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Short Communication

Photosynthetic Adaptation by *Synechococcus leopoliensis* in Response to Exogenous Dissolved Inorganic Carbon[1]

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ABSTRACT

Synechococcus leopoliensis **was grown over a wide range of dissolved inorganic carbon (DIC) concentrations (4–25,000 micromolar) which were obtained by varying culture pH (6.2–9.6) and the CO_2 concentration of the gas stream (36–50,000 microliters per liter). The [DIC] required to half-saturate photosynthesis ($K_{1/2}^{DIC}$) was found to vary depending upon the ambient DIC concentration at which the cells were grown. Low [DIC] grown cells exhibited low values of $K_{1/2}^{DIC}$ (4.7 micromolar) whereas cells grown at high [DIC] exhibited high values of $K_{1/2}^{DIC}$ (1–2.5 millimolar). Intermediate concentrations of DIC produced intermediate values. Changes in $K_{1/2}^{DIC}$ appeared to be solely a function of [DIC] and were independent of both culture pH and CO_2 concentration. As changes in $K_{1/2}^{DIC}$ occur in response to DIC concentrations commonly found in natural systems we suggest this adaptation may be of ecological significance.**

The photosynthetic properties of algal cells are greatly influenced by the levels of CO_2 on which they are grown. Cells grown on elevated levels of CO_2 (1–5%) exhibit high values of $K_{1/2}^{DIC}$[2] and hence show a much reduced ability to photosynthesize at low DIC when compared to air-grown cells (1, 2, 6, 7). While there is unquestionably a difference in the DIC concentration in cultures growing under either air or high CO_2 bubbling, the actual concentration and form of DIC has either not been reported or not rigorously controlled. Recently, the use of DIC-limited chemostats has provided results showing that CO_2 concentrations above air levels are not required to produce cells with $K_{1/2}^{DIC}$ values typical of 'high CO_2 cells' (11). Under conditions where growth was DIC limited the cells exhibit an average $K_{1/2}^{DIC}$ of 4.7 μM whereas under conditions of DIC-sufficiency the kinetics of photosynthesis with respect to DIC are multiphasic and $K_{1/2}^{DIC}$ values average 1100 μM (11, 17, 18). No steady state intermediates were observed between these extremes.

In the present study we examine the role of exogenous CO_2 and DIC concentration in determining $K_{1/2}^{DIC}$. The results provide some basic information on the adaptive significance associated with changes in $K_{1/2}^{DIC}$ and the role of medium CO_2 concentration in regulating adaptation to low carbon.

MATERIALS AND METHODS

Stock cultures of *Synechococcus leopoliensis* (UTEX 625) were grown axenically as previously described (11). Experimental cultures were grown in a cylindrical, water-jacketed culture flask (160 ml) equipped with a glass fritted bubbler. Culture flasks were equipped with a sampling port closed with a serum stopper thereby enabling sterile sampling. Culture medium (buffered with 50 mM 1,3-bis[tris(hydroxymethyl)methylamino]-propane, BTP) was adjusted to predetermined pH with HCl. Cultures were bubbled (1.6 L/min) with filter sterilized CO_2 free air enriched to a variety of CO_2 concentrations (37 μl CO_2/L, 494 μl CO_2/L, or 50,000 μl CO_2/L) and stirred vigorously with a magnetic stirrer. Cells in midlog phase were harvested by centrifugation, washed twice and resuspended in low [DIC] BTP buffer (15–30 μM), at culture pH, containing 5 mM NaCl (11). The resulting suspension was placed in a Clark type O_2 electrode (Hansatech Ltd., Kings Lynn, Norfolk, U.K.) and the kinetics of photosynthesis with respect to DIC ($K_{1/2}^{DIC}$) were determined as previously described (11, 18). DIC-limited chemostats (pH stat controlled) were established as previously outlined (19).

The theoretical concentration of DIC in the culture medium was calculated using the CO_2 concentration in the gas stream, medium pH, the dissociation constants tabulated by Buch (3) and the equations outlined by Stumm and Morgan (14). Actual DIC concentrations were measured as previously described (11).

RESULTS AND DISCUSSION

The high bubbling rate and vigorous stirring resulted in culture [DIC] which was in close agreement with theoretical predictions (Fig. 1). This indicated that culture [CO_2] was in equilibrium with the gas stream.

P_{max} values ranged from 100 to 500 μmol $O_2 \cdot mg^{-1}$ Chl $a \cdot h^{-1}$ and showed a pH optimum of about 8.2 (data not shown). At [DIC] below about 40 μM, $K_{1/2}^{DIC}$ was low (4.7 ± 0.5 μM) and constant (Fig. 2; 11). At [DIC] above about 2 mM, $K_{1/2}^{DIC}$ was high and unaffected by further increases in carbon. Intermediate levels of [DIC] produced cells with intermediate values of $K_{1/2}^{DIC}$ (Fig. 2). The role of ambient CO_2 concentration in the induction of high affinity kinetics was examined by growing cells at near air levels of CO_2 (494 μl/L) over a range of pH (6.3–9.6). The resulting $K_{1/2}^{DIC}$ values ranged from 56 to 2093 μM, respectively (Fig. 2) indicating that ambient CO_2 concentration *per se* is not a deciding factor in determining $K_{1/2}^{DIC}$. Values for $K_{1/2}^{DIC}$ in the range of 2000 μM have not previously been reported for cells grown at air levels of CO_2. Our ability to generate these cells is a result of maintaining high ambient [DIC] through rapid bubbling (1.6 L/min) and elevated pH. As a control for pH dependent effects we grew *S. leopoliensis* in DIC-limited chemostats (D =

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[2] Abbreviations: $K_{1/2}^{DIC}$, DIC concentration required to half-saturate photosynthesis; [DIC], dissolved inorganic carbon concentration; D, chemostat dilution rate (d^{-1}).

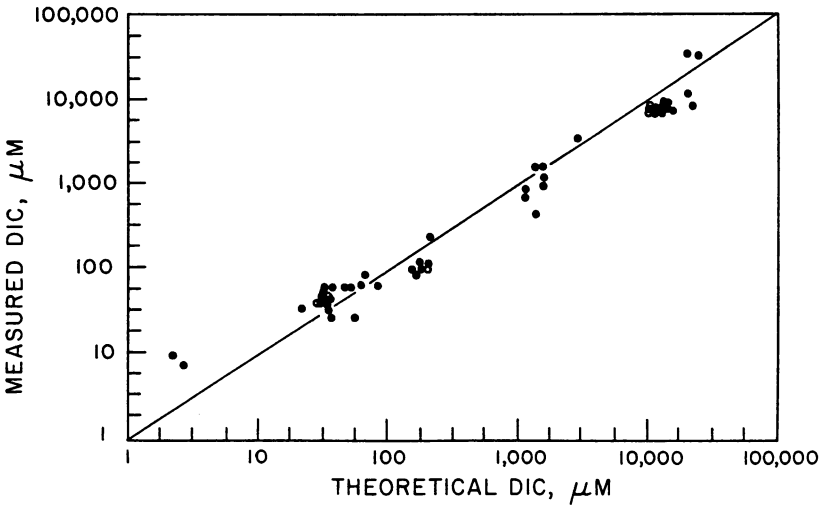

FIG. 1. Measured [DIC] in cultures of *S. leopoliensis* at several pH values (6.2–9.6) and gas stream CO_2 concentrations (36–50,000 μl/L) as a function of the theoretical [DIC]. The correlation coefficient between log measured and log theoretical DIC was 0.99. Open circles represent two coincident points.

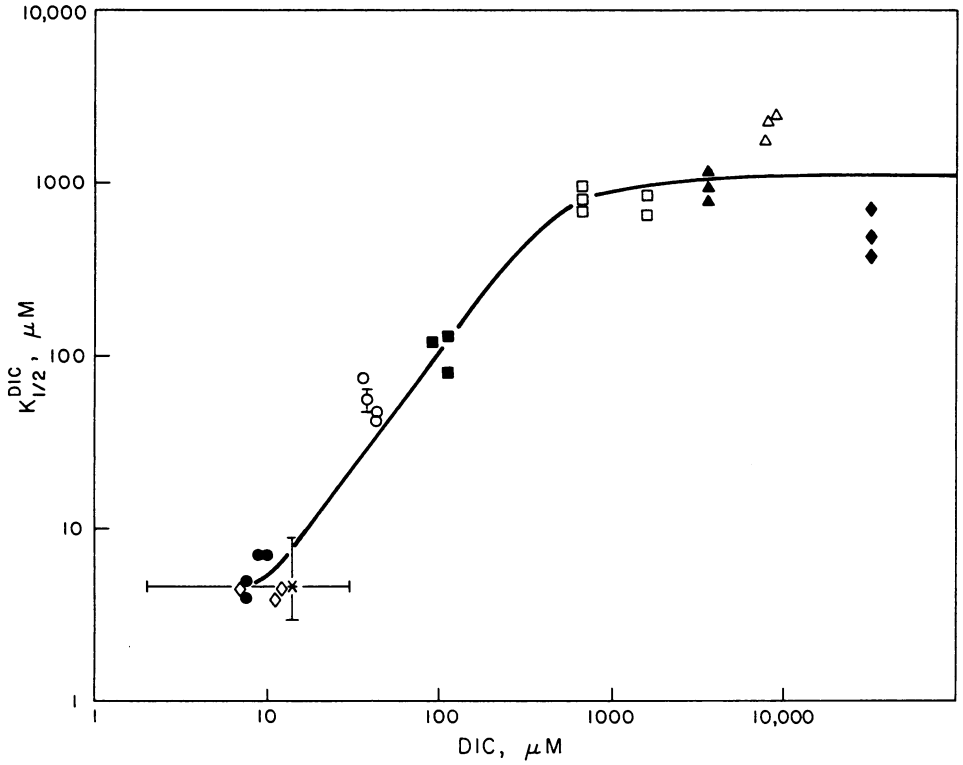

FIG. 2. Half saturation constants for photosynthesis with respect to [DIC] ($K_{1/2}^{DIC}$) as a function of the [DIC] during culture growth. Standard deviation is shown for a representative set of points. [DIC] was varied by altering the culture pH and the CO_2 concentration of the gas stream. pH 6.3–6.5, CO_2 = 37 μl/L, (●); pH 6.3–6.5, CO_2 = 494 μl/L, (○); pH 7.3–7.4, CO_2 = 494 μl/L (■); pH 8.2–8.3, CO_2 = 494 μl/L, (□); pH 8.6, CO_2 = 494 μl/L, (▲); pH 9.2–9.4, CO_2 = 494 μl/L, (△). pH 7.5, CO_2 = 50,000 μl/L (♦). DIC-limited, chemostat grown cells (D = 0.5 d^{-1}) at pH 6.2, 7.5, and 10 (◇). The asterisk (*) represents the mean, and the bars, the associated range of data reported by Miller *et al.* (11) from steady state, carbon limited, chemostat cultures of *S. leopoliensis* grown at pH 9.6.

0.5 d^{-1}; ambient [DIC] = 10.2 ± 2.6 μM) at pH 6.2, 7.5, and 10. The $K_{1/2}^{DIC}$ values were independent of pH (Table I) and the resulting changes in [CO_2]. This confirms that the effects reported in Figure 2 are a sole result of changes in culture [DIC] and are independent of both pH and [CO_2] over the range evaluated.

Based on these results we suggest that high CO_2 cells reported in previous studies (1, 2, 6, 7) are the laboratory counterparts of algae growing in natural systems at high [DIC]. Furthermore, the air-grown cells, particularly those at high pH, may only exhibit partially induced high affinity photosynthetic kinetics. In order to assure that fully induced high affinity kinetics are attained, DIC-limited chemostat cultures should be employed (Fig. 2; 11, 18).

Table I. *Effect of pH on the $K_{1/2}^{DIC}$ of Chemostat Grown, DIC-limited S. leopoliensis and the Calculated Equilibrium CO_2 Concentration*

pH was maintained using a pH stat with a titrant of 0.5 N HCl. The steady state growth rate was 0.5 d^{-1} and the average [DIC] was 10.2 ± 2.6 μM.

	Steady State pH		
	6.2	7.5	10.0
$K_{1/2}^{DIC}$ (μM)	4.5 ± 0.6	3.5 ± 0.7	4.5 ± 0.6
[CO_2] (μM)	5.84	0.64	1.4×10^{-3}

The observation that $K_{1/2}^{DIC}$ is independent of pH (6.2–10; Table I) at constant [DIC] is interesting as the CO_2/HCO_3^- ratio varies from approximately 1.3 to 2.1 × 10^{-4} over this range. To maintain a constant $K_{1/2}^{DIC}$ over this range in pH, CO_2 transport (2, 8, 20) would have to be favored at low pH and HCO_3^- transport at high (2, 4, 5, 9–11, 13). This hypothesis is currently under evaluation.

In our previous studies employing DIC limited chemostats (11, 18), we showed a sharp transition in $K_{1/2}^{DIC}$ (from 4.7 to about 1100 μM) as DIC-limited growth rates increased from ≤1.7 d^{-1} to ≥1.7 d^{-1}. The lack of $K_{1/2}^{DIC}$ values intermediate between these

extremes can be explained by the nature of steady state chemostat culture (19). In such systems the algae must maintain a growth rate equivalent to the culture dilution rate and therefore establish an exogenous DIC concentration accordingly (19). Long-term stability in chemostats can therefore only be maintained when $K_{1/2}^{DIC}$ is either fully induced or repressed. The present study has shown that under conditions of intermediate [DIC] *S. leopoliensis* can exist in a wide range of physiological intermediates between the fully induced and repressed states. The levels of DIC resulting in these intermediates have been reported in natural systems (12, 15, 16). Consequently, as [DIC] decreases below growth saturating levels *S. leopoliensis* can adapt by lowering its $K_{1/2}^{DIC}$. Only at DIC concentrations below 20 to 40 μM would growth become DIC limited (11, 18).

We have shown that $K_{1/2}^{DIC}$ values are a function of the [DIC] at which cells are grown, thus underscoring the importance of controlling and measuring [DIC] during preconditioning experiments. In addition, we have provided evidence that ambient CO_2 levels are not responsible for inducing this adaptation to low DIC. As the reported changes in $K_{1/2}^{DIC}$ occur at environmental concentrations of dissolved inorganic carbon this is an adaptation which may be of ecological significance.

LITERATURE CITED

1. BADGER MR, A KAPLAN, JA BERRY 1978 A mechanism for concentrating CO_2 in *Chlamydomonas reinhardii* and *Anabaena variabilis*. Arch Biochem Biophys 201: 247–254
2. BADGER MR, TJ ANDREWS 1982 Photosynthesis and inorganic carbon usage by the marine cyanobacterium *Synechococcus* sp. Plant Physiol 70: 251–261
3. BUCH K 1960 Dissoziation der Kohlensaure, gleichgewichteund Puffersytema. *In* W Ruhland, ed, Encyclopedia of Plant Physiol, Vol 1. Springer-Verlag, Berlin, p 5
4. ESPIE GS, KS GEHL, GW OWTTRIM, B COLMAN 1984 Inorganic carbon utilization by cyanobaceria. *In* C Sybesma, ed, Advances in Photosynthetic Research, Vol 3. Martinus Nijhoff/Junk, Netherlands, pp 457–460
5. KAPLAN A, MR BADGER, JA BERRY 1980 Photosynthesis and the intracellular inorganic carbon pool in the bluegreen alga *Anabaena variabilis*: response to external CO_2 concentration. Planta 149: 219–226
6. MARCUS Y, D ZENVIRTH, E HAREL, A KAPLAN 1982 Induction of HCO_3^- transporting capability and high photosynthetic affinity to inorganic carbon by low concentration of CO_2 in *Anabaena variabilis*. Plant Physiol 69: 1008–1012
7. MARCUS Y, E HAREL, A KAPLAN 1983 Adaptation of the cyanobacterium *Anabaena variabilis* to low CO_2 concentration in their environment. Plant Physiol 71: 208–210
8. MILLER AG, DT CANVIN 1985 Distinction between HCO_3^- and CO_2-dependent photosynthesis in the cyanobacterium *Synechococcus leopoliensis* based on the selective response of HCO_3^- transport to Na^+. FEBS Lett 187: 29–32
9. MILLER AG, B COLMAN 1980 Evidence for HCO_3^- transport by the blue-green alga (cyanobacterium) *Coccochloris peniocystis*. Plant Physiol 65: 397–402
10. MILLER AG, B COLMAN 1980 Active transport and accumulation of bicarbonate by a unicellular cyanobacterium. J Bacteriol 143: 1253–1259
11. MILLER AG, DH TURPIN, DT CANVIN 1984 Growth and photosynthesis of the cyanobacterium *Synechococcus leopoliensis* in HCO_3^--limited chemostats. Plant Physiol 75: 1064–1070
12. SCHINDLER DW, EJ FEE 1973 Diurnal variation of dissolved inorganic carbon and its use in estimating primary production and CO_2 invasion in Lake 227. J Fish Res Board Can 30: 1501–1510
13. SHELP BJ, DT CANVIN 1984 Evidence for bicarbonate accumulation by *Anacystis nidulans*. Can J Bot 62: 1398–1403
14. STUMM W, JJ MORGAN 1981 Aquatic Chemistry, Ed 2. John Wiley & Sons, New York, pp 174–181
15. TALLING JF 1973 The application of some electrophysiological methods to the measurement of photosynthesis and respiration in freshwaters. Freshwater Biol 3: 335–362
16. TALLING JF 1976 The depletion of carbon dioxide from lake water by phytoplankton. J Ecol 64: 79–121
17. TURPIN DH, AG MILLER, DT CANVIN 1984 Carboxysome content of *Synechococcus leopoliensis* (Cyanophyta) in response to inorganic carbon. J Phycol 20: 249–253
18. TURPIN DH, AG MILLER, JS PARSLOW, IR ELRIFI, DT CANVIN 1985 Predicting the kinetics of dissolved inorganic carbon limited growth from the short-term kinetics of photosynthesis in *Synechococcus leopoliensis* (Cyanophyta). J Phycol. In press
19. TURPIN DH, AG MILLER, DT CANVIN 1985 Chemostats in the study of inorganic carbon metabolism in microalgae. *In* WJ Lucas, JA Berry, eds, Inorganic Carbon Uptake by Aquatic Photosynthetic Organisms. American Society of Plant Physiologists, Rockville, MD, pp 437–448
20. VOLOKITA M, D ZENVIRTH, A KAPLAN, L REINHOLD 1984 Nature of the inorganic carbon species actively taken up by the cyanobacterium *Anabaena variabilis*. Plant Physiol 76: 599–602