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Membrane adenosine triphosphatase in synchronous cultures of *Rhodobacter sphaeroides*

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Studies of intracytoplasmic membrane biogenesis utilizing synchronized cultures of *Rhodobacter sphaeroides* have revealed that most intracytoplasmic membrane proteins accumulate continuously throughout the cell cycle while new phospholipid appears discontinuously within the intracytoplasmic membrane. The resulting changes in the structure of the membrane lipids was proposed to influence the activities of enzymes associated with the intracytoplasmic membranes (Wraight, C.A., Leuking, D.R., Fraley, R.T. and Kaplan, S. (1978) J. Biol. Chem. 253, 465–471). We have extended the study of intracytoplasmic membrane biogenesis in *R. sphaeroides* to include the membrane adenosine triphosphatase. The membrane bound Mg^{2+}-dependent, oligomycin-sensitive adenosine triphosphatase activity was measured throughout the cell cycle for steady-state synchronized cells of *R. sphaeroides* and found to accumulate discontinuously. Following treatment with an uncoupling reagent (2,4-dinitrophenol) the intracytoplasmic membrane associated adenosine triphosphatase activity was stimulated uniformly in membranes isolated at different stages of the cell cycle. The adenosine triphosphatase was also measured by quantitative immunoblots utilizing specific antibody to compare the enzyme activity and enzyme protein mass. Immunologic measurement of the adenosine triphosphatase in isolated membranes indicated a constant ratio of enzyme to chromatophore protein exists during the cell cycle in contrast to the discontinuous accumulation of adenosine triphosphatase activity. These results are discussed in light of the cell-cycle specific synthesis of the intracytoplasmic membrane.

Introduction

Rhodobacter sphaeroides is a typical Gram-negative bacterial cell when grown aerobically, but under anaerobic conditions it will develop the ability to grow photosynthetically. Induction of photosynthetic growth under anaerobic conditions results in the synthesis of a new internal membrane system. This new intracytoplasmic membrane (ICM) arises as invaginations of the existing cytoplasmic membrane and the amount of intracytoplasmic membrane synthesized is within limits, inversely proportional to light intensity. Upon cell disruption the intracytoplasmic membrane can be isolated as uniform closed vesicles termed chromatophores which are oriented inside-out relative to the cytoplasmic membrane and contain all of the necessary photosynthetic complexes and related activities associated with the

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light reactions of photosynthesis. Control of membrane biosynthesis and the ability to isolate the photosynthetic membranes make *R. sphaeroides* an excellent system for the study of membrane biogenesis [1–3].

A variety of methods have been applied to the study of intracytoplasmic membrane biosynthesis in photosynthetically growing cultures of *R. sphaeroides.* Employing density shift experiments [4], the intracytoplasmic membrane was observed to replicate in a semi-conservative manner, thereby eliminating the possibility that 'old' and 'new' intracytoplasmic membrane exist together in the same cell. A series of studies using steady-state photosynthetically grown synchronous cell cultures of *R. sphaeroides* reveal: continuous insertion of new membrane protein, chiefly photosynthetic complexes and cytochromes into the preexisting intracytoplasmic membrane [5]; new phospholipid insertion into the intracytoplasmic membrane only near the time of cell division [6]; large cellular increases in the rate of phospholipid synthesis after cell division [7]; an increase of phospholipid exchange protein activity just before cell division [8]; cell cycle changes in protein to phospholipid ratios corresponding precisely to changes in chromatophore phospholipid membrane structure [6] and correspondence of physical membrane changes with the numbers of intramembranous particles, i.e. photosynthetic complexes [9]. Membrane bound enzyme activities, such as succinate dehydrogenase and NADH dehydrogenase were shown to vary discontinuously in synchronously dividing photosynthetic populations [5]. Other enzyme activities, such as succinyl-CoA thiokinase, δ-aminolevulinic acid synthetase and δ-aminolevulinic acid dehydrase, have also been shown to accumulate discontinuously during the cell cycle [10]. These enzymes are involved in tetrapyrrole synthesis whereas heme and bacteriochlorophyll accumulate continuously during the cell cycle [5].

Synchronously growing cultures have been useful to the study of membrane biogenesis in a variety of other organisms. Membrane lipids have been reported to accumulate discontinuously in *Escherichia coli* [11], *Bacillus megaterium* [12], and in yeast [13]. But, the discontinuous accumulation of phospholipids in *E. coli* was reported to be strain dependent [14]. Discontinuous accumulation of membrane associated enzyme activities has been reported in a number of organisms [15,16]. Yet total membrane protein is found to be synthesized at linear rates in *E. coli* [17] and *Bacillus subtilis* [14]. The continuous synthesis of cytochromes *b*, *c*, and aa_3 in yeast have also been reported but the functional activity of the cytochrome aa_3 appears to accumulate discontinuously [18]. Measurements of enzymatic activity as distinct from the physical accumulation of a particular membrane protein has usually been carried out with cytochromes.

Since the membrane associated Mg^{2+}-ATPase is a key enzyme in both respiratory and photosynthetic growth we have used it to extend the study of *R. sphaeroides* membrane synthesis. In the present report we have measured the membrane bound ATPase activity on chromatophores isolated from synchronously growing cultures of *R. sphaeroides.* Antibody specific for the F_1 region of the *E. coli* ATPase was used to distinguish between levels of activity and actual enzyme accumulation. The results are discussed relative to what is known of intracytoplasmic membrane biosynthesis in *R. sphaeroides.*

Materials and Methods

Cell growth. R. sphaeroides strain 2.4.1 was grown photoheterotrophically on succinic acid minimal medium A of Sistrom [19], by sparging with 95% nitrogen, 5% carbon dioxide at 33°C under a light intensity of 10 W/m^2. *E. coli* strain JM83 was grown on LB at 37°C on a gyratory shaker.

Synchronous cultures. A stationary phase cycling technique [20] as modified [19], was used to synchronize cultures of *R. sphaeroides.* Cultures were sampled at 20-min intervals and 400–1000 cells were counted per sample with a phase contrast microscope using a Petroff-Houser chamber.

Membrane preparation. Samples of *R. sphaeroides* cells were washed and resuspended in 9 ml of TM buffer (50 mM Tris-HCl (pH 8.0)/1 mM $MgCl_2$) and frozen at −20°C. The cells were broken by two passages through a French pressure cell at a pressure of 15000 $lbs/inch^2$. The cell lysate was cleared by centrifugation at $12000 \times g$ for 20 min. The supernatant was then layered on a

60% sucrose cushion and centrifuged at 200 000 × *g* for 1 h in a 50 Ti rotor at 5°C. The supernatant was removed and the particulate material remaining on the cushion was removed and brought to 2 ml volume by addition of TM buffer, and labeled crude membrane. After retaining a sample of crude membranes for assay (0.2 ml), the remaining crude membrane was brought to a total volume of 3 ml to reduce the sucrose concentration to approx. 10% and the crude membrane fraction was layered on a discontinuous sucrose gradient (20% : 40% in TM buffer). The sucrose gradient was prepared by a layer of 20% sucrose in TM buffer (1 ml) upon a layer of 40% sucrose in TM buffer (1 ml) and the sample was placed upon the 20% sucrose layer and centrifuged in a Beckman SW55 rotor at 360 000 × *g* for 2 h at 5°C. The membrane band at the 20%/40% interface was removed and retained as the chromatophore preparation. The membranes from each sample of cells were prepared as described above and all ATPase assays were carried out within 24 h of cell breakage.

Chromatophores were prepared as described above from asynchronously grown cells and the ATPase was removed from the chromatophores as previously described [21]. The chromatophores were passed through a French pressure cell at 20 000 lbs/inch^2 after the TM buffer was adjusted to 1 mM ethylenediaminetetracetic acid (EDTA). The membranes were then pelleted onto a 60% sucrose cushion by centrifugation at 200 000 × *g* for 1 h at 5°C. The supernatant, which now contained the released F_1-ATPase complex, was removed and the membranes were resuspended in normal TM buffer and assayed for activity. These ATPase depleted membranes were used in Western blot experiments described in the text. *E. coli* membranes were prepared by washing and resuspending the cells in TM buffer. The cells were passed through a French pressure cell at 10 000 lbs/inch^2. The cell lysate was cleared by centrifugation at 12 000 × *g* for 20 min. The supernatant was layered on a 60% sucrose cushion and centrifuged at 200 000 × *g* for 1 h in a 50 Ti rotor at 5°C. The supernatant was removed and the particulate material residing on the cushion was retained as the *E. coli* membranes.

Enzyme assay. ATPase assays were carried out as previously described [22,23], by incubation of the sample in TM buffer containing 1 mM ATP for 1 h at 30°C before addition of trichloroacetic acid to 10%. The assay conditions gave a linear response of product (released phosphate) with respect to the amount of enzyme added and time of incubation. The sucrose present in the membrane preparations had no effect upon the ATPase activity. The ATPase inhibitors, oligomycin or *N,N'*-dicyclohexylcarbodiimide, were dissolved in ethanol or dimethylsulfoxide before addition to the ATPase assay and appropriate solvent controls were utilized. Units of enzyme activity are reported as μmoles phosphate released per h. It was determined that maximal activation of the membrane-bound ATPase by 2,4-dinitrophenol was at 0.8 mM. This activity was independent of sample protein concentration.

Western blots. Membrane samples were solubilized as previously described [24]. Electrophoresis was carried out as described [25] using 10% polyacrylamide gels. The transfer and washing of the nitrocellulose was performed [26] as modified [27]. Kodak XAR-5 film was preflashed and used to develop the blots [28]. The film was scanned by a Hoeffer densitometer as previously described [29]. The conditions of protein load per lane or the time of exposure of the film were always compared from the same blot since background varied for each blot. Conditions were determined such that the autoradiogram signal response was linearly dependent upon the amount of protein sample and film exposure time.

Analysis procedures. Protein assays were carried out as described by Lowry et al. [30] using bovine serum albumin as a standard. The method of Bartlett [31] was used to quantitate lipid phosphorus. Bacteriochlorophyll was quantified by measuring $A_{775\,nm}$ [32].

Materials. The antibody was raised in rabbtis against the F_1-ATPase of *E. coli* and was kindly provided by D. Klionsky and Dr. R. Simoni [33,34]. The *Staphylococcus* protein A was purchased from Sigma Chemical Co. and radioiodinated with sodium [^{125}I]iodide purchased from New England Nuclear. Iodogen was from Pierce Chemical Co. and used according to the method of Fraker and Speck [35]. The adenosine triphosphate, oligomycin, and dicyclohexylcarbodiimide were purchased from Sigma Chemical Co.

Results

Membrane preparation from synchronously growing culture of R. sphaeroides

Photosynthetically growing cultures of *R. sphaeroides* were synchronized by the stationary phase cycling technique [20], as modified [19]. Previous studies employing synchronously grown *R. sphaeroides* have shown that cell mass, bulk soluble protein, and bulk membrane protein increased exponentially while cell number and DNA accumulation increases step-wise during growth [19]. Membranes were prepared from both synchronously grown cultures (Fig. 1) and also from asynchronously grown cultures (Fig. 2) for comparison. Total cell membrane or crude membranes were prepared by methods routinely employed for the study of the intracytoplasmic membrane [19]. The only alteration in the procedure of membrane preparation was the elimination of EDTA from the buffers and the addition of $MgCl_2$ in order to maximize retention of the ATPase on the membrane [21]. Protein and bacteriochlorophyll content of the membranes, as well as ATPase activity, were used to verify the enrichment for photosynthetic membranes in the fractions designated chromatophores. Most of the bacteriochlorophyll was recovered in the chromatophore fraction (76%), as was also 9% of the whole cell protein. These recoveries are similar to recoveries obtained by others [19] from synchronous cultures, in which 65% of the whole cell bacteriochlorophyll was recovered in the chromatophore fraction as was 14% of the recovered protein. Using sucrose gradient centrifugation, 45% of the total ATPase activity in the chromatophore fraction was recovered from total crude cell membranes. It should be recognized that while this procedure separates the chromatophores from the outer membrane it does not resolve chromatophores from the bulk of the cytoplasmic membrane, which may also contain ATPase activity. However, the specific activity of the ATPase in the chromatophore fraction is enriched compared to crude membranes by a factor of two. The membrane ATPase activity was absolutely dependent upon the presence of divalent cations such as Mg^{2+} as has been previously reported [21]. When the chromatophores were treated with oligomycin (62 μm) only 25% of the original ATPase activity remained whereas crude membranes retained 30% of the original ATPase activity under similar conditions of treatment. Inhibition of the ATPase activity by treatment with dicyclohexylcarbodiimide (1.45 μm) resulted in only 72% of the original activity remaining. These inhibitor profiles of the ATPase are consistent with other reports on the *R. sphaeroides* chromatophore ATPase [22].

Total ATPase activity of both crude membranes and chromatophores isolated from both synchronously growing cultures (Fig .1) and asynchronously growing cultures (Fig. 2) was determined. The slopes of the lines for Figs. 1c,d and 2c,d were derived using both a least-squares analysis of the data points as well as an analysis of variance. In the curves derived for the synchronous cultures, the points assigned to the plateaus showed a very large degree of variance when compared to the points used to determine the slope of the curve. Membrane ATPase activity increased discontinuously in the synchronous culture, when measured as either total cellular ATPase per ml of culture (Fig. 1c) or as total chromatophore ATPase per ml culture (Fig. 1d). These results should be compared with those obtained from the asynchronously grown control culture (Figs. 2c,d), where no evidence of a stepwise increase in activity was apparent. These experiments were repeated several times with identical results. Further, each experimental point is the result of several independent assays performed to insure that the activity response was linear with respect to both time and protein used in the assay.

When the specific ATPase activity (ATPase activity per mg total chromatophore protein) was plotted for either the asynchronous control culture (Fig. 2f) or the synchronous culture (Fig. 3a) a straight line of constant and similar value was obtained. This was to be expected despite the fact that a linear protein enrichment was observed for the intracytoplasmic membrane over the course of the cell cycle, i.e. during the plateau period between the increases in cell number. The very minor oscillations that might have been observed were dampened by the calculation of ATPase specific activity, i.e. normalizing the fluctuation of activity to mg of protein. However, during the plateau period both cell number and chromatophore

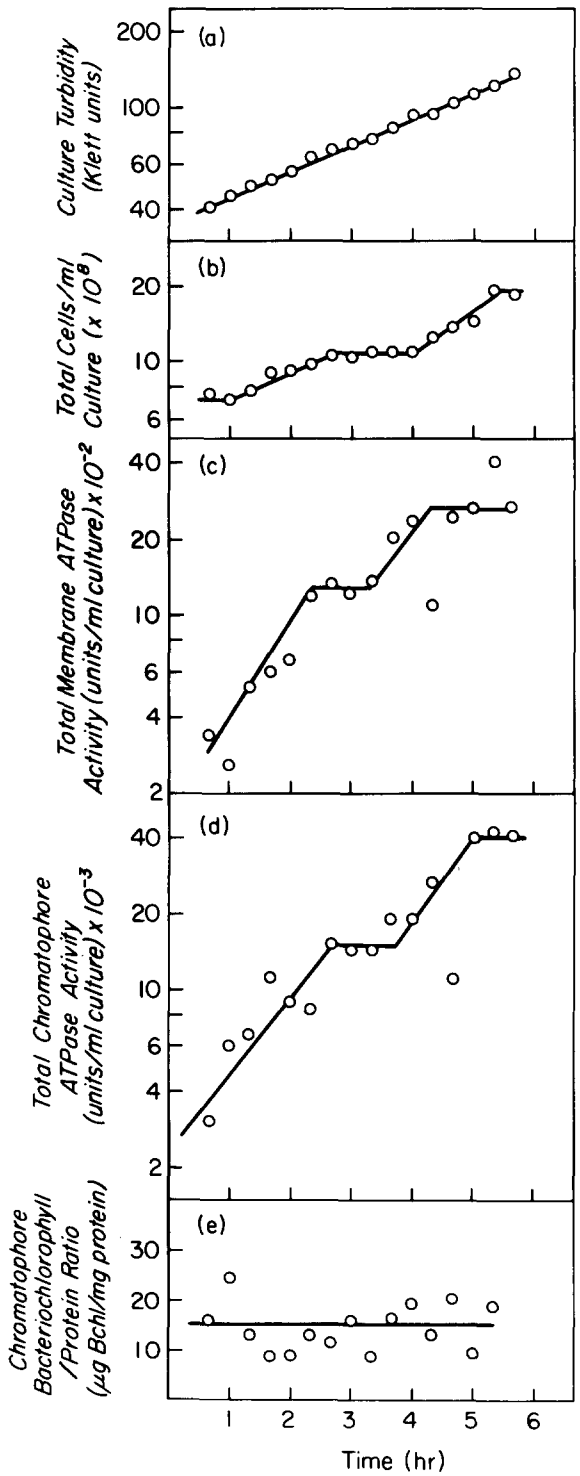
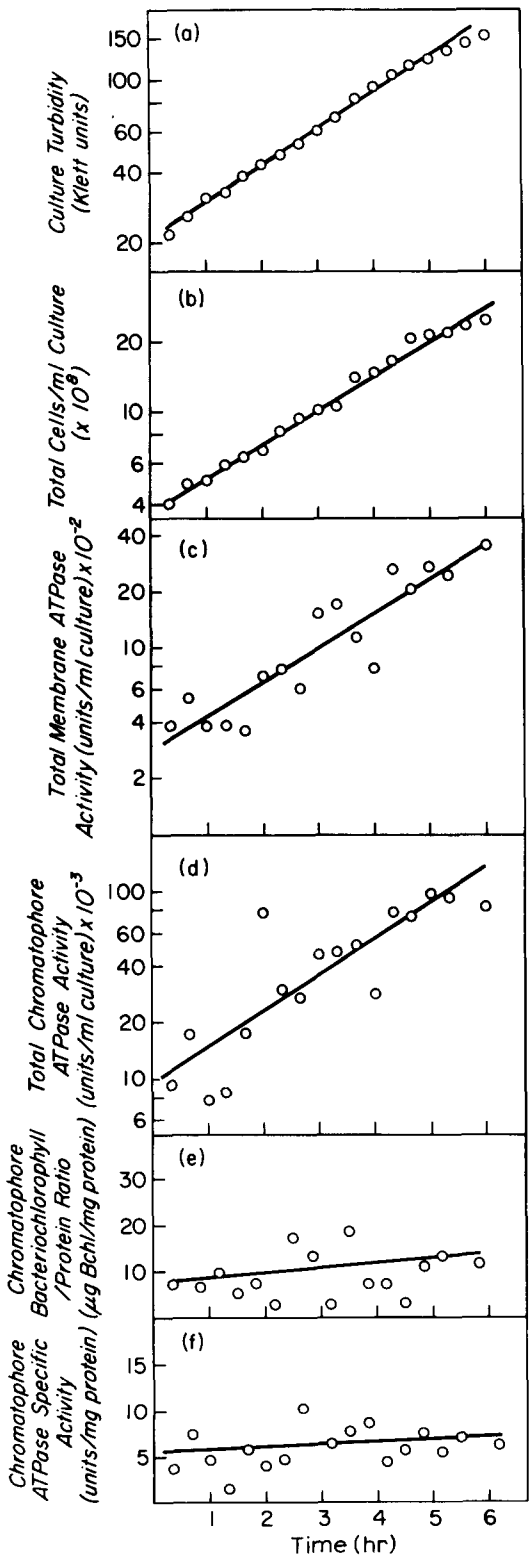


Fig. 1. Membrane bound ATPase activity in synchronously growing culture of *R. sphaeroides*. The method of culture synchrony, membrane preparation, and ATPase assay are described in Experimental Procedures. (a) Culture turbidity; (b) cell number per ml of culture; (c) crude membrane ATPase activity per ml of culture; (d) chromatophore ATPase activity per ml of culture; and (e) ratio of the bacteriochlorophyll to protein content of the chromatophores.

ATPase activity remained constant. This observation requires explanation if we are to presume the amount of ATPase protein increased together with the amount of total chromatophore protein during the plateau. This will be addressed in a later section. The values depicted in Figs. 2f and 3a were obtained from the ATPase activities and protein concentrations used in the presentation of Figs. 2d,e and 1d,e, respectively. The bacteriochlorophyll/protein ratio of the isolated chromatophores from the synchronous cultures was found to remain constant throughout the cell cycle as was expected for a culture growing in steady-state [5]. Chromatophore ATPase activity from asynchronously grown cells increased continuously together with cell number (Fig. 2). On the other hand, the ATPase activity/unit chromatophore protein (specific activity) of isolated chromatophores from the synchronous culture displayed in Fig. 1, was found to remain constant during the cell cycle (Fig. 3), despite the continuous enrichment of the intracytoplasmic membrane with membrane protein during the course of the cell cycle. Chromatophores isolated from the synchronous culture were found to have a maximal protein/phospholipid ratio immediately prior to cell division (Fig. 3). The observed cell cycle specific changes in the protein/phospholipid ratios, shown in Fig. 3, of the isolated chromatophores are consistent with previously reported results [6]. It is important to reemphasize the cyclical change in protein to phospholipid ratio. This change represents an independent measure of the synchrony of the cell population.

ATPase activity in the presence of an uncoupling reagent

Observations of a cell cycle specific change in the total amount of ATPase activity may be due to either a cell cycle specific modulation of the ATPase activity or a discontinuous rate of enzyme synthesis and/or assembly. The activity of several membrane associated ATPases are sensitive to



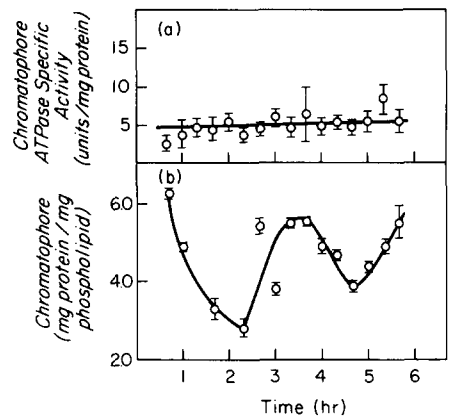


Fig. 3. Comparison of the ATPase specific activity and protein to phospholipid ratio of chromatophores derived from synchronously grown cells. The chromatophore specific activity was derived from results presented in Fig. 1 panels (d) and (e). The protein to phospholipid ratio for chromatophores analyzed from Fig. 1 was determined as described in Experimental Procedures. The error bars indicate one standard deviation from the mean of each set of samples. The line drawn to best fit the data points in panel (a) has a coefficient of correlation of 0.93. If a line were used to best fit the data in panel (b) it would have a coefficient of correlation of 0.28.

their intermembrane environment [36–38] as is the *R. sphaeroides* ATPase activity, as evidenced by the activation of the ATPase by detergents [21] or uncoupling agents [22]. To determine if the characteristics of the chromatophore ATPase activation changed during the cell cycle, we assayed the ATPase in the presence of the uncoupling reagent 2,4-dinitrophenol (DNP).

Application of 2,4-dinitrophenol resulted in an increase in ATPase activity, both in chromatophores and crude membranes isolated from asynchronous as well as synchronously grown cells. The ATPase activity was observed to be stimulated maximally at 0.8 mM 2,4-dinitrophenol regardless of the stage of the cell cycle from which the membranes were isolated. When chromatophores isolated from asynchronously growing cultures were measured for ATPase activity in the

Fig. 2. ATPase activity measured in membranes from an asynchronously dividing culture of *R. sphaeroides*. See the legend to Fig. 1 for a description of panels (a) to (e), (f) chromatophore ATPase specific activity.

presence of 2,4-dinitrophenol, the ATPase activity increased an average of 22% over that of the control lacking 2,4-dinitrophenol. However, the ratio of the chromatophore ATPase activity plus 0.8 mM 2,4-dinitrophenol to that minus 2,4-dinitrophenol (Fig. 4) remained relatively constant during the cell cycle.

Immunologic measurement of the ATPase throughout the cell cycle

Measuring the total activity of the membrane associated ATPase is not necessarily a reflection of the physical mass of the enzyme present, if the enzyme activity is being modulated in some manner. Likewise, the accumulation of ATPase activity cannot necessarily be correlated with the synthesis of the ATPase during the cell cycle. Therefore, immunological methods were used to measure the concentration of chromatophore

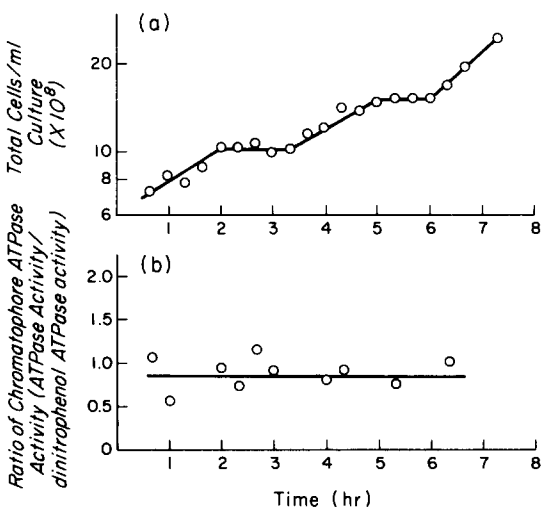
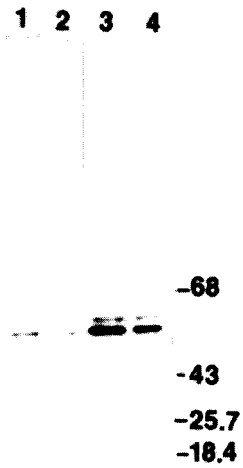


Fig. 4. Effect of an uncoupling reagent upon the ATPase activity of chromatophores isolated from a synchronously growing culture. Chromatophores were prepared from cells sampled at the indicated times from the culture whose growth is displayed in panel (a). Synchronization of the culture and chromatophore isolation was carried out as described in Experimental Procedures. Chromatophore ATPase activity was measured with or without 0.8 mM 2,4-dinitrophenol as described in the text. The lower panel (b) presents the ratio of, chromatophore ATPase activity/chromatophore ATPase activity with 0.8 mM 2,4-dinitrophenol, for the various chromatophore samples from the culture. The line drawn in panel (b) to best fit the data points has a coefficient of correlation of 0.85.

ATPase independent of the ATPase activity. The antibody used was raised against the *E. coli* F_1-ATPase. As shown in the autoradiogram of the Western blot (Fig. 5, lanes 3 and 4), the antibody reacted predominately with *E. coli* α and β subunits, as judged from the bands of M_r of 55000 and 50000, respectively. Longer exposures of the X-ray film resulted in the visualization of other reactive polypeptide bands of sizes appropriate to the smaller subunits of the *E. coli* F_1-ATPase. The antibody cross-reacted with only one *R. sphaeroides* chromatophore protein, the molecular weight of which was approximately equal to that of the *E. coli* β-subunit (Fig. 5, lanes 1 and 2). We believe the cross-reacting polypeptide to be the β-subunit of the *R. sphaeroides* ATPase, since htis is consistent with the similarity of the β-subunit sequence of the photosynthetic bacterium *Rhodopseudomonas blastica* [39,40], and other species of bacteria having highly conserved regions within the β-subunit. Further, this antibody preparation will precipitate the β-subunit synthesized in vitro from a clone of *R. blastica* DNA containing the *atp* operon [39]. We have demonstrated (unpublished results) that the *R. blastica* and *R. sphaeroides* DNA sequences corresponding to the F_1 portions of the ATPase share substantial sequence homology. Taken together, all of these findings strongly suggest that the Anti-F_1 immunoglobulin fraction prepared against the *E. coli* ATPase cross-reacts with the β-subunit of the *R. sphaeroides* enzyme. Pre-immune serum did not cross-react when used in immunoblots of either *E. coli* or *R. sphaeroides* (data not shown). When the ATPase activity was removed from the chromatophores by treatment with EDTA, there was an accompanying reduction of the antibody reaction as measured by immunoblots using the washed chromatophores. Autoradiograms of the immunoblots employing different amounts of chromatophore protein were densitometrically scanned (Fig. 5). A linear densitometric signal per μg of protein was obtained between 1 and 15 μg of chromatophore protein (Fig. 5a). In addition, the time of exposure of the X-ray film to the autoradiogram yielded a linear densitometric response as a function of the time of exposure (Fig. 5b).

Chromatophores isolated from the cells derived from the cultures shown in Figs. 1 and 2 were



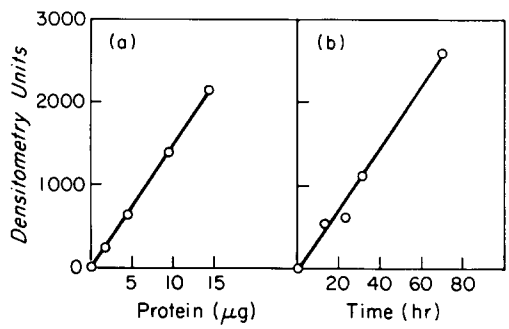


Fig. 5. Quantitation of the ATPase by immunoblot analysis. Isolated membrane samples were solubilized, subjected to SDS-polyacrylamide gel electrophoresis on a 10% SDS-polyacrylamide gel, and then electrophoretically transferred to nitrocellulose paper as described in Experimental Procedures. Samples were *E. coli* membranes (5 μg protein) (lanes 3 and 4) and *R. sphaeroides* chromatophores (10 μg protein) (lanes 1 and 2). The nitrocellulose was probed with antibody raised against the F_1 portion of the *E. coli* ATPase and the bound antibody was visualized using ^{125}I-labeled *Staphylococcus aureus* protein A. The autoradiograms were scanned by densitometry to evaluate conditions for linear densitometric response as a function of chromatophore protein sample (panel a) and response as a function of autoradiogram exposure time (panel b) using 7.5 μg of chromatophore protein.

solubilized, subjected to electrophoresis on SDS-polyacrylamide gels and used for immunoblot assays employing conditions which yield a linear response of the autoradiogram to the amount of protein present. Immunoblots were carried out using chromatophores derived from both the synchronous culture (Fig. 1) as well as chromatophores derived from asynchronous cultures (Fig. 2). The densitometric units per unit chromatophore protein were measured from autoradiograms of immunoblots of chromatophores from asynchronously growing cultures (Fig. 6a) or from synchronously growing culture (Fig. 6b). Since the non-specific background and age of the ^{125}I-labeled protein A differed for the experiments of Fig. 6, panel (a) from those of Fig. 6, panel (b), the absolute densitometric units are smaller in panel (b). From the results shown in Fig. 6, it is readily apparent that the amount of the β-subunit of the chromatophore-bound ATPase and, therefore, presumably the amount of ATPase, per μg of chromatophore membrane protein remains constant throughout the cell cycle. We have calculated that approx. 2–5% of the total chromatophore protein is ATPase. This result is identical to the results shown in Fig. 3 when measuring ATPase-

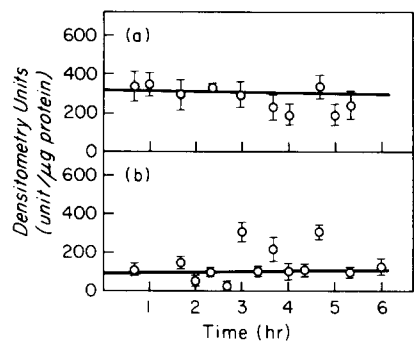


Fig. 6. Immunoblot analysis of the chromatophore ATPase from asynchronous and synchronously growing cultures. Chromatophores were isolated and immunoblots were carried out as described in Experimental Procedures using the appropriate conditions established in Fig. 5. Densitometric units per μg of protein were determined for chromatophore samples prepared from asynchronously grown cultures (panel a), or synchronously grown cultures (panel b). The chromatophores assayed in panel (a) were isolated from the asynchronous culture shown in Fig. 2. The chromatophores assayed in panel (b) were isolated from the synchronous culture reported in Fig. 1. The error bars indicate one standard deviation from the mean for each set of determinations. Lines were drawn as a best fit of the sample points with a coefficient of correlation for panel (a) of 0.82 and for panel (b) of 0.88.

specific activity but should be contrasted to the results presented in Fig. 1 for total chromatophore ATPase activity which does not show a continuous increase throughout the cell cycle.

Discussion

This study extends earlier observations made on the assembly of the intracytoplasmic membrane in synchronously dividing cultures of *R. sphaeroides*. This organism has been extensively used for the study of membrane biogenesis, since the intracytoplasmic membrane system which can be readily manipulated both in its synthesis and structure, and the cells can be readily synchronized by stationary phase cycling. Utilizing synchronously growing cells, the majority of intracytoplasmic membrane protein was shown, in previous studies, to be assembled into pre-existing intracytoplasmic membrane continuously throughout the cell cycle [19]. In addition to the bacteriochlorophyll binding proteins, the *b* and *c* type cytochromes also accumulate continuously [5]. These earlier studies also reveal the discontinuous accumulation of phospholipids into the intracytoplasmic membrane [9] as a function of the stage in the cell cycle. This results in a cell cycle specific fluctuation in the protein to phospholipid ratio which has been correlated with major changes in membrane lipid structure as well as with the physical structure of the membrane [6,9].

Previous studies of synchronously dividing cultures of *R. sphaeroides* suggest that the functions of some membrane proteins were correlated with changes in the lipid/protein ratio of the intracytoplasmic membrane during the cell cycle [5]. This study also examined the activities of two dehydrogenases found in the membranes from synchronously dividing cultures [5]. To determine if the discontinuous appearance of enzyme activity in *R. sphaeroides* during the cell cycle was unique to the intracytoplasmic membrane dehydrogenases, we examined the membrane bound proton-ATPase on the intracytoplasmic membrane from synchronous cultures. This ATPase has been characterized as one of the key enzymatic components of the intracytoplasmic membrane [41] and is similar to other bacterial ATPases. Since some ATPase components are integral membrane proteins, the activity of this enzyme may be influenced by changing lipid/protein ratios of the intracytoplasmic membrane, especially since this enzyme has been implicated as existing in different conformations [42,43] or activity states [44].

The total membrane bound ATPase activity in synchronously dividing cells accumulatd discontinuously during the cell cycle. The total activity on crude membranes or on purified chromatophores increased step-wise concomitant with cell division resulting in a constant membrane ATPase activity per cell throughout the cell cycle. Only two other chromatophore enzyme activities that have been studied, succinate dehydrogenase and NADH dehydrogenase, also display a discontinuous accumulation of activity during the cell cycle. Yet, the specific activity of the chromatophore ATPase remained constant throughout the cell cycle. Given these results, the chromatophore ATPase specific activity might have been expected to decrease during the cell cycle due to the continuous accumulation of protein into the intracytoplasmic membrane during the cell cycle [19] when coupled to the stepwise change in ATPase activity. Therefore, we suggest that the activity of the chromatophore ATPase is being modulated such that the higher protein to phospholipid ratio of the chromatophore results in a progressively lower ATPase activity, despite the continuous accumulation of new ATPase protein. When the protein to phospholipid ratio is lowest concomitant with cell division [6], the activity of the ATPase is at its highest per unit of enzyme mass. At the present time, we have no evidence that additional protein components may be involved in regulating the ATPase activity in *R. sphaeroides* in a cell cycle-dependent manner.

Modulation of the activity of membrane enzymes by phospholipids or by the physical state of the membrane has been reported [36–38]. Enzymes such as ATPase [45,46] or the mitochondrial β-hydroxybutyrate dehydrogenase [47] are activated by particular types of lipid. Similarly, mitochondrial cytochrome oxidase activity parallels the increases in cardiolipin during the cell cycle [48]. However, in previous studies it was found that all the major phospholipids of *R. sphaeroides* are synthesized at a constant relative

rate throughout the cell cycle [7]. This would apepar to preclude the possibility of the ATPase activity being altered by any specific phospholipid species. However, it is possible that microheterogeneity of the membrane phospholipid species exists, i.e. specific species of lipids associating with the ATPase. Indeed, an *R. sphaeroides* F_0-F_1-ATPase preparation has been reported to contain a lipid species different from that of the normal bulk membrane lipid components [49].

The enzyme activity of numerous integral membrane proteins is altered by changes in the physical characteristics of their membranes [36–38]. Changes in the activity of the calcium-ATPase is apparently dependent upon the total levels of membrane phospholipids which may influence the binding of specific lipids [50]. Cytochrome oxidase apparently exhibits preferential interactions with some phospholipids. At low lipid/protein ratios (less than 0.2% mg lipid/mg of protein) when all lipid is immobilized the cytochrome oxidase exhibits low activity. A more mobile phospholipid phase occurs at higher lipid/protein ratios and higher oxidase activities results [51,52]. The lipid/protein ratio, in vivo, of the *R. sphaeroides* intracytoplasmic membrane varies during the cell cycle from 0.25 to 0.45 mg lipid/mg protein. Therefore, it is distinctly possible that enzymes associated with the intracytoplasmic membrane may have altered activities due to the alterations in lipid/protein ratio during the cell cycle. These shifts in intracytoplasmic membrane phospholipid structure might also result in passive permeability changes in the intracytoplasmic membrane since *E. coli* cell membranes become leaky when the phospholipid becomes immobile at temperatures near the lipid phase transition. If the membrane increases in passive ion permeability, then the stimulation of the *R. sphaeroides* ATPase by uncoupling reagents, might vary as a function of the membrane lipid/protein ratio during the cell cycle. However, we found no evidence of cell cycle specific response of the ATPase to 2,4-dinitrophenol. To monitor the accumulation of the ATPase during the cell cycle by a method independent of enzyme activity, antibody against *E. coli* F_1-ATPase was used. The results indicated that a constant amount of ATPase was present per unit of chromatophore protein throughout the cell cycle. The continuous accumulation of the ATPase protein during the cell cycle is similar to the continuous accumulation of other major membrane proteins of the intracytoplasmic membrane [5].

The accumulation of light-harvesting complexes, reaction center, cytochrome *b*, cytochrome *c*, and the ATPase of the *R. sphaeroides* ICM is continuous when measured, respectively, by spectroscopy(s), SDS-polyacrylamide gel electrophoresis [11], or immunoblots. Yet, three enzyme activities; succinate dehydrogenase, NADH dehydrogenase, and ATPase, accumulate discontinuously on the intracytoplasmic membrane during the cell cycle. Only the ATPase has been measured by two independent methods and the results suggest a continuous accumulation of the enzyme with a discontinuous accumulation of enzyme activity during the cell cycle. Therefore, the possibility of a cell cycle specific modulation of enzyme activity of the ATPase exists and by analogy, also of the succinate and NADH dehydrogenases of the intracytoplasmic membrane.

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References

1 Kaplan, S. (1978) in The Photosynthetic Bacteria (Clayton, R.K. and Sistrom, W.R., eds.), pp. 809–839, Plenum Press, New York
2 Kaplan, S. and Arntzen, C.J. (1982) in Photosynthesis (Govindjee, ed.), pp. 65–154, Academic Press, New York
3 Ohad, I. and Drews, G. (1982) in Photosynthesis (Govindjee, ed.), pp. 89–140, Academic Press, New York
4 Kosakowski, M.H. and Kaplan, S. (1974) J. Bacteriol. 118, 1144–1157
5 Wraight, C.A., Lueking, D.R., Fraley, R.T. and Kaplan, S. (1978) J. Biol. Chem. 253, 465–471
6 Fraley, R.T., Lueking, D.R. and Kaplan, S. (1979) J. Biol. Chem. 254, 1980–1986
7 Cain, B.D., Deal, C.D., Fraley, R.T. and Kaplan, S. (1981) J. Bacteriol. 145, 1154–1166

8 Tai, S.-P., Hoger, J.H. and Kaplan, S. (1986) Biochim. Biophys. Acta 859, 198–208
9 Yen, G.S.L., Cain, B.D. and Kaplan, S. (1984) Biochim. Biophys. Acta 777, 41–55
10 Ferretti, J.J. and Gray, E.D. (1968) J. Bacteriol. 95, 1400–1406
11 Hakenbeck, R. and Messer, W. (1977) J. Bacteriol. 129, 1234–1238
12 Daniel, M.J. (1969) Biochem. J. 115, 697–701
13 Cottrell, S.F., Getz, G.S. and Rabinowitz, M. (1981) J. Biol. Chem. 256, 10973–10978
14 Joseleau-Petit, D., Kepes, F. and Kepes, A. (1984) Eur. J. Biochem. 139, 605–611
15 Sargent, M.G. (1973) J. Bacteriol. 116, 397–409
16 Edward, S.C., Spode, J.A. and Jones, C.W. (1978) Biochem. J. 172, 253–260
17 Churchward, G.G. and Holland, I.B. (1976) J. Mol. Biol. 105, 245–261
18 Cottrell, S.F., Rabinowitz, M. and Getz, G.S. (1975) J. Biol. Chem. 250, 4087–4094
19 Fraley, R.T., Leuking, D.R. and Kaplan, S. (1978) J. Biol. Chem. 253, 458–464
20 Cutler, R.G. and Evans, J.E. (1966) J. Bacteriol. 91, 469–476
21 Reed, D.W. and Raveed, D. (1972) Biochim. Biophys. Acta 283, 79–91
22 Muller, H., Neufang, H. and Knobloch, K. (1982) Eur. J. Biochem. 127, 559–566
23 Chen, P.S., Toribara, T.Y. and Warner, H. (1956) Anal. Chem. 28, 1756–1758
24 Cohen, L.K. and Kaplan, S. (1981) J. Biol. Chem. 256, 5901–5908
25 Laemmli, U.K. and Favre, N. (1973) J. Mol. Biol. 80, 575–599
26 Burnette, W.N. (1981) Anal. Biochem. 112, 195–203
27 Kranz, R.G. and Gennis, R.B. (1982) Anal. Biochem. 127, 247–257
28 Laskey, R.A. (1984) Methods Enzymol. 65, 363–371
29 Chory, J., Donohue, T.J., Varga, A.R., Staehelin, L.A. and Kaplan, S. (1984) J. Bacteriol. 159, 540–554
30 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
31 Bartlett, G.R. (1959) J. Biol. Chem. 234, 400–408
32 Clayton, R.K. (1966) Photochem. Photobiol. 5, 669–677
33 Klionsky, D.J., Brusilow, W.S.A. and Simoni, R.D. (1983) J. Biol. Chem. 258, 10136–10143
34 Aris, J.P. and Simoni, R.D. (1983) J. Biol. Chem. 258, 14599–14609
35 Fraker, P.J. and Speck, J.C. (1978) Biochem. Biophys. Res. Commun. 80, 849–857
36 Johannsson, A., Keightley, C.A., Smith, G.A., Richards, C.D., Hesketh, T.R. and Metcalfe, J.C. (1981) J. Biol. Chem. 256, 1643–1650
37 Johannsson, A., Smith, G.A. and Metcalfe, J.C. (1981) Biochim. Biophys. Acta 641, 416–421
38 Knowles, A.F., Kundrach, A., Racker, E. and Khorana, H.G. (1975) J. Biol. Chem. 250, 1809–1813
39 Tybulewicz, V.L.J., Falk, G. and Walker, J.E. (1984) J. Mol. Biol. 179, 188–214
40 Dunn, S.D., Tozer, R.G., Antczak, D.F. and Haprel, L.A. (1985) J. Biol. Chem. 260, 10418–10425
41 McCarty, R.E. and Carmeli, C. (1982) in Photosynthesis (Govindjee, ed.), pp. 647–695, Academic Press, Inc., New York
42 Saphon, S. and Graber, P. (1978) Z. Naturforsch. 33c, 421–427
43 Casadio, R. and Wagner, R. (1985) Biochim. Biophys. Acta 809, 215–227
44 Saphon, S., Jackson, J.B. and Witt, H.T. (1975) Biochim. Biophys. Acta 408, 67–82
45 McMurchie, E.J., Abeywardena, M.Y., Charnock, J.S. and Gibson, R.A. (1983) Biochim. Biophys. Acta 760, 13–24
46 Caffrey, M. and Feigenson, G.W. (1981) Biochemistry 20, 1949–1961
47 McIntyre, J.O., Warg, C. and Fleischer, S. (1979) J. Biol. Chem. 254, 5199–5207
48 Cottrell, S.F., Getz, G.S. and Rabinowitz, M. (1981) J. Biol. Chem. 256, 10973–10978
49 Kaiser, I. and Oelze, J. (1980) Arch. Microbiol. 126, 195–200
50 Bennett, J.P., McGill, K.A. and Warren, G.B. (1980) Curr. Top. Membranes Transp. 14, 127–164
51 Chuang, T.F. and Crane, F.L. (1973) J. Bioenerg. 4, 563–578
52 Jost, P., Griffith, O.H., Capaldi, R.A. and Vanderkooi, G. (1973) Biochim. Biophys. Acta 311, 141–152