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Cell-Cycle-Specific Oscillation in the Composition of Chromatophore Membrane in *Rhodospirillum rubrum*

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Synchrony in phototrophic cultures of *Rhodospirillum rubrum* was induced by stationary-phase cycling or by alterations in light intensity. Intracytoplasmic chromatophore membranes were prepared by differential centrifugation. Analysis of the composition of chromatophores obtained from cells at different times indicated that the protein/bacteriochlorophyll *a* ratio was constant throughout the cell cycle but that the protein/phospholipid ratio oscillated. This cell-cycle-dependent fluctuation in chromatophore membrane composition was reflected in the buoyant densities of the isolated chromatophores.

Rhodospirillum rubrum is a facultatively photosynthetic bacterium of the family *Rhodospirillaceae*. Members of this group, including *R. rubrum* and *Rhodopseudomonas sphaeroides*, are under study as models of membrane structure and formation (24, 33). In these organisms the photosynthetic apparatus is localized in an intracytoplasmic chromatophore membrane that is absent from cells grown under nonphotosynthetic conditions, i.e., high aeration. Kaplan and co-workers have evaluated chromatophore membrane formation in synchronously growing cell cultures of *Rhodopseudomonas sphaeroides* (19–21, 31, 37, 38). These investigators have shown that the components of the chromatophore membrane are inserted in a noncoordinate manner. While proteins (19) and photopigments (37) are inserted continuously throughout the cell cycle, phospholipids are inserted at a specific point in the cell cycle (31). As a result of this cell-cycle-specific accumulation of phospholipids in the chromatophore membrane, there is a fluctuation in the ratio of protein to phospholipids (20, 31) in the isolated chromatophore membranes and in their buoyant densities (20). This fluctuation in chromatophore membrane composition is reflected in structural variation. Membrane fluidity evaluated by fluorescence polarization by α-parinaric acid oscillated with the protein/phospholipid ratio (21). Yen et al. (38) applied freeze-fracture electron microscopy to analyze membrane structure throughout the cell cycle. The particle density of the protoplasmic face of the chromatophore membrane correlated with the protein/lipid ratio.

The relationship between cell cycle and membrane composition and structure has not been evaluated in other photosynthetic bacteria. To analyze the molecular architecture of *R. rubrum* membranes, it was necessary to determine whether the structure and composition of *R. rubrum* membranes are affected by cell-cycle-specific events. This study of chromatophore membranes obtained from synchronized *R. rubrum* demonstrated cyclic fluctuation in chromatophore membrane composition.

MATERIALS AND METHODS

Growth of organism and induction of synchrony. *R. rubrum* S1 was grown in the medium of Ormerod et al. (34) modified by the substitution of 9.5 mM $(NH_4)_2SO_4$ for glutamate. Cultures were incubated in the light (approximately 2,000 lx unless otherwise specified) at 30°C in completely filled screw-cap vessels or in 500-, 1,000-, or 9,000-ml glass bottles (152 by 457 mm). Culture growth was monitored at A_{680} with a Beckman DU spectrophotometer (Beckman Instruments, Inc., Palo Alto, Calif., modified by Update Instruments, Madison, Wis.). Populations of synchronously dividing cells were obtained by the stationary-phase cycling procedure of Cutler and Evans (16). Cultures adapted to logarithmic asynchronous growth in the medium of Ormerod et al. were allowed to complete approximately 0.5 mass doublings after the cessation of logarithmic growth. The cells (100 ml) of this first stationary-phase cycling were suspended in 900 ml of the medium, and incubation was continued. When an optical density equivalent to that of the first culture was reached, cells (900 ml) of this second stationary-phase cycling were suspended in 8,100 ml of the medium. Cells of this last culture were found to possess a high degree of division synchrony through the two subsequent division cycles monitored (Fig. 1) and were used for membrane preparation.

Synchronously dividing cells were also obtained by the light-induced method of Lueking et al. (30). An asynchronous high-light-adapted (2,000 lx) culture (9,000 ml) growing exponentially was subjected to an abrupt light decrease to 500 lx, which resulted in a cessation of culture growth. The cell number became fixed at its pretransition value for a period of 4 h after the shift to low light, whereupon it exhibited a marked (19%) abrupt increase. Cells were kept at this low light intensity for 1.5 h after this increase, at which time they were returned to high-intensity light (2,000 lx). Upon reexposure to high-intensity light, immediate exponential increases in culture turbidity and total cellular protein were observed. In contrast, the increase in cell number was discontinuous and characteristic of a synchronously dividing population (Fig. 2).

Cell enumeration and chromatophore preparation. Culture samples (200 ml) were removed at 30-min intervals and immediately iced. A portion was washed and suspended in water for determination of total cellular protein. For cell counts, 1 ml of the culture was transferred to a tube containing 1 ml of 1% (wt/vol) formaldehyde and held at 4°C. A 10-μl volume of 1% crystal violet was added to stain the cells. Cell counts were done with a Neubauer hemacytometer counting chamber, and approximately 500 cells were counted per determination. One drop of each sample was air dried on a slide for cell length measurements

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performed with a calibrated ocular micrometer. The lengths of 40 cells selected at random were determined for each sample.

To obtain chromatophore membranes, the remaining cells were harvested, washed once in 10 mM Tris hydrochloride (pH 7.5), and resuspended in 2 ml of the buffer. This and all subsequent procedures, unless otherwise specified, were performed at 0 to 4°C. Washed cells were disrupted essentially as described by Lueking et al. (31) by sonication for 2 min (35% efficiency; relative output, 0.5), using a Sonic 300 Dismembrator (Artek Systems Corp., Farmingdale, N.Y.). The disrupted cells were incubated in the presence of RNase A and DNase I (40 U each) (Sigma Chemical Co., St. Louis, Mo.) and 5 mM $MgSO_4$ for 30 min at room temperature. Unbroken cells and debris were removed by centrifugation at 12,000 × *g* for 10 min. The resulting supernatant fraction was centrifuged at 106,000 × *g* for 1 h in a Beckman 50Ti rotor to recover chromatophore membranes. The resulting chromatophores were washed once, resuspended in buffer, and used directly for the determination of membrane density or dialyzed exhaustively against deionized water before chemical analysis.

Determination of chromatophore density. The equilibrium buoyant densities of chromatophores were determined as adapted from Kosakowski and Kaplan (27). Chromatophores prepared as described above were suspended in 5% (wt/wt) CsCl in 10 mM Tris hydrochloride (pH 7.5) and layered onto 7-ml preformed linear CsCl gradients. Centrifugation was at 32,500 rpm in a Beckman 50Ti rotor for 42 h. After centrifugation, the gradients were fractionated with a Buchler Autodensiflow II (Buchler Instruments Div., Nuclear-Chicago Corp., Fort Lee, N.J.) coupled to an ISCO model 1200 (ISCO, Lincoln, Nebr.) fraction collector. The refractive index of each fraction was determined with a Bausch & Lomb (Bausch & Lomb, Inc., Rochester, N.Y.) refractometer, and the density was calculated as described before (27).

Preparation of chromatophores by sucrose density gradient centrifugation. Cells from an asynchronously, exponentially growing cell culture were harvested, washed, and disrupted in a French pressure cell, and crude membranes were recovered as described previously (32). Chromatophores were resolved from crude membranes by centrifugation for 2.5 h at 25,000 rpm (113,000 × *g*) in a Beckman SW27 rotor in an L-8 ultracentrifuge through a gradient of 10 to 60% (wt/wt) sucrose. Chromatophores were recovered from gradient fractions by centrifugation for 90 min at 50,000 rpm (226,000 × *g*) in a Beckman 50Ti rotor. A buffer of 10 mM *N*-2-hydroxy-ethylpiperazine–*N'*-2-ethanesulfonic acid (pH 7.5) was used throughout these procedures. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profile of these density gradient purified chromatophores was compared with that of the chromatophores purified by differential centrifugation.

Analytical procedures. Chromatophore protein content was determined by the method of Lowry et al. (29) modified as described previously (11, 12). The bacteriochlorophyll *a* (BCHL) content of chromatophores was determined from the A_{770} of acetone-methanol (7:2 [vol/vol]) extracts (10) with the extinction coefficient of Clayton (8). Chromatophore phospholipids were extracted by the method of Blight and Dyer (3) as described by Ames (1). Lipid phosphorus determinations were performed as described by Chen et al. (6) on lipid extracts that were wet ashed by the method of Bartlett (2). Lipid phosphorus values were multiplied by a factor of 25 to calculate the quantity of phospholipid (31). Whole-cell protein was determined by the Lowry method (29) on cell suspensions that had been treated at 60°C in 1 N NaOH for 15 min (31).

SDS-PAGE was performed as adapted from Laemmli (28). The running gel (12% acrylamide, 0.378 M Tris hydrochloride [pH 8.8], and 0.02% SDS) was polymerized in the presence of 0.04% ammonium persulfate and 6.6 mM tetramethyethylenediamine. The stacking gel (6% acrylamide, 0.125 M Tris hydrochloride [pH 6.8], and 0.2% SDS) was polymerized in the presence of 0.1% ammonium persulfate and 6.6 mM tetramethylethylenediamine. The sample contained 0.125 M Tris hydrochloride (pH 6.8), 4% SDS, 21% glycerol, 0.1 M dithiothreitol, and 0.0005% bromophenol blue. Electrophoresis was conducted with a Bio-Rad model 360 miniature vertical slab cell at a constant current of 20 mA. Gels were fixed in a destain solution of methanol-water-glacial acetic acid (5:5:1 [vol/vol]), stained in 0.25% Coomassie brilliant blue (prepared in destain/solution) for 4 h, and destained by repeated washes in a destain solution.

RESULTS

The usefulness of the stationary-phase cycling technique for inducing synchrony in phototrophic bacteria has been previously demonstrated for cultures of *Rhodopseudomonas sphaeroides* (18–21, 26, 31, 37, 38). This technique proved to be very useful for obtaining division synchrony in *R. rubrum* (Fig. 1). Exponential increases in cell mass (optical density) (Fig. 1a) and total cellular protein (Fig. 1b) were observed during synchronous growth. The occurrence of synchronous division was determined by enumeration of cells (Fig. 1c). The measurement of cell length provided additional verification of division synchrony, as increases and decreases in mean cell length (Fig. 1d) corresponded with the times of cell division as determined by cell number. Differences between peak and trough mean cell lengths were shown to be statistically significant ($P < 0.01$).

While the stationary-phase cycling method provided for good division synchrony, a drawback seemed to be that all cells were not dividing. Cell counts (Fig. 1c) indicated that only about 22 to 23% of the cells were dividing. This percentage remained constant for both divisions monitored. Increases in culture turbidity (20 to 21%) and total cellular protein (20 to 23%) (Fig. 1a and b) during each division cycle corresponded to this. While the length of the largest cell was 1.92 times that of the shortest cell, the difference between the low mean cell length and the high mean cell length represented a difference of 21%, which corresponded well to these other values. This proportion of dividing to nondividing cells was reproducible in several trials of cell synchrony.

The light-dark technique used by Lueking et al. for inducing synchrony in cultures of *Rhodopseudomonas sphaeroides* (30) was also successful for obtaining division synchrony in *R. rubrum*. Exponential increases in culture turbidity and total cellular protein were observed (Fig. 2a) concurrent with discontinuous increases in cell number which were consistent with division synchrony (Fig. 2b).

As in cultures synchronized by stationary-phase cycling, nondividing cells were also detected when synchrony was induced by alteration of light intensity. Increases in culture turbidity and total cellular protein (Fig. 2a) corresponded to the 28% increase in cell number (Fig. 2b). Viable cell counts (data not shown) demonstrated that 95 to 100% of the cells measured by the direct cell counts were viable, indicating that a large population of the cells in synchronous cultures were viable but nongrowing.

The chromatophore preparations were analyzed by SDS-

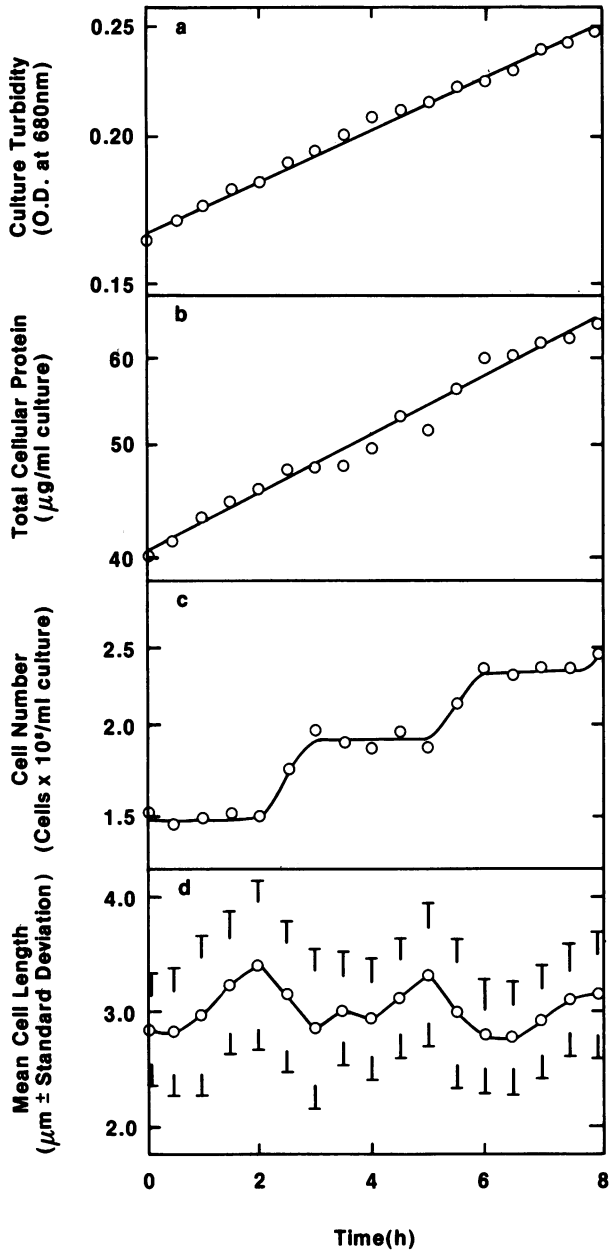

FIG. 1. Changes during stationary-phase-cycling-induced synchronous growth of *R. rubrum*. (a) Culture turbidity; (b) total cellular protein; (c) cell number; (d) mean cell length. O. D., Optical density.

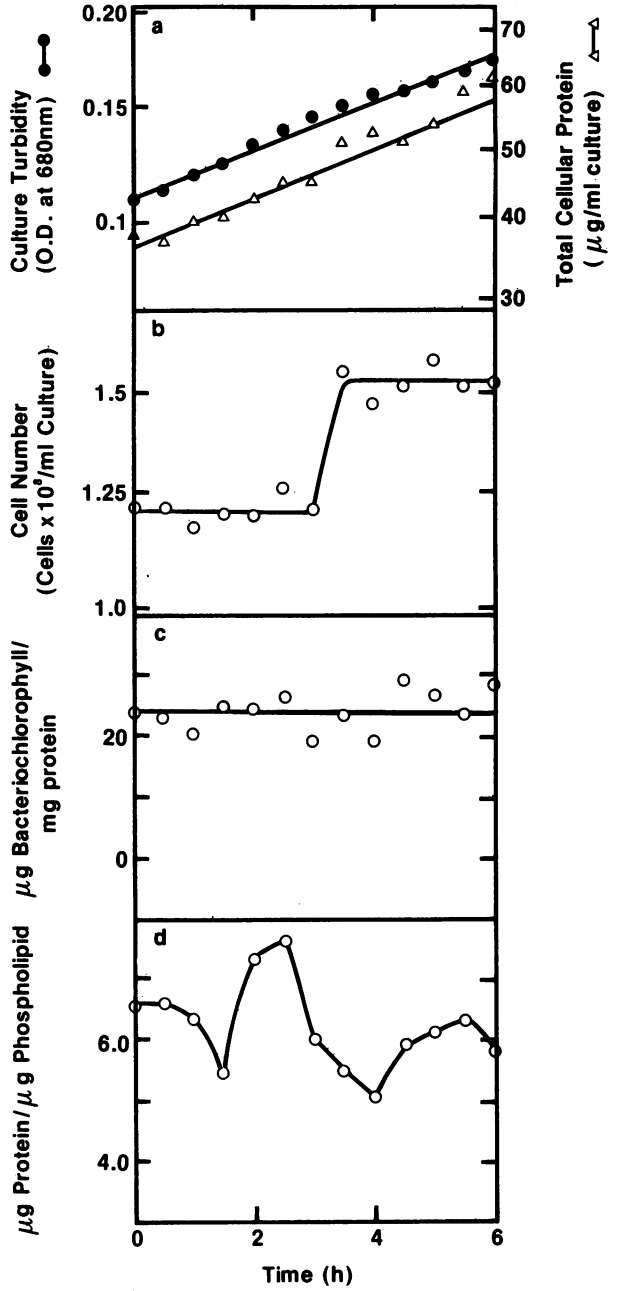

FIG. 2. Changes during light-induced synchronous growth of *R. rubrum*. (a) Increases in cellular mass and total cellular protein and (b) increases in cell number; (c) BCHL/protein ratio of chromatophores; (d) protein/phospholipid ratio of chromatophores. O.D., Optical density.

PAGE. SDS-PAGE profiles of chromatophores were identical at all stages in the cell cycle (data not shown). The polypeptide patterns of chromatophores were obtained from dividing cells (2.5 h) and those at midcycle (3.5 h) (Fig. 3). These SDS-PAGE profiles were found to be nearly identical to each other and to those of chromatophores purified from asynchronously growing cultures by sucrose density gradient centrifugation (Fig. 3); this latter finding supports the adequacy of the preparations in this study. No bands corresponding to outer membrane, purified according to Collins and Niederman (13), were detected. Electron micrographs of negatively stained preparations (micrographs not shown) indicated that the chromatophores were free from other structures, including cell wall and outer membrane, and were similar to other preparations (14, 23, 25).

The protein, phospholipid, and BCHL contents of chromatophores from various stages in the cell cycle were evaluated. The protein/phospholipid ratio of the chromatophores isolated from stationary-phase-induced synchronous cells varied within the cell division cycle (Fig. 4a). This pattern, as well as the absolute ratios, is similar to that reported for *Rhodopseudomonas sphaeroides* (20, 31). The BCHL/protein ratio of the chromatophores remained essentially constant throughout the cell cycle (Fig. 4b). This is

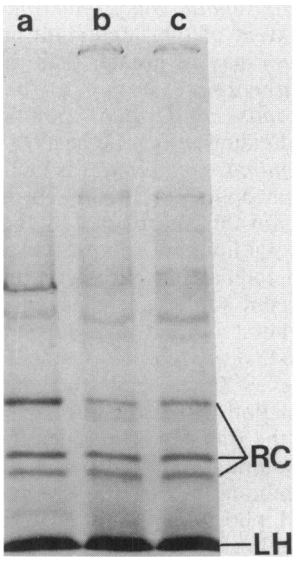

FIG. 3. SDS-PAGE analysis of chromatophore membranes. Chromatophore membranes were prepared from (a) asynchronous cells, (b) synchronous cells harvested at 3.5 h, and (c) synchronous cells harvested at 2.5 h. Asynchronous cell chromatophores were prepared by density gradient centrifugation. Synchronous cell chromatophores were prepared by differential centrifugation (see text) from stationary-phase-cycling-induced cells. LH, Peptides of light-harvesting antennas; RC, peptides of photochemical reaction center.

consistent with results reported for *Rhodopseudomonas sphaeroides* (37). The pattern of fluctuation of the protein/phospholipid ratio of chromatophores from light-induced synchronous cells paralleled that of stationary-phase-induced cells (Fig. 2d); likewise, the BCHL/protein ratio of these chromatophores remained essentially constant throughout the cell cycle (Fig. 2c). The relative BCHL content and protein/phospholipid ratio of chromatophores isolated from light-induced synchronous cells were greater than the corresponding values for chromatophores from stationary-phase-cycling synchronous cells. This was probably a result of the lower light intensity to which the former cultures were exposed.

The equilibrium buoyant densities of the chromatophores varied with the protein/phospholipid ratio (Fig. 4c). This was also observed for light-induced synchronous cell chromatophores (data not shown). The density values reported (Fig. 4c) were determined for the chromatophore band identified as the peak of A_{880}, which is the $A_{maximum}$ for BCHL in *R. rubrum*. In addition, a denser shoulder of A_{880} also exhibited a fluctuation in density in phase with the major band (data not shown); the presence of this shoulder may be attributable to the use of chromatophores prepared by differential centrifugation.

In contrast to the results obtained with synchronous cultures, chromatophores isolated from an asynchronous culture had a constant (correlation coefficient, 0.99) protein/phospholipid ratio (Fig. 5d). As was the case with the synchronous cell chromatophores, a constant BCHL/protein ratio was also observed (Fig. 5c).

DISCUSSION

The technique of stationary-phase cycling produced a high degree of synchrony in dividing cells in phototrophic cultures of *R. rubrum* (Fig. 1), as did the light-dark technique

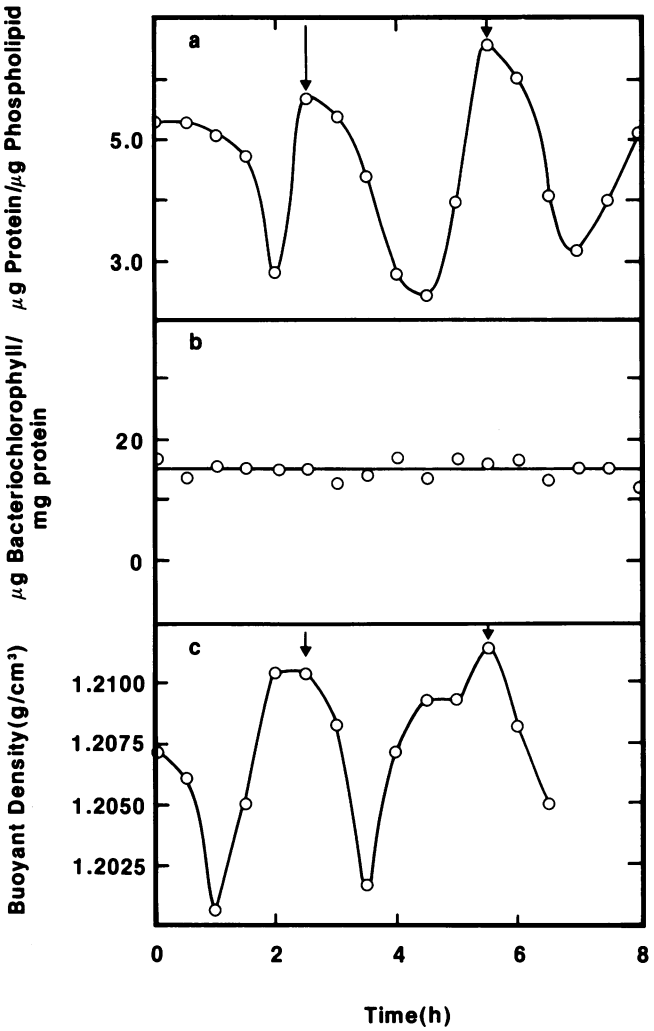

FIG. 4. Composition of chromatophore membranes in synchronously (stationary-phase-cycling induced) growing *R. rubrum*. (a) Protein/phospholipid ratio; (b) BCHL/protein ratio; (c) equilibrium buoyant density. Arrows indicate time of cell division.

(Fig. 2), although both resulted in a low percentage of dividing cells. It is possible that a selection method (17, 22) for achieving cell synchrony in cultures of *R. rubrum* would result in a greater percentage of dividing cells. However, despite the failure of these induction methods to establish cultures in which all cells were dividing, cells obtained from these cultures demonstrated cell-cycle-specific changes. In view of the presence of nondividing (nongrowing) cells, the extent of the cell-cycle-specific variation observed would have been diminished. For this reason, the extreme values for protein/phospholipid contents and buoyant densities may be minimal since measurements included nondividing as well as dividing cells. However, this conclusion is valid only if dividing and nondividing cells were disrupted equally well by sonication. Cells of *Escherichia coli* poised for cell division (i.e., long cells) have been shown to be five to six times more susceptible to sonic disruption than cells that have just completed division (i.e., short cells) (7). That populations of cells in the present study contained both dividing and nondividing cells is consistent with cell number increases and with the measurements of cell length. The mean cell lengths, which included data from nondividing cells, varied

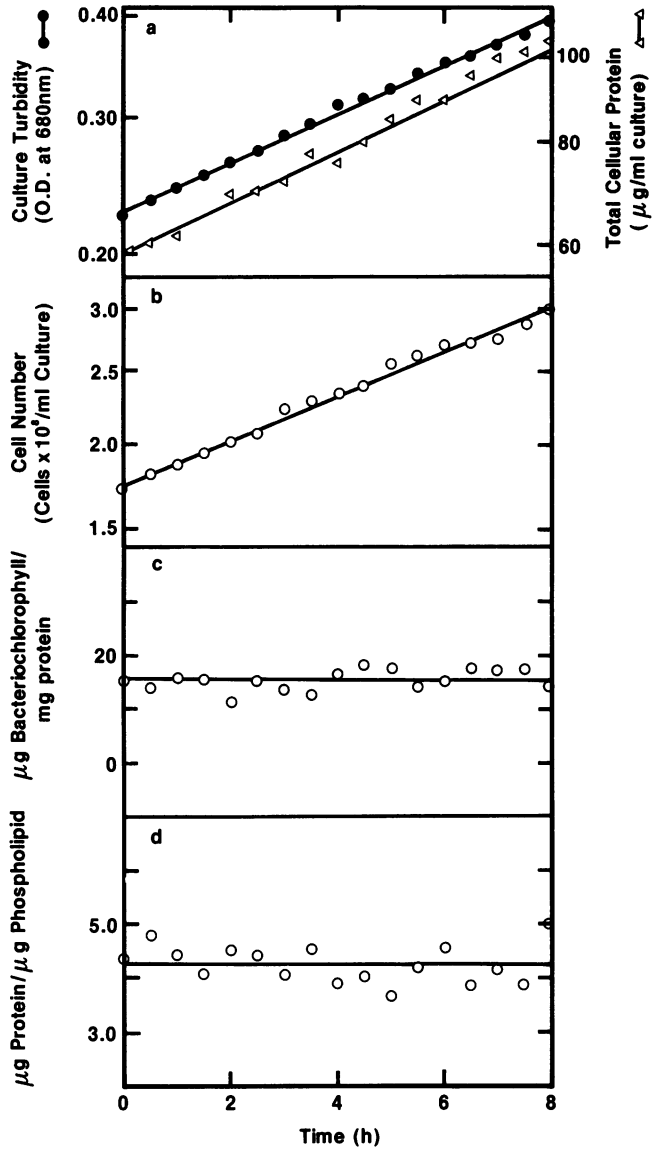

FIG. 5. Changes during asynchronous growth of *R. rubrum*. (a) Increases in cellular mass and total cellular protein; (b) cell number; (c) BCHL/protein ratio of chromatophores (correlation coefficient = 0.99); (d) protein/phospholipid ratio of chromatophores (correlation coefficient = 0.99). O. D., Optical density.

by 21%, while the extremes, which would have been determined from dividing cells, differed by 92%. A doubling in length would be expected for cells undergoing binary fission.

The analysis of numerous samples and the low recovery from sucrose density gradients necessitated the use for this study of chromatophores prepared by differential centrifugation. This method has been used by others for analysis of chromatophores from *Rhodopseudomonas sphaeroides* (27). SDS-PAGE analysis indicated that the chromatophores obtained by differential centrifugation were essentially identical to those obtained by sucrose density gradient centrifugation (Fig. 3).

The extent of fluctuation in the protein/lipid contents (Fig. 2d and 4a) and buoyant densities (Fig. 4c) of chromatophore membranes from synchronized cells was consistent with the findings of Kaplan and co-workers, who observed such changes in *Rhodopseudomonas sphaeroides* (20, 31). However, the real extent of the fluctuation in the protein/lipid ratio in *R. rubrum* may be greater than that in *Rhodopseudomonas sphaeroides*, because the synchronized *Rhodopseudomonas sphaeroides* populations contained few, if any, nondividing cells. The basis of this phenomenon in *Rhodopseudomonas sphaeroides* is cell-cycle-specific accumulation of phospholipid in the chromatophore membrane. Evidence for this is provided by the observation that the increase in total lipid phosphorus is stepwise, while the increase in total cellular protein is continuous (31). This is supported by recent studies of Knacker et al. (26) demonstrating that a burst of phospholipid synthesis occurs in *Rhodopseudomonas sphaeroides* just before cell division. Pulse-chase studies have suggested that the cell-cycle-specific accumulation of lipid in the chromatophore membrane is due to the transfer of lipid synthesized at another site (4). Phospholipid synthesis has been localized in the cytoplasmic membrane of *Rhodopseudomonas sphaeroides* (5, 15, 35), and phospholipid transfer activity has been demonstrated (9, 36).

In the present study of *R. rubrum* chromatophore membranes, a continuous exponential increase in total cellular protein was demonstrated (Fig. 1b and 2a), while a decrease in the protein/phospholipid ratio was correlated with a specific time in the cell cycle (Fig. 2d and 4a). It is possible that cell-cycle-specific accumulation of phospholipid was reponsible for this oscillation in chromatophore membrane composition in *R. rubrum*. The recognition of the fluctuation in the protein/lipid ratio in the chromatophore membrane of *R. rubrum* necessitates an evaluation of the effect of this compositional variation in studies of membrane structure.

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