JOURNAL OF BACTERIOLOGY, Feb. 1986, p. 620–624
0021-9193/86/020620-05$02.00/0
Copyright © 1986, American Society for Microbiology
Vol. 165, No. 2

Independent Regulation of Synthesis of Form I and Form II Ribulose Bisphosphate Carboxylase-Oxygenase in *Rhodopseudomonas sphaeroides*

YVES JOUANNEAU AND F. ROBERT TABITA*

Center for Applied Microbiology and Department of Microbiology, The University of Texas at Austin, Austin, Texas 78712-1095

Received 16 September 1985/Accepted 15 November 1985

Ribulose 1,5-bisphosphate carboxylase-oxygenase (RuBPC-O) activity was greatly enhanced when *Rhodopseudomonas sphaeroides* was grown in a mineral salts medium supplied with 1.5% CO_2 in hydrogen. Analysis of cell extracts by sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicated that cells growing on 1.5% CO_2 in H_2 specifically accumulated RuBPC-O polypeptides. Quantitative immunological determinations revealed that accumulation of form I and form II RuBPC-O closely correlates with the increase of specific activity. However, the two enzymes appeared to be derepressed at different levels. Upon transfer from heterotrophic to autotrophic (1.5% CO_2) growth conditions, the intracellular form I RuBPC-O concentration was augmented 17-fold, whereas the form II RuBPC-O content increased only fourfold. As a result, the form I-form II ratio changed from 0.5 to about 2.0. Since this change in the RuBPC-O ratio occurred in the early stage of growth, it suggests that form I RuBPC-O is required for growth under drastic CO_2 limitation. The difference in the extent of derepression of form I and form II RuBPC-O also indicates that the synthesis of each enzyme is regulated somewhat independently of the other.

Ribulose 1,5-bisphosphate carboxylase-oxygenase (RuBPC-O) is a bifunctional enzyme which catalyzes the initial reaction of the two competing metabolic pathways of photosynthetic carbon assimilation and photorespiratory glycolate metabolism. Because this enzyme is such a poor catalyst, organisms which depend on CO_2 for carbon must synthesize enormous quantities of RuBPC-O (21). This is particularly evident in *Rhodospirillum rubrum*, where up to 50% of the soluble protein is found to be RuBPC-O when cells are grown photolithotrophically under an atmosphere of hydrogen and CO_2, with CO_2 supplied at levels of 2% or less of the total gas mixture (18, 19).

Rhodopseudomonas sphaeroides, another member of the family *Rhodospirillaceae*, presents an interesting situation. Two different forms of RuBPC-O are found in *R. sphaeroides* (4) and in the closely related *R. capsulata* (5, 20). Form I RuBPC-O resembles the enzyme found in eucaryotes and most procaryotes in that it is a very large molecule with a native molecular weight of about 550,000 and is composed of eight large (catalytic) and eight small subunits. The form II enzyme, on the other hand, resembles the *Rhodospirillum rubrum* enzyme in that it is an aggregate of large subunits only and has a native molecular weight of about 290,000 (6a). Antiserum to the form I or form II enzyme does not cross-react with the heterologous enzyme (4, 6). The large and small subunits of the form I enzyme have been separated, and detailed peptide mapping experiments have shown that there are significant differences in the primary structure of the catalytic subunits of the form I and form II RuBPC-O from *R. sphaeroides*, suggesting that these proteins are products of different genes (6a). In addition, form II RuBPC-O has been cloned (3, 11, 16) and expressed in *Escherichia coli* (11, 16; F. R. Tabita, J. L. Gibson, W. J. Mandy, and R. G. Quivey, Jr., Bio/Technology, in press). Recently, Weaver and Tabita (24) isolated several mutants that were impaired in the regulation of RuBPC-O production in this organism. Studies with these mutants suggested that the form I and form II enzymes might be under independent control (24).

In this paper, the regulation of form I and form II RuBPC-O synthesis in *R. sphaeroides* is reported. The levels of derepression of the two forms of carboxylase were followed by quantitative immunological analyses. It is shown that carbon limitation caused a differential derepression of both carboxylase enzymes and resulted in the preferential accumulation of form I RuBPC-O in derepressed cells.

MATERIALS AND METHODS

Reagents. All biochemicals were purchased from the 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. sphaeroides* HR was derived from ATCC 17023 (24). The organism was cultured photoheterotrophically in Ormerod medium (13) with 0.2% malate as the electron donor as previously described (4). All culture media were supplemented with 15 μg of biotin per liter, 1 mg each of nicotinic acid and thiamine hydrochloride, and 0.1% $NaHCO_3$. Cultures of 800 ml were inoculated with 20 ml of late-logarithmic-phase malate-grown *R. sphaeroides*. Anaerobiosis was maintained by bubbling cultures with oxygen-free argon. Growth vessels used were 1- and 2-liter Blake rectangular bottles (Kimble; Owens-Illinois, Inc., Toledo, Ohio).

R. sphaeroides was also cultured under photolithotrophic conditions in a mineral salts medium lacking bicarbonate, supplemented with the vitamins and with 10 μM $NiCl_2 \cdot 6H_2O$. Cultures were bubbled with 1.5% CO_2 in H_2 at the rate of approximately 2.25 liters of gas per h per liter of culture. Hydrogen and carbon dioxide gases were mixed with rotameters (Matheson Scientific, Inc., Elk Grove Vil-

* Corresponding author.

lage, Ill.). Final CO_2 levels were determined by gas chromatography as previously described (18).

All cultures were grown at 30°C in temperature-controlled water baths illuminated by four 60-W incandescent light bulbs (Soft-White; General Electric Co., Schenectady, N.Y.) on each side of the bath about 15 cm from the growth vessels. Growth was followed by measuring the light scattering at 650 nm.

Preparation of cell extracts. Extracts were prepared from culture samples, washed, and concentrated 10 to 40 times to a final volume of 1.0 ml in Tris-EDTA buffer (50 mM Tris sulfate, 10 mM EDTA [pH 8.0]) and stored frozen at −20°C. Thawed cells were treated with lysozyme (0.125 mg/ml) for 20 min at 30°C and then sonicated three times for 30 s at 15 W with the microtip of a sonifier (model 350; Branson Sonic Power Co., Danbury, Conn.). For purification purposes, greater amounts of extract were prepared by following the same procedure. Cell debris was removed by centrifugation for 10 min at 12,400 × *g* in a Microfuge 12 (Beckman Instruments, Inc., Fullerton, Calif.) or at 10,000 × *g* in a centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.). The recovery of RuBPC-O in these crude extracts was between 90 and 100% as judged by the ratio of carboxylase activity in cell extracts and in whole cells. Cell extracts were freed of most membrane fragments by heat treatment at 50°C for 10 min in the presence of 50 mM $MgCl_2$, followed by centrifugation (4).

RuBPC-O purification. Form I and form II RuBPC-O from the heat-treated crude extract were separated by affinity chromatography on a green A agarose column (Amicon Corp., Lexington, Mass.). Adsorption of the proteins on the column was carried out in a Tris-EDTA buffer (25 mM Tris hydrochloride [pH 7.3], 1 mM EDTA) containing 5 mM 2-mercaptoethanol, 10 mM $MgCl_2$, and 50 mM $NaHCO_3$. After extensive washing, form II RuBPC-O was eluted with 10 mM 6-phosphogluconate in the same buffer. The column was washed again with the same buffer containing 0.25 M NaCl, and then form I RuBPC-O was eluted with a gradient of 0.3 to 0.5 M NaCl. Fractions of each protein were pooled, dialyzed, and concentrated on an ultrafiltration membrane (PM10; Amicon). Form I RuBPC-O was further purified by gel filtration on a Bio-gel A 0.5-m column (2.6 by 100 cm; Bio-Rad Laboratories, Richmond, Calif.). Form I and form II RuBPC-O were estimated to be 95 and 90% homogeneous after sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. These preparations were used as standards for rocket electroimmunoassay (EIA). For the preparation of antibodies, form II RuBPC-O was purified to homogeneity by preparative electrophoresis at pH 7.0 as previously described (4), and the pure form II RuBPC-O was localized by its carboxylase activity and recovered from polyacrylamide gel slices by electroelution (2). Form I RuBPC-O was purified by established procedures (4).

Ribulosebisphosphate carboxylase assays. Ribulosebisphosphate carboxylase activity was measured at pH 7.2, either in culture samples by whole cell assay (22) or in cell extracts and purified preparations as previously described (25). A unit of ribulosebisphosphate carboxylase activity is defined as the amount of enzyme needed to fix 1 μmol of CO_2 per min at 30°C. Enzyme-specific activity is expressed as units per 1.0 light scattering unit at 650 nm in whole-cell preparations or as units per milligram of protein in cell extracts.

Preparations of antibodies. Antisera were raised in rabbits injected subcutaneously with homogeneous form I and form II RuBPC-O as previously described (4).

Quantitation of form I and form II RuBPC-O in cell extracts. The amount of form I and form II enzymes present in cell extracts was determined by rocket EIA and by competitive enzyme-linked immunosorbent assay (ELISA).

The rocket EIA method, described by Laurell (9), was used with some modifications (8). Immunoplates were prepared by mixing, at 55°C, 1% (wt/vol) melted agarose with either form I (0.3% vol/vol) or form II antiserum (0.5%, vol/vol). Agarose also contained calcium acetate (0.5 mM) and Tris-acetate buffer (pH 8.6) (15). Electrophoresis was carried out in this same buffer at 4 V/cm overnight. Three dilutions of the standards were run at the same time. Quantitation of form I and form II RuBPC-O antigens was made by measurement of the height of the rockets and by comparison with a calibration curve drawn from the standard rockets. No cross-reactivity was detected in control experiments when pure antigens were run against heterologous antisera. The form I enzyme was also quantitated by competitive ELISA as previously described (1). Cuvettes (Immunolon; Dynatech Laboratories, Inc., Alexandria, Va.) were first coated by the addition of 200 ng of form I protein in 0.1 ml of coating buffer (1). Then 10 μl of anti-form I serum, diluted 5×10^{-4}, was added to 0.1 ml of an appropriate dilution of the samples, and 100 μl of the mixture was transferred to a coated cuvette and incubated for 2 h at room temperature. Cuvettes were then incubated for 1 h with 0.1 ml of alkaline phosphatase-goat anti-rabbit immunoglobulin G conjugate (Sigma) diluted 10^{-3}. Cuvettes were developed with 100 μl of alkaline phosphatase substrate, as previously described (1). Data were linearized by using the Logit transformation (17).

Other analyses. SDS-polyacrylamide electrophoresis was performed as previously described (10). Two-dimensional gel electrophoresis was done by standard procedures (12). Protein in extracts was estimated by the method of Lowry in the presence of 1% SDS (14). Protein concentrations of pure form I and form II enzymes were measured by the biuret method (23). Bovine serum albumin was used as a standard in both protein assays. Based on the biuret determination, 1 mg of solution per ml of form I and form II enzymes displayed absorbances of 1.42 and 1.01 to 1.05, respectively, at 280 nm.

Bacterial density was followed by reading the light scattering at 650 nm of cultures sampled.

RESULTS

RuBPC-O levels in malate- and CO_2-H_2-grown *R. sphaeroides*. Low levels of RuBPC-O were found in malate-grown *R. sphaeroides*, consistent with earlier results from this laboratory (4). However, cells initially grown on malate and subsequently transferred to a 1.5% CO_2-H_2 atmosphere showed a marked increase in the level of RuBPC-O (Fig. 1). This response is similar to that seen with *Rhodospirillum rubrum* (18) and, as already pointed out for *Rhodospirillum rubrum*, the CO_2 percentage in the H_2-CO_2 gas mixture is critical for obtaining maximal levels of RuBPC-O. Cultures of *R. sphaeroides*, sparged with 2.5% CO_2 in H_2, displayed activities about two-thirds lower than those grown under 1.5% CO_2. As indicated in Fig. 1, the increase of RuBPC-O specific activity occurred in the mid-logarithmic phase of growth and slowed as cells entered the stationary phase. Subsequent experiments were directed at determining whether this increase in carboxylase activity was a result of new protein synthesis or activation of preformed enzyme.

Intracellular accumulation of form I and form II RuBPC-O. In the experiment shown in Fig. 1, cells were taken at

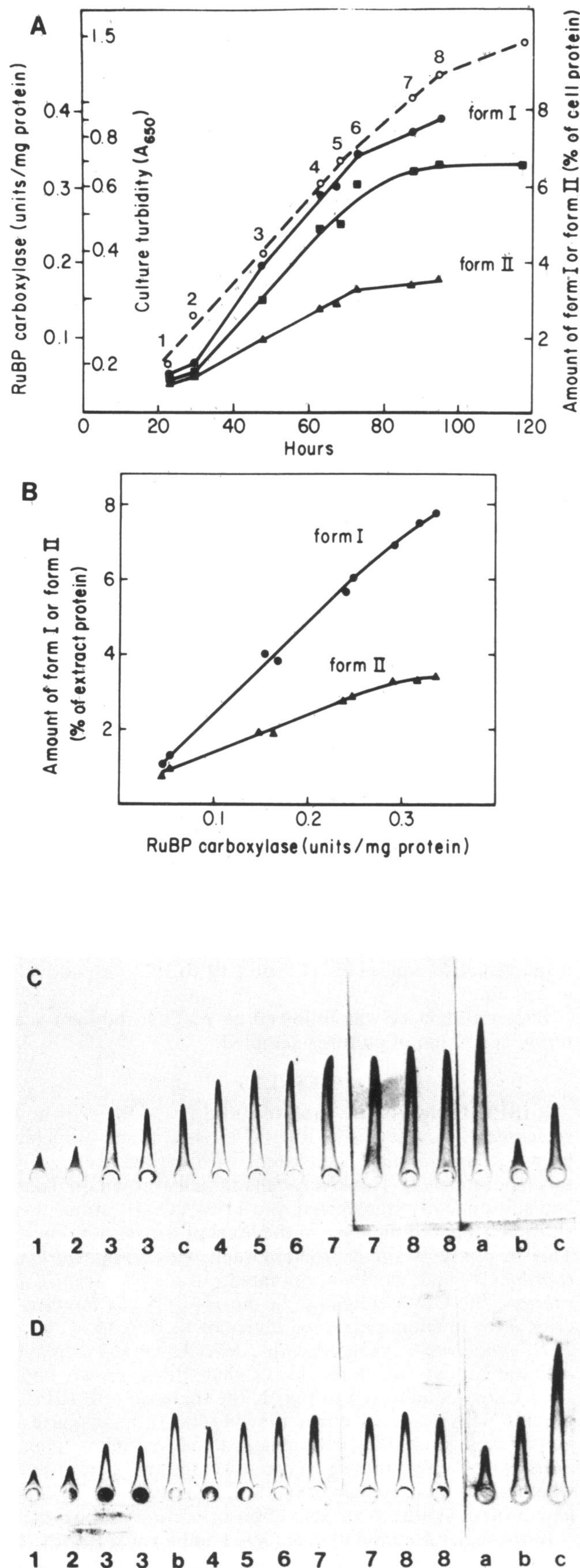

FIG. 1. Time course of ribulosebisphosphate (RuBP) carboxylase activity and form I and form II RuBPC-O accumulation in extracts of cells growing photolithotrophically (1.5% CO_2 in H_2). (A) At time zero, the culture was inoculated with malate-grown cells and then sparged with a CO_2-H_2 gas mixture (1.5% CO_2) throughout growth. At the times indicated, samples were analyzed for cell density at 650 nm (O), ribulosebisphosphate carboxylase activity (■), and the relative amounts of form I (●) and form II (▲) enzymes determined by rocket EIA. (B) Relationship between the relative amounts of form I and form II RuBPC-O and ribulosebisphosphate carboxylase specific activity. Curves are plotted according to the data shown in panel A. (C) Rocket EIA measurement of form I RuBPC-O. Standards contained purified form I enzyme (in micrograms): a, 1.4; b, 0.25; c, 0.63. (D) Rocket EIA measurement of form II RuBPC-O. Standards contained purified form II enzyme (in micrograms): a, 0.27; b, 0.54; c, 0.86. Numbers refer to the sampling order in panel A. In both panels C and D, cell extracts were made equivalent to the total amount of protein (1.5 mg/ml); 10 μl of extract was applied per well.

intervals during growth, and the protein content was analyzed by SDS-polyacrylamide gel electrophoresis. One-dimensional SDS-polyacrylamide gel electrophoresis revealed that form I subunits selectively accumulated in extracts of CO_2-H_2-grown cells (data not shown). The form II subunit, as resolved by two-dimensional gel electrophoresis, also appeared much more abundant in CO_2-H_2-grown cells than in malate-grown cells (Fig. 2).

The amount of form I and form II RuBPC-O in autotrophically grown cells was further investigated by quantitative immunological methods. Cell extracts, made equivalent with respect to the concentration of total protein, were compared by rocket EIA of each individual RuBPC-O by using the respective specific antisera. Autotrophic growth under CO_2 limitation promoted the accumulation of both form I and form II RuBPC-O inside the bacteria (Fig. 1). The amount of each holoenzyme, calculated by referring to standards of pure protein (Fig. 1C and D), is reported as a percentage of all the extract protein (Fig. 1A and B). The results show a close correlation between the increase in specific activity and the accumulation of form I and form II carboxylase proteins (Fig. 1A). This correlation is illustrated in Fig. 1B where the amount of form I and form II enzymes is plotted versus the specific carboxylase activity. From the accumulated percentages of the form I and form II enzymes, it can be calculated that the average specific activity of either enzyme was around 2.7 U/mg of carboxylase protein. This value is in good agreement with the activities reported for the pure enzyme (4). It should be noted that the concentrations of both form I and form II RuBPC-O increased dramatically in the early stage of growth, form I increasing four times and form II increasing 2.5 times within a single doubling time (between 30 and 56 h of growth). As the culture approached the stationary phase, the amount of form II RuBPC-O apparently reached a maximum of around 3.5 to 4% of the total extract protein, whereas the form I percentage culminated at values twice as high. To confirm these results by an independent immunological assay, the amount of form I RuBPC-O was also quantitated by the sensitive, competitive ELISA technique. A standard curve for the form I enzyme was generated, allowing the quantitation of as little as 10 ng of form I protein. Because of the potential cross-reaction between form II and anti-form I antiserum, we tested the interference of form II enzyme in the form I assay. We found no detectable competition until the concentration of form II enzyme reached 10 μg/ml. Since assays

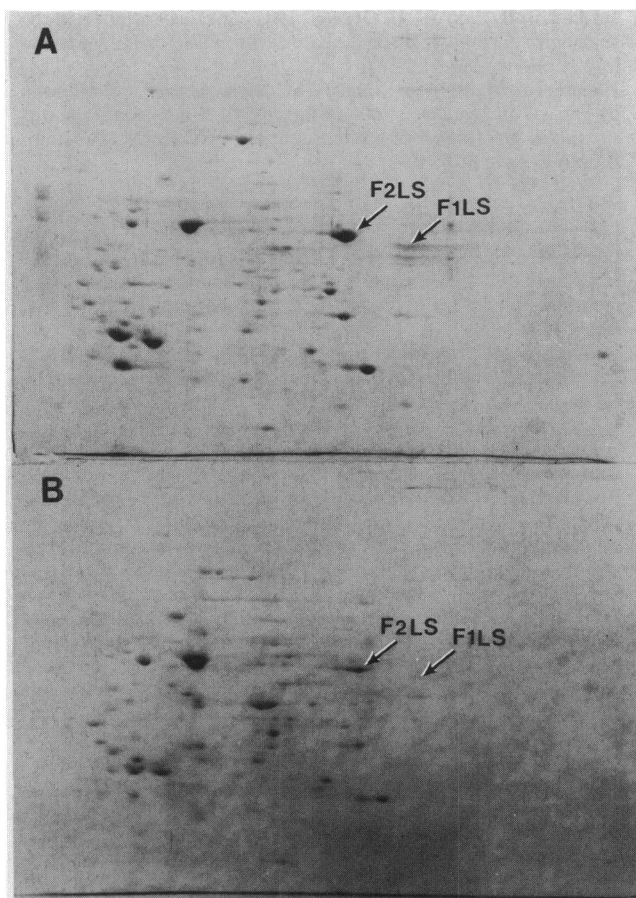

FIG. 2. Two-dimensional electrophoretic analysis of extracts from H_2-1.5% (CO_2)- (A) and malate- (B) grown cells. Arrows show the positions of the form I large subunit (F1LS) and the form II large subunit (F2LS). The form I small subunit was not resolved by this gel system. The pH gradient for isoelectric focusing ranged from pH 2.7 to 6.6.

were performed in the range of 0.1 to 1.0 μg/ml and the form II-form I ratio in extracts was never found to be higher than 2, we neglected any contribution of form II enzyme. Samples from the experiment shown in Fig. 1 were assayed in duplicates of three dilutions. Values reported in Table 1 are in fairly good agreement with the rocket EIA data. Significant variations were observed at the lower percentages of form I RuBPC-O, which corresponded to amounts at the limit of detection of the rocket EIA method. Therefore, the ELISA determinations are probably more reliable in those cases. Based on these data we calculated the ratio of form I-form II RuBPC-O on a protein basis. This ratio doubled in the very early stages of growth and then reached an equilibrium when form I RuBPC-O was about twice as abundant as form II RuBPC-O. Interestingly, this ratio was reversed in cells grown on malate as carbon source (Table 2). Upon transferring cells from heterotrophic to autotrophic growth conditions, the percentage of form I RuBPC-O in the protein extract increased 17-fold, whereas the form II content was augmented only fourfold. The average increase of RuBPC-O protein, about 11-fold, matched quite well with the observed increase of carboxylase specific activity (Table 2). These results suggest that the increase of RuBPC-O activity which occurred upon the imposition of a photolithotrophic gas

TABLE 1. Comparison of form I and form II RuBPC-O levels in *R. sphaeroides* cells grown autotrophically (1.5% CO_2 in H_2)[a]

Time after inoculation (h)	Amt of ribulosebisphosphate carboxylase (μg)/100 μg of cell protein by indicated test			Form I-form II ratio[c]
	Level of form I enzyme by:		Level of form II enzyme by EIA	
	ELISA[b]	EIA		
24	0.72 ± 0.12	1.05	0.83	1.07
30	0.84 ± 0.17	1.36	1.02	1.08
48	4.05 ± 0.5	3.95	1.90	2.10
63.5	6.3 ± 0.7	5.7	2.83	2.12
68	5.9 ± 1.0	6.1	2.90	2.07
73	6.9 ± 1.0	6.9	3.27	2.11
88	7.3 ± 0.5	7.35	3.35	2.18
95	8.0 ± 1.1	7.7	3.41	2.30

[a] The inoculum was taken from a culture grown under photoheterotrophic conditions, with malate as the electron donor.
[b] Values are averages calculated from six replicates.
[c] Calculated as the ratio of micrograms of form I (mean of ELISA and EIA values) over micrograms of form II enzyme.

atmosphere (at low levels of CO_2) was caused by the synthesis of new RuBPC-O protein.

DISCUSSION

In this investigation, we have shown that *R. sphaeroides*, much like *Rhodospirillum rubrum* (18), responded to photolithotrophic growth at low concentrations of gaseous CO_2 by substantially increasing the levels of RuBPC-O. In the case of *R. sphaeroides*, however, a much more complicated situation exists since this organism synthesizes two apparently distinct forms of enzyme (4). The large subunit of the form I enzyme is not readily discernible in one-dimensional SDS gels unless the cells are grown photolithotrophically in a low-CO_2-H_2 atmosphere or photoheterotrophically with a reduced carbon source such as butyric acid as the electron donor (4, 21). The form I large subunit was also undetectable in extracts from malate-grown cells by two-dimensional gel electrophoresis. Recently, it was found that *R. capsulata* also apparently synthesized no detectable form I RuBPC-O in cells grown heterotrophically or with high levels of CO_2 (20). In the present study, however, by using specific and sensitive immunological techniques, we measured substantial amounts of form I RuBPC-O in cells grown photoheterotrophically on malate. Form I polypeptides accounted for about 0.5% of the cell extract protein based on both ELISA and rocket EIA determinations. This relatively low level is likely to explain

TABLE 2. Comparison of RuBPC-O levels in photoheterotrophically grown (malate) and photolithotrophically grown (1.5% CO_2 in H_2) *R. sphaeroides*[a]

Carbon source	Sp act (U/mg of protein)	Amt of antigen (% extract protein)[b]		Form I-form II ratio
		form I	form II	
Malate	0.032 ± 0.01	0.49 ± 0.05	0.92 ± 0.04	0.53
1.5% CO_2 in H_2	0.37 ± 0.05	8.4 ± 0.8	4.0 ± 0.5	2.10

[a] Cells were grown to stationary phase, and extracts were prepared as described in Materials and Methods.
[b] The EIA procedure was used to quantify the amounts of form I and form II RuBPC-O.

the previous inability to detect form I RuBPC-O with less sensitive techniques. These results indicate that form I RuBPC-O synthesis is not completely repressed in heterotrophically grown cells. Under the same growth conditions, form II RuBPC-O is twice as abundant in cell extracts on a protein basis. Upon transfer to a photolithotrophic minimal medium under low CO_2 (1.5%), form I and form II RuBPC-O accumulated up to 17- and fourfold greater amounts than levels in malate-grown cells, respectively, in close correlation with the increase of carboxylase specific activity. Form I RuBPC-O became the more abundant carboxylase species (on a protein basis), presumably because the enzymes are synthesized at different rates. It can be calculated that the form I and form II RuBPC-O intracellular concentrations increased at a rate of 1.6 and 0.5 μg/h per mg of protein, respectively, in the early stages of growth. Although form I RuBPC-O has a larger molecular weight than the form II enzyme (550,000 and 290,000, respectively), the rate of form I RuBPC-O synthesis appears to be twice as high as that of form II RuBPC-O on a molar basis. These results indicate that the two forms of carboxylase present in *R. sphaeroides*, although both derepressed under CO_2 limitation, have somewhat independent mechanisms of regulation. The predominant synthesis of form I RuBPC-O suggests that this enzyme is required for growth at low levels of CO_2. In agreement with this hypothesis, form I RuBPC-O has a relatively higher affinity for CO_2 than the form II enzyme (7; J. L. Gibson and F. R. Tabita, unpublished observations).

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant GM 24497 from the National Institutes of Health, by grant 83-CRCR-1-1344 from the U.S. Department of Agriculture, and by grant F-691 from the Robert A. Welch Foundation. Y.J. also acknowledges the award of a NATO fellowship.

LITERATURE CITED

1. **Bassett, C. L., C. A. Rinehart, and J. R. W. Rawson.** 1985. Immunological determination of phosphoenolpyruvate carboxylase and the large and small subunits of ribulose 1,5-bisphosphate carboxylase in leaves of the C_4 plant pearl millet. Plant Physiol. **77:**828–832.
2. **Braatz, J. A., and K. R. McIntire.** 1977. A rapid and efficient method for the isolation of proteins from polyacryl-amide gels. Prep. Biochem. **7:**495–509.
3. **Fornari, C. S., and S. Kaplan.** 1983. Identification of nitrogenase and carboxylase genes in the photosynthetic bacteria and cloning of a carboxylase gene from *Rhodopseudomonas sphaeroides*. Gene **25:**291–299.
4. **Gibson, J. L., and F. R. Tabita.** 1977. Different molecular forms of D-ribulose 1,5-bisphosphate carboxylase from *Rhodopseudomonas sphaeroides*. J. Biol. Chem. **252:**943–949.
5. **Gibson, J. L., and F. R. Tabita.** 1977. Isolation and preliminary characterization of two forms of ribulose 1,5-bisphosphate carboxylase from *Rhodopseudomonas capsulata*. J. Bacteriol. **132:**818–823.
6. **Gibson, J. L., and F. R. Tabita.** 1977. Characterization of antiserum directed against form II ribulose 1,5-bisphosphate carboxylase from *Rhodopseudomonas sphaeroides*. J. Bacteriol. **131:**1020–1022.

6a. **Gibson, J. L., and F. R. Tabita.** 1985. Structural differences in the catalytic subunits of form I and form II ribulose 1,5-bisphosphate carboxylase/oxygenase from *Rhodopseudomonas sphaeroides*. J. Bacteriol. **164:**1188–1193.

7. **Jordan, D. B., and W. L. Orgren.** 1981. Species variation in the specificity of ribulose bisphosphate carboxylase/oxygenase. Nature (London) **291:**513–515.
8. **Jouanneau, Y., B. Wong, and P. M. Vignais.** 1985. Stimulation by light of nitrogenase synthesis in cells of *Rhodopseudomonas capsulata* growing in N-limited continuous cultures. Biochim. Biophys. Acta **808:**149–155.
9. **Laurell, C. B.** 1966. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal. Biochem. **15:**45–52.
10. **Lugtenberg, B., T. Meijers, R. Peters, P. van der Hoek, and L. van Alpen.** 1975. Electrophoretic resolution of the "major outer membrane protein" of *E. coli* into bands. FEBS Lett. **58:**254–258.
11. **Muller, E. D., J. Chory, and S. Kaplan.** 1985. Cloning and characterization of the gene product of the form II ribulose-1,5-bisphosphate carboxylase gene of *Rhodopseudomonas sphaeroides*. J. Bacteriol. **161:**469–472.
12. **O'Farrell, P. H.** 1975. High resolution two-dimensional electrophoresis of proteins. J. Biol. Chem. **250:**4007–4021.
13. **Ormerod, J. G., K. D. Ormerod, and H. Gest.** 1961. Light dependent utilization of organic compounds and photoproduction of hydrogen by photosynthetic bacteria: relationships with nitrogen metabolism. Arch. Biochem. Biophys. **94:**449–463.
14. **Peterson, G. L.** 1977. A simplification of the protein assay method of Lowry *et al.* which is more generally applicable. Anal. Biochem. **83:**346–356.
15. **Plumley, F. G., and G. W. Schmidt.** 1983. Rocket and crossed immunoelectrophoresis of proteins solubilized with sodium dodecyl sulfate. Anal. Biochem. **134:**86–95.
16. **Quivey, R. G., Jr., and F. R. Tabita.** 1984. Cloning and expression in *Escherichia coli* of the form II ribulose 1,5-bisphosphate carboxylase/oxygenase gene from *Rhodopseudomonas sphaeroides*. Gene **31:**91–101.
17. **Rodbard, D., and D. M. Hutt.** 1974. Statistical analysis of radioimmunoassay and immunoradiometric (labelled antibody) assays, p. 165–192. *In* Radioimmunoassay and related procedures in biology and medicine, vol. 1. International Atomic Energy Agency, Vienna.
18. **Sarles, L. S., and F. R. Tabita.** 1983. Derepression of the synthesis of D-ribulose 1,5-bisphosphate carboxylase/oxygenase from *Rhodospirillum rubrum*. J. Bacteriol. **153:**458–464.
19. **Schloss, J. V., E. F. Phares, M. V. Long, I. L. Norton, C. D. Stringer, and F. C. Hartman.** 1979. Isolation, characterization, and crystallization of ribulosebisphosphate carboxylase from autotrophically grown *Rhodospirillum rubrum*. J. Bacteriol. **137:**490–501.
20. **Shively, J. M., E. Davidson, and B. L. Marrs.** 1984. Derepression of the synthesis of the intermediate and large forms of ribulose 1,5-bisphosphate carboxylase/oxygenase in *Rhodopseudomonas capsulata*. Arch. Microbiol. **138:**233–236.
21. **Tabita, F. R.** 1981. Molecular regulation of carbon dioxide assimilation in autotrophic microorganisms, p. 70–81. *In* H. Dalton (ed.), Microbial growth on C1 compounds. Heydon & Sons, London.
22. **Tabita, F. R., P. Caruso, and W. Whitman.** 1978. Facile assay of enzymes unique to the Calvin cycle in intact cells, with special reference to ribulose 1,5-bisphosphate carboxylase. Anal. Biochem. **84:**462–472.
23. **Watters, C.** 1978. A one-step biuret assay for protein in the presence of detergent. Anal. Biochem. **88:**695–698.
24. **Weaver, K. E., and F. R. Tabita.** 1983. Isolation and partial characterization of *Rhodopseudomonas sphaeroides* mutants defective in the regulation of ribulose bisphosphate carboxylase/oxygenase. J. Bacteriol. **156:**507–515.
25. **Whitman, W., and F. R. Tabita.** 1976. Inhibition of ribulose 1,5-bisphosphate carboxylase by pyridoxal 5'-phosphate. Biochem. Biophys. Res. Commun. **71:**1034–1039.