. Biophys. Acta **75**:312–323.
5. **Cogdell, R. J., J. G. Lindsay, J. Valentine, and I. Durant.** 1982. A further characterisation of the B890 light-harvesting pigment-protein complex from *Rhodospirillum rubrum* strain S1. FEBS Lett. **150**:151–154.
6. **Cohen-Bazire, G., and R. Kunisawa.** 1963. The fine structure of *Rhodospirillum rubrum*. J. Cell. Biol. **16**:401–419.
7. **Cohen-Bazire, G., W. R. Sistrom, and R. Y. Stanier.** 1957. Kinetic studies of pigment synthesis by non-sulfur purple bacteria. J. Cell. Comp. Physiol. **49**:25–68.
8. **Collins, M. L. P., D. E. Mallon, and R. A. Niederman.** 1980. Assessment of *Rhodopseudomonas sphaeroides* chromatophore membrane asymmetry through bilateral antiserum adsorption studies. J. Bacteriol. **143**:221–230.
9. **Collins, M. L. P., and S. T. Remson.** 1984. Immunogold detection of chromatophore antigens on the surface of *Rhodopseudomonas sphaeroides* spheroplasts. Curr. Microbiol. **11**:269–274.
10. **Collins, M. L. P., and M. R. J. Salton.** 1979. Solubility characteristics of *Micrococcus lysodeikticus* membrane components in detergents and chaotropic salts analyzed by immunoelectrophoresis. Biochim. Biophys. Acta **553**:40–53.
11. **Elferink, M. G. L., K. J. Hellingwerf, P. A. M. Michels, H. G. Seyen, and W. N. Konings.** 1979. Immunochemical analysis of membrane vesicles and chromatophores of *Rhodopseudomonas sphaeroides* by crossed immunoelectrophoresis. FEBS Lett. **107**:300–307.
12. **Frens, G.** 1973. Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. Nature (London) Phys. Sci. **241**:20–22.
13. **Golecki, J. R., and J. Oelze.** 1975. Quantitative determination of cytoplasmic membrane invaginations in phototrophically growing *Rhodospirillum rubrum*. J. Gen. Microbiol. **88**:253–258.
14. **Goodman, S. L., G. M. Hodges, L. K. Trejdosiewicz, and D. C. Livingston.** 1979. Colloidal gold probes—a further evaluation. Scanning Electron Microsc. **3**:619–628.
15. **Guan, T., A. Ghosh, and B. K. Ghosh.** 1985. Immunoelectron microscopic double labeling of alkaline phosphatase and penicillinase with colloidal gold in frozen thin sections of *Bacillus licheniformis* 749/C. J. Bacteriol. **164**:107–113.
16. **Hodges, G. M., M. A. Smolira, and D. C. Livingston.** 1984. Scanning electron microscope immunocytochemistry in practice, p. 189–233. *In* J. M. Polak and I. M. Varndell (ed.), Immunolabeling for electron microscopy. Elsevier Science Publishing Inc., New York.
17. **Horisberger, M.** 1983. Colloidal gold as a tool in molecular biology. Trends Biochem. Sci. **8**:395–397.
18. **Horisberger, M., and J. Rosset.** 1977. Colloidal gold, a useful marker for transmission and scanning electron microscopy. J. Histochem. Cytochem. **25**:295–305.
19. **Inamine, G. S., and R. A. Niederman.** 1982. Development and growth of photosynthetic membranes of *Rhodospirillum rubrum*. J. Bacteriol. **150**:1145–1153.
20. **Inamine, G. S., J. Van Houten, and R. A. Niederman.** 1984. Intracellular localization of photosynthetic membrane growth initiation sites in *Rhodopseudomonas sphaeroides*. J. Bacteriol. **158**:425–429.
21. **Jackowski, S., H. H. Edwards, D. Davis, and C. O. Rock.** 1985. Localization of acyl carrier protein in *Escherichia coli*. J. Bacteriol. **162**:5–8.
22. **Kaplan, S., and C. J. Arntzen.** 1982. Photosynthetic membrane structure and function, p. 65–151. *In* Govindjee (ed.), Photosynthesis, vol. 1. Energy conversion by plants and bacteria. Academic Press, Inc., New York.
23. **Kozuka, S., Y. Yasuda, and K. Tochikubo.** 1985. Ultrastructural localization of dipicolinic acid in dormant spores of *Bacillus subtillis* by immunoelectron microscopy with colloidal gold particles. J. Bacteriol. **162**:1250–1254.
24. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
25. **Lommen, M. A. J., and J. Takemoto.** 1978. Comparison, by freeze-fracture electron microscopy, of chromatophores, spheroplast-derived membrane vesicles, and whole cells of *Rhodopseudomonas sphaeroides*. J. Bacteriol. **136**:730–741.
26. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
27. **Luft, J. H.** 1961. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. **9**:409–414.
28. **Mueller, P. R., and M. L. P. Collins.** 1983. Identification of two distinct lactate dehydrogenases in *Rhodospirillum rubrum*. J. Bacteriol. **153**:1562–1566.
29. **Myers, C. R., G. Bergtrom, S. M. Crook, D. R. Boyer, and M. L. P. Collins.** 1986. Post-embedding direct immunogold detection of a protein antigen in insect tissue. J. Histochem. Cytochem. **34**:221–226.
30. **Myers, C. R., and M. L. P. Collins.** 1986. Cell-cycle-specific oscillation in the composition of chromatophore membrane in *Rhodospirillum rubrum*. J. Bacteriol. **166**:818–823.
31. **Niederman, R. A., D. E. Mallon, and J. J. Langan.** 1976. Membranes of *Rhodopseudomonas sphaeroides*. IV. Assembly of chromatophores in low-aeration cell suspensions. Biochim. Biophys. Acta **440**:429–447.
32. **Niederman, R. A., D. E. Mallon, and L. C. Parks.** 1979. Membranes of *Rhodopseudomonas sphaeroides*. VI. Isolation of a fraction enriched in newly synthesized bacteriochlorophyll *a*-protein complexes. Biochim. Biophys. Acta **555**:210–220.
33. **Noel, H., M. Van Der Rest, and G. Gingras.** 1972. Isolation and partial characterization of a P870 reaction center complex from wild type *Rhodospirillum rubrum*. Biochim. Biophys. Acta **275**:219–230.
34. **Oelze, J.** 1978. Proteins exposed at the surface of chromatophores of *Rhodospirillum rubrum*. Biochim. Biophys. Acta **509**:450–461.
35. **Oelze, J., and G. Drews.** 1972. Membranes of photosynthetic bacteria. Biochim. Biophys. Acta **265**:209–239.
36. **Ohad, I., and G. Drews.** 1982. Biogenesis of the photosynthetic apparatus in prokaryotes and eukaryotes, p. 89–140. *In* Govindjee (ed.), Photosynthesis: development, carbon metabolism, and plant productivity, vol. 2. Academic Press, Inc., New York.
37. **Ormerod, J. G., K. S. Ormerod, and H. Gest.** 1961. Light-dependent utilization of organic compounds and photoproduction of molecular hydrogen by photosynthetic bacteria; relationships with nitrogen metabolism. Arch. Biochem. Biophys. **94**:449–463.
38. **Picorel, R., G. Belanger, and G. Gingras.** 1983. Antenna holochrome B880 of *Rhodospirillum rubrum* S1. Pigment, phospholipid, and polypeptide composition. Biochemistry **22**: 2491–2497.
39. **Remsen, C. C.** 1978. Comparative subcellular architecture of photosynthetic bacteria, p. 31–60. *In* R. K. Clayton and W. R. Sistrom (ed.), The photosynthetic bacteria. Plenum Publishing Corp., New York.
40. **Reynolds, E. S.** 1963. The use of lead citrate at high pH as an electronopaque stain in electron microscopy. J. Cell Biol. **17**:208–212.
41. **Russo, G. M., and J. L. Van Etten.** 1985. Synthesis and localization of a development-specific protein in sclerotia of *Sclerotinia sclerotiorum*. J. Bacteriol. **163**:696–703.

42. **Scholes, P., P. Mitchell, and J. Moyle.** 1969. The polarity of proton translocation in some photosynthetic microorganisms. Eur. J. Biochem. **8**:450–454.
43. **Sherwood, J. E., J. M. Vasse, F. B. Dazzo, and G. L. Truchet.** 1984. Development and trifoliin A-binding ability of the capsule of *Rhizobium trifolii*. J. Bacteriol. **159**:145–152.
44. **Slater, M. L., B. Bowers, and E. Cabib.** 1985. Formation of septum-like structures at locations remote from the budding sites in cytokinesis-defective mutants of *Saccharomyces cerevisiae*. J. Bacteriol. **162**:763–767.
45. **Swafford, J. R., P. J. Malloy, and H. C. Reeves.** 1983. Immunochemical localization of NADP-specific isocitrate dehydrogenase in *Escherichia coli*. Science **221**:295–296.
46. **Swafford, J. R., H. C. Reeves, and R. Brandsch.** 1985. Localization of the enantiozymes of 6-hydroxy-nicotine oxidase in *Arthrobacter oxidans* by electron immunochemistry. J. Bacteriol. **163**:792–795.
47. **Thorell, J. I., and B. G. Johansson.** 1971. Enzymatic iodination of polypeptides with ^{125}I to high specific activity. Biochim. Biophys. Acta **251**:363–369.
48. **Tinglu, G., A. Ghosh, and B. K. Ghosh.** 1984. Subcellular localization of alkaline phosphatase in *Bacillus licheniformis* 749/C by immunoelectron microscopy with colloidal gold. J. Bacteriol. **159**:668–677.
49. **Vadeboncoeur, C., H. Noel, L. Poirier, Y. Cloutier, and G. Gingras.** 1979. Photoreaction center of photosynthetic bacteria. I. Further chemical characterization of the photoreaction center from *Rhodospirillum rubrum*. Biochemistry **18**:4301–4307.
50. **Vos-Scheperkeuter, G. H., E. Pas, G. J. Brakenhoff, N. Nanninga, and B. Witholt.** 1984. Topography of the insertion of lamB protein into the outer membrane of *Escherichia coli* wild-type and *lac-lamB* cells. J. Bacteriol. **159**:440–447.
51. **Zurrer, H., M. Snozzi, K. Hanselmann, and R. Bachofen.** 1977. Localisation of the subunits of the photosynthetic reaction centers in the chromatophore membrane of *Rhodospirillum rubrum*. Biochim. Biophys. Acta **460**:273–279.