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Glycolaldehyde Inhibits CO_2 Fixation in the Cyanobacterium *Synechococcus* UTEX 625 without Inhibiting the Accumulation of Inorganic Carbon or the Associated Quenching of Chlorophyll *a* Fluorescence[1]

Anthony G. Miller* and David T. Canvin

Department of Biology, Queen's University, Kingston, Ontario, Canada, K7L 3N6

ABSTRACT

When studying active CO_2 and HCO_3^- transport by cyanobacteria, it is often useful to be able to inhibit concomitant CO_2 fixation. We have found that glycolaldehyde was an efficient inhibitor of photosynthetic CO_2 fixation in *Synechococcus* UTEX 625. Glycolaldehyde did not inhibit inorganic carbon accumulation due to either active CO_2 or HCO_3^- transport. When glycolaldehyde (10 millimolar) was added to rapidly photosynthesizing cells, CO_2 fixation was stopped within 15 seconds. The quenching of chlorophyll *a* fluorescence remained high (≥ 82% control) when CO_2 fixation was completely blocked by glycolaldehyde. This quenching was relieved upon the addition of a glucose oxidase oxygen-trap. This is consistent with our previous finding that *q*-quenching in the absence of CO_2 fixation was due to O_2 photoreduction. Photosynthetic CO_2 fixation was also inhibited by D,L-glyceraldehyde but a sixfold higher concentration was required. Glycolaldehyde acted much more rapidly than iodoacetamide (15 seconds *versus* 300 seconds) and did not cause the onset of net O_2 evolution often observed with iodoacetamide. Glycolaldehyde will be a useful inhibitor when it is required to study CO_2 and HCO_3^- transport without the complication of concomitant CO_2 fixation.

The investigation of active CO_2 and HCO_3^- transport by cyanobacteria is aided when the photosynthetic fixation of the accumulated CO_2 is inhibited (8, 9, 11, 13, 14). In most past studies this has been done by adding iodoacetamide or by using a mutant that is unable to fix CO_2 at air levels of CO_2 (8, 9, 11, 13, 14). In both cases, the transport of CO_2 and HCO_3^- continued quite normally (8, 9, 11, 13). Iodoacetamide probably inhibits CO_2 fixation by inhibiting triose phosphate isomerase (3), but since iodoacetamide is quite a strong alkylating agent this may not be its only site of action. A rapid inhibition of CO_2 fixation with iodoacetamide is hard to achieve because the inhibition takes about 5 min to be complete at the concentration (3.3 mM) normally used (8, 9, 11, 13, 14). In spite of these drawbacks, iodoacetamide has been a useful inhibitor of CO_2 fixation during studies of C_i[2] transport (8, 9, 11, 13, 14).

The E_1 mutant of *Anacystis nidulans* R2 can only grow on high levels of CO_2 (7). When cells of this mutant are bubbled with CO_2 at or below air levels, the high affinity C_i transport capacity develops, but the accumulated C_i cannot be fixed (7). This inability to fix the accumulated C_i may be due to an inability of the cells to catalyze the intracellular conversion of HCO_3^- to CO_2 (2). The E_1 mutant has been useful in studies of active C_i transport (13). One drawback with such mutants is that the cells must be grown on higher than normal CO_2 concentrations and then allowed to develop the high affinity C_i transport capacity by aerating the cells overnight (13). As controls, one must use the wild-type cells grown and treated in the same fashion. In most studies it is more convenient to be able to observe C_i transport processes in the absence and presence of CO_2 fixation in the same cell suspension.

D,L-Glyceraldehyde is known to inhibit CO_2 fixation in isolated chloroplasts (19) and in cyanobacteria (17). Shelp and Canvin (17) found that active C_i transport by *Synechococcus* UTEX 625 proceeded normally in the presence of 25 mM D,L-glyceraldehyde even though CO_2 fixation was completely inhibited. Romero *et al.* (15) used D,L-glyceraldehyde to demonstrate that NO_3^- transport by *A. nidulans* required concomitant CO_2 fixation. Stokes and Walker (19) showed that, in isolated chloroplasts, D,L-glyceraldehyde blocked the conversion of triose phosphate to ribulose-1,5-bisphosphate. The drawback with D,L-glyceraldehyde as an inhibitor is that high concentrations (>25 mM) are required, and for best results a 15 min dark incubation is needed in the case of *Synechococcus* sp. For C_i transport studies, an inhibitor of CO_2 fixation is most useful if it can be added to cells in the light when they are at their CO_2 compensation point.

Sicher (18) has demonstrated that glycolaldehyde acts similarly to D,L-glyceraldehyde in isolated chloroplasts of barley, but at an order of magnitude lower concentration. We report in this paper that glycolaldehyde is an effective inhibitor of CO_2 fixation in *Synechococcus* UTEX 625. It does not inhibit

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[2] Abbreviations: C_i, inorganic carbon; BTP, 1,3-bis[tris(hydroxymethyl)methylamino]propane; CA, carbonic anhydrase; F_M, maximum fluorescence yield; F_0, fluorescence yield in the absence of actinic light; F_v, variable fluorescence ($F_M - F_0$); Q_A, primary electron accepting plastoquinone of PSII.

CO_2 or HCO_3^- transport or the associated quenching of Chl *a* fluorescence.

MATERIALS AND METHODS

Organism and Growth Conditions

The unicellular cyanobacterium *Synechococcus* UTEX 625, obtained from the University of Texas Culture Collection (Austin, TX), was grown with air-bubbling as previously described (5, 6).

Experimental Conditions

Prior to experiments, cells were washed with 25 mM BTP/23.5 mM HCl buffer (pH 8) as previously described (5, 6). Experiments were conducted in thermostatted cuvettes at 30°C, and light was provided by a quartz-halogen projector lamp at 88 $\mu E \cdot m^{-2} \cdot s^{-1}$. This photon flux density is subsaturating for CO_2 fixation but allows the C_i-dependent quenching of Chl *a* fluorescence to be readily measured. Higher photon flux densities progressively reduce the amount of quenching since *q*-quenching is the major component (10). The Chl concentration was 7 to 9 $\mu g \cdot mL^{-1}$.

Measurement of C_i Uptake

Membrane inlet mass spectrometry was used to monitor C_i uptake (1, 9). Carbonic anhydrase (25 $\mu g \cdot mL^{-1}$) was added to the cell suspension so that the dissolved CO_2 was in equilibrium with the HCO_3^-. Under these circumstances measurement of CO_2 uptake by MS represents uptake of total C_i (CO_2 + HCO_3^-) (1, 9). Any ambiguities due to release of respiratory $^{12}C_i$ were avoided by the use of $^{13}C_i$ (9, 11).

The $K_2\,^{13}CO_3$ (99 atom % ^{13}C) was purchased from MSD Isotopes (Montreal, Quebec).

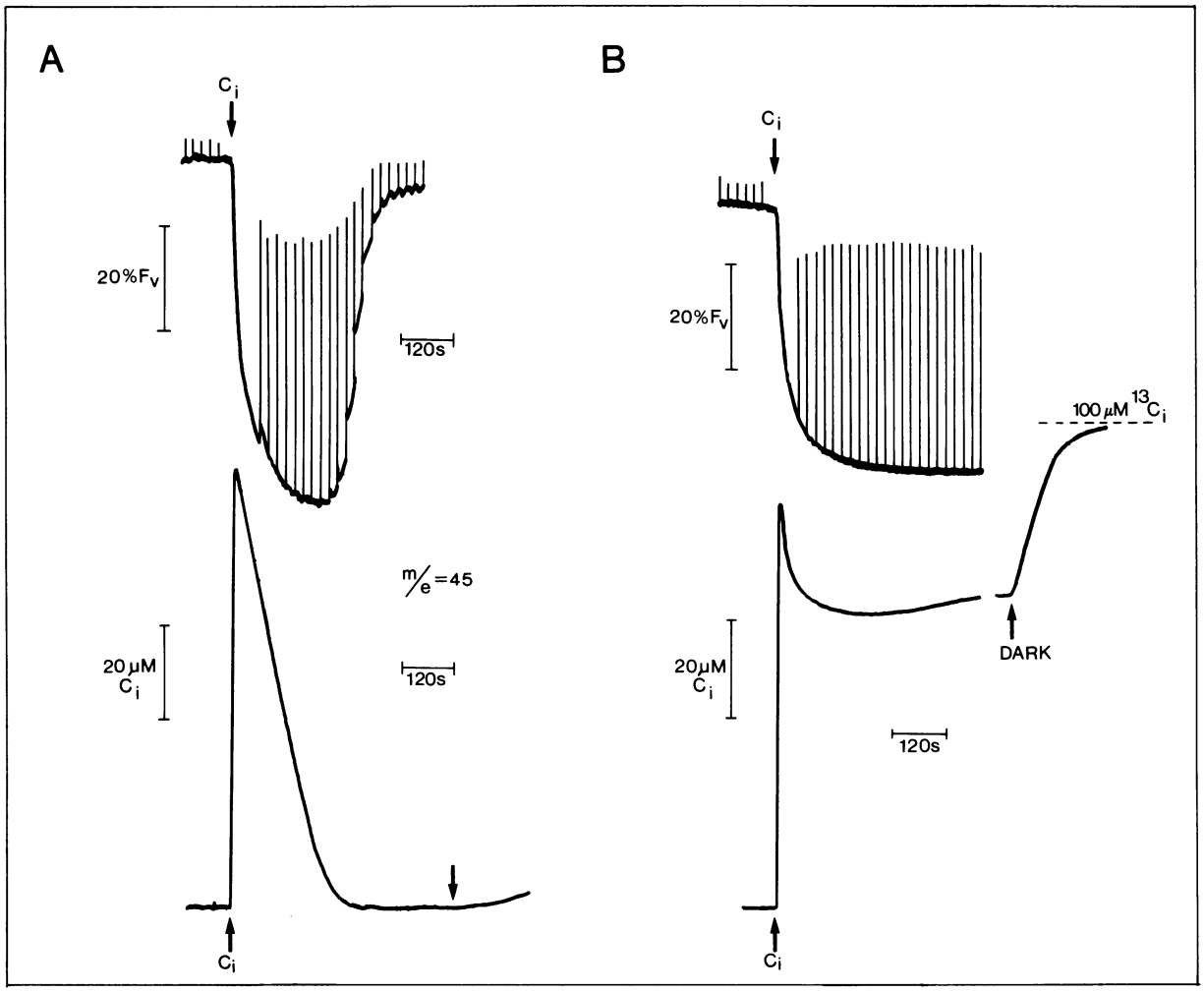

Figure 1. Effect of 10 mM glycolaldehyde upon C_i uptake and C_i-dependent quenching of Chl *a* fluorescence emission. Cells (8.7 μg Chl $\cdot mL^{-1}$) in 25 mM BTP/HCl (pH 8.0) with CA (25 $\mu g \cdot mL^{-1}$) but lacking added NaCl were allowed to reach the CO_2-compensation point. The PFD was 150 $\mu E \cdot m^{-2} \cdot s^{-1}$. Fluorescence emission (*upper traces*) was measured with the PAM fluorimeter. Every 20 s the cells were illuminated with a saturating flash (1600 $\mu E \cdot m^{-2} \cdot s^{-1}$) of about 500 ms duration. The $^{13}C_i$ was added as 100 μM $K_2{}^{13}CO_3$. The $^{13}C_i$ uptake was measured by MS (*lower traces*). A, No glycolaldehyde added. B, 10 mM glycolaldehyde added after the CO_2 compensation point had been reached. *Arrow* in lower trace of A indicates darkness.

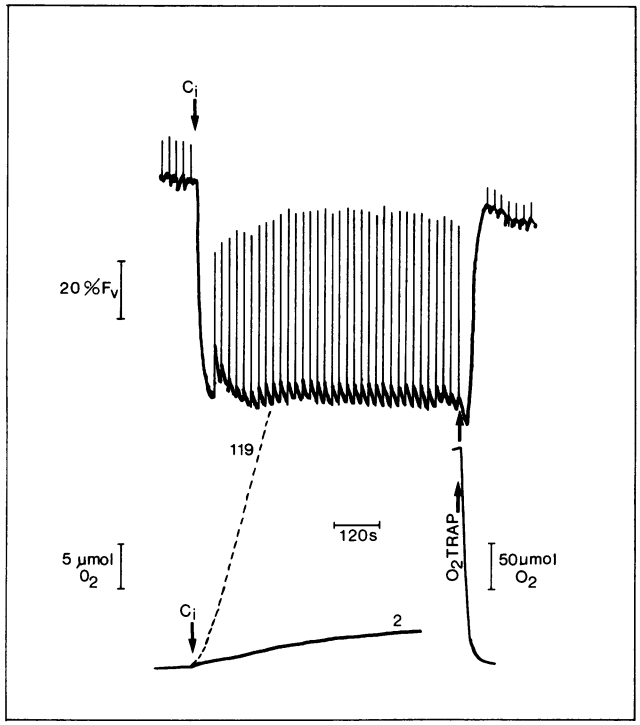

Figure 2. Inhibition of O_2 evolution after addition of 10 mM glycolaldehyde at the CO_2-compensation point. Cells (8.6 μg Chl·mL^{-1}) in 25 mM BTP/HCl (pH 8.0) with 25 mM NaCl were allowed to reach the CO_2-compensation point. The PFD was 88 μE·m^{-2}·s^{-1}. Fluorescence emission in the presence of glycolaldehyde (*upper trace*) measured as described for Fig. 1. The O_2 evolution (*lower traces*) was measured with a Clark-type electrode. The figures next to the traces show the photosynthetic rate in μmol·mg^{-1}Chl·h^{-1}. The C_i was added as 250 μM K_2CO_3. The cells were incubated in the absence (*dotted line*, O_2 evolution) or presence (*solid lines*) of 10 mM glycolaldehyde. The O_2 trap consisted of 10 mM glucose, glucose oxidase (100 μg·mL^{-1}) and catalase (50 μg·mL^{-1}). The change in O_2 concentration following addition of the O_2 trap was measured at 1/10 × the sensitivity used to measure the photosynthetic O_2 evolution.

Chl *a* Fluorescence Yield

The fluorescence yield of Chl *a* was monitored with a pulse amplitude modulated fluorimeter (PAM-101, H. Walz, Effeltrich, D-8521, FRG) as previously described (10, 11, 16). The degree of quenching is calculated as a percentage of the change in fluorescence yield measured when dark-adapted cells are illuminated with the actinic light source in the absence of C_i. At the CO_2-compensation the fluorescence yield observed during a saturating light flash (1 s duration) is very close to that seen after the addition of DCMU (our unpublished data). This value is called F_M (8, 10, 11). When dark-adapted cyanobacteria are illuminated, the majority of the increase in fluorescence yield is due to a state 2 to state 1 transition (4, 12). This means that the F_0 measured for dark-adapted cells is lower than for light-adapted cells (4, 12). Even though the magnitude of F_0 changes upon illumination, the dark-adapted F_0 value still serves a useful baseline for calculating the degree of fluorescence quenching observed upon C_i addition (8).

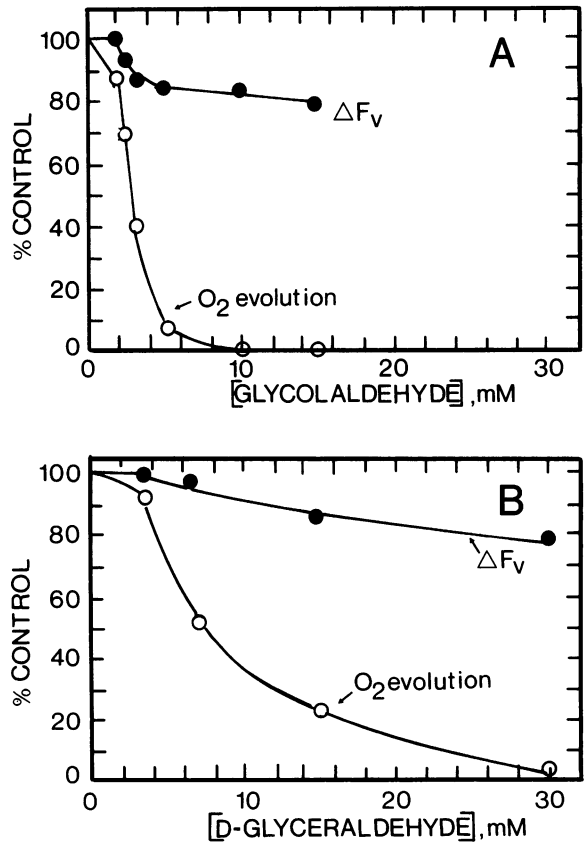

Figure 3. Effect of glycolaldehyde concentration (A) or D-glyceraldehyde concentration (B) upon C_i-dependent fluorescence quenching and O_2 evolution. Cells were incubated in 25 mM BTP/HCl (pH 8.0) with 25 mM NaCl and allowed to reach the CO_2 compensation point. The PFD was 88 μE·m^{-2}·s^{-1}. Then O_2 evolution was initiated by the addition of 250 μM K_2CO_3. After steady-state O_2 evolution had been attained, glycolaldehyde (A) or D,L-glyceraldehyde (B) was added. In the latter case, it has been assumed that D-glyceraldehyde was the isomer responsible for the inhibition of CO_2 fixation. The degree of fluorescence quenching observed after addition of the 250 μM K_2CO_3, but before inhibitor addition, ranged from 75 to 80% of F_v defined as F_M–F_0 (8). After the addition of glycolaldehyde (A) or D,L-glyceraldehyde (B) the degree of quenching was reduced and has been expressed as a percentage of the quenching (100%) observed before inhibitor addition. The steady-state rates of O_2 evolution before inhibitor addition ranged from 101 to 123 μmol O_2·mg^{-1}Chl.h^{-1}.

RESULTS AND DISCUSSION

Cells were allowed to deplete the buffer of C_i, as monitored by a rise in the Chl *a* fluorescence yield to a stable value close to F_m (Fig. 1). The buffer contained CA (25 μg·mL^{-1}) without 25 mM NaCl, so that mainly CO_2 transport occurred (9). Very similar results were obtained when both CA and 25 mM NaCl were present, so that both CO_2 and HCO_3^- transport occurred (data not shown). The addition of 10 mM glycolaldehyde at the CO_2-compensation point had very little effect upon the Chl *a* fluorescence yield (data not shown). The addition of 100 μM $^{13}C_i$ in the absence of glycolaldehyde resulted in a quenching of Chl *a* fluorescence as C_i was accumulated within the cells (Fig. 1A). The fluorescence yield recovered as the C_i was consumed by CO_2 fixation (Fig. 1A). After fluorescence

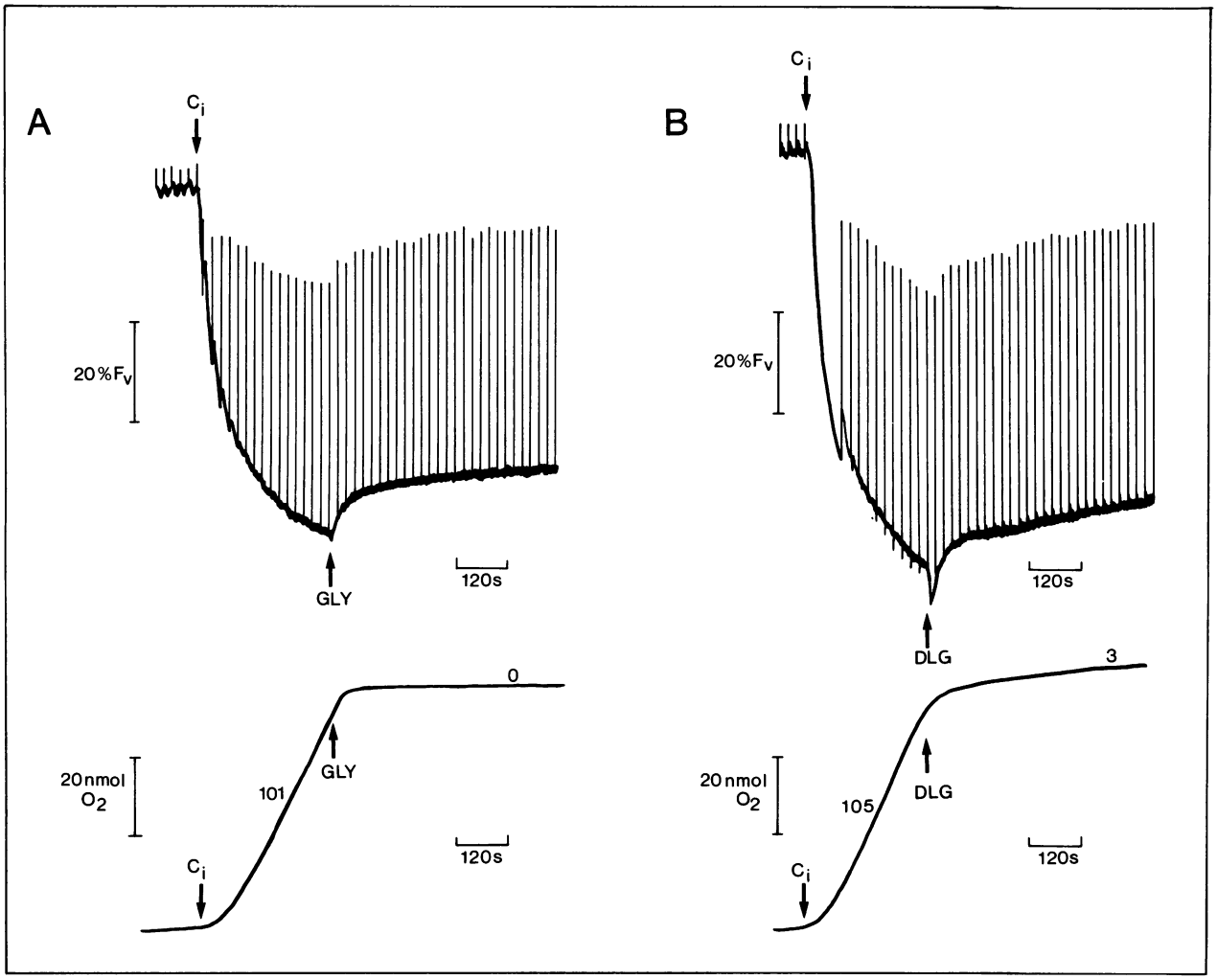

Figure 4. Effect of 10 mM glycolaldehyde or 60 mM D,L-glyceraldehyde when added during steady-state O_2 evolution. Cells were incubated in 25 mM BTP/HCl (pH 8.0) with 25 mM NaCl and allowed to reach the CO_2 compensation point. Fluorescence emission (*upper traces*) and O_2 evolution (*lower traces*) were monitored as previously described. Rates of photosynthesis in $\mu mol \cdot mg^{-1}Chl \cdot h^{-1}$ are shown next to the traces. The C_i was added as 250 μM K_2CO_3. At the times indicated by the arrows either 10 mM glycolaldehyde (GLY) (A) or 60 mM D,L-glyceraldehyde (DLG) (B) were added.

yield had recovered to a value close to the original F_M value (Fig. 1A), the actinic light was turned off. Very little leakage of $^{13}C_i$ from the cells was seen (Fig. 1A), indicating that most of the added $^{13}C_i$ had been fixed into organic compounds.

When 100 μM $^{13}C_i$ was added to cells at the CO_2-compensation point, in the presence of 10 mM glycolaldehyde, both $^{13}C_i$ uptake and quenching of Chl *a* occurred (Fig. 1B). The $^{13}C_i$ taken up by the cells was quantitatively released back into the medium when the actinic light was turned off (Fig. 1B), demonstrating that the glycolaldehyde had completely inhibited C_i fixation. In this experiment, the initial rate of $^{13}C_i$ transport in the presence of glycolaldehyde was about 560 $\mu mol \cdot mg^{-1}Chl \cdot h^{-1}$ and the concentration of $^{13}C_i$ within the cells about 38 mM. The rates of $^{13}C_i$ transport were similar in the absence or presence of the 10 mM glycolaldehyde (Fig. 1). Since the C_i added in the presence of glycolaldehyde was transported and accumulated by the cells but was not consumed by C_i fixation, the Chl *a* fluorescence remained quenched as long as the actinic light was kept on (Fig. 1B). Illumination of the cells with saturating flashes of white light of 1 s duration serves to fully reduce Q_A and overcome the quenching due to oxidized Q_A (16). During such flashes, the fluorescence yield recovered by more than 70%, both in the absence or presence of glycolaldehyde (Fig. 1), indicating that much of the quenching caused by the addition of C_i was due to development of oxidized Q_A. The same conclusion was reached from studies using iodoacetamide to inhibit CO_2 fixation (10). From the data (Fig. 1B) obtained in the presence of glycolaldehyde, it can be calculated that the degree of Chl *a* fluorescence quenching correlates with the degree of C_i accumulation and not the rate of C_i transport. Again, similar conclusions were reached when iodoacetamide was used (11).

Further evidence for the inhibition of CO_2 fixation, but not the quenching of Chl *a* fluorescence, was obtained when O_2 evolution was monitored (Fig. 2) instead of C_i disappearance (Fig. 1). Glycolaldehyde was added to a final concentration

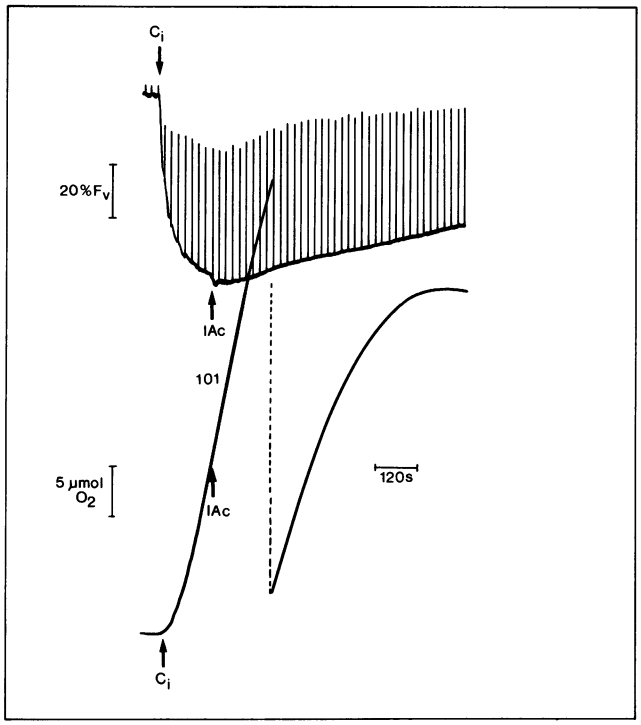

Figure 5. Effect of iodoacetamide when added during steady-state O_2 evolution. Cells, incubated in 25 mM BTP/HCl (pH 8.0), were allowed to reach the CO_2-compensation point. The PFD was 88 $\mu E \cdot m^{-2} \cdot s^{-1}$. Fluorescence emission (*upper trace*) and O_2 evolution (*lower trace*) were measured as previously described. Rates of photosynthesis in $\mu mol \cdot mg^{-1}Chl \cdot h^{-1}$ are shown next to the trace. The O_2 evolution was initiated by the addition of 250 μM K_2CO_3. The iodoacetamide (IAc) was added to yield a final concentration of 3.3 mM.

of 10 mM at the CO_2-compensation point and then 250 μM C_i was added (Fig. 2). In the presence of glycolaldehyde, O_2 evolution was inhibited by more than 98% but very substantial quenching of Chl *a* fluorescence still occurred (Fig. 2). Most of the quenching was transiently relieved by a 1 s flash of saturating light (Fig. 2). We have previously shown that oxidation of Q_A can occur in such cases, when CO_2 fixation is inhibited, because linear electron flow serves to photoreduce O_2 (10). Thus, as expected, when O_2 was removed, by the addition of a glucose/glucose oxidase trap, very little quenching of Chl *a* fluorescence occurred when electron flow to CO_2 was blocked by glycolaldehyde (Fig. 2).

The effect of glycolaldehyde concentration on C_i-dependent O_2 evolution and Chl fluorescence quenching has been examined (Fig. 3A). These results were obtained when the glycolaldehyde was added during steady state photosynthesis, but similar effects of concentration were seen when it was added at the CO_2 compensation point (data not shown). Glycolaldehyde at 5 mM inhibited O_2 evolution by more than 90% but fluorescence quenching upon the addition of C_i was only 15% lower than in the absence of glycolaldehyde (Fig. 3A). D-L-Glyceraldehyde at 5 mM was considerably less effective as an inhibitor of CO_2 fixation, as monitored by O_2 evolution (Fig. 3B). Assuming that D-glyceraldehyde was the active isomer, then a concentration of 25 mM (Fig. 3B) was required to inhibit O_2 evolution to the same level as that seen with only 5 mM glycolaldehyde (Fig. 3A). Thus glycolaldehyde is the more effective inhibitor of CO_2 fixation in cyanobacteria, as Sicher (18) found for spinach chloroplasts and barley protoplasts.

The addition of 10 mM glycolaldehyde caused a rapid inhibition of steadystate O_2 evolution (Fig. 4A). The quenching of Chl *a* fluorescence was somewhat reduced but remained very high (Fig. 4A). Addition of 60 mM D-L-glyceraldehyde gave similar results (Fig. 4B). The component of chlorophyll *a* fluorescence quenching that was due to Q_A oxidation, *q*-quenching, increased somewhat when CO_2 fixation was inhibited (Fig. 4). A more detailed account of the nature of C_i-dependent quenching of Chl *a* fluorescence in cyanobacteria is in preparation (AG Miller, DT Canvin, unpublished results).

Iodoacetamide has been used extensively to inhibit CO_2 fixation without inhibiting C_i transport (8, 10, 11, 13, 14). When added during steady state photosynthesis, however, it does not rapidly inhibit CO_2 fixation (Fig. 5), and it can induce net O_2 uptake in the light.

The results demonstrate that glycolaldehyde is a useful inhibitor of CO_2 fixation (Figs. 1–4) during studies of C_i transport (Fig. 1). It is preferable to iodoacetamide or D-L-glyceraldehyde as it inhibits more quickly or at lower concentration. The results also further validate the use of Chl *a* fluorescence quenching as a monitor of C_i accumulation (Fig. 1) (11).

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