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# Derepression of the Synthesis of D-Ribulose 1,5-Bisphosphate Carboxylase/Oxygenase from *Rhodospirillum rubrum*

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The synthesis of ribulose 1,5-bisphosphate carboxylase/oxygenase in *Rhodospirillum rubrum* was greatly influenced by the conditions of culture. When grown photolithotrophically in an atmosphere containing low levels of $CO_2$ (1.5 to 2%), enzyme synthesis was derepressed, with the result that the enzyme comprised up to 50% of the soluble protein of the cells as determined by immunological quantitation. This response was not observed when *R. rubrum* was grown photolithotrophically in an atmosphere of 5% $CO_2$ in hydrogen. Similarly, the derepression of ribulose 1,5-bisphosphate carboxylase/oxygenase was observed in photoheterotrophically (butyrate)-grown cultures only after the $HCO_3^-$ supply was nearly exhausted. The increase in enzyme activity observed in derepressed cultures was not paralleled by an increase in the in vivo $CO_2$ fixation rate. Apparently, *R. rubrum* derepresses the synthesis of ribulose 1,5-bisphosphate carboxylase/oxygenase when exposed to low $CO_2$ concentrations to scavenge the limited $CO_2$ available to such cultures.

Ribulose 1,5-bisphosphate (Rbu-$P_2$) carboxylase/oxygenase (3-phospho-D-glycerate carboxylase [dimerizing], E.C. 4.1.1.39) is one of the few enzymes capable of catalyzing two distinct reactions, namely, the carboxylation or oxygenolysis of ribulose bisphosphate. Since the reactions catalyzed represent the first steps of two competing pathways of photosynthetic metabolism, there is considerable interest in all aspects of control.

Anderson and Fuller (1) originally found that the level of Rbu-$P_2$ carboxylase/oxygenase in *Rhodosspirillum rubrum* is greatly influenced by cultural conditions. Over 30-fold-higher levels of enzyme are obtained when *R. rubrum* is grown with molecular hydrogen as the electron donor and $CO_2$ as the carbon source compared with photoheterotrophic growth on malate (1). When cells are grown with a reduced fatty acid (i.e., butyrate) in the presence of bicarbonate, derepressed levels of Rbu-$P_2$ carboxylase/oxygenase are obtained, such that this enzyme represents from 6 to 8% of all of the soluble protein (15). Interestingly, synthesis takes place only after the active growth of *R. rubrum* ceases in the butyrate-bicarbonate medium (13). Phosphoribulokinase (ATP:D-ribulose-5-phosphate 1-phosphotransferase, E.C. 2.7.1.19), the enzyme which catalyzes the synthesis of the $CO_2$ acceptor, ribulose bisphosphate, is synthesized in parallel with Rbu-$P_2$ carboxylase/oxygenase under photoheterotrophic growth conditions (13).

Recently, it was reported that levels of Rbu-$P_2$ carboxylase/oxygenase representing up to 40% of the soluble protein of *R. rubrum* may be obtained upon repeated transfer (10 to 14 weeks) of cells grown under a $CO_2/H_2$ atmosphere (12). Thus, the levels of Rbu-$P_2$ carboxylase/oxygenase obtained by these investigators in *R. rubrum* are similar to the levels found in the chloroplasts of eucaryotic cells. It is not clear, however, whether the extraordinarily high levels of enzyme obtained in *R. rubrum* by Schloss et al. (12) represent the natural selection of a mutant during this rather long (14-week) incubation period or whether some form of adaptation to the growth conditions occurs with the concurrent overproduction of Rbu-$P_2$ carboxylase/oxygenase. In this investigation, we examined the physiological conditions important for the synthesis of Rbu-$P_2$ carboxylase/oxygenase in *R. rubrum* and show that this organism and perhaps other members of the *Rhodospirillaceae* produce extremely high levels of enzyme only in an environment where the carbon dioxide level is diminished.

## MATERIALS AND METHODS

**Reagents.** All biochemicals were purchased from Sigma Chemical Co., St. Louis, Mo. All common compounds were of reagent-grade quality; [$^{14}$C]$NaHCO_3$ (20 mCi/mmol) was purchased from Amersham Corp., Arlington Heights, Ill.

**Organism and growth.** *R. rubrum* standard (S-1) was cultured photoheterotrophically in Ormerod medium

with 0.4% butyrate or 0.4% malate as the electron donor as previously described (4, 15). All culture media were further supplemented with 15 μg of biotin per liter and 0.1% $NaHCO_3$. Cultures of 1 liter were inoculated with 20 ml of late-logarithmic-phase malate-grown *R. rubrum*. Anaerobiosis was maintained by completely filling the 1-liter screw-cap Erlenmeyer flasks.

*R. rubrum* was also cultured under photolithotrophic conditions in a mineral salts medium lacking bicarbonate and supplemented with 15 μg of biotin per liter and 10 μM $NiCl_2$. Cultures were bubbled with the indicated atmosphere of $CO_2$ and $H_2$ at a rate of approximately 2.25 liters of gas per h/liter of culture. Hydrogen and carbon dioxide gases were mixed with Matheson rotameters. Final $CO_2$ levels were determined with an Antek thermal conductivity gas chromatograph (model 461 LP) fitted with a Porapak N column. Volumes of 800 ml were grown in 1-liter Roux flasks. The pH of the culture was maintained at 6.5 with sterile 10 N NaOH.

All cultures were illuminated by banks of 60-W soft-white incandescent light bulbs. The temperature was maintained at approximately 30°C by oscillating fans and temperature-controlled water baths.

Growth was followed by measuring the absorption at 650 nm.

**Enzyme assay.** Rbu-$P_2$ carboxylase/oxygenase was assayed by the whole cell assay previously described (14). A cell suspension was treated with toluene, the enzyme was activated by a 5-min incubation with 20 mM $NaHCO_3$ and 10 mM $Mg^{2+}$, and the reaction was initiated with Rbu-$P_2$. The incorporation of $NaH^{14}CO_3$ into acid-stable 3-phosphoglyceric acid was measured with a Beckman LS-100C liquid scintillation counter. One unit of Rbu-$P_2$ carboxylase activity is defined as the amount of enzyme needed to fix 1 μmol of $CO_2$ per min. Enzyme activity is expressed as units per gram (dry weight). An absorbance of 1.0 at 650 nm corresponds to 0.36 g (dry weight) per liter for malate-grown and autotrophically grown cultures; for butyrate-$HCO_3^-$-grown cultures, an absorbance of 1.0 corresponds to 0.43 g (dry weight) per liter.

**Electrophoresis.** Cultures were harvested and washed one time with TEMMB buffer (20 mM Tris-$SO_4$, 1 mM EDTA, 10 mM $MgCl_2$, 5 mM β-mercaptoethanol, 50 mM $NaHCO_3$ [pH 8.0]), and the cells were broken by two passages through a French pressure cell at 10,000 lb/in$^2$. Membranes and other debris were removed by centrifugation for 120 min at 40,000 rpm in a Beckman model 65 ultracentrifuge with a 70 Ti rotor. High-speed supernatants were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 1.5-mm slab gels by the procedure of Lugtenberg et al. (11). Slab gels were stained with 0.1% Coomassie blue R in 25% isopropanol–10% acetic acid and destained with 10% acetic acid–10% isopropanol. Protein was determined by the method of Lowry et al. (2, 10) or by the procedure of Bradford (5), with crystalline bovine serum albumin as the protein standard.

**Radial immunodiffusion.** Antisera were raised in a New Zealand white female rabbit injected subcutaneously with 2 to 3 mg of Rbu-$P_2$ carboxylase suspended in Freund incomplete adjuvant. After two weekly injections, the rabbit was bled, and the blood was allowed to clot at 4°C overnight. The clot was removed by centrifugation, and the crude antiserum was used for immunodiffusion. The antiserum was diluted 1:15 in saline borate agar (1 mM boric acid, 160 mM NaCl, 1% Noble agar) at 50°C. This mixture of buffer, antisera, and agar was poured onto a glass plate (8 by 10 cm), and 5-μl wells were punched into the agar matrix. Immunodiffusion was conducted by introducing 5 μl of antigen into each well, and the amount of antigen (Rbu-$P_2$ carboxylase) was quantified by measuring the diameter of the precipitin circle after 18 h. Purified Rbu-$P_2$ carboxylase from *R. rubrum* was used to construct a standard curve relating the logarithm of the concentration of Rbu-$P_2$ carboxylase to the square of the diameter of the precipitin circle.

**Incorporation of $NaH^{14}CO_3$.** Screw-cap Erlenmeyer flasks (500 ml) containing the butyrate-bicarbonate media were inoculated with 8 ml of late-logarithmic-phase malate-grown *R. rubrum*. Radioactive $NaH^{14}CO_3$ (500 μCi) was added to each 500-ml flask. Samples were removed every 12 h and acidified for 12 h with 0.1 ml of 50% acetic acid to drive off unfixed $CO_2$. A tissue solubilizer (0.2 ml of Soluene 350; Packard Instrument Co., Inc., Rockville, Md.) and a scintillation cocktail (3 ml of Insta-Gel; Packard Instrument Co., Inc.) were added, and samples were counted with a Beckman LS-100C liquid scintillation counter. Background activity, taken as incorporation at time zero, was subtracted in all calculations.

**Rate of $NaH^{14}CO_3$ incorporation.** Samples of *R. rubrum* grown under various conditions were removed from the culture vessels at a time when enzyme activity was maximally derepressed. In vivo $CO_2$ fixation assays were performed by the following method: a 5-ml sample was washed two times in media which lacked bicarbonate and placed in an anaerobic vial flushed with $H_2$. Then 20 mM [$^{14}$C]sodium bicarbonate (specific radioactivity, 0.25 μCi/μmol) was added to each vial. Vials were placed on a lighted Warburg apparatus with continuous shaking at 30°C. At frequent time intervals, 0.5-ml samples were removed and immediately acidified with 0.2 ml of 50% acetic acid. The acidified sample remained uncovered overnight to drive off unfixed $CO_2$, and then 200 μl of Soluene 350 and 3 ml of Insta-Gel were added to 200-μl samples, which were then counted. Illumination with 30-W incandescent bulbs 8 cm beneath each flask was determined to be optimal in these experiments.

## RESULTS

**Rbu-$P_2$ carboxylase/oxygenase levels as a function of growth.** It has repeatedly been observed that *R. rubrum* produces low amounts of Rbu-$P_2$ carboxylase/oxygenase when cultured with electron donors more oxidized than the intracellular milieu, such as malate (15; Fig. 1). However, if a more reduced electron donor, such as butyrate, is supplied, significant synthesis of Rbu-$P_2$ carboxylase/oxygenase takes place after exponential growth ceases, which would be 7 days in the experiment depicted in Fig. 1. There was an enormous difference in the levels of enzyme obtained under photolithotrophic conditions when cells were grown under either a 2 or 5 to 6% $CO_2$-$H_2$ atmosphere, and Rbu-$P_2$ carboxyl-

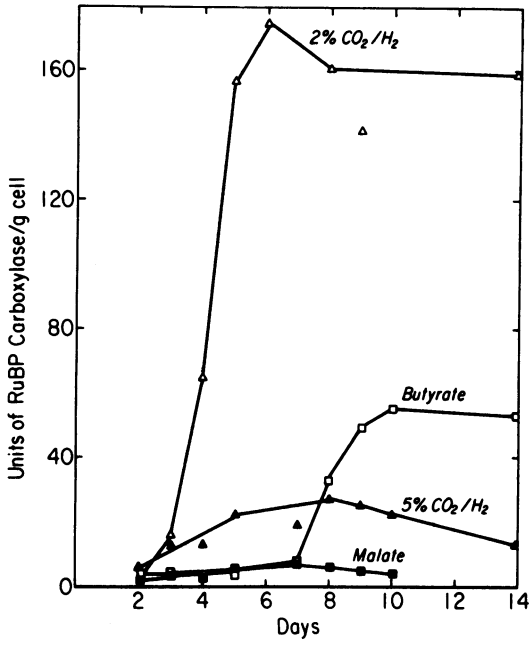


FIG. 1. Levels of *R. rubrum* Rbu-$P_2$ carboxylase/oxygenase during growth in various media. Cells were grown in a mineral salts basal medium under a gas atmosphere of either 2 or 5% $CO_2$ in hydrogen or in a mineral salts basal medium containing either 0.4% butyrate–0.1% bicarbonate or 0.4% malate–0.1% bicarbonate in completely filled, anaerobic vessels. Activity is expressed as units per gram (dry weight).

ase/oxygenase levels steadily increased during the time cells were actively growing, particularly the cells grown under a 2% $CO_2$ atmosphere. In all cases, there was no apparent loss of activity with time after the cells had synthesized maximal levels of enzyme. It should also be pointed out that the doubling time of *R. rubrum* grown under the 2% $CO_2$-$H_2$ atmosphere was the same as that of cells grown under the higher (5 or 6%) $CO_2$-$H_2$ atmosphere, namely, about 16 to 20 h in these vessels; yet, there was a five- to sixfold increase in the level of Rbu-$P_2$ carboxylase/oxygenase when cells were supplied with 2% $CO_2$-$H_2$ (Fig. 1). We reproducibly obtained between 150 and 200 U of enzyme per g (dry weight) of cells when 20 ml of malate-grown *R. rubrum* was transferred to 800 ml of minimal media under the 2% $CO_2$-$H_2$ atmosphere. Schloss et al., with a 50-liter culture, obtained about 50 U/g (dry weight) of cells in one experiment with a 3.75% inoculum and less than 10 U/g (dry weight) of cells with a 10% inoculum after transferring malate-grown cells to the 2% $CO_2$-$H_2$ atmosphere (12). Certainly, we have not observed the rather lengthy adaptation period of 14 weeks previously reported (12). In a separate experiment, we varied the bubbling rate of a culture grown in a 2% $CO_2$-$H_2$ atmosphere. It was found that, after 5 days of growth, cells that were bubbled at a rate of 0.5 liter of gas per h/liter of culture had 174 U of enzyme per g (dry weight) of cells; cells bubbled with gas at a rate of 1.25 liters of gas per h/liter of culture exhibited 161 U/g (dry weight) of cells, and cells bubbled at a rate of 9.0 liters of gas per h/liter of culture had 134 U of enzyme per g (dry weight) of cells. At the lower bubbling rate of 0.5 liter of gas per h/liter of culture, the amount of $CO_2$ supplied to the cells was growth limiting. However, at bubbling rates of between 1 and 2.25 liters of gas per h/liter of culture, the amount of enzyme produced and the rate and extent of growth were optimized for both the 2% $CO_2$-$H_2$- and 5% $CO_2$-$H_2$-grown cells.

When a batch culture of *R. rubrum* grown under an atmosphere of 6.4% $CO_2$-$H_2$ was shifted to an atmosphere of 1.5% $CO_2$-$H_2$, Rbu-$P_2$ carboxylase activity increased immediately after the concentration of $CO_2$ was reduced (Fig. 2A). In a control culture maintained continuously under 6.4% $CO_2$-$H_2$, Rbu-$P_2$ carboxylase activity increased very slowly with growth, reaching about 30 U/liter. When grown under 1.5% $CO_2$-$H_2$, enzyme activity increased five- to sixfold, reaching 155 U/liter after 6 days of growth. A culture grown under an atmosphere of 1.5% $CO_2$-$H_2$ until enzyme activity was derepressed (optical density at 650 nm, 1.25) was shifted to an atmosphere of 6.4% $CO_2$-$H_2$. Rbu-$P_2$ carboxylase synthesis ceased shortly after the change in $CO_2$ concentration. Yet the amount of total enzyme units remained relatively constant, indicating that the enzyme was diluted out as growth continued. An immediate decrease in enzyme activity, characteristic of enzyme inactivation, was not observed. Rather, the effect of $CO_2$ concentration on the regulation of Rbu-$P_2$ carboxylase appears to occur at the level of enzyme synthesis.

The differential rate of enzyme synthesis in cultures grown with different levels of $CO_2$ is depicted in Fig. 2B. Note that *R. rubrum* grown under 1.5% $CO_2$-$H_2$ synthesized Rbu-$P_2$ carboxylase at a much faster rate than when grown on 6.4% $CO_2$-$H_2$. A culture shifted from 6.4 to 1.5% $CO_2$-$H_2$ increased the rate of synthesis immediately after the decrease in $CO_2$ concentration and reached a rate of synthesis identical to the culture maintained continuously on 1.5% $CO_2$-$H_2$. A culture shifted from 1.5 to 6.4% $CO_2$-$H_2$ exhibited an immediate decrease in the apparent rate of enzyme synthesis.

**Stimulation of Rbu-$P_2$ carboxylase/oxygenase activity in butyrate-bicarbonate medium.** To further study the effects of $CO_2$ concentration on the levels of enzyme activity obtained, we followed the incorporation of $NaH^{14}CO_3$ in *R.*

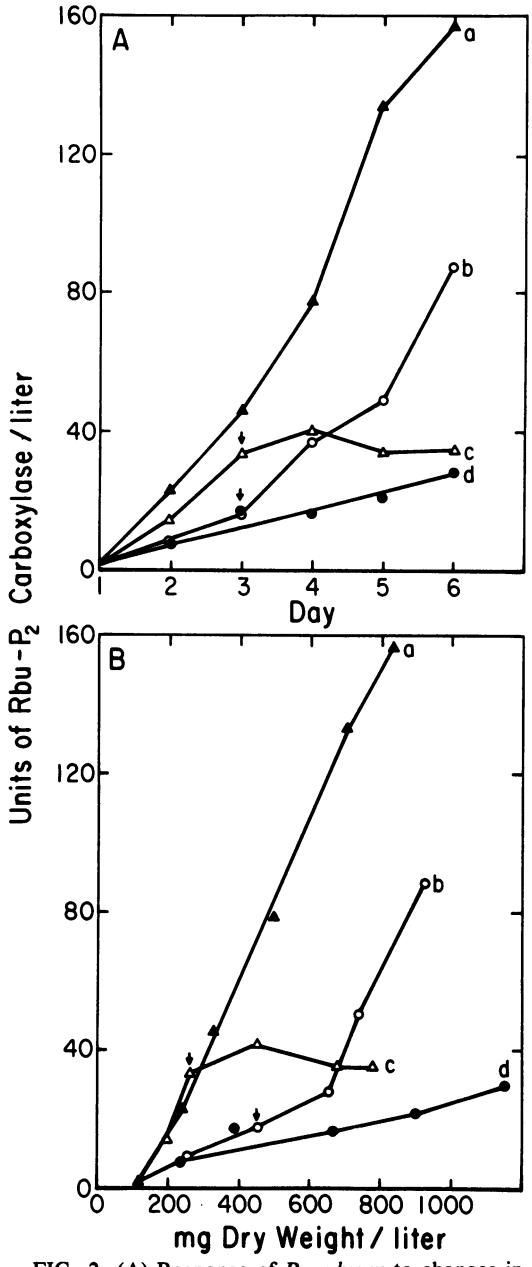


FIG. 2. (A) Response of *R. rubrum* to changes in the concentration of $CO_2$ supplied to photolithotrophically grown cells. Rbu-$P_2$ carboxylase/oxygenase activity is expressed as units per liter of culture as a function of the time of growth. (B) Differential rate of Rbu-$P_2$ carboxylase/oxygenase synthesis in photolithotrophically grown *R. rubrum* at different concentrations of $CO_2$. A culture was maintained for (a) 6 days in 1.5% $CO_2$ in hydrogen; (b) 3 days in 6.4% $CO_2$ in hydrogen, then shifted to an atmosphere of 1.5% $CO_2$ in hydrogen; (c) 3 days in 1.5% $CO_2$ in hydrogen, then shifted to an atmosphere of 6.4% $CO_2$ in hydrogen; (d) 6 days in an atmosphere of 6.4% $CO_2$ in hydrogen. The arrows indicate the times at which the $CO_2$ concentration was either decreased (b) or increased (c).

*rubrum* grown in the butyrate-bicarbonate medium. Under these conditions, the maximum growth rate was about one generation per 24 h, and the incorporation of $^{14}C$-labeled bicarbonate closely paralleled growth (Fig. 3). The original concentration of $NaHCO_3$ in the medium was 11.8 mM. After 10 μmol of bicarbonate per ml had been fixed into organic carbon by the cells, leaving a free concentration of 1.8 μmol of bicarbonate per ml in the culture medium, growth ceased. Rbu-$P_2$ carboxylase activity increased only after the concentration of bicarbonate in the medium had decreased to about 2 mM. This stimulation of Rbu-$P_2$ carboxylase activity occurred simultaneously with the depletion of bicarbonate in the medium (Fig. 3) and appeared to be a response to severe bicarbonate ($CO_2$) limitation.

**Derepression of Rbu-$P_2$ carboxylase/oxygenase synthesis.** To determine whether the high levels of Rbu-$P_2$ carboxylase activity were due to increased synthesis of the enzyme or to activation of the preformed enzyme, we subjected the soluble portion of crude extracts of *R. rubrum* grown under different cultural conditions to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. When purified *R. rubrum* Rbu-$P_2$ carboxylase was used as a standard, it was evident that cells which exhibited an increase in enzyme activity showed a correspondingly high increase in the proportion of Rbu-$P_2$ carboxylase found in the crude extract. Crude extracts were made from *R. rubrum* grown on various media when the enzyme activity was at the highest level. It is apparent that there was a large increase in the amount of protein which comigrated with authentic *R. rubrum* enzyme when extracts from butyrate-bicarbonate-grown cells were compared with extracts obtained from cells grown on malate (Fig. 4). Similarly, the extraordinary levels of Rbu-$P_2$ carboxylase activity found in cells grown photolithotrophically under an atmosphere of 1.5 to 2% $CO_2$-$H_2$ compared with those cells grown with 5 to 6% $CO_2$-$H_2$ showed a corresponding increase in the amount of protein comigrating with purified Rbu-$P_2$ carboxylase (Fig. 4).

To quantitate the amount of Rbu-$P_2$ carboxylase in the soluble fraction of the cells, we subjected crude extracts of *R. rubrum* grown under different cultural conditions to radial immunodiffusion. The results of this quantitative analysis (Table 1) are consistent with the sodium dodecyl sulfate-polyacrylamide gel electrophoresis results. When grown under conditions of carbon dioxide limitation (1.5% $CO_2$-$H_2$), Rbu-$P_2$ carboxylase comprised up to 50% of the soluble protein of the cell. In contrast, when grown in an atmosphere of 6.4% $CO_2$-$H_2$, only 4% of the soluble protein was Rbu-$P_2$ carboxyl-

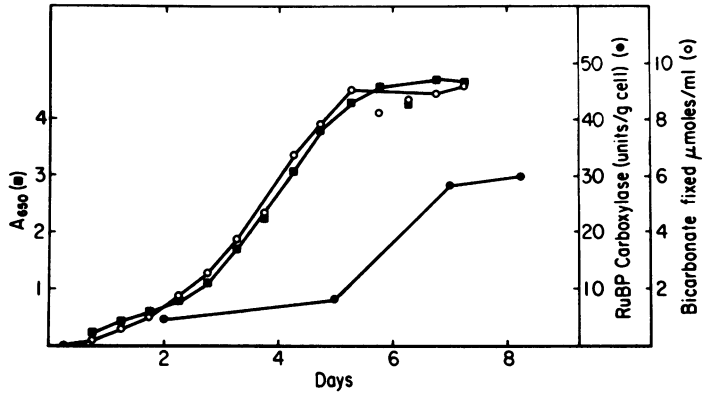


FIG. 3. Determination of the level of Rbu-$P_2$ carboxylase/oxygenase activity and the amount of bicarbonate fixed by a culture of *R. rubrum* grown in a butyrate-bicarbonate medium. The amount of bicarbonate incorporated and the activity of Rbu-$P_2$ carboxylase/oxygenase were determined as described in the text. The original concentration of bicarbonate in the medium was 11.8 mM. $A_{650}$, Absorbance at 650 nm.

ase. Similarly, Rbu-$P_2$ carboxylase comprised 14% of the soluble protein of butyrate-$HCO_3^-$-grown cells and only 0.3% of the soluble protein of malate-grown cells. These results suggest that a derepression of the synthesis of Rbu-$P_2$ carboxylase as a function of the level of $CO_2$ dissolved in the medium is responsible for the increase in enzyme activity.

**Rate of $NaHCO_3$ incorporation.** *R. rubrum* has the same growth rate when grown on 2% $CO_2$ and $H_2$ as when grown on 5% $CO_2$ and $H_2$. Thus, the five- to sixfold increase in Rbu-$P_2$ carboxylase activity at low $CO_2$ concentrations does not seem to significantly affect the rate of in vivo $CO_2$ fixation by the organism. Samples were thus taken from each of the four growth conditions at a time when Rbu-$P_2$ carboxylase synthesis was maximally expressed, and in vivo $CO_2$ fixation assays were performed. The rate at which the radioactive bicarbonate was fixed into organic carbon by the organism was followed over 15 min (Table 2). Note that when enzyme synthesis was derepressed, as measured by a whole cell Rbu-$P_2$ carboxylase assay, there did not appear to be an increase in the rate of in vivo $CO_2$ fixation. When the organism was grown under 1.5% $CO_2$-$H_2$, the $CO_2$ fixation rate per g (dry weight) was only 5% of the catalytic capacity of the enzyme. When grown on malate, the amount of Rbu-$P_2$ carboxylase activity per g (dry weight) was nearly equivalent to the in vivo $CO_2$ fixation rate. It is worth noting that the rate of $CO_2$ fixation in autotrophically grown cells is very close to what is theoretically needed to support the observed growth rate in these cultures. The in vivo $CO_2$ fixation rate for photoheterotrophically grown cells was somewhat lower, presumably because a significant proportion of the organic carbon is derived from either the butyrate or malate in the medium.

## DISCUSSION

The results presented in this investigation demonstrate that the levels of Rbu-$P_2$ carboxyl-

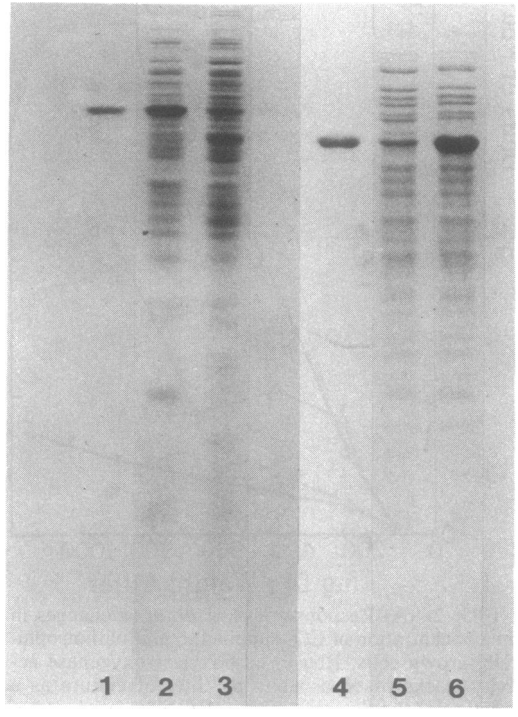


Fig. 4. Sodium dodecyl sulfate-slab gel electrophoresis of extracts obtained from *R. rubrum* grown under various conditions. Extracts were prepared from the soluble fraction of cells as described in the text. Lanes 1 and 4 contain homogeneous *R. rubrum* Rbu-$P_2$ carboxylase/oxygenase. Lanes 2 and 3 contain 20 μg of protein from butyrate- and malate-grown cells, respectively. Lanes 5 and 6 contain 30 μg of protein from cells grown in a 5 or a 2% $CO_2$-$H_2$ atmosphere, respectively.

TABLE 1. Quantitative radial immunodiffusion analysis of the amount of Rbu-$P_2$ carboxylase/oxygenase present under different growth conditions

| Growth substrate[a] | Sp act (U/mg of protein) | Protein (mg/ml) | Rbu-$P_2$ carboxylase (mg/ml) (RID)[b] | % Soluble protein as Rbu-$P_2$ carboxylase |
|---|---|---|---|---|
| Malate | 0.017 | 8.45 | 0.03 | 0.36 |
| Butyrate-$HCO_3^-$ | 0.600 | 7.9 | 1.10 | 13.9 |
| 6.4% $CO_2$-$H_2$ | 0.177 | 6.2 | 0.24 | 3.9 |
| 1.5% $CO_2$-$H_2$ | 1.68 | 6.3 | 3.16 | 50.2 |

[a] Extracts were prepared from the soluble fraction of cells as described in the text.
[b] RID, Quantitative radial immunodiffusion analysis of the amount of Rbu-$P_2$ carboxylase/oxygenase present.

ase/oxygenase in *R. rubrum* greatly depend on the concentration of carbon dioxide supplied to the cells. Furthermore, this increased activity is paralleled by an increase in the amount of Rbu-$P_2$ carboxylase/oxygenase actually synthesized by the organism, with this enzyme produced at a level which represents up to 50% of the soluble protein under conditions of carbon dioxide deprivation. We further show that the adaptation occurs within the first transfer of heterotrophically grown cells to photolithotrophic growth conditions and not after 10 to 14 weeks of growth and transfer as previously reported (12). Such a response to nutrient (carbon dioxide) limitation is by no means unprecedented since various degrees of derepression of Rbu-$P_2$ carboxylase/oxygenase synthesis have been observed in the hydrogen-oxidizing bacterium *Alcaligenes eutrophus* (7), the obligate chemolithotroph *Thiobacillus neapolitanus* (3), the cyanobacterium *Anacystis nidulans* (8), and the facultative autotroph *Pseudomonas oxalaticus* OXI (6). The extraordinarily high amounts of Rbu-$P_2$ carboxylase/oxygenase that may be obtained by *R. rubrum* (50% of the total soluble protein), however, represent the greatest commitment to the synthesis of this enzyme by any procaryote thus far reported. Indeed, another photosynthetic procaryote, *Rhodopseudomonas sphaeroides*, also responds to low levels of $CO_2$ and produces levels of enzyme comparable to that obtained by *R. rubrum* (F. R. Tabita and F. Waddill, unpublished data).

TABLE 2. Comparison of Rbu-$P_2$ carboxylase activity with the actual rate of in vivo $CO_2$ fixation

| Growth substrate | Rbu-$P_2$ carboxylase activity[a] (μmol of $CO_2$ fixed per min/g [dry wt]) | Total in vivo $CO_2$ fixation rate[b] (μmol of $CO_2$ fixed per min/g [dry wt]) |
|---|---|---|
| 1.5% $CO_2$-$H_2$ | 276.0 | 12.6 |
| 5.5% $CO_2$-$H_2$ | 50.3 | 12.8 |
| Butyrate-$HCO_3^-$ | 42.0 | 4.5 |
| Malate | 5.7 | 4.5 |

[a] Rbu-$P_2$ carboxylase was determined with whole cells treated with toluene as described in the text.
[b] Rate at which living cells incorporated $^{14}CO_2$ into organic carbon. The assay is described in the text.

At this time, there is no indication that either activation or inactivation of the preformed enzyme is an important mode of control; rather, the presence of excess $CO_2$ appears to signal a repression in the synthesis of Rbu-$P_2$ carboxylase/oxygenase.

The enzyme activities observed in vitro are far in excess of what is theoretically necessary to support either the observed growth rate or the observed in vivo $CO_2$ fixation rate. It is possible that *R. rubrum* and perhaps other members of the *Rhodospirillaceae* produce phenomenal levels of Rbu-$P_2$ carboxylase under conditions of carbon limitation to scavenge the relatively sparse amounts of $CO_2$ that are available. Since the *R. rubrum* enzyme has a relatively low affinity (high $K_m^{cat}$) for $CO_2$ (12, 13, 15, 16), perhaps the organism compensates for $CO_2$ limitation by synthesizing large amounts of Rbu-$P_2$ carboxylase/oxygenase to maintain adequate growth rates. An increase in the activity or the amount of the enzyme would be necessary to maintain the same rate of $CO_2$ fixation when the substrate $CO_2$ (bicarbonate) is not present at saturating levels.

Metabolic control in *R. rubrum* may not be mediated entirely by the level of carbon dioxide that is available for growth. It is conceivable that other factors, such as the concentration of reduced pyridine nucleotides, might be involved in the regulation of enzyme synthesis (9). In any case, it may now be possible to probe the nature of control at the molecular level through the manipulation of $CO_2$-limited chemostat cultures of *R. rubrum*.

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