JOURNAL OF BACTERIOLOGY, Nov. 1979, p. 452-458
0021-9193/79/11-0452/07$02.00/0

Vol. 140, No. 2

Carbon Dioxide Assimilation in Cyanobacteria: Regulation of Ribulose 1,5-Bisphosphate Carboxylase

F. ROBERT TABITA* AND CLARE COLLETTI

Department of Microbiology, The University of Texas at Austin, Austin, Texas 78712

Received for publication 28 June 1979

Cyanobacteria assimilate carbon dioxide through the Calvin cycle and therefore must regulate the activity of ribulose 1,5-bisophosphate carboxylase. Using an in situ assay, as well as measuring the activity in crude, partially purified, and homogeneous preparations, we can show that a number of phosphorylated intermediates exert a regulatory role. Three diverse organisms, *Agmenellum quadruplicatum, Aphanocapsa* 6714, and *Anabaena* sp. CA, were studied, and it was found that the in situ and cell-free carboxylase activities were particularly affected by low levels of phosphogluconate and reduced nicotinamide adenine dinucleotide phosphate. There was a marked activation by these ligands when the inactive enzyme was assayed in the presence of low levels of bicarbonate, a result significantly different from a previous report. Moreover, the fully activated enzyme was inhibited by phosphogluconate. In situ *Anabaena* CA carboxylase activity exhibited a particular capacity for activation by phosphogluconate and reduced nicotinamide adenine dinucleotide phosphate. However, activation of the crude, partially purified, or homogeneous *Anabaena* CA carboxylase by phosphogluconate and reduced nicotinamide adenine dinucleotide phosphate was significantly decreased when compared with enzyme activity in permeabilized cells. It appears that the microenvironment or the conformation of the enzyme within the cell may be significantly different from that of the isolated enzyme.

Cyanobacteria (blue-green algae) form organic matter by assimilating carbon dioxide. The enzyme which catalyzes this primary CO_2 assimilation is ribulose 1,5-bisphosphate (Rbu-P_2) carboxylase (3-phospho-D-glycerate carboxylase [dimerizing]; EC 4.1.1.39). Various phosphorylated intermediates, particularly those characteristic of photosynthetic metabolism, have been shown to either inhibit or activate the carboxylase. Inhibition by 6-phosphogluconate, NADPH, or fructose bisphosphate is pronounced when the enzyme is activated by preincubation at high levels of bicarbonate (20 to 50 mM) in the presence of magnesium (3, 7, 21). In contrast, upon incubation at low levels of bicarbonate (about 1 mM), the carboxylase is relatively inactive, and phosphorylated metabolites (particularly NADPH and phosphogluconate) markedly activate the inactive enzyme if included in the preincubation mixture (4-6, 8, 9, 15, 18). Such behavior yielded a plausible hypothesis for the physiological regulation of this key enzyme based upon the known concentrations and fluctuations of these metabolites within the chloroplast (1, 14).

The cyanobacteria are procaryotic organisms that resemble the chloroplasts of photosynthetic eucaryotes in that they possess the capacity to split water and thus evolve oxygen via photosystem II-directed photosynthesis. Moreover, kinetic studies of $^{14}CO_2$ fixation and ^{32}P incorporation into *Aphanocapsa* strains 6308 and 6714 (16, 17) showed a rapid increase in the concentration of phosphogluconate and changes in the concentrations of sugar mono- and diphosphates when cells were transferred from the light to the dark. This transition also caused an immediate cessation of biosynthetic CO_2 fixation. Thus, since phosphogluconate was found to be a potent competitive inhibitor for Rbu-P_2 carboxylase and since the levels increased so dramatically in the dark, it was proposed that this metabolite might be the physiological regulator preventing CO_2 fixation in the dark (21). Indeed, preliminary studies did indicate that the cyanobacterial enzyme is inhibited by phosphogluconate at high bicarbonate concentrations (21), a result recently corroborated by Codd and Stewart (10). However, Codd and Stewart found that the *Aphanocapsa* 6308 enzyme was not activated by phosphogluconate at 1 mM HCO_3^-, suggesting that the cyanobacterial enzyme might be unique in its activation properties, despite the structural resemblance of the *Aphanocapsa* enzyme to the

higher plant carboxylase (10). With these results in mind, we sought in this study to investigate further the regulatory properties of the cyanobacterial Rbu-P_2 carboxylase. We examined the properties of the enzyme within the cell, using a recently devised whole-cell carboxylase assay (20), as well as the properties of crude, partially purified, and homogeneous carboxylases, to examine the possibility that the basic regulatory properties of the enzyme might change upon isolation or purification or both.

MATERIALS AND METHODS

Growth of organisms. Two unicellular organisms and one filamentous cyanobacterium were used in the present investigation. *Agmenellum quadruplicatum* strain PR-6 (ATCC 27264), a marine coccoid organism, and *Aphanocapsa* strain 6714 (ATCC 27178) were the unicellular organisms employed; *Anabaena* strain CA (ATCC 33047) is a filamentous organism capable of high rates of growth and nitrogen fixation (19). Strain PR-6 was grown in ASP-2 medium at 39°C according to the procedures described previously (26). Strain 6714 was grown in Cg-10 medium (28) at 30°C. *Anabaena* CA was cultured under nitrogen-fixing conditions at 39°C as reported previously (19). All cultures were bubbled with 1% CO_2 in air for maximum and reproducible growth rates, which were generation times of 3.5 h for PR-6, 6 h for 6714, and 4.5 h for CA. All cultures were illuminated by four F36T12/D/HO fluorescent lights, two on each side of the culture bath, 15 cm from the lamp center to the growth tube center, at an incident light intensity of approximately 15,500 ergs/cm^2 × s.

Preparation of cells. Logarithmic-phase cells were harvested by centrifugation, washed in a 10 mM 4-morpholinepropanesulfonic acid (MOPS)–KOH–1 mM EDTA buffer at pH 7.8, and suspended in 100 mM MOPS–KOH (pH 7.8) containing 10 mM EDTA. Cells were usually concentrated from 5- to 10-fold over the density of the original culture. The procedures for toluene treatment and subsequent assay of whole cells were exactly as previously described (20).

Preparation of cell extracts. Cells were harvested and washed once in distilled water. The cells were suspended in 2 volumes of 20 mM Tris-SO_4, (pH 8.0) containing 1 mM EDTA, 5 mM 2-mercaptoethanol, 10 mM $MgCl_2 \cdot 6H_2O$, and 50 mM $NaHCO_3$ (TEMMB buffer) and passed twice through a chilled French pressure cell at 20,000 lb/in^2. The extract was then centrifuged for 15 min at 27,000 × *g*, and the pellet was discarded. The supernatant fluid was subsequently centrifuged at 100,000 × *g* for 60 min, and the resulting membrane fraction was discarded. The 100,000 × *g* supernatant fraction was the source of crude Rbu-P_2 carboxylase and was used in several experiments after dialysis against a large excess of TEMMB buffer. In some cases, the crude Rbu-P_2 carboxylase was acid precipitated as described earlier (25) to remove virtually all phycocyanin.

Purification of Rbu-P_2 carboxylase. Rbu-P_2 carboxylase was purified from extracts of *Anabaena* CA by centrifuging dialyzed ammonium sulfate fractions into a sucrose density gradient (0.2 to 0.8 M) (22, 25, 26). The enzyme was homogeneous by the criterion of polyacrylamide disc gel electrophoresis and was found to contain two subunits upon dissociation and subsequent electrophoresis in gels polymerized with sodium dodecyl sulfate. This preparation of homogeneous carboxylase had a specific activity of 0.6 μmol of CO_2 fixed per min per mg of enzyme when fully activated and assayed for 5 min at 30°C. The ratio of the absorbance at 280 nm to the absorbance at 260 nm was about 1.9, indicating the absence of nucleic acids or nucleotides.

Enzyme assay. The assay for Rbu-P_2 carboxylase, using either whole cells or crude, partially purified, or homogeneous enzyme, was as described earlier (29). Assays were performed in 64 mM MOPS–KOH, pH 7.6, after preincubation for 10 min at 30°C in the presence of this buffer plus 10 mM magnesium acetate, 1 mM EDTA, and, depending on the experiment, either 1 or 20 mM $NaHCO_3$. After the preincubation, toluene-treated whole cells, crude enzyme, or purified enzyme was assayed at either 1 or 20 mM $NaHCO_3$ by initiating the reaction with 0.8 mM Rbu-P_2. Effectors at the indicated concentrations were present during the preincubation and in the assay.

Recovery of reduced pyridine nucleotide. To determine whether NADPH was oxidized during the incubation with toluene-treated whole cells, the following experiment was performed. Test tube cultures (30 ml each) of strains PR-6, 6714, and CA were grown to absorbances at 650 nm of 0.9, 2.5, and 1.7, respectively. The cells were washed, suspended, and treated with toluene as previously described (20), and 180 μl of these cells was added (to yield a total volume of 2.25 ml) to a solution containing 61.6 mM MOPS–KOH (pH 7.6), 10 mM magnesium acetate, 20 mM $NaHCO_3$, 0.8 mM Rbu-P_2, and 0.6 mM NADPH at 30°C. Another 180-μl batch of cells was added to the same mixture in the absence of NADPH. At the desired time intervals, 250 μl of the cell suspension was removed from each incubation tube and immediately chilled in an ice bath.These 250-μl samples were then centrifuged in a Clay Adams Sero-Fuge (Parsippany, N.J.) centrifuge to pellet the cells. The supernatant was carefully removed, and 200 μl of it was added to 800 μl of 10 mM MOPS–KOH buffer, pH 7.6. For each time interval, the cell supernatant from cells incubated in the absence of NADPH served as a blank for the cell supernatant incubated in the presence of NADPH. The absorbance of the solution at 340 nm was used to determine the extent of NADPH oxidation; the concentration of NADPH remaining was calculated from the extinction coefficient, 6.22 cm^2/μmol.

RESULTS

The development of the whole-cell Rbu-P_2 carboxylase assay (20) allows the measurement of the activity and regulation of this key enzyme within its native environment, that is, within the cell, although it is undoubtedly true that some metabolites leak out of the cell. Nevertheless, it is obvious that macromolecular constituents remain associated with the cell (11, 13) and that certain low-molecular-weight metabolites also

are retained since there are some endogenous whole-cell phosphoribulokinase and Rbu-P_2 carboxylase activities in the absence of added sugar phosphate (≤2%) (20; unpublished observations). Thus, the whole-cell assay is the most convenient and accurate method for measuring activity in the absence of potential artifacts of isolation. In all cases, the data discussed in this investigation refer to Rbu-P_2-dependent CO_2 fixation.

With all three organisms studied, carboxylase activity was obviously activated by many of the phosphorylated intermediates at 1 mM bicarbonate (Table 1). In particular, phosphogluconate and NADPH were the most effective regulator metabolites, with the *Anabaena* CA enzyme under the greatest metabolic control, i.e., approximately four- and fivefold stimulation by phosphogluconate and NADPH, respectively. Other differences exist: 40 μM phosphogluconate activated the *Agmenellum* and *Anabaena* enzymes more strongly than did the higher concentration of 1 mM, whereas the opposite was true for the *Aphanocapsa* enzyme; fructose bisphosphate had little effect on the *Aphanocapsa* enzyme; ATP, ADP, and AMP were all effective regulators at 1 mM for the *Anabaena* carboxylase, whereas for the other organisms, ADP at 1 mM was effective only for the *Agmenellum* enzyme. NAD or NADH (data not shown) were without significant effect.

When the enzyme was fully activated at 20 mM bicarbonate, the regulation by these metabolites markedly changed, consistent with results previously obtained for the higher plant enzyme (7). Phosphogluconate and NADPH inhibited, as did fructose 1,6-bisphosphate and fructose-6-phosphate. ATP at 1 mM inhibited the *Agmenellum* enzyme somewhat, activated the *Anabaena* enzyme, and had virtually no effect on the *Aphanocapsa* enzyme. ADP and AMP were slightly inhibitory for the *Agmenellum* and *Aphanocapsa* enzymes, whereas NAD had no effect in all cases (Table 1).

The results in Table 1 show that NADPH and phosphogluconate obviously are important regulator ligands. Thus, subsequent experiments were directed at more closely defining the action of these metabolites. Figure 1 depicts the concentration dependence of phosphogluconate on

TABLE 1. *Regulation of whole-cell Rbu-P_2 carboxylase activity*[a]

Effector[b]	Concn (mM)	Activity (%)[c]					
		Agmenellum strain PR-6		*Aphanocapsa* strain 6714		*Anabaena* strain CA	
		20 mM HCO_3^-	1 mM HCO_3^-	20 mM HCO_3^-	1 mM HCO_3^-	20 mM HCO_3^-	1 mM HCO_3^-
PGN	1	29	148	45	180	53	342
	0.04	91	195	89	161	100	391
NADPH	1	60	196	77	206	79	532
	0.04	85	116	99	101	117	223
NADP	1	85	161	109	110	100	190
	0.04	95	116	100	103	100	90
FDP	1	72	162	89	129	73	196
	0.04	109	131	90	113	105	122
F6P	1	65	121	74	112	66	148
	0.04	95	105	86	110	103	101
ATP	1	73	116	108	106	131	167
	0.04	90	108	91	107	138	100
ADP	1	83	151	83	104	100	238
	0.04	89	103	97	93	115	105
AMP	1	79	113	90	106	100	219
	0.04	84	113	100	102	123	105
NAD	1	90	103	97	103	100	95
	0.04	94	103	97	114	108	100

[a] Cells were harvested, washed, and toluene-treated as described in the text. One hundred percent activity represents control without effector.

[b] Effector ligands were incubated with the toluene-treated cells for 10 min at 30°C at the appropriate concentration of bicarbonate in the presence of 20 mM magnesium acetate. Carboxylase activity was initiated with 0.8 mM Rbu-P_2, and the reaction was terminated after 5 min. PGN, 6-Phospho-D-gluconate; FDP, fructose 1,6-bisphosphate; F6P, fructose-6-phosphate.

[c] The control activities are as follows: for strain PR-6, 0.25 and 4.9 nmol of CO_2 fixed per assay at 1 and 20 mM HCO_3^-, respectively; for strain 6714, 0.125 and 1.28 nmol of CO_2 fixed per assay at 1 and 20 mM HCO_3^-, respectively; and for strain CA, 5.85 and 0.42 nmol of CO_2 fixed per assay at 20 and 1 mM HCO_3^-, respectively.

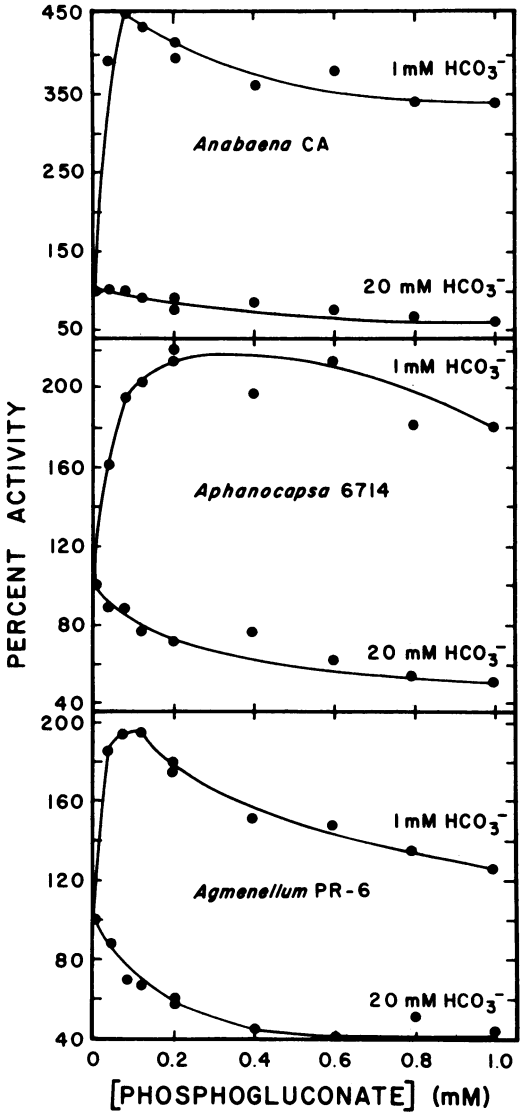

FIG. 1. *Regulation of Rbu-P2 carboxylase by phosphogluconate in permeabilized cyanobacteria at 1 and 20 mM HCO_3^-. One hundred percent activities are as follows: for strain PR-6, 3.8 and 0.20 nmol of CO_2 fixed per assay at 20 and 1 mM HCO_3^-, respectively; for strain 6714, 1.64 and 0.4 nmol of CO_2 fixed per assay at 20 and 1 mM HCO_3^-, respectively; and for strain CA, 2.9 and 0.14 nmol of CO_2 fixed per assay at 20 and 1 mM HCO_3^-, respectively.*

whole-cell Rbu-P_2 carboxylase from the three organisms at both 1 and 20 mM bicarbonate. In agreement with the results of Table 1, the *Anabaena* CA carboxylase was more effectively activated at 1 mM bicarbonate than were the enzymes from *Agmenellum* and *Aphanocapsa;* we observed that the maximum extent of activation for the *Anabaena* enzyme at 80 μM phosphogluconate was 4.5-fold, whereas the *Aphanocapsa* and *Agmenellum* enzymes were activated approximately 2-fold. Moreover, the *Aphanocapsa* enzyme was maximally activated over a range of phosphogluconate concentrations (0.2 to 0.6 mM), whereas both the *Anabaena* and *Agmenellum* enzymes showed rather sharp peaks of activation at about 0.08 to 0.12 mM (Fig. 1). Again, in agreement with earlier findings (10, 21) and the results shown in Table 1, the whole-cell activities of all three enzymes were inhibited by phosphogluconate when the enzyme was fully activated, that is, when it was preincubated and assayed in the presence of 20 mM bicarbonate (Fig. 1).

The concentration dependence of NADPH regulation on whole-cell carboxylase activity is shown in Fig. 2. With all three organisms, the activation at 1 mM bicarbonate showed a saturation effect, with maximum activity at about 1 mM NADPH. Again, the whole-cell activity of *Anabaena* CA was the most strongly regulated, reaching over a fivefold stimulation as compared with about a twofold activation of the *Agmenellum* and *Aphanocapsa* whole-cell carboxylase activities. That NADPH was probably the actual pyridine nucleotide responsible for the regulatory effects noted in Table 1 and Fig. 2 was shown by a recovery experiment in which toluene-treated cyanobacteria failed to metabolize or otherwise oxidize this nucleotide. Moreover, when NADP and NADPH were tested over a wide range of equimolar concentrations, the *Anabaena* CA carboxylase was clearly preferentially activated by the reduced pyridine nucleotide. In addition, experiments performed in absolute darkness gave the same results as assays performed under normal laboratory lighting conditions.

Regulation of crude, partially purified, and homogeneous Rbu-P_2 carboxylases. To supplement the whole-cell regulatory studies, we performed experiments first with crude 100,000 × *g* supernatant fractions and second with enzyme that had been partially purified through the acid precipitation step. Apparently, acidification causes an alteration in the quaternary structure of the *Aphanocapsa* 6308 enzyme (10). However, NADPH and phosphogluconate had identical effects upon crude or partially purified carboxylases from each organism. Corroborating the whole-cell data, NADPH and phosphogluconate significantly activated the enzymes, particularly the *Anabaena* CA enzyme, at 1 mM bicarbonate (Table 2). Interestingly, the magnitude of the activation observed in this experiment was considerably less than that obtained

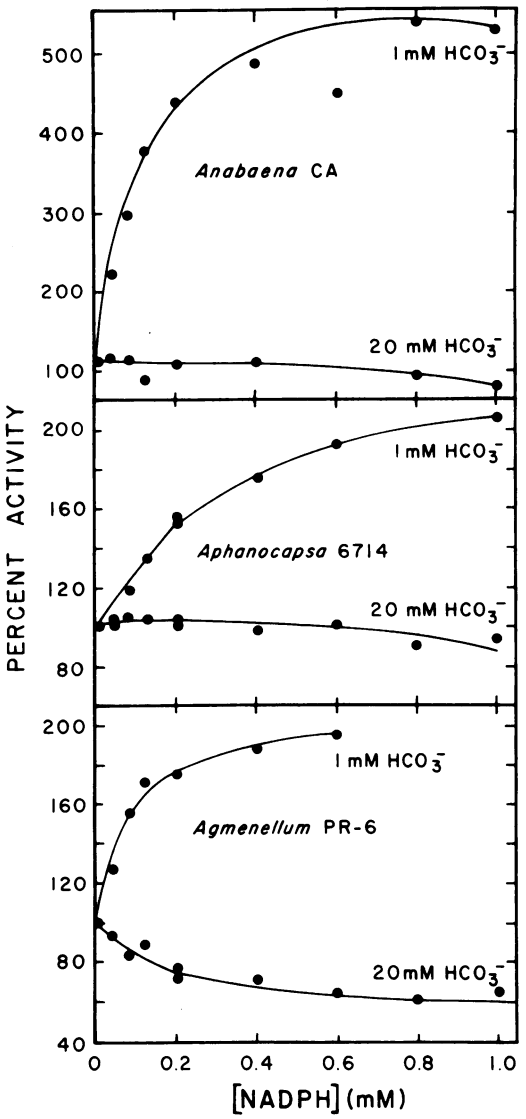

FIG. 2. *Regulation of Rbu-P₂ carboxylase by NADPH in permeabilized cyanobacteria at 1 and 20 mM HCO_3^-. One hundred percent activities are as follows: for strain CA, 7.6 and 0.38 nmol of CO_2 fixed per assay at 20 and 1 mM HCO_3^-, respectively; for strain 6714, 6.0 and 0.41 nmol of CO_2 fixed per assay at 20 and 1 mM HCO_3^-, respectively; and for strain PR-6, 2.72 and 0.35 nmol of CO_2 fixed per assay at 20 and 1 mM HCO_3^-, respectively.*

with permeabilized *Anabaena* CA cells (Table 1 and Fig. 1 and 2). Low concentrations (80 μM) of phosphogluconate were slightly inhibitory to the *Agmenellum* and *Anabaena* CA crude and partially purified enzymes when assayed at 20 mM HCO_3^-. To further corroborate the regulatory effects noted with permeabilized cells and cell extracts, the homogeneous Rbu-P_2 carboxylase from *Anabaena* CA was activated by both phosphogluconate and NADPH at 1 mM bicarbonate, whereas only phosphogluconate concentrations above 0.6 mM were inhibitory at 20 mM bicarbonate (Fig. 3). NADPH had no effect when assayed in the presence of 20 mM bicarbonate. Again, the extent of activation seen at 1 mM bicarbonate did not approach that obtained with toluene-treated whole cells.

DISCUSSION

The regulation of Rbu-P_2 carboxylase activity has provoked considerable interest since it is the first enzyme in the competing pathways of photosynthetic carbon dioxide assimilation and photorespiratory carbon dioxide dissipation. The higher plant enzyme has been shown to be modulated by several metabolites common to intermediary metabolism, metabolites which either activate or inhibit activity, depending on whether the enzyme is in a conformationally active or inactive state (7). Both the activation and inhibition sites are localized on the large subunits, since the *Rhodospirillum rubrum* Rbu-P_2 carboxylase, a dimeric protein devoid of small subunits, is similar to the structurally complex plant enzyme in its regulatory response, with the exception of a lack of inhibition by up to 1 mM phosphogluconate at 20 mM bicarbonate (6, 23; W. B. Whitman, M. N. Martin, and F. R. Tabita, J. Biol. Chem., in press). Recently, it was shown that several cyanobacterial carboxylases structurally resemble the plant enzyme and are comprised of both large and small subunits (10, 27). It was also reported that the acid precipitation step previously used in the purification of the cyanobacterial enzyme is responsible for the lack of small subunits previously noted (10). Certainly, the nonessentiality of small subunits in catalysis is thus confirmed (23; Whitman et al., in press).

Despite the structural similarity of the *Aphanocapsa* carboxylase to the enzyme from leaf tissue of higher plants, Codd and Stewart (10) were unable to demonstrate activation by phosphogluconate when the enzyme was in its inactive state, i.e., when incubated and assayed in the presence of 1 mM bicarbonate. They were able, however, to demonstrate phosphogluconate inhibition at a high concentration (20 mM) of bicarbonate. Indeed, inhibition was enhanced at 1 mM bicarbonate after up to 5 min of preincubation in the presence of phosphogluconate (10). Thus, the enzyme from *Aphanocapsa* 6308 appeared anomalous in its response to at least one effector at a low concentration of bicarbonate, but responded similarly to other cyanobacterial (21), plant (3, 5, 7), and bacterial (12, 18, 21, 24) carboxylases at a high concentration of

TABLE 2. *Regulation of crude and partially purified cyanobacterial Rbu-P_2 carboxylase*[a]

Effector[b]	Activity (%)[c]					
	Agmenellum PR-6		*Aphanocapsa* 6714		*Anabaena* CA	
	20 mM HCO_3^-	1 mM HCO_3^-	20 mM HCO_3^-	1 mM HCO_3^-	20 mM HCO_3^-	1 mM HCO_3^-
NADPH	ND[d]	165	99	167	112	374
NADP	97	109	107	116	102	169
Phosphogluconate	73	157	104	141	90	333

[a] Experiments with strain PR-6 are the average of duplicate assays performed with five different crude extracts; for strain 6714, duplicate assays were performed with five different crude extracts and four distinct preparations purified through the acid precipitation step (25); experiments with strain CA are the average of duplicate assays performed with six different crude extracts and six distinct preparations partially purified through the acid precipitation step (25).

[b] NADPH and NADP are at 1 mM, and phosphogluconate is at 80 μM.

[c] The control activities are as follows: for strain PR-6, 0.28 and 1.62 nmol of CO_2 fixed per assay at 1 and 20 mM HCO_3^-, respectively; for strain 6714, 2.5 and 19.3 nmol of CO_2 fixed per assay at 1 and 20 mM HCO_3^-, respectively; and for strain CA, 0.19 and 7.0 nmol of CO_2 fixed per assay 1 and 20 mM, respectively.

[d] ND, Not determined.

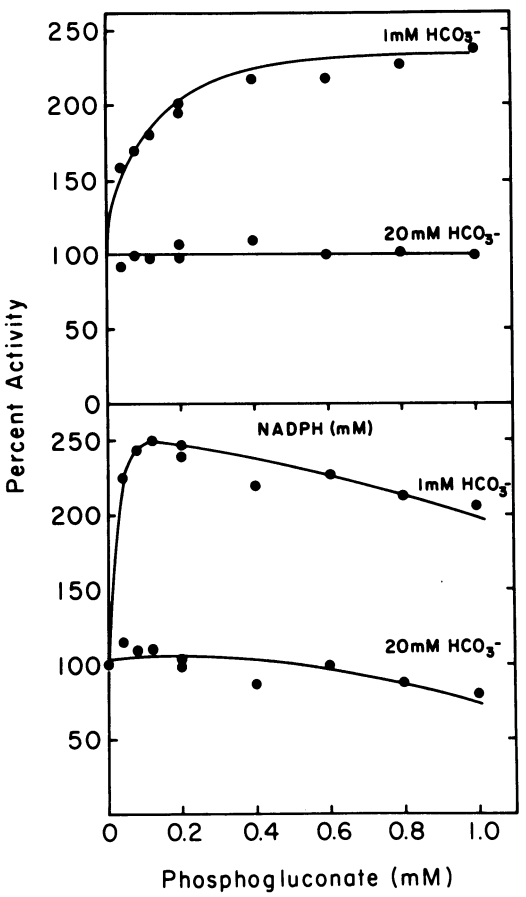

FIG. 3. *Regulation of homogeneous Anabaena CA Rbu-P_2 carboxylase by NADPH and phosphogluconate at 1 and 20 mM HCO_3^-. One hundred percent activities are 6.08 and 0.56 nmol of CO_2 fixed per assay at 20 and 1 mM HCO_3^-, respectively.*

bicarbonate. Unless the carboxylases from *Aphanocapsa* strains 6308 and 6714 are not comparable, the low specific activity of the *Aphanocapsa* preparation (10) (0.04 μmol of CO_2 fixed per min per mg when calculated from the activity shown for homogeneous fractions of the sucrose gradient, assuming that an optical density at 280 nm of 1 is about 1 mg of protein per ml) may be responsible for this unusual behavior.

Previous studies have shown that permeabilized whole cells of a variety of photosynthetic species catalyze a stoichiometric conversion of Rbu-P_2 to phosphoglyceric acid, and the total activity measured with these cells closely approximates the activity obtained with crude extracts (20). Thus, this mode of assay allows the investigator to measure carboxylase activity in situ. Interestingly, in this study, we clearly show that the in situ *Anabaena* CA Rbu-P_2 carboxylase activity is significantly more sensitive to carboxylase effectors than is the homogeneous or freshly prepared crude enzyme. This increased sensitivity to phosphogluconate and NADPH is not an artifact of the assay system, but may reflect the microenvironment of the enzyme in situ. Thus, the regulatory response with permeabilized *Anabaena* CA cells resembles the enhanced allosteric regulation of both threonine deaminase and acetohydroxy acid synthase obtained with permeabilized *Escherichia coli* K-12 (2). The basis for the apparent enhanced regulation of in situ *Anabaena* CA Rbu-P_2 carboxylase activity is under investigation. Certainly, it is clear that fluctuations in the cellular level of key metabolites such as phosphogluconate and NADPH may exert a fine control over CO_2 assimilation in the cyanobacteria.

ACKNOWLEDGMENTS

This investigation was supported by National Science Foundation grant PCM 78-15111 and Public Health Service grant GM 24497 from the National Institutes of Health.

LITERATURE CITED

1. **Bassham, J. A., and M. Kirk.** 1968. Dynamic metabolic regulation of the photosynthetic carbon reduction cycle, p. 365–378. *In* K. Shibata, A. Takamiya, A. T. Jagendorf, and R. C. Fuller (ed.), Comparative biochemistry and biophysics of photosynthesis. University of Tokyo Press, Tokyo.
2. **Blatt, J. M., and J. H. Jackson.** 1978. Enhanced allosteric regulation of threonine deaminase and acetohydroxy acid synthase from *Escherichia coli* in a permeabilized-cell assay system. Biochim. Biophys. Acta **526**: 267–275.
3. **Bowes, G., and W. L. Ogren.** 1972. Oxygen inhibition and other properties of soybean ribulose 1,5-diphosphate carboxylase. J. Biol. Chem. **247**:2171–2176.
4. **Buchanan, B. B., and P. Schurmann.** 1973. Regulation of ribulose 1,5-diphosphate carboxylase in the photosynthetic assimilation of carbon dioxide. J. Biol. Chem. **248**:4956–4964.
5. **Chollet, R., and L. L. Anderson.** 1976. Regulation of ribulose 1,5-bisphosphate carboxylase-oxygenase activities by temperature pretreatment and chloroplast metabolites. Arch. Biochem. Biophys. **176**:344–351.
6. **Christeller, J. T., and W. A. Laing.** 1978. A kinetic study of ribulose bisphosphate carboxylase from the photosynthetic bacterium *Rhodospirillum rubrum*. Biochem. J. **173**:467–473.
7. **Chu, D. K., and J. A. Bassham.** 1973. Activation and inhibition of ribulose 1,5-diphosphate carboxylase by 6-phosphogluconate. Plant Physiol. **52**:373–379.
8. **Chu, D. K., and J. A. Bassham.** 1974. Activation of ribulose 1,5-diphosphate carboxylase by nicotinamide adenine dinucleotide phosphate and other chloroplast metabolites. Plant Physiol. **54**:556–559.
9. **Chu, D. K., and J. A. Bassham.** 1975. Regulation of ribulose 1,5-diphosphate carboxylase by substrates and other metabolites. Plant Physiol. **55**:720–726.
10. **Codd, G. A., and W. D. P. Stewart.** 1977. D-Ribulose 1,5-diphosphate carboxylase from the blue-green alga *Aphanocapsa* 6308. Arch. Microbiol. **113**:105–110.
11. **DeSmet, M. J., J. Kingma, and B. Witholt.** 1978. The effect of toluene on the structure and permeability of the outer and cytoplasmic membranes of *Escherichia coli*. Biochim. Biophys. Acta **506**:64–80.
12. **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.
13. **Jackson, R. W., and J. A. DeMoss.** 1965. Effects of toluene on *Escherichia coli*. J. Bacteriol. **90**:1420–1425.
14. **Jensen, R. G., and J. T. Bahr.** 1977. Ribulose 1,5-bisphosphate carboxylase-oxygenase. Annu. Rev. Plant Physiol. **28**:379–340.
15. **Lawlis, V. B., G. L. R. Gordon, and B. A. McFadden.** 1978. Regulation of activation of ribulose bisphosphate carboxylase from *Pseudomonas oxalaticus*. Biochem. Biophys. Res. Commun. **84**:699–705.
16. **Pelroy, R. A., and J. A. Bassham.** 1972. Photosynthetic and dark carbon metabolism in unicellular blue-green algae. Arch. Mikrobiol. **86**:25–38.
17. **Pelroy, R. A., G. A. Levine, and J. A. Bassham.** 1976. Kinetics of light-dark CO_2 fixation and glucose assimilation by *Aphanocapsa* 6714. J. Bacteriol. **128**:633–643.
18. **Purohit, K., and B. A. McFadden.** 1979. Ribulose 1,5-bisphosphate carboxylase and oxygenase from *Thiocapsa roseopersicina*: activation and catalysis. Arch. Biochem. Biophys. **194**:101–106.
19. **Stacey, G., C. Van Baalen, and F. R. Tabita.** 1977. Isolation and characterization of a marine *Anabaena* sp. capable of rapid growth on molecular nitrogen. Arch. Microbiol. **114**:197–201.
20. **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.
21. **Tabita, F. R., and B. A. McFadden.** 1972. Regulation of ribulose 1,5-diphosphate carboxylase by 6-phospho-d-gluconate. Biochem. Biophys. Res. Commun. **48**:1153–1160.
22. **Tabita, F. R., and B. A. McFadden.** 1974. One-step isolation of microbial ribulose 1,5-diphosphate carboxylase. Arch. Mikrobiol. **99**:231–240.
23. **Tabita, F. R., and B. A. McFadden.** 1974. D-Ribulose 1,5-diphosphate carboxylase from *Rhodospirillum rubrum*. I. Levels, purification and effects of metallic ions. J. Biol. Chem. **249**:3453–3459.
24. **Tabita, F. R., and B. A. McFadden.** 1976. Molecular and catalytic properties of ribulose 1,5-bisphosphate carboxylase from the photosynthetic extreme halophila *Ectothiorhodospira halophila*. J. Bacteriol. **126**:1271–1277.
25. **Tabita, F. R., S. E. Stevens, Jr., and J. L. Gibson.** 1976. Carbon dioxide assimilation in blue-green algae: initial studies on the structure of ribulose 1,5-bisphosphate carboxylase. J. Bacteriol. **125**:531–539.
26. **Tabita, F. R., S. E. Stevens, Jr., and R. Quijano.** 1974. D-ribulose 1,5-diphosphate carboxylase from blue-green algae. Biochem. Biophys. Res. Commun. **61**:45–52.
27. **Takabe, T., M. Nishimura, and T. Akazawa.** 1976. Presence of two subunit types in ribulose 1,5-bisphosphate carboxylase from blue-green algae. Biochem. Biophys. Res. Commun. **68**:537–544.
28. **Van Baalen, C.** 1967. Further observations on growth of single cells of coccoid blue-green algae. J. Phycol. **3**: 154–157.
29. **Whitman, W., and F. R. Tabita.** 1976. Inhibition of D-ribulose 1,5-bisphosphate carboxylase by pyridoxal 5'-phosphate. Biochem. Biophys. Res. Commun. **71**:1034–1039.