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Short Communication

Adaptation of the Cyanobacterium *Anabaena variabilis* to Low CO_2 Concentration in Their Environment[1]

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ABSTRACT

The rate of adaptation of high CO_2 (5% v/v CO_2 in air)-grown *Anabaena* to a low level of CO_2 (0.05% v/v in air) was determined as a function of O_2 concentration. Exposure of cells to low (2.6%) O_2 concentration resulted in an extended lag in the adaptation to low CO_2 concentration. The rate of adaptation following the lag was not affected by the concentration of O_2. The length of the lag period is markedly affected by the O_2/CO_2 concentration ratio, indicating that the signal for adaptation to low CO_2 may be related to the relative rate of ribulose-1,5-bisphosphate carboxylase/oxygenase activities, rather than to CO_2 concentration proper. This suggestion is supported by the observed accumulation of phosphoglycolate following transfer of cells from high to low CO_2 concentration.

The adaptive transformation that green algae and cyanobacteria undergo following transfer from high to low CO_2 concentration (5% and 0.03% v/v CO_2 in air, respectively) involves metabolic and structural changes. The ability to concentrate Ci[2] within the cells (2, 3, 9, 14) and the activity of several enzymes (7, 15, 17) increase during the adaptation to low CO_2 conditions. Electron microscope studies of high and low CO_2-adapted cells indicate differences in cell wall structure in *Anabaena* (13) and an increased number of mitochondria as well as a change in their location in low CO_2-grown *Scenedesmus* (12).

These alterations in structure and metabolic activities result in increased apparent photosynthetic affinity to the Ci in the medium (2, 9), decrease of O_2 inhibition of photosynthesis (4, 18), and increased resistance to photoinhibition (8).

The mechanism by which a single environmental condition, namely CO_2 concentration in the medium, induces these changes is not yet understood. The adaptation process involves protein synthesis and is light dependent (13). The excretion of glycolate, when high CO_2-adapted cells are transferred to low CO_2 conditions (6), indicates alteration of the relative rates of RubisP carboxylase/oxygenase activities (10). The adaptation to low CO_2 level could be induced by a change in the level of a product of the RubisP carboxylation/oxygenation reaction. Alternatively, the cells might directly monitor the concentration of inorganic carbon. In order to examine the two possibilities, we determined the effect of CO_2/O_2 concentration ratio on the rate of adaptation of *Anabaena* to low CO_2 levels.

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[2] Abbreviations: Ci, inorganic carbon; RubisP, ribulose-1,5-bisphosphate.

MATERIALS AND METHODS

Anabaena variabilis strain M-3 (Tokyo University collection) was grown in the presence of 5% v/v CO_2 in air and harvested as described previously (13). Adaptation to low CO_2 level was induced by aerating the cells with different mixtures of CO_2, O_2, and N_2 using two mixing pumps (Wösthoff, Bochum, F.R.G.).

The apparent photosynthetic affinity to Ci was determined from the dependence of the rate of O_2 evolution (O_2 electrode; Rank Brothers, Cambridge, U.K.) on the concentrations of Ci in the medium (9). Intracellular Ci concentration as well as CO_2 fixation were simultaneously determined using the filtering centrifugation technique previously described (9, 11, 13).

The concentrations of glycolate and P-glycolate within the cells were determined as follows: cells were collected by filtration, transferred into cold $HClO_4$ (7%), and kept on ice for 15 min. The pH was then adjusted to 7.5 by KOH, followed by centrifugation to remove the precipitate of $KClO_4$. The supernatant was loaded on Dowex 50 (H^+ form, 200–400 mesh) columns (Bio-Rad Econo-Columns, 0.7 × 4 cm). The eluant not retained by the column was collected and loaded on a Dowex 1 formate (100–200 mesh) 1 × 20 cm column. The column was washed with 50 ml distilled H_2O and eluted with a linear gradient of 0 to 0.1 M HCl at a flow rate of 0.35 ml/min. Fractions were collected and their pH adjusted to 10.2. The concentration of glycolate in these samples was determined by the method of Calkins (5) before and after the treatment with alkaline phosphatase. The latter was performed by incubating 200-μl aliquots with 3.8 units of alkaline phosphatase (from bovine intestine, Sigma) at 37°C for 1 h. Fractions which cochromatographed with P-glycolate and showed Calkins reaction for glycolate only after the alkaline phosphatase treatment were regarded as containing P-glycolate. Measurements of the intracellular volume of the cells were performed as described previously (9) enabling estimation of the concentration of P-glycolate within the cells.

RESULTS AND DISCUSSION

Three criteria were used to determine the extent of adaptation of high CO_2-grown cells to low CO_2 level: the apparent photosynthetic affinity to Ci; the accumulation of Ci within the cell following exposure to 50 μM $NaH^{14}CO_3$ for 30 s; and the accumulation of acid stable ^{14}C (photosynthetic products) during that time. The extent of accumulation of Ci within the cells (Fig. 1a) increased during the adaptation to low CO_2 level, resulting in an enhanced rate of CO_2 fixation (Fig. 1b) and increased apparent photosynthetic affinity to Ci (lower $K_{1/2}$) in the medium (Fig. 1c). Exposure of the cells to a low concentration of O_2 (2.6%) resulted in an extended lag in the process of adaptation. The rate of adaptation following this lag period was not affected by the concentration of O_2. The extended lag in adaptation at low O_2

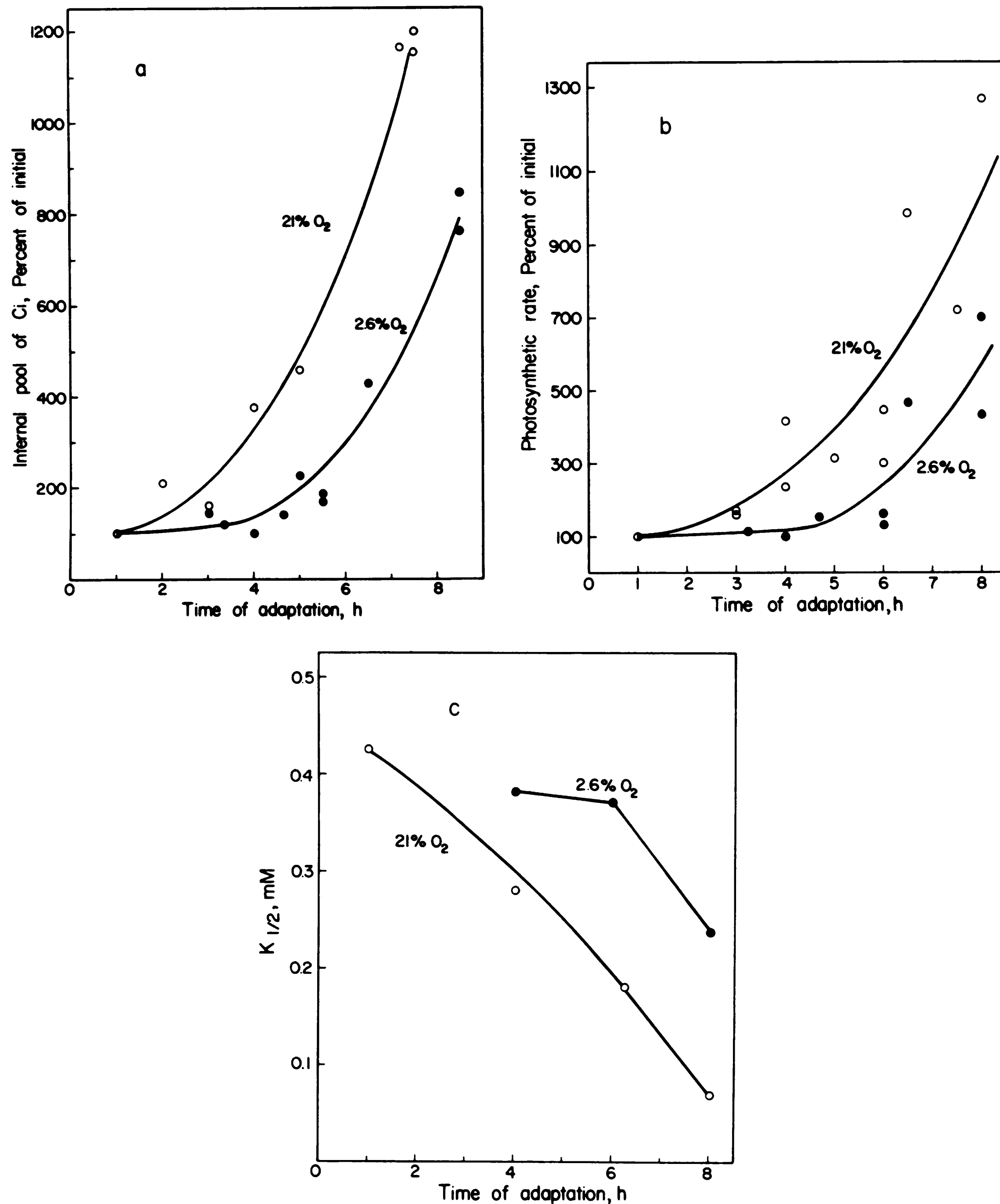

FIG. 1. The rate of adaptation of high CO_2-grown *Anabaena* to low CO_2 conditions as affected by O_2 concentration. Cells were exposed to 0.05% CO_2 and 21% or 2.6% O_2 at zero time. Aliquots of 200 µl cell suspensions (1–3 µg Chl) were withdrawn at different times as indicated and incubated in microfuge tubes in the presence of 50 µM $NaH^{14}CO_3$ (0.37 µCi/µmol) for 30 s. The extent of accumulation of Ci (a) and acid stable ^{14}C (b) were determined by the filtering centrifugation technique (9, 13). 100% corresponded to 0.4 to 0.6 mM Ci and 10 to 20 µmol C mg^{-1} Chl h^{-1} in (a) and (b), respectively. c, Photosynthetic apparent affinity ($K_{1/2}$ is the concentration of Ci in the medium which yielded half maximum rate of photosynthesis) was determined in the O_2 electrode on 2 ml of cell suspension (6–9 µg Chl/ml). Experiments were conducted at 30°C, 10 m/cm^{-2} (400–700 nm).

concentration cannot be attributed to an alteration of the rate of respiration as the latter was hardly affected in the O_2 concentrations used (results not shown). The dependence of the length of the lag period on the O_2 concentration, at constant CO_2 level, indicates that the cells respond to the CO_2/O_2 concentration ratio rather than to CO_2 level proper.

To calculate the rates of RubisP carboxylase/oxygenase reactions, at varying external O_2 and CO_2 concentrations, their intracellular concentration should be estimated. It is assumed that with the cells used in these experiments (absence of heterocysts) the gradient of O_2 concentration between the site of its formation in photosynthesis and the medium is small compared to its background concentration (16). *Anabaena* cells are capable of concentrating Ci within the cells. The internal concentration of CO_2 however, cannot be evaluated from the internal Ci concentration and from the pH since the species of Ci are not in equilibrium (9). The concentration of CO_2 at the carboxylation site has been estimated from comparison of the rate of photosynthesis in intact

Table I. *Length of the Lag Period in Adaptation to Low CO_2 Level in Anabaena as Affected by the Concentration of CO_2 and O_2 in the Medium*

$[CO_2]_e$[a]	$[O_2]_e$	$[CO_2]_i$	$[O_2]_i$	V_c/V_o	Lag Period
%		μM			h
5	21	1,222	201	202	∞
0.03	21	13	201	2.1	2
0.05	21	19.8	201	3.3	2
0.05	2.6	19.8	25	26.8	4–5
0.001	21	0.4	201	0.07	0.5–1
0.001	4	0.4	38	0.35	4–5

[a] Symbols *e* and *i* are the external and calculated internal concentrations, respectively. V_c/V_o is the ratio of the expected rates of RubisP carboxylation (V_c) and oxygenation (V_o). The rates of carboxylation (V_c) and of oxygenation (V_o) were calculated assuming that CO_2 and O_2 compete on the same catalytic site of RubisP carboxylase/oxygenase. Thus:

$$V_c/V_o = \frac{V^c_{max}[CO_2]/K_m{}^c(1 + [O_2]/K_i{}^o) + [CO_2]}{V^o_{max}[O_2]/K_m{}^o(1 + [CO_2]/K_i{}^c) + [O_2]}$$

where $K_m{}^c$ and $K_m{}^o$ are the Michaelis coefficients of CO_2 and O_2, respectively, and $K_i{}^c$ and $K_i{}^o$ are the inhibition coefficients of CO_2 on oxygenation and O_2 on carboxylation, respectively; V^c_{max} and V^o_{max} are the maximal rates of carboxylation and oxygenation, respectively. The V_c/V_o ratios were calculated based on kinetic parameters for RubisP carboxylase/oxygenase isolated from *A. variabilis* [1]. The lag periods were calculated from experiments such as those presented in Figure 1a.

cells as affected by the external Ci concentration, with the kinetics of carboxylation of isolated RubisP carboxylase (1), assuming that the kinetic parameters of RubisP carboxylase/oxygenase *in vivo* are similar to those determined *in vitro*.

Data presented in Table I clearly indicate that, with one exception to be discussed below, the length of the lag in adaptation to low CO_2 level is strongly affected by the V_c/V_o ratio rather than by CO_2 concentration proper. The higher V_c/V_o, the longer the lag period. Exposure of cells to low O_2 and CO_2 concentrations (4% and 0.001%, respectively), conditions under which the expected V_c/V_o ratio is relatively low, resulted in an extended lag period (4–5 h). Raising O_2 concentration to 21% under the same CO_2 concentration shortened the lag significantly (0.5–1 h). This may indicate that the induction of adaptation to low CO_2 conditions is dependent on a product of the RubisP oxygenase reaction. The relatively long lag period at low O_2 and CO_2 conditions may be due to the low rate of oxygenation under these conditions, resulting in slow accumulation of the 'inducing' agent. Verification of this suggestion may be obtained in experiments in which the concentration of O_2 is raised above the ambient one at a given CO_2 level. However, adaptation to low CO_2 level in the presence of high O_2 concentration (~40%), is strongly inhibited due to the formation and accumulation of peroxides which severely damage the photosynthetic apparatus and the capability to accumulate Ci in cyanobacteria (8).

Glycolate excretion following transfer of high CO_2-adapted cells to low CO_2 conditions indicates that V_o has been increased (10). The addition of glycolate (10 mM) to high CO_2-adapted cells does not induce adaptation to low CO_2 level (results not shown). Contrary to glycolate, *Anabaena* cells are impermeable to phosphoglycolate, the product of RubisP oxygenase. Considerable amounts of phosphoglycolate could be detected after exposure of high CO_2-grown *Anabaena* cells to CO_2-free air for 30 min. Intracellular concentrations of 2 to 4 mM phosphoglycolate were determined after its isolation by ion exchange chromatography. The phosphoglycolate content of *Anabaena* cells grown under low CO_2 concentration (bubbling with air) was too low to be detected by the technique used here (lower than 0.25 μM). These observations suggest that phosphoglycolate is indeed the signal for adaptation to low CO_2 conditions, although a definite conclusion must await further confirmation.

The fact that different CO_2/O_2 concentration ratios affected the length of the lag period and not the rate of adaptation to low CO_2 level that follows, suggests that the process is controlled by the rate of accumulation of the inducing agent possibly to a threshold level. The V_c/V_o ratio is continuously altered during the course of adaptation to low CO_2 concentrations due to the increasing internal concentration of Ci (Fig. 1a). Therefore, the changing level of the inducing agent might also serve as a signal for termination of the process. These possibilities are presently under investigation.

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