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H_2 Metabolism in the Photosynthetic Bacterium *Rhodopseudomonas capsulata:* Production and Utilization of H_2 by Resting Cells

PETER HILLMER AND HOWARD GEST*

Photosynthetic Bacteria Group, Department of Microbiology, Indiana University, Bloomington, Indiana 47401

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Photoproduction of H_2 and activation of H_2 for CO_2 reduction (photoreduction) by *Rhodopseudomonas capsulata* are catalyzed by different enzyme systems. Formation of H_2 from organic compounds is mediated by nitrogenase and is not inhibited by an atmosphere of 99% H_2. Cells grown photoheterotrophically on C_4 dicarboxylic acids (with glutamate as N source) evolve H_2 from the C_4 acids and also from lactate and pyruvate; cells grown on C_3 carbon sources, however, are inactive with the C_4 acids, presumably because they lack inducible transport systems. Ammonia is known to inhibit N_2 fixation by photosynthetic bacteria, and it also effectively prevents photoproduction of H_2; these effects are due to inhibition and, in part, inactivation of nitrogenase. Biosynthesis of the latter, as measured by both H_2 production and acetylene reduction assays, is markedly increased when cells are grown at high light intensity; synthesis of the photoreduction system, on the other hand, is not appreciably influenced by light intensity during photoheterotrophic growth. The photoreduction activity of cells grown on lactate + glutamate (which contain active nitrogenase) is greatly activated by NH_4^+, but this effect is not observed in cells grown with NH_4^+ as N source (nitrogenase repressed) or in a Nif^- mutant that is unable to produce H_2. Lactate, malate, and succinate, which are readily used as growth substrates by *R. capsulata* and are excellent H donors for photoproduction of H_2, abolish photoreduction activity. The physiological significances of this phenomenon and of the reciprocal regulatory effects of NH_4^+ on H_2 production and photoreduction are discussed.

Numerous procaryotes can utilize and produce H_2 through the action of hydrogenases (4, 9, 14), which have been often likened (e.g., see [10, 18]) to reversible hydrogen electrodes in which a metal such as platinum serves as the catalyst. The hydrogenases of certain kinds of bacteria may in fact act essentially in this fashion in vivo. For example, the oxidation-reduction aspects of carbohydrate fermentations by clostridia can be rationalized by assuming that one function of clostridial hydrogenase is to aid, through reversible activity, in regulating the final balancing of electron flow transactions (9). It has become evident, however, that in various other organisms different enzyme systems may be responsible for H_2 oxidation and formation. This appears to be so for nonsulfur purple photosynthetic bacteria. Many such organisms display the two capacities under discussion, in that they can use H_2 as a reductant for photoautotrophic growth and also can produce H_2 as a major product during photoheterotrophic growth under certain circumstances. *Rhodopseudomonas capsulata* typifies bacteria of this kind; in this organism, the in vivo photoproduction of H_2 is catalyzed by nitrogenase, whereas the utilization of H_2 as a biosynthetic reductant is effected by another kind of (classical) hydrogenase (25).

The regulatory systems that govern the direction of H_2 metabolism are of great interest from a physiological standpoint and, in this paper, we report relevant studies with *R. capsulata*. Our findings and others in the literature indicate that *R. capsulata* and related bacteria employ controls which ensure that when readily utilizable organic H donors are supplied, the system that catalyzes light-dependent reduction of CO_2 with H_2 (photoreduction) becomes inoperative. Another major regulatory device centers on the effects of NH_4^+. Thus, NH_4^+ acts as a reciprocal regulator in that it is a potent inhibitor of H_2 production from organic compounds (and of N_2 fixation) and, by acting in

this way, permits photoreduction of CO_2 with H_2 for biosynthesis with NH_4^+ as the N source.

MATERIALS AND METHODS

Bacterial strains. Except for mutant W15, the strains of *R. capsulata* used are described in the preceding paper (11). Strains Z-1, J2, and L_1 have wild-type characteristics in respect to production and utilization of H_2; W15 is a Nif^- mutant incapable of light-dependent production of H_2 (25).

Growth media. Unless otherwise noted, cultures were grown in the standard medium described in (11), in which the C and N sources are 30 mM DL-lactate and 7 mM L-glutamate.

Growth of cells. All cultures were grown photosynthetically (anaerobically). Precultures, to provide inocula for experimental cultures, were ordinarily cultivated as specified by Hillmer and Gest (11). Cells used for manometric and other experiments were usually grown in completely full prescription bottles (190-ml capacity); the bottles were closed with solid rubber stoppers, each fitted with a narrow-gauge hypodermic needle to permit gas escape. The bottles were incubated in a glass-sided water bath (35°C) and illuminated by a bank of Lumiline incandescent lamps; light intensity, ca. 1,000 ft-c (10,800 lux).

Preparation of resting-cell suspensions. Cultures were centrifuged, and the cell pellets were resuspended in a solution (mineral base) having the same composition as the growth medium, except that lactate and glutamate were omitted and 30 mM K phosphate buffer (pH 6.9) was added. Before use for resuspension of cells, mineral base solutions were gassed with argon for 10 min, and the cell suspensions were kept under anaerobic conditions until delivery into the Warburg vessels.

Manometric methods. In all experiments, each Warburg vessel contained cells in a final fluid volume of 2.5 ml; substrates (in small volumes) were tipped in from side arms in the usual fashion. Unless otherwise specified, the center well contained 0.5 ml of diethanolamine + CO_2 "buffer" (prepared as described in reference 13) and a folded filter paper. The diethanolamine buffer maintains a constant CO_2 tension in the system, and the pressure changes observed in the present experiments are due to production or utilization of molecular hydrogen. During addition of cell suspensions, etc., to the vessels, the latter were continuously gassed with argon, i.e., until connection with the manometers. The vessels were shaken at 35°C in a Warburg bath (GME-Lardy Warburg Apparatus model RWBP-3) manufactured by Gilson Medical Electronics, Middleton, Wis. In this bath, each vessel can be illuminated from below by a 30-W reflector flood lamp that moves in synchrony with the vessel; light intensity, ca. 1,300 ft-c (14,000 lux).

(i) **Photoproduction of H_2.** The vessels were gassed with 1% CO_2 in helium for 10 min and, after 10 min of further equilibration, substrate was tipped in and illumination was begun (dark controls and vessels without substrates were included in the trials).

(ii) **Light-dependent reduction of CO_2 with H_2.** (This is hereafter referred to as photoreduction.) For such experiments, the gas phase was 1% CO_2 in H_2. After gassing and further equilibration, illumination was begun. Ordinarily, some gas production is observed for about 30 min and, after this had ceased, substrates (ammonium sulfate, etc.) were tipped into the main compartment of the vessel. Controls were as in the H_2 photoproduction assays. Since the diethanolamine buffer maintains a constant CO_2 tension, photoreduction activity is measured in terms of H_2 utilization. Control experiments verified that, in the absence of CO_2, H_2 is not consumed by *R. capsulata* suspensions.

Acetylene reduction assay (nitrogenase activity). Small glass jars (35-ml capacity) fitted with serum bottle caps were flushed with argon for 10 min, and a portion of a resting-cell suspension supplemented with 90 μmol of DL-lactate (3-ml volume) was then injected into each vessel. After temperature equilibration (35°C) for 5 min, in the Warburg bath, 3 ml of acetylene (from a Matheson Co. gas cylinder; purity, 99.5%) was injected into the gas phase. Following 5 min of further equilibration, illumination (ca. 1,000 ft-c [10,800 lux]) was started. The jars were shaken in the same fashion as the Warburg vessels, and, at suitable times, 0.5-ml samples of gas phase were removed with gas-tight syringes. The samples were analyzed for ethylene by gas chromatography (room temperature) using a Hewlett-Packard model 402 analyzer; the 2.7-m glass column was filled with Porapack N. Appropriate controls (kept dark, or minus lactate) were included.

Other determinations. Bacterial and ammonia concentrations were measured as described in the preceding paper (11). Bacteriochlorophyll (BChl) concentrations in cells were determined by extraction of the pigment with cold acetone-methanol (7:2, vol/vol); in each instance, 0.5 g (wet weight) of cells was extracted twice, each time with 2 ml of solvent, and absorbancy at 775 nm of the combined extracts was measured in a spectrophotometer (Zeiss PMQ2); the concentration of BChl was calculated using an extinction coefficient of 75 mM^{-1} cm^{-1} (2).

RESULTS

H_2 production from C_3 and C_4 organic substrates. Non-nitrogenous compounds known to be readily metabolized by growing cells of *R. capsulata* (11) were tested as substrates for photoproduction of H_2 by resting suspensions. For these experiments, cells were grown with the individual substrates listed in Table 1 plus L-glutamate (7 mM), and their capacities for producing H_2 from C_3 and C_4 substrates were measured. It can be seen that cells grown on C_4 dicarboxylic acids evolved H_2 from these compounds, and from lactate and pyruvate. In contrast, bacteria grown on lactate or pyruvate produce H_2 from these substrates, but not from the C_4 acids. Similar results were observed in experiments with the L_1 strain grown on glyc-

TABLE 1. *Photoproduction of H_2 from organic acids by resting cells of R. capsulata*[a]

Cells grown on:	Rate of H_2 production (μl/h per mg [dry wt] of cells) from:				
	Lactate	Pyruvate	Malate	Fumarate	Succinate
Lactate	75	61	0	0	0
Pyruvate	22	41	0	0	0
Glycerol	72	72	0	0	0
Malate	113	83	58	72	87
Fumarate	95	120	10	25	38
Succinate	72	61	40	46	71

[a] *R. capsulata* Z-1 was used, except for the experiments with glycerol; in the latter instance, strain L_1 was employed. Precultures were grown for at least 10 generations on the organic compound specified (30 mM DL-lactate, pyruvate, DL-malate, fumarate, succinate, or 56 mM glycerol) with 7 mM L-glutamate as N source; and these were employed for inoculation of experimental cultures using corresponding media. The Warburg vessels contained 2.5 to 3.0 mg of harvested cells (dry weight) in 2.5 ml of mineral base, and 90 μmol of substrate were tipped in at zero time.

erol + glutamate; that is, such cells produce H_2 from lactate and pyruvate (also from glycerol), but not with the C_4 acids as substrates. This pattern suggests that utilization of C_4 dicarboxylic acids by *R. capsulata* is dependent on inducible transport systems. Gibson (8) has described evidence for separate uptake systems for pyruvate and C_4 dicarboxylic acids in the related photosynthetic bacterium *R. spheroides*, and specific inducible transport systems for the C_4 acids have been observed in various other bacteria (see [1]).

H_2 production under an atmosphere of hydrogen. Resting cells of *Rhodospirillum rubrum* exhibit active photoproduction of H_2 under an atmosphere of 100% H_2 (5, 6). Similarly, it was found that cells of *R. capsulata* produce H_2 from lactate at the same rates under atmospheres of 1% CO_2 + 99% H_2 and 1% CO_2 + 99% He (Fig. 1). This is an indication that the *R. capsulata* nitrogenase, which catalyzes H_2 formation, cannot function as a "biosynthetic hydrogenase" (i.e., for generating reducing power from H_2 for reduction of CO_2, etc.).

Significant rates of endogenous production of H_2 have been noted in experiments with various purple bacteria (7, 16, 22), but the rates observed with *R. capsulata*, grown as specified, are quite low (Fig. 1). Early studies (22) with *R. rubrum* and *Rps. gelatinosa* showed that continuous removal of metabolic CO_2, by absorption with alkali in the center well of the Warburg vessel, frequently caused significant (sometimes great) reduction in rates (and yields) of H_2 formation. This was also found with *R. capsulata* cells under atmospheres of 100% H_2 or He; using the diethanolamine + CO_2 buffer method, however, characteristic rates of H_2 evolution are reproducibly observed.

Effect of ammonium salts on H_2 production. As in other nitrogen fixation systems (e.g., see [3]), excess ammonia represses nitrogenase synthesis in photosynthetic bacteria (6, 20). This repression is directly reflected in the inability of ammonia-grown cells to photoproduce H_2 (16, 17). In *R. rubrum* grown so as to contain active nitrogenase, addition of NH_4^+ causes a rapid inhibition of both N_2 fixation and photoproduction of H_2 from organic compounds (6, 19); similar observations have been reported for H_2 formation by *R. gelatinosa* (22). The effect of NH_4^+ on production of H_2 by resting cells of *R. capsulata*, derived from lactate + glutamate medium, is shown in Fig. 2. Formation of H_2 was immediately abolished by 0.5 mM and higher concentratons of NH_4^+; in other experiments, inhibition by as little as 0.1 mM NH_4^+ could be detected. Hydrogen evolution resumes after the NH_4^+ has been consumed, and the length of the lag is proportional to the quantity of NH_4^+ added. These results corre-

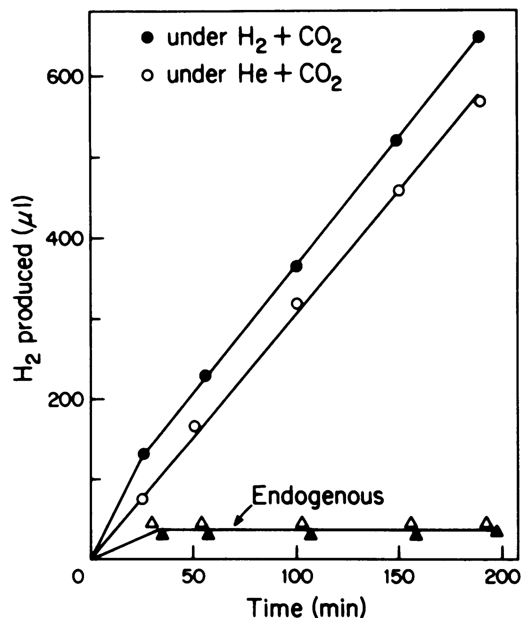

FIG. 1. *Photoproduction of H_2 by resting cells of R. capsulata J2; a 99% H_2 atmosphere does not inhibit. The cells were grown in the standard lactate + glutamate medium; cell quantity, 2.5 mg (dry weight); substrate, 90 μmol of DL-lactate; gas phase, 1% CO_2 in He or H_2, as indicated. Endogenous rates: (▲) under H_2 + CO_2, (△) under He + CO_2.*

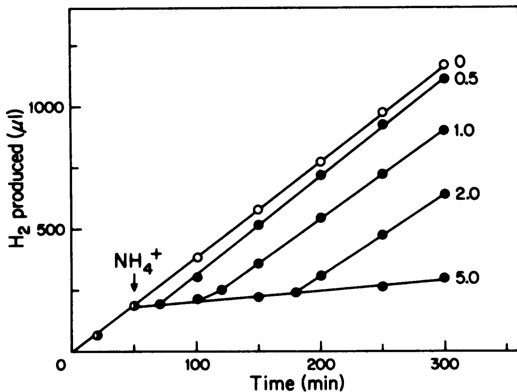

FIG. 2. *Inhibition of photoproduction of H_2 by NH_4^+. Experimental details as in Fig. 1. The vessels were gassed with 1% CO_2 in He, and ammonium sulfate was added after 50 min of illumination in the presence of lactate; the concentrations of NH_4^+ (millimolar) are indicated (0, no NH_4^+ added).*

spond closely with the observations of Schick (19) on inhibition of N_2 fixation by NH_4^+ in *R. rubrum*.

Metabolic capacities of cells grown at different light intensities. Cells were grown at various light intensities to the stationary phase and harvested, and their capacities were measured in respect to photoproduction of H_2 (from lactate), acetylene reduction, and photoreduction of CO_2 (with H_2). The results depicted in Fig. 3 show that increase of light intensity during growth specifically leads to increased abilities to produce H_2 and reduce acetylene; these activities changed in parallel fashion, as expected, and were saturated at ca. 1,000 ft-c (10,800 lux). On the other hand, photoreduction activity remained essentially constant. These results reinforce others which clearly indicate that production and utilization of H_2 are catalyzed by different systems. In this connection, it is of interest that a more limited early study (23) of the effects of light intensity during growth on H_2 metabolism in *R. rubrum* showed grossly similar results; thus, the development pattern of hydrogenase activity with ferricyanide as electron acceptor was distinctly different from that of H_2 producton activity (see reference 17 for similar observations). The striking effect of light intensity during growth on activity of the nitrogenase-hydrogenase system in *R. capsulata* is evidently independent of total BChl synthesis (note that, as light intensity diminishes, the increase of BChl content is largely due to increased synthesis of light-harvesting pigment).

Figure 4 shows the results of an abrupt increase in light intensity on development of H_2 production capacity. Two comparable experiments were performed with cells at different stages of growth, and we first consider log-phase cells. Duplicate cultures (30 mM lactate + 10 mM glutamate medium) were grown at ca. 225-ft-c (2,500-lux) light intensity until mid-log phase; at zero time, the light intensity was increased to 1,300 ft-c (ca. 14,000 lux) and chloramphenicol (CAM; 10 μg/ml final concentration) was added to one culture (●). Incubation of both cultures was continued (at high light intensity) and, at intervals, samples of cells were removed for determination of H_2 production activity (by suspended resting cells). In the absence of CAM, the increase of light intensity led to a rapid and substantial increase in activity, which remained at an elevated level for many hours. Addition of CAM, on the other hand, resulted in a rapid and complete loss of H_2 production activity. In the comparable experiment with cells grown to the stationary phase (in 30 mM lactate + 4 mM glutamate medium) before light intensity increase, the effect of the latter was much less pronounced, and the inactivating effect of CAM addition, though evident, was also diminished. The results of other experiments indicate that the effect of CAM is probably due to inactivation of nitrogenase by NH_4^+ that accumulates from glutamate deamination when protein synthesis is inhibited by the antibiotic; similar inactivations attributable to NH_4^+ have been reported in other orga-

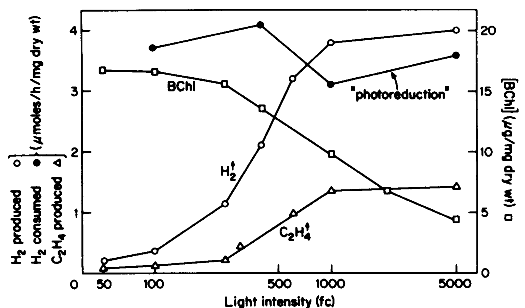

FIG. 3. *Effects of light intensity during growth on capacities for photoproduction of H_2, acetylene reduction, and photoreduction of CO_2. Cells of R. capsulata J2 were grown in standard lactate + glutamate medium at the light intensities indicated, and harvested in the stationary growth phase (at an absorbancy [at 660 nm] of ca. 3.5). H_2 production assays: 3 to 7 mg (dry weight) of cells; 90 μmol of DL-lactate; gas phase, 1% CO_2 in He. Acetylene reduction assays: 1.7 to 4.7 mg (dry weight) of cells; rates of ethylene production are given. Photoreduction assays: 3.7 to 4.6 mg (dry weight) of cells, supplemented with 14 mM NH_4^+; gas phase, 1% CO_2 in H_2. Note the expected effect of light intensity during growth on BChl content of the cells (□).*

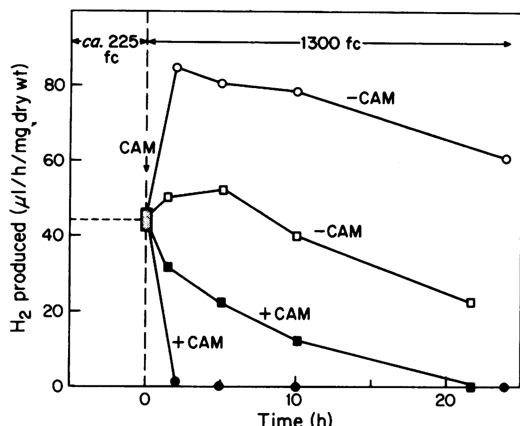

FIG. 4. *Effects of increase in light intensity and of CAM addition on H_2 production capacity in growing cultures of R. capsulata. Two pairs of cultures of R. capsulata J2 were grown (at 35°C) photosynthetically in 30 mM DL-lactate medium, one set with 10 mM L-glutamate as N source, and the other with 4 mM glutamate, at a light intensity of ca. 225 ft-c (2,500 lux). When the 10 mM glutamate cultures (○, ●) were in mid-log phase, the light intensity was suddenly increased to 1,300 ft-c (ca. 14,000 lux) and, at the same time, CAM (10 µg/ml) was added to one of the pair; the 4 mM glutamate cultures (□, ■) were similarly treated when they reached stationary phase. Before and after the treatments indicated, samples of the cultures were periodically removed, and the cells were harvested for determination of their H_2 production capacities with lactate as substrate (during sample removal, the cultures were gassed with 5% CO_2 in argon to maintain anaerobiosis).*

nisms (15, 24). Formation of free ammonia subsequent to CAM addition in the experiment of Fig. 4 (log-phase cells) occurred with the kinetics shown in Fig. 5. In the experiment with stationary-phase cells (Fig. 4), CAM was added after glutamate had been exhausted; accordingly, in this instance, it is presumed that NH_4^+ was generated in lesser quantity from endogenous amino acids, etc.

If the light intensity effects observed in Fig. 4 (without CAM) were due to some kind of direct activation, similar results would be expected with both log- and stationary-phase cells. Since the effect of light intensity was much smaller with stationary-phase cells, it appears that illumination affects the system through its influence on protein synthesis.

Utilization of H_2 for CO_2 reduction (photoreduction). Representative strains of all types of photosynthetic bacteria can catalyze light-dependent reduction of CO_2 with H_2 (16), and most isolates of *R. capsulata* can grow photoautotrophically on CO_2 + H_2 with ammonium salts as the N source (26). Under an atmosphere of CO_2 + H_2, illuminated resting cells of *R. capsulata* (derived from lactate + glutamate medium) display a phase of endogenous production of H_2 which is followed by a low rate of photoreduction activity (Fig. 6). As in the experiments of Schick (21) with *R. rubrum*, a high photoreduction rate becomes evident after addition of NH_4^+. The accelerated rate of H_2 consumption, induced by NH_4^+ supplementation, was the same for all ammonia concentrations tested (0.3 to 2.4 mM), and the duration of the high rate was dependent on the quantity of NH_4^+ added (Fig. 6). The rapid photoreduction rate abruptly decreases to a secondary rate, which is influenced by the quantity of NH_4^+ initially added; presumably, the transition occurs upon exhaustion of the NH_4^+. Progressively higher secondary rates are observed with increasing NH_4^+ concentrations, and it seemed possible that this effect is somehow related to the inhibitory and inactivating effects of NH_4^+ on nitrogenase-hydrogenase.

Under certain conditions, *R. capsulata* produces NH_4^+ by deamination of amino acids (11), and it would be expected that certain amino acids can substitute for ammonia as stimulators of photoreduction activity. This was found to be the case with glutamine, glutamate, aspartate, and alanine, as illustrated in Fig. 7. In the experiment shown, a series of identical Warburg vessels was used, each containing 6 µmol of L-glutamine in a side arm. At zero

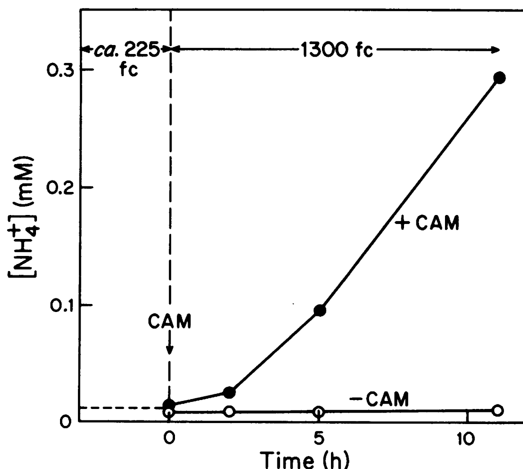

FIG. 5. *NH_4^+ production in cultures of R. capsulata after simultaneous increase in light intensity and addition of CAM. The supernatant fluids from the cell samples collected in the experiment of Fig. 4 (10 mM glutamate cultures) were assayed for NH_4^+ concentration using an ammonia electrode.*

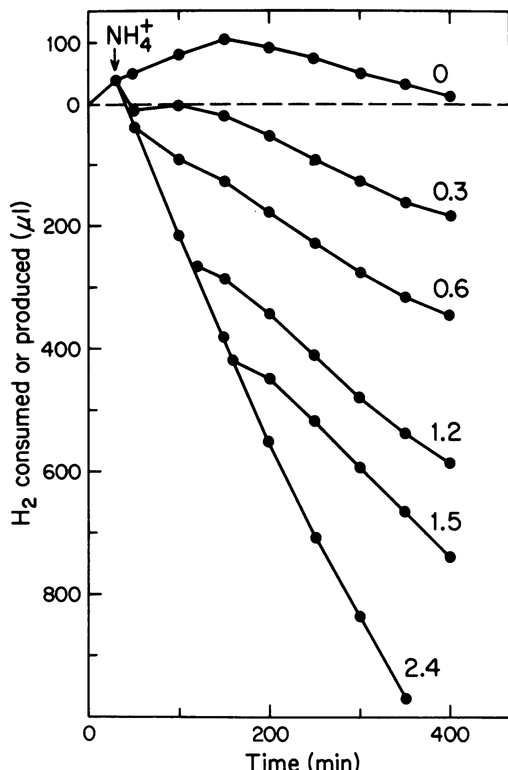

FIG. 6. *Stimulation of photoreduction activity of R. capsulata by ammonium ions. R. capsulata J2 was grown in standard lactate + glutamate medium, and photoreduction activity was assayed in the usual manner (gas phase, 1% CO_2 in H_2). Thirty minutes after illumination was begun, ammonium sulfate was added to give the initial NH_4^+ concentrations indicated (millimolar); in each instance, the cell quantity was 3 mg (dry weight). Note that in the control* (O) *a low rate of photoreduction activity (scale below ordinate zero) was observed after a phase of endogenous H_2 production (scale above ordinate zero).*

time, illumination was begun and, after 30 min, the glutamine was added to the cells. Hydrogen gas exchange was followed and, at intervals, vessels were sacrificed for determination of NH_4^+ concentrations in the suspensions. From the composite results, it can be seen that H_2 consumption began immediately after glutamine addition, and accelerated at a rate coordinate with the formation of ammonia. The highest photoreduction rate coincided with the peak of NH_4^+ formation and, after exhaustion of the ammonia, the rate of H_2 utilization shifted to a slower secondary rate as in the experiments of Fig. 6. It is evident that concentrations of NH_4^+ less than 0.1 mM affect both endogenous photoproduction of H_2 and photoreduction.

Photoreduction in a Nif⁻ mutant incapable of producing H_2. The relationships between photoproduction of H_2 and photoreduction activity can be seen more clearly from a comparison of activities of wild-type cells (strain J2) and a Nif⁻ mutant (W15) devoid of nitrogenase-hydrogenase activity; relevant data are summarized in Table 2. Both types of cells were grown with lactate, and either ammonium sulfate or L-glutamate as the N source, and the hydrogen metabolism of the harvested cells was examined in particular respect to the effects of ammonia and lactate.

(i) Cells grown with glutamate as N source. The wild type shows the already-described effect of NH_4^+ on photoreduction activity and addition of lactate results in the expected photoproduction of H_2. The low endogenous photoreduction rate (column 0) suggests the possibility that, under these conditions, there is an approximate balance between H_2 utilization

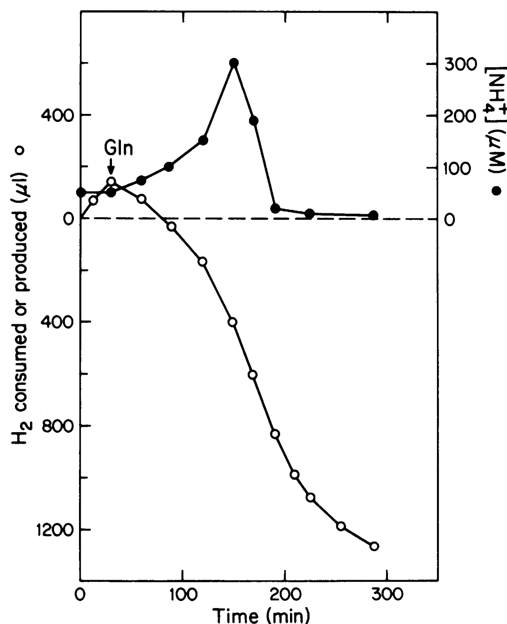

FIG. 7. *NH_4^+ production during stimulation of photoreduction by L-glutamine. Experimental details essentially as in Fig. 6, except that 6 μmol of freshly prepared L-glutamine (Gln) were added, rather than ammonium sulfate, and the cell quantity in each Warburg vessel was 6 mg (dry weight). The data shown are the composite results from a set of 10 identical vessels; at intervals, individual vessels were sacrificed for determination of NH_4^+ concentration (vessel contents were centrifuged, and the supernatant fluids were assayed using an ammonia electrode). As in the experiment of Fig. 6, some endogenous H_2 production was observed before addition of glutamine.*

TABLE 2. *Effects of NH_4^+ and lactate on photoreduction activities of cell suspensions of R. capsulata wild-type strain J2 and Nif$^-$ mutant W15* [a]

Cells	Rate of H_2 consumption[b] in the presence of:		
	0[c]	NH_4^+	Lactate
Wild type (J2) grown on:			
NH_4^+	42	42	0[d]
Glutamate	4	68	87[b]
Mutant W15 (Nif$^-$) grown on:			
NH_4^+	51	75	0
Glutamate	52	77	0

[a] Precultures were grown in the standard lactate + glutamate medium, and experimental cultures were grown on 30 mM DL-lactate + 7 mM ammonium sulfate or 7 mM L-glutamate, as indicated. The Warburg vessels contained 3 to 7 mg (dry weight) of harvested cells in 2.5 ml of mineral base, and were all gassed with 1% CO_2 in H_2. In this particular experiment, illumination was begun after temperature equilibration, and substrates (17.5 μmol of ammonium sulfate, 90 μmol of DL-lactate, or a corresponding small volume of mineral base [0]) were added after 30 min.

[b] Values are expressed as microliters per hour per milligram (dry weight) of cells and are for H_2 utilization, except in the trial with J2 cells grown with glutamate as N source and supplied with lactate; in the latter instance, H_2 was produced at the rate indicated.

[c] 0 designates controls in which neither NH_4^+ nor lactate was added.

[d] In this trial, H_2 production commenced after a lag of ca. 3 h.

and production. In the Nif$^-$ mutant, high photoreduction activity is observed in the absence of NH_4^+, and addition of the latter stimulates somewhat; photoproduction of H_2 from lactate does not occur. Note, however, that in the presence of lactate, photoreduction activity is completely inhibited.

(ii) Cells grown with ammonia as N source. Wild-type cells grown this way show good photoreduction activity, and this is unaffected by addition of NH_4^+. Similar results are seen with the mutant but, in this case, ammonia stimulates (as with glutamate grown cells). Since NH_4^+ represses synthesis of the nitrogenase system, photoproduction of H_2 is not seen with wild-type or mutant cells. Rather, addition of lactate abolishes photoreduction activity.

The effect of lactate on photoreduction activity is of particular interest. The experiments with W15 show clearly that lactate addition shuts off reduction of CO_2 with H_2 even in cells that do not have the ability to produce H_2. Since malate and succinate were observed to have the same effect (in cells grown on C_4 dicarboxylic acids), it is likely that the inhibition is due to some common metabolite derived from the organic acids in question.

DISCUSSION

R. capsulata, and related bacteria, displays a remarkably facile metabolism in regard to energy conversion mechanisms, N and C metabolism, and capabilities in generating reducing power from diverse sources. This plasticity implies the operation of sophisticated and complex regulatory mechanisms, and this is especially apparent in connection with the controls purple bacteria employ for coordinating electron transport and adenosine 5′-triphosphate (ATP) regeneration (i.e., the bioenergetic machinery) with biosynthetic activity. The capacity of *R. capsulata* to use H_2 for CO_2 reduction and to evolve H_2 when supplied with preformed organic compounds enhances its metabolic versatility, and these processes are closely integrated with nitrogen assimilation.

From the present and other investigations, it is evident that utilization of H_2 as a biosynthetic reductant of CO_2 and production of H_2 are catalyzed by different enzyme systems; also, NH_4^+, in addition to being a readily utilizable N source, is an important regulatory signal for H_2 production, and indirectly for H_2 utilization. Thus, NH_4^+ rapidly inhibits both N_2 fixation and the energy-dependent nitrogenase-mediated production of H_2 from organic compounds. These effects can be interpreted as devices for conserving ATP and reducing power under conditions in which the cell has the potential for rapid growth on preformed organic substrates. When fixed N is available in the form of certain amino acids and the energy supply (photophosphorylation) is not limiting, the H_2-evolving function of nitrogenase appears to provide a means of coping with excessive fluxes of ATP and reducing power; under conditions of energy limitation, however, the energy-dependent formation of H_2 may present a metabolic burden (11).

A substantially different picture is observed in the photoautotrophic (photoreduction) pattern of growth and metabolism. *R. capsulata* grows readily on CO_2 + H_2 with NH_4^+ (or N_2) as N source, and the photoreduction system is also developed in cells grown photoheterotrophically (in our experiments, with lactate as a C source; note, however, that Klemme [12] has observed that H_2-dependent reductions of nicotinamide adenine dinucleotide and cytochrome *c* by chromatophores of *R. capsulata* are considerably more active with preparations from cells grown photoautotrophically). The role of NH_4^+ in regulation of photoreduction activity is particularly evident in cells grown so as to have an

active H_2-evolving system (i.e., nitrogenase synthesis derepressed). In such cells, NH_4^+ (or amino acids that yield NH_4^+) inhibits H_2 production and thereby permits operation of the photoreduction system. The activating effect of NH_4^+ is absent or much diminished in extent in wild-type cells grown on NH_4^+ (i.e., in cells that have no nitrogenase-hydrogenase activity) and in the W15 mutant, which does not synthesize nitrogenase even under nonrepressing conditions (with glutamate as N source).

It appears that the reciprocal control effects of NH_4^+ on photoreduction and photoproduction of H_2 are designed to maximize the efficiency and economy of biosynthesis with the raw materials available. The striking inhibitory effect of lactate and other organic acids on photoreduction can also be understood, in terms of this general rationale, as replacement of the energy-expensive reduction of CO_2 to cell materials with more economic biosyntheses starting from assimilable organic compounds. (Schick [21] has similarly observed that the rate of photoreduction by cells of *R. rubrum* is markedly decreased by addition of L-malate.) The molecular bases for the effects under discussion are still largely unknown. It seems likely to us that the influence of organic compounds and NH_4^+ on photoreduction, and of NH_4^+ on photoproduction of H_2, are probably attributable, in part at least, to effects on electron flow to and from pyridine nucleotides, which play a central role in the metabolism of all types of cells.

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LITERATURE CITED

1. **Bisschop, A., H. Doddema, and W. N. Konings.** 1975. Dicarboxylic acid transport in membrane vesicles from *Bacillus subtilis*. J. Bacteriol. 124:613-622.
2. **Clayton, R. K.** 1963. Toward the isolation of a photochemical reaction center in *Rhodopseudomonas spheroides*. Biochim. Biophys. Acta 75:312-323.
3. **Drozd, J. W., R. S. Tubb, and J. R. Postgate.** 1972. A chemostat study of the effect of fixed nitrogen sources on nitrogen fixation, membranes and free amino acids in *Azotobacter chroococcum*. J. Gen. Microbiol. 73:221-232.
4. **Gest, H.** 1954. Oxidation and evolution of molecular hydrogen by microorganisms. Bacteriol. Rev. 18:43-73.
5. **Gest, H., and M. D. Kamen.** 1949. Photoproduction of molecular hydrogen by *Rhodospirillum rubrum*. Science 109:558-559.
6. **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.
7. **Gest, H., J. G. Ormerod, and K. S. Ormerod.** 1962. Photometabolism of *Rhodospirillum rubrum:* light-dependent dissimilation of organic compounds to carbon dioxide and molecular hydrogen by an anaerobic citric acid cycle. Arch. Biochem. Biophys. 97:21-33.
8. **Gibson, J.** 1975. Uptake of C_4 dicarboxylates and pyruvate by *Rhodopseudomonas spheroides*. J. Bacteriol. 123:471-480.
9. **Gray, C. T., and H. Gest.** 1965. Biological formation of molecular hydrogen. Science 148:186-192.
10. **Green, D. E., and L. H. Stickland.** 1934. Studies on reversible dehydrogenase systems. I. The reversibility of the hydrogenase system of *Bact. coli*. Biochem. J. 28:898-900.
11. **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.
12. **Klemme, J.-H.** 1969. Studies on the mechanism of NAD-photoreduction by chromatophores of the facultative phototroph, *Rhodopseudomonas capsulata*. Z. Naturforsch. Teil B 24:67-76.
13. **Krebs, H. A.** 1951. The use of 'CO_2 buffers' in manometric measurements of cell metabolism. Biochem. J. 48:349-359.
14. **Mortenson, L. E., and J.-S. Chen.** 1974. Hydrogenase, p. 231-282. *In* J. B. Neilands (ed.), Microbial iron metabolism. Academic Press Inc., New York.
15. **Neilson, A. H., and S. Nordlund.** 1975. Regulation of nitrogenase synthesis in intact cells of *Rhodospirillum rubrum:* inactivation of nitrogen fixation by ammonia, L-glutamine, and L-asparagine. J. Gen. Microbiol. 91:53-62.
16. **Ormerod, J. G., and H. Gest.** 1962. Hydrogen photosynthesis and alternative metabolic pathways in photosynthetic bacteria. Bacteriol. Rev. 26:51-66.
17. **Ormerod, J. G., K. S. Ormerod, and H. Gest.** 1961. Light-dependent utilization of organic compounds and photoproduction of molecular hydrogen by photosynthetic bacteria; relationships with nitrogen metabolism. Arch. Biochem. Biophys. 94:449-463.
18. **Peck, H. D., Jr., and H. Gest.** 1956. A new procedure for assay of bacterial hydrogenases. J. Bacteriol. 71:70-80.
19. **Schick, H.-J.** 1971. Substrate and light dependent fixation of molecular nitrogen in *Rhodospirillum rubrum*. Arch. Mikrobiol. 75:89-101.
20. **Schick, H.-J.** 1971. Interrelationship of nitrogen fixation, hydrogen evolution and photoreduction in *Rhodospirillum rubrum*. Arch. Mikrobiol. 75:102-109.
21. **Schick, H.-J.** 1971. Regulation of photoreduction in *Rhodospirillum rubrum* by ammonia. Arch. Mikrobiol. 75:110-120.
22. **Siegel, J. M., and M. D. Kamen.** 1951. Studies on the metabolism of photosynthetic bacteria. VII. Comparative studies on the photoproduction of H_2 by *Rhodopseudomonas gelatinosa* and *Rhodospirillum rubrum*. J. Bacteriol. 61:215-228.
23. **Stiffler, H. J., and H. Gest.** 1954. Effects of light intensity and nitrogen growth source on hydrogen metabolism in *Rhodospirillum rubrum*. Science 120:1024-1026.
24. **Strandberg, G. W., and P. W. Wilson.** 1968. Formation of the nitrogen-fixing enzyme system in *Azotobacter vinelandii*. Can. J. Microbiol. 14:25-31.
25. **Wall, J. D., P. F. Weaver, and H. Gest.** 1975. Genetic transfer of nitrogenase-hydrogenase activity in *Rhodopseudomonas capsulata*. Nature (London) 258:630-631.
26. **Weaver, P. F., J. D. Wall, and H. Gest.** 1975. Characterization of *Rhodopseudomonas capsulata*. Arch. Microbiol. 105:207-216.