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Effect of Light on Nitrogenase Function and Synthesis in *Rhodopseudomonas capsulata*

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The metabolic versatility of the purple nonsulfur photosynthetic bacteria permits the expression of either a phototrophic or a dark aerobic mode of growth. These organisms also possess nitrogenase activity which may function under semiaerobic conditions. On the basis of these important properties, the light dependence of nitrogenase function and synthesis in *Rhodopseudomonas capsulata* was investigated. Nitrogenase activity was strictly dependent on light; no activity was observed in the dark, even when energy (ATP) was supplied by oxidative phosphorylation. It was concluded that the low-potential reducing agent required by the nitrogenase-catalyzed reaction could only be generated by a photochemical reaction. Nitrogenase biosynthesis was also largely dependent on light; however, a small amount of synthesis was observed in resting cells incubated in the dark. Resting cells prepared from dark-grown cultures synthesized nitrogenase at high rates upon illumination. The highest stability of nitrogenase in these resting cells was observed when suspensions were exposed to a diurnal pattern of illumination rather than continous light. Although nitrogenase function and synthesis are closely coupled to photosynthetic activity, the biosyntheses of bacteriochorophyll and nitrogenase are independent of each other and are most probably subject to different regulatory mechanisms by light.

N_2 fixation is now well established as a widespread phenomenon among phototrophic bacteria (21). Since these organisms are largely dependent on light to meet their energy requirements, their ability to fix N_2 would appear to be strictly light dependent. However, the basic requirements for nitrogenase activity, namely, ATP and a low-potential reducing agent, can be met by dark reactions, as evidenced by the fact that a great number of N_2 fixers are not phototrophs. Indeed, even some photosynthetic organisms, like the blue-green algae (cyanobacteria) (9), can fix nitrogen at significant rates over long periods in the absence of light. In phototrophic bacteria, it is presently not known how tightly nitrogen fixation is coupled to photosynthetic activity. There have been some reports (16, 18, 24, 26) of low nitrogenase activities in the dark, whereas little is known about the effect of light on nitrogenase synthesis (13). Clearly, this aspect of phototrophic metabolism requires further elucidation. Purple nonsulfur bacteria provide excellent material for such an investigation, since they are facultative anaerobes and phototrophs, i.e., they display facile interchange between light anaerobic and dark aerobic growth modes. Furthermore, an initial report (11) that nitrogen fixation in *Rhodospirillum rubrum* is somewhat oxygen resistant has been recently extended to include *Rhodopseudomonas palustris* (29) and *Rhodopseudomonas capsulata* (18, 20, 21). Therefore, these organisms might, in theory, be expected to drive the nitrogenase reaction via a dark aerobic metabolism. The present study used these somewhat flexible growth characteristics of the purple nonsulfur bacterium *R. capsulata* to examine how strictly both nitrogenase biosynthesis and nitrogenase activity are coupled to the photosynthetic mode of life.

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MATERIALS AND METHODS

Bacterial strain and culture. *R. capsulata* strain B10 was a generous gift from the Photosynthetic Bacteria Group, Department of Microbiology, Indiana University, Bloomington, Ind.

Cultures were grown at 30°C in a mineral salts medium (12, 28) supplemented with DL-lactate (30 mM) and L-glutamate (7 mM). Anaerobic cultures were grown in modified 50-ml syringes (12) illuminated by 100-W incandescent lamps (ca. 10,000 lx). Dark aerobic cultures (200 ml) were grown on a rotatory shaker (200 strokes per min) in 1-liter Erlenmeyer flasks covered with aluminium foil and stoppered with

sterile rubber septa. Known quantities of oxygen were injected into the flasks, which had been previously gassed with argon. The concentration of oxygen in the gas phase was determined at the beginning and at the end of the culture period by the same gas-chromatographic procedure as that used for hydrogen assay (17).

Resting-cell preparation. Resting cells were prepared as follows. Cultures were centrifuged (25,000 × *g*, 30 min), washed once with the mineral salts medium (12, 28) (devoid of carbon and nitrogen sources), recentrifuged as above, and resuspended in the same medium. The resting-cell suspensions were stored in the dark under argon at 0°C until required.

Nitrogenase induction in resting cells. Dark-grown resting-cell suspensions (20 to 30 ml) were added to 100-ml Erlenmeyer flasks, gassed with argon, and stoppered with rubber septa. The flasks were shaken at 30°C in a Warburg bath (B. Braun AG, Melsungen, West Germany) uniformly illuminated by 14 40-W incandescent lamps from below (ca. 5,000 lx.). Samples of 1 ml were withdrawn at intervals with a syringe and injected into 4-ml vials for assay of nitrogenase activity.

Nitrogenase assay. Assays were carried out in either 12-ml vials containing 2 ml of cell suspension or 4-ml vials containing 1 ml of the suspension. Reaction mixtures were supplemented with lactate (final concentration, 20 mM) and chloramphenicol (final concentration 20 μg/ml). Vials were fitted with rubber septa, thoroughly gassed with argon, and incubated on an illuminated Warburg water bath as described above. Cells from dark-grown cultures were incubated in the dark in the presence of chloramphenicol (20 μg/ml) for 10 min before illumination was started. After 5 min of equilibration, acetylene was injected into the gas phase (1 ml of acetylene in 12-ml flasks, 0.3 ml in 4-ml flasks). At intervals, aliquots (25 μl) were withdrawn from the gas phases of the vials and injected into a Hewlett Packard 5750 gas chromatograph equipped with a flame ionization detector; the 1.5-m stainless-steel column (diameter, 3.2 mm) was filled with Porapak R (80 to 100 mesh). The carrier gas was nitrogen (60 ml/min), and the oven temperature was 70°C. Under these conditions, the retention times of ethylene and acetylene were 80 and 90 s, respectively. Calibration with pure acetylene and ethylene showed that the amount of ethylene produced by the nitrogenase reaction could be calculated easily and reliably by using the equation: C_2H_4 produced = C_2H_2 added initially × [C_2H_4 peak height/(C_2H_2 peak height + C_2H_4 peak height)].

Cell mass determination. Bacterial growth was followed by measuring the optical density of the cell suspension at 660 nm. Dry weight determinations were carried out according to a previously described technique (12). Protein in whole cells was assayed as follows. A 0.1-ml amount of cholate (4%, wt/vol) and 0.8 ml of NaOH (10%, wt/vol) were added to 0.1 ml of cell suspension. The mixture was boiled for 15 min in a water bath, and the proteins were subsequently assayed with the Folin reagent (19). Numerous parallel determinations of the optical density at 660 nm, dry weight, and protein concentration, under different culture conditions, in different media, and during complete growth cycles, have shown that these three parameters (optical density at 660 nm: milligrams of dry weight per milliliter: milligrams of protein per milliliter) are consistently in the ratio of 10:5:2 within less than 10% error.

Other determinations. ATP (14), ADP, and AMP (15) were determined by enzymatic assays carried out on perchloric acid extracts subsequently neutralized with KOH. For bacteriochlorophyll determination, 1 or 2 ml of cell suspension was centrifuged for 7 min at 25,000 × *g*. The pellet was extracted with 2 ml of cold acetone-methanol (7:2, vol/vol), and the absorbance of the extract was measured at 775 nm. The concentration of bacteriochlorophyll was calculated, using an extinction coefficient of 75 mM^{-1} cm^{-1} (3).

RESULTS

Light dependence of nitrogenase activity. Maximum nitrogenase activity was observed under anaerobic conditions in the light (Fig. 1). Due to the high energy requirements of nitrogenase, dark nitrogenase activity would require an alternative energy source to light, namely, dark respiration. Under such conditions, the level of oxygen must be sufficient to permit significant energy supply while avoiding total inhibition or inactivation of nitrogenase. Under our experimental conditions, an oxygen concentration of 10% in the gas phase caused no greater than a 30 to 40% inhibition of nitrogenase activity (Fig. 1). Since this experiment was carried out in the presence of C_2H_2, we verified that dark aerobic metabolism was unaffected by acetylene: the latter compound did not inhibit dark aerobic growth or respiration of resting-cell suspensions. With respect to energy supply, it has been previously demonstrated (25) that dark respiration can sustain energy levels as high as does photosynthesis in *R. rubrum*. In similar fashion, we determined the adenine nucleotide content of cells (Table 1) incubated under the conditions shown in Fig. 1. It was evident that under an atmosphere containing 10% oxygen in the dark, the cells contained twice as much ATP as, and had a significantly higher energy charge (1) than, cells incubated anaerobically in the dark (Table 1). The aerobic conditions used here did not provide as high an energy charge as did illumination (Table 1), presumably due to oxygen limitation. However, it is apparent that the experimental conditions established in Fig. 1 satisfy the requirements discussed above in that (i) nitrogenase was only partially inhibited by oxygen and (ii) the oxygen tension was sufficient to sustain a high energy charge. Figure 1 clearly demonstrates that when illumination was suppressed, nitrogenase activity ceased completely within 3 to 4 min and only resumed some 10 min after reillumination (Fig. 1, curves 1 and 2). The provision of an energy supply via respiration

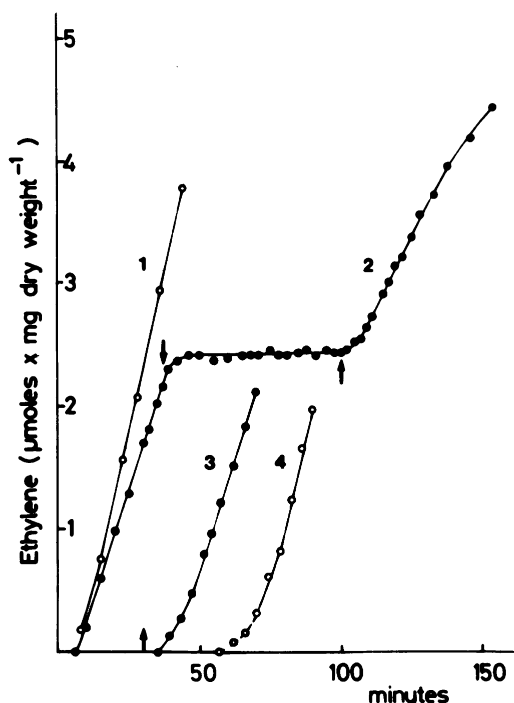

FIG. 1. *Effect of light and darkness on acetylene reduction by whole cells of R. capsulata. Resting cells from a culture grown anaerobically in the light were incubated in stoppered 12-ml flasks, gassed with argon, and shaken in a photosynthetic water bath (ca. 5,000 lx, 30°C). Reaction mixtures (2 ml) consisted of 2 mg of bacterial dry weight, 40 μg of chloramphenicol, and 20 μmol of lactate. The reaction was started by injecting 1 ml of acetylene. Two flasks (curves 1 and 2) were illuminated at time zero; flask 2 was darkened after 30 min and reilluminated 70 min later; two others (curves 3 and 4) were maintained in the dark, then illuminated after 30 min and then continually sampled.* (○) *Argon,* (●) *argon + 10% O_2. The arrows indicate light on* (↑) *and light off* (↓).

during the period of darkness was unable to replace light. Similarly, when cells were first maintained in the dark, no ethylene was produced until after the light had been switched on (Fig. 1, curves 3 and 4). It is of note that ethylene production commenced significantly earlier in the presence of oxygen than under anaerobic conditions after a dark incubation period. This presumably resulted from the lower energy charge generated by cells incubated anaerobically in the dark (Table 1). Such cells would require a longer exposure to light before the energy needs of the nitrogenase reaction could be met.

Nitrogenase biosynthesis in resting cells. Photosynthetic bacteria grown anaerobically in the light develop high nitrogenase activities (12, 20). We observed that *R. capsulata* did not grow in the dark with N_2 as the sole nitrogen source, even with as little as 1 or 2% oxygen in the gas phase. Cells grown under the same conditions with glutamate replacing N_2 as the nitrogen supply contained no nitrogenase activity, although care was taken in subsequent preparative procedures to exclude light, which would favor enzyme synthesis. The level of ammonia (assayed as described by Chaykin [2]) found in such cultures was similar to that found in light-grown cultures (less than 20 μM), which display high nitrogenase activity. Resting cells prepared from dark-grown cultures lacked nitrogenase activity and thus provided excellent material for a study of nitrogenase biosynthesis. We conducted analyses of extracts from cells (wild-type and Nif$^-$ strains), cultured under widely differing conditions, by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. These confirmed that the appearance of nitrogenase activity during illumination of dark-grown resting cells was concomitant with the synthesis of the nitrogenase protein components. Maxiumum nitrogenase biosynthesis occurred under anaerobic light conditions (Table 2). The inhibitory effect of chloramphenicol indicated that active protein synthesis was taking place. The suppression of illumination had a similar effect to that of chloramphenicol (Table 2), although it is noteworthy that some small synthesis of nitrogenase apparently took place under dark conditions. Indeed, this synthesis was stimulated slightly when 5% oxygen was added to the gas phase (Table 2). Higher oxygen levels (10 to 20%) suppressed dark nitrogenase synthesis (data not shown). This result is somewhat in contrast to the situation found in cultures growing in the dark in the presence of oxygen, where no nitrogenase synthesis was apparent.

Nitrogenase biosynthesis in dark-grown resting cells was extremely active upon exposure to light. Such cell preparations rapidly displayed activities similar to those present in photosynthetically grown cells. The increase in nitrogenase activity could be stopped at any stage by adding chloramphenicol, indicating a continuous synthesis of the enzyme (Fig. 2A). The suppression of illumination (Fig. 2B) had the same effect as the addition of chloramphenicol. Nitrogenase synthesis resumed rapidly upon illumination after a period of time in the dark (Fig. 3).

We further showed that not only were resting-cell suspensions, which are devoid of growth substrate, capable of synthesizing large amounts of nitrogenase during a few hours but that they could maintain this activity for a prolonged period of time, depending on the conditions of

TABLE 1. *Relative AMP, ADP, and ATP contents and energy charges (1) of R. capsulata resting cells under different metabolic conditions*[a]

Conditions	No. of flasks assayed	Energy charge		
		$\frac{ATP + 0.5\ ADP}{ATP + ADP + AMP}$	$\frac{ATP}{ATP + ADP + AMP}$	$\frac{ATP}{ADP}$
Light anaerobic	5	0.74 ± 0.03	0.60 ± 0.04	2.4 ± 0.4
Dark + 10% O_2	4	0.58 ± 0.03	0.40 ± 0.04	1.3 ± 0.2
Dark anaerobic	6	0.43 ± 0.02	0.20 ± 0.04	0.4 ± 0.1

[a] Resting cells from a culture grown anaerobically in the light were incubated in stoppered 12-ml flasks and shaken in a photosynthetic water bath (5,000 lx, 30°C). Reaction mixtures (2 ml) consisted of 7 mg of bacterial dry weight, 40 μg of chloramphenicol, and 20 μmol of lactate. Oxygen was added to the gas phase as indicated. After 20 min of incubation, 0.5 ml of 70% perchloric acid was added to each flask. Further treatment and nucleotide assays were carried out as described in the text.

TABLE 2. *Nitrogenase synthesis in dark-grown resting cells of R. capsulata*[a]

Conditions	% of nitrogenase activity	
	Expt 1	Expt 2
Light	100[b]	100[c]
Light + CAM	16.8	12.6
Dark	20.6	14.7
Dark + CAM		14.7
Dark + 5% O_2	29.7	20.7
Dark + 5% O_2 + CAM	12.1	10.2

[a] Resting cells (1 mg of bacterial dry weight per ml) were prepared from a culture grown in the dark under 5% oxygen. These cells contained no nitrogenase activity. Incubations were carried out under argon in 12-ml stoppered flasks containing 2 ml of resting cell suspension, oxygen, and chloramphenicol (CAM, 40 μg) as indicated in the table. Where required, flasks were covered with foil to exclude light. After a 3-h incubation in a photosynthetic bath (ca. 5,000 lx, 30°C), enzyme synthesis was terminated by adding CAM (40 μg) to the appropriate flasks. All flasks were regassed with argon, and 0.8 ml of acetylene was added to each flask for determination of nitrogenase activity in the light.

[b] 100% represents 57.9 nmol of C_2H_2 reduced per min per mg of dry wt.

[c] 100% represents 36.2 nmol of C_2H_2 reduced per min per mg of dry wt.

illumination (Fig. 3). Cell suspensions incubated under continuous light showed maximum nitrogenase synthesis after 20 h. Prolonged incubation under these conditions revealed a steady decline in the nitrogenase activity of these cells (Fig. 3). It is of interest that not only were cells incubated under an alternating light-dark pattern capable of synthesizing the same level of nitrogenase as under the continuous light treatment but also the nitrogenase synthesized under these conditions showed a higher activity over a longer period of time (Fig. 3). There was also an apparent stability of previously light-synthesized enzyme when cells were incubated for long periods in the dark. Such resting cells were able to resynthesize nitrogenase upon the introduction of light after a 48-h period of darkness (Fig. 3). We did not detect any decrease in cell viability under all experimental conditions throughout the duration of the incubations. Similarly, the ammonia concentration was found to remain constant, at very low levels (10 to 15 μM). These results indicate that cell lysis and ammonia release were of little importance in this experiment.

The rate of nitrogenase synthesis induced in resting cells upon illumination depends on their previous dark growth history. It has been shown that photosynthetic membranes and bacteriochlorophyll are synthesized in the dark under low oxygen concentrations by *Rhodopseudomonas sphaeroides* (5) and by *R. capsulata* (7). Our data revealed that light-dependent nitrogenase synthesis was faster in cells grown in the dark under lower oxygen partial pressure and thus possessing a higher bacteriochlorophyll content (Fig. 4). This was most likely due to their better ability to harvest light energy and to use it for their biosynthetic needs.

Nitrogenase and bacteriochlorophyll synthesis. Since nitrogenase activity and synthesis show such a dependency on light, it is tempting to speculate that nitrogenase biosynthesis is more or less tightly coupled to the biosynthesis of the photosynthetic apparatus. However, several lines of evidence indicate that this is not the case. Photosynthetic membranes and bacteriochlorophyll are actively synthesized in the dark under low oxygen tensions (5, 7), whereas nitrogenase is not (see above). In addition, Hillmer and Gest (13) have shown that, conversely to the bacteriochlorophyll content, the nitrogenase activity in photosynthetically grown cells increases when the light intensity during growth is increased. The experiment shown in Fig. 5 further demonstrates kinetically that the synthesis of nitrogenase and bacterio-

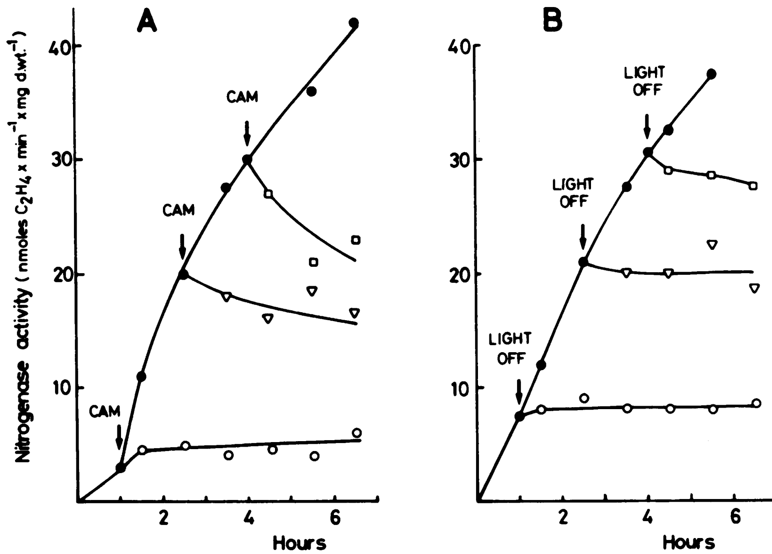

FIG. 2. *Effect of light interruption or chloramphenicol (CAM) addition on nitrogenase synthesis in resting cells of R. capsulata. Resting cells (1.1 mg of dry weight per ml) from a culture grown in the dark under an atmosphere containing 10% oxygen in argon were transferred into eight 100-ml conical flasks (20 ml of resting cell suspension per flask), gassed with argon, and shaken in a photosynthetic water bath (5,000 lx, 30°C). At intervals indicated in the figure, one flask was wrapped in aluminium foil (B) and chloramphenicol (20 µg/ml, final concentration) was added to another flask (A). Samples of 1 ml were withdrawn as indicated for nitrogenase assay.*

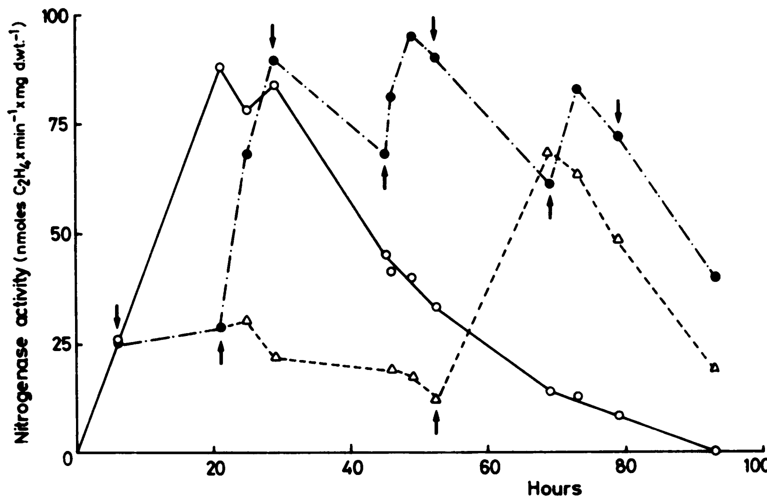

FIG. 3. *Synthesis and stability of nitrogenase in resting-cell suspensions of R. capsulata incubated under differing light regimes. Resting-cell suspensions (0.6 mg of bacterial dry weight per ml) were prepared from a culture grown in the dark under 5% oxygen. The suspension (30 ml) was placed in 100-ml stoppered conical flasks, gassed with argon, and shaken in a photosynthetic water bath (ca. 5,000 lx, 30°C). Samples were withdrawn at the times indicated in the figure, and nitrogenase activities were determined as described in the text. The lighting regimes used were alternating light and dark periods (●), continous light (○), and light (6 h) - dark (48 h) - light (△). The arrows indicate light on (↑) and light off (↓).*

chlorophyll are independent. Cells cultured in the dark under low oxygen tension possessed a high bacteriochlorophyll content but no nitrogenase activity. Upon exposure to high light intensity and anaerobic conditions, they grew rapidly and synthesized nitrogenase at a rapid

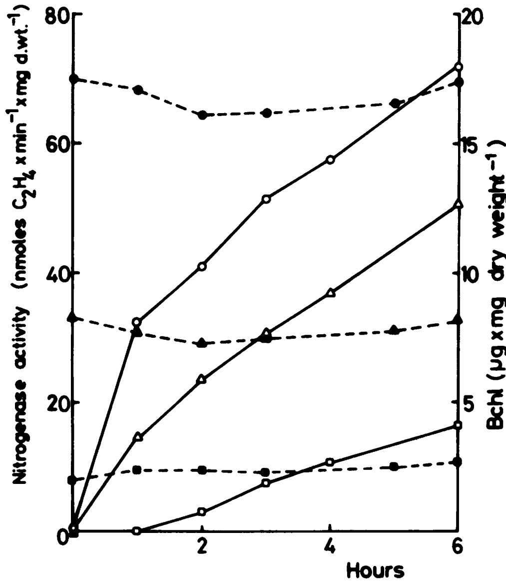

FIG. 4. *Light-dependent synthesis of nitrogenase in resting cell suspensions of R. capsulata prepared from cultures grown under different oxygen tensions in the dark. Resting cell suspensions (ca. 1 mg of bacterial dry weight per ml) were prepared from cultures grown in the dark under oxygen tensions of 2% (circles); 17% (triangles) and 31% (squares), respectively. The suspensions (20 ml) were placed in 100-ml stoppered conical flasks, gassed with argon, and shaken in a photosynthetic water bath (ca. 5,000 lx, 30° C). Samples were withdrawn at the times indicated in the figure, and nitrogenase activities (open symbols) and bacteriochlorophyll (Bchl) contents (closed symbols) were determined as described in the text.*

rate. However, very little bacteriochlorophyll synthesis took place under these conditions (Fig. 5). The doubling times for cell mass and nitrogenase and bacteriochlorophyll syntheses were 2.5, 1.0 and 8.5 h respectively (Fig. 5), indicating that nitrogenase synthesis was nearly 10 times faster than bacteriochlorophyll synthesis. In a converse experiment (not shown), we observed active bacteriochlorophyll synthesis occurring under conditions where no nitrogenase synthesis was taking place. It is thus apparent that bacteriochlorophyll and nitrogenase syntheses are independent of each other.

DISCUSSION

To date, there have been conflicting reports as to whether dark metabolism can sustain nitrogenase activity in purple nonsulfur bacteria. On the one hand, rates of $^{15}N_2$ fixation in the dark have been reported to range from 5% of the rates under illumination in *R. rubrum* (16) to the high value of 40% in *R. capsulata* (18). In addition, when N_2 consumption by *R. rubrum* was measured manometrically, the activity continued for some 20 min in the dark after a period of illumination (24). It has been recently reported (26) that several species of purple nonsulfur bacteria were able to grow on N_2 aerobically in the dark. In contrast, $^{15}N_2$ fixation in *R. rubrum* has been shown to stop abruptly after the onset of darkness (23). Our results (Fig. 1) are in agreement with the latter report. By using the acetylene reduction assay, we have shown that there is no nitrogenase activity in *R. capsulata* in the absence of light, even under conditions where oxygen is present to sustain oxidative phosphorylation and thus meet the energy requirement of the nitrogenase reaction. Since the basic requirements of this reaction are ATP and a low-potential reducing agent, we conclude that the generation of the reducing agent is light dependent. In purple nonsulfur bacteria, the reduction of low-potential electron carriers has been considered to occur via an energy-dependent reverse electron flow, energy being provided by cyclic photophosphorylation (10). Indeed, the high oxidation-reduction potentials (−50 to −150 mV) of the primary electron

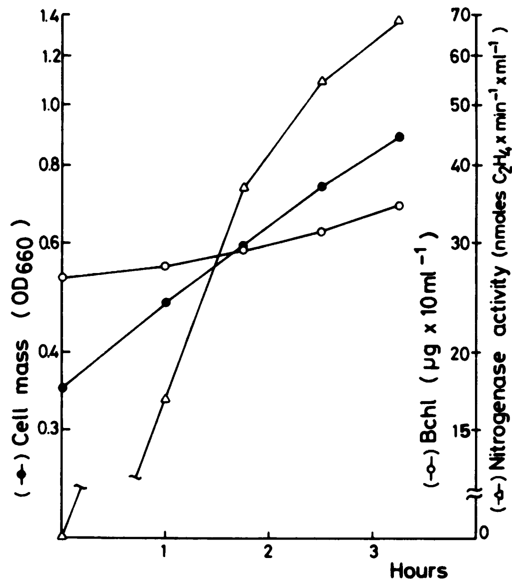

FIG. 5. *Synthesis of nitrogenase and bacteriochlorophyll (Bchl) in growing cells of R. capsulata. Cells growing in the dark under an atmosphere containing 3% oxygen in argon were transferred into a stoppered 100-ml conical flask, gassed with argon, and shaken in a photosynthetic water bath (ca. 10,000 lx, 30°C). Samples were withdrawn as indicated in the figure for turbidity (●), Bchl (○), and nitrogenase (△) measurements.*

acceptors measured in purple nonsulfur bacteria (22) would make it unlikely that the physiological reducing agent for nitrogenase ($E_0 \simeq -400$ mV) could be directly generated by light. However, recent kinetic studies (8) strongly suggest that bacteria of the genus *Rhodopseudomonas* may contain photoreducible species of much lower oxidation-reduction potentials (−500 to −600 mV). This would mean that electrons could be fed to nitrogenase directly by the photochemical reactions. The fact that nitrogenase activity is totally dependent on light, as we have demonstrated above, does favor such a mechanism rather than an energy-dependent reverse electron flow which would be dependent on either light or dark metabolism.

It is of interest that nitrogenase activity has been found in *Rhodopseudomonas acidophila* under both light and dark aerobic conditions (26). This is quite contradictory with our results, and to date we can offer no better explanation than a species difference.

One of the most interesting properties of nitrogenase biosynthesis in *R. capsulata* is the extremely active synthesis of this enzyme observed in dark-grown resting cells once they are exposed to light. In the absence of exogenous nitrogen and carbon sources, nitrogenase synthesis proceeds at a rate such that within 3 to 4 h, the specific activity of this enzyme is equivalent to that found in light-grown cultures. Such a rapid synthesis would be expected to be of little benefit to the cell. It appears that in the absence of factors which repress nitrogenase synthesis, e.g., ammonia, darkness, and high oxygen levels, uncontrolled synthesis of the enzyme takes place. This finding agrees with previous results demonstrating that in *Clostridium pasteurianum* nitrogenase synthesis is regulated by ammonia, but not by N_2, the physiological substrate of nitrogenase (6). This explanation may possibly be extended to include the apparent discrepancy that we have observed for nitrogenase synthesis under dark conditions. Growing cultures, supplied with their necessary carbon and nitrogen sources, synthesize nitrogenase only under illumination. Due to the extreme energy demands for growth, synthesis of the high-energy-requiring nitrogenase enzyme is probably under strict control, particularly under growth in the dark, where oxygen levels may be limiting. In resting-cell suspensions, however, the absence of repressor agents may allow the expression of an energy-dependent synthesis of nitrogenase in the dark. The provision of such an energy supply via bacterial respiration may explain the small stimulation of dark nitrogenase synthesis in resting cells, as observed in the present study (Table 2). Thus, it is highly probable that nitrogenase biosynthesis in resting cells is not subject to the stricter control measures present under conditions of growth.

We have found that resting cells of *R. capsulata* retain the ability to synthesize nitrogenase over long time periods under continuous or discontinuous illumination. It is of special interest that the activity is consistently higher under intermittent illumination. Indeed, this situation may more closely resemble the natural growth situation, namely, growth under a daily light cycle and most probably under low levels of substrate. This property could prove useful in future attempts to use purple nonsulfur bacteria as producers of hydrogen (21) or ammonia (27). According to the results shown in Fig. 3, such systems would be viable under discontinuous illumination. It appears that nitrogenase is synthesized and is stable whether substrate is present (13) or absent (Fig. 3). Hence, the provision of substrate at irregular intervals should still allow expression of nitrogenase activity. Although still in the stage of basic research, such systems can already be foreseen to enjoy a great versatility (21).

Although nitrogenase activity as well as biosynthesis are closely linked to photosynthetic activity in *R. capsulata*, we have confirmed that biosyntheses of bacteriochlorophyll and nitrogenase are independent of each other. This may be more readily understood from a physiological viewpoint. Bacteriochlorophyll synthesis in the dark, although energy consuming, is advantageous in allowing rapid transition to the photosynthetic mode of growth during the natural diurnal cycle: indeed, it has been shown that when cells devoid of bacteriochlorophyll are illuminated, they show lags of several hours before they can start photosynthetic growth (5). On the other hand, when cells already containing bacteriochlorophyll are illuminated, they very actively synthesize nitrogenase (Fig. 2 through 4) and can thus use N_2 as a nitrogen supply within 15 to 30 min. As to the effect of light intensity, it has long been known that the increased synthesis of bacteriochlorophyll at low light intensities represents an adaptation to the lowering of the incident energy flux (4). Conversely, nitrogenase biosynthesis increases with an augmentation of incident light intensity, thus following a general increase in metabolic activity and growth rate (13).

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LITERATURE CITED

1. **Atkinson, D. E.** 1968. The energy charge of the adenylate pool as a regulatory parameter. Interaction with feedback modifiers. Biochemistry 7:4030–4034.
2. **Chaykin, S.** 1969. Assay of nicotinamide deaminase. Determination of ammonia by the indophenol reaction. Anal. Biochem. 31:375–382.
3. **Clayton, R. K.** 1963. Toward the isolation of a photochemical reaction center in *Rhodopseudomonas spheroides*. Biochim. Biophys. Acta 75:312–323.
4. **Cohen-Bazire, G., and R. Kunisawa.** 1960. Some observations on the synthesis and function of the photosynthetic apparatus in *Rhodospirillum rubrum*. Proc. Natl. Acad. Sci. U.S.A. 46:1543–1553.
5. **Cohen-Bazire, G., W. R. Siström, and R. Y. Stanier.** 1957. Kinetic studies of pigment synthesis by non-sulfur purple bacteria. J. Cell. Comp. Physiol. 49:25–68.
6. **Daesch, G., and L. E. Mortenson.** 1972. Effect of ammonia on the synthesis and function of the N_2-fixing system in *Clostridium pasteurianum*. J. Bacteriol. 110:103–109.
7. **Drews, G., H. H. Lampe, and R. Ladwig.** 1969. Die Entwicklung des Photosyntheseapparates in Dunkelkulturen von *Rhodopseudomonas capsulata*. Arch. Mikrobiol. 65:12–28.
8. **Dutton, P. L., R. C. Prince, D. M. Tiede, K. M. Petty, K. J. Kaufmann, T. L. Netzel, and P. M. Rentzepis.** 1977. Electron transfer in the photosynthetic reaction center. Brookhaven Symp. Biol. 28:213–237.
9. **Fay, P.** 1976. Factors influencing dark nitrogen fixation in a blue-green alga. Appl. Environ. Microbiol. 31: 376–379.
10. **Gest, H.** 1972. Energy conversion and generation of reducing power in bacterial photosynthesis. Adv. Microb. Physiol. 7:243–282.
11. **Gest, H., M. D. Kamen, and H. M. Bregoff.** 1950. Studies on the metabolism of photosynthetic bacteria. V. Photoproduction of hydrogen and nitrogen fixation by *Rhodospirillum rubrum*. J. Biol. Chem. 182: 153–170.
12. **Hillmer, P., and H. Gest.** 1977. H_2 metabolism in the photosynthetic bacterium *Rhodopseudomonas capsulata*: H_2 production by growing cultures. J. Bacteriol. 129:724–731.
13. **Hillmer, P., and H. Gest.** 1977. H_2 metabolism in the photosynthetic bacterium *Rhodopseudomonas capsulata*: production and utilization of H_2 by resting cells. J. Bacteriol. 129:732–739.
14. **Jaworek, D., W. Gruber, and H. U. Bergmeyer.** 1974. Adenosine-5′-triphosphate determination with 3-phosphoglycerate kinase, p. 2097–2101. *In* H. U. Bergmeyer (ed.), Methods of enzymatic analysis, 2nd ed., vol. 4. Academic Press, Inc., New York.
15. **Jaworek, D., W. Gruber, and H. U. Bergmeyer.** 1974. Adenosine-5′-diphosphate and adenosine-5′-monophosphate, p. 2127–2131. *In* H. U. Bergmeyer (ed.), Methods of enzymatic analysis, 2nd ed., vol. 4. Academic Press, Inc., New York.
16. **Kamen, M. D., and H. Gest.** 1949. Evidence for a nitrogenase system in the photosynthetic bacterium *Rhodospirillum rubrum*. Science 109:560.
17. **Kelley, B. C., C. M. Meyer, C. Gandy, and P. M. Vignais.** 1977. Hydrogen recycling by *Rhodopseudomonas capsulata*. FEBS Lett. 81:281–285.
18. **Kobayashi, M., and M. Z. Haque.** 1971. Contribution to nitrogen fixation and soil fertility by photosynthetic bacteria. Plant Soil Special volume:443–456.
19. **Lowry, D. 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.
20. **Meyer, J., B. C. Kelley, and P. M. Vignais.** 1978. Aerobic nitrogen fixation by *Rhodopseudomonas capsulata*. FEBS Lett. 85:224–228.
21. **Meyer, J., B. C. Kelley, and P. M. Vignais.** 1978. Nitrogen fixation and hydrogen metabolism in photosynthetic bacteria. Biochimie 60:245–260.
22. **Parson, W. W., and R. J. Cogdell.** 1975. The primary photochemical reaction of bacterial photosynthesis. Biochim. Biophys. Acta 416:105–149.
23. **Pratt, D. C., and A. W. Frenkel.** 1959. Studies on nitrogen fixation and photosynthesis of *Rhodospirillum rubrum*. Plant Physiol. 34:333–337.
24. **Schick, H. J.** 1971. Substrate and light dependent fixation of molecular nitrogen in *Rhodospirillum rubrum*. Arch. Mikrobiol. 75:89–101.
25. **Schön, G.** 1969. Der Einfluss der Kulturbedingungen auf den ATP-, ADP, un AMP-Spiegel bei *Rhodospirillum rubrum*. Arch. Mikrobiol. 66:348–364.
26. **Siefert, E.** 1976. Nitrogen fixation in facultative aerobic *Rhodospirillaceae* with photosynthetic or respiratory energy generation. p. 149–151. *In* G. A. Codd and W. D. P. Stewart (ed.), Second International Symposium on Photosynthetic Procaryotes, Dundee.
27. **Weare, N. M., and K. T. Shanmugam.** 1976. Photoproduction of ammonium ion from N_2 in *Rhodospirillum rubrum*. Arch. Microbiol. 110:207–213.
28. **Weaver, P. F., J. D. Wall, and H. Gest.** 1975. Characterization of *Rhodopseudomonas capsulata*. Arch. Miciobiol. 105:207–216.
29. **Zumft, W. F., and F. Castillo.** 1978. Regulatory properties of the nitrogenase from *Rhodopseudomonas palustris*. Arch. Microbiol. 117:53–60.