Ethoxyzolamide Inhibition of CO₂-Dependent Photosynthesis in the Cyanobacterium Synechococcus PCC7942

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ABSTRACT

Cells of the cyanobacterium, Synechococcus PCC7942, grown under high inorganic carbon (C₄) conditions (1% CO₂ pH 8) were found to be photosynthetically dependent on exogenous CO₂. This was judged by the fact that they had a similar photosynthetic affinity for CO₂ (Kₐ₄[CO₂] of 3.4–5.4 micromolar) over the pH range 7 to 9 and that the low photosynthetic affinity for CO₂ measured in dense cell suspensions was improved by the addition of exogenous carbonic anhydrase (CA). The CA inhibitor, ethoxyzolamide (EZ), was shown to reduce photosynthetic affinity for CO₂ in high C₄ cells. The addition of 200 micromolar EZ to high C₄ cells increased Kₐ₄(CO₂) from 4.6 micromolar to more than 155 micromolar at pH 8.0, whereas low C₄ cells (grown at 30 microliters CO₂ per liter of air) were less sensitive to EZ. EZ inhibition in high and low C₄ cells was largely relieved by increasing exogenous C₄ up to 100 micromolar. Lipid soluble CA inhibitors such as EZ and chlorozolamide were shown to be the most effective inhibitors of CO₂ usage, whereas water soluble CA inhibitors such as methazolamide and acetazolamide had little or no effect. EZ was found to cause a small drop in photosystem II activity, but this level of inhibition was not sufficient to explain the large effect that EZ had on CO₂ usage. High C₄ cells of Anabaena variabilis M3 and Synechocystis PCC6803 were also found to be sensitive to 200 micromolar EZ. We discuss the possibility that the inhibitory effect of EZ on CO₂ usage in high C₄ cells of Synechococcus PCC7942 may be due to inhibition of a ‘CA-like’ function associated with the CO₂ utilizing C₄ pump or due to inhibition of an internal CA activity, thus affecting CO₂ supply to ribulose bisphosphate carboxylase-oxygenase.

It is now well documented that cyanobacteria possess an inducible CO₂ concentrating mechanism (CCM) which functions to elevate internal CO₂ levels around Rubisco (5, 6, 10, 13, 17). Both CO₂ and HCO₃⁻ can be transported and/or utilized by the CCM (6, 7, 24), but the ability to utilize HCO₃⁻ is most apparent when cells are grown at very low levels of C₄ (7, 15). Although most research has centered upon characterizing the high affinity HCO₃⁻ uptake system, it has been known for some time that CO₂ is often a more efficient substrate for the CCM than HCO₃⁻ (6, 7, 24) Nevertheless, the most recent model for the CCM postulates a pivotal role for a HCO₃⁻ transporter on the plasma membrane (24). In this proposal the ability to utilize CO₂ is accommodated for by the addition of a ‘CA-like’ moiety that converts CO₂ to HCO₃⁻ in close proximity to the HCO₃⁻ pump. It was assumed that CO₂ acts as a better substrate due to a higher diffusion rate into the periplasmic space than the charged HCO₃⁻ ion. The best evidence for this model is that in low CO₂ grown cells of Anabaena variabilis M3, a low concentration (10 μM) of the CA inhibitor, EZ, inhibits CO₂ uptake more than HCO₃⁻ uptake (24). Unfortunately this type of evidence is not generally applicable to other cyanobacteria, such as Synechococcus sp., which are virtually unaffected by 10 to 100 μM EZ.

Recent studies have verified that low CO₂ grown cyanobacteria possess the ability to transport or utilize both CO₂ and HCO₃⁻ (1, 7). More important, it has been shown that cells of Synechococcus PCC6301 grown at high CO₂ retain the ability to take up CO₂ while losing the capacity for HCO₃⁻ uptake (7). This CO₂ uptake activity predominates in cells grown in media (pH 8.2) equilibrated with CO₂ levels above normal air levels (>350 μL/L), whereas maximum induction of HCO₃⁻ uptake capacity requires CO₂ levels of less than 50 μL/L in the gas phase (7). They suggest, in contrast to Volokita et al. (24), that a CO₂ utilizing C₄ pump may be the primary component of the CCM and that HCO₃⁻ utilization would require ‘front end’ conversion of HCO₃⁻ to CO₂ in the vicinity of the C₄ pump.

The studies described in this paper were aimed at extending the characterization of the ecologically important CO₂ utilization system in Synechococcus PCC7942. We show that CO₂ utilization can be severely inhibited by high concentrations of the CA inhibitor, EZ, with high CO₂ grown cells being more sensitive to EZ than low CO₂ grown cells. We also show that EZ inhibits CO₂ utilization in other cyanobacterial strains, namely Anabaena variabilis M3 and Synechocystis PCC6803.

MATERIAL AND METHODS

Growth Conditions

Cells of Synechococcus PCC7942 (Anacystis nidulans R2), Anabaena variabilis M3, and Synechocystis PCC6803 were
grown in BG11 media (21) buffered with 10 mM 1,3-bis(tris(hydroxymethyl)methylamino)propane (pH 8.0). Cells were grown in batch culture in large test-tubes (195 × 35 mm; 100 mL) illuminated by a combination of Gro-Lux and white fluorescent tubes (60 μE·m⁻²·s⁻¹; 30°C). Equilibrium between CO₂ in the gas phase and total CO₂ in the media was assured by vigorous aeration, using gas distribution tubes, and by harvesting cells (in log phase) before a Chl density of 3 μg/mL was reached (7). High C₄ cells were grown with 1% CO₂ (v/v) of air) in the gas phase and ‘low C₄’ cells with 30 to 50 ppm CO₂ in the gas phase. Culture pH remained constant at pH 8.0 when Chl densities were kept below 5 μg/mL.

**Photosynthetic Measurements**

The photosynthetic response of cyanobacteria to added inorganic carbon (as NaHCO₃) was measured using a Clarke-type O₂ electrode (Hansatech, Kings Lynn, Norfok, UK) maintained at 30°C and illuminated with a quartz halogen projector lamp at 250 μE·m⁻²·s⁻¹. Cells were collected by centrifugation (5 min; 4000g; 25°C) and washed twice and resuspended in low CO₂ containing (<30 μM C₄) BG11 media buffered with 20 mM 1,3-bis(tris(hydroxymethyl) methylamino)propane-HCl at pH 7.0, 8.0, or 9.0. Most experiments were performed at pH 8.0 and with a Chl density in the O₂ electrode cuvette of 1.5 to 2.5 μg/mL. Low C₄ cells were allowed to run out of C₄ in the cuvette before additions of NaHCO₃ were made. This was not usually necessary with high C₄ cells. In calculation of K₀₅ (C₄) values from the photosynthetic response to C₄, rates of O₂ evolution at zero C₄ were assumed to be due to respiratory CO₂ efflux (23). In such cases, rates were extrapolated to zero and the small C₄ value was added in determining K₀₅ (C₄).

Chl a was determined by the method of Wintermans and de Mots (25). EZ (obtained from Sigma; 1978 catalog) additions were made from 100 mM stock solutions dissolved in DMSO. When used at a final concentration of 0.2% (v/v), DMSO had no apparent effect on the photosynthetic response to C₄ (results not shown). Concentrations above 0.2% had a small effect on photosynthesis and PSII electron transport (see text). PSII electron transport in whole cells was measured as O₂ evolution in the presence 1 mM DMQ as described by Ogawa et al. (18).

**RESULTS**

**CO₂ Dependent Photosynthesis in High C₄, Grown Cells.**

Some of the photosynthetic characteristics of high C₄ and low C₄ cells of *Synechococcus* PCC7942, assayed at different pH are shown in Table I. Over the pH range 7 to 9, high C₄ cells had virtually the same photosynthetic affinity for CO₂ (i.e. K₀₅ (CO₂) of 3.4–5.4 μM). This indicates that high C₄ cells have a strong dependence on CO₂ species for photosynthesis.

Low C₄ cells displayed a constant and high photosynthetic affinity for C₄ (K₀₅ (C₄) of 10–11 μM) over the pH range 7 to 9 (Table I), indicating the ability to utilize both HCO₃⁻ and CO₂. This is consistent with the ability of low C₄ cells to utilize HCO₃⁻ and CO₂ which is now well established (5–7, 10, 24).

**Table 1. Photosynthetic Parameters of High C₄ and Low C₄, Grown Cells**

<table>
<thead>
<tr>
<th>Cells</th>
<th>K₀₅(C₄)μM</th>
<th>K₀₅(CO₂)μM</th>
<th>Pₘₐₓ (μmol O₂·mg Chl⁻¹·h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High C₄</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 7.0</td>
<td>24</td>
<td>4.2</td>
<td>298.0</td>
</tr>
<tr>
<td>pH 8.0</td>
<td>262</td>
<td>5.4</td>
<td>310.6</td>
</tr>
<tr>
<td>pH 9.0</td>
<td>1700</td>
<td>3.4</td>
<td>272.7</td>
</tr>
<tr>
<td>Low C₄</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 7.0</td>
<td>9.5</td>
<td>1.65</td>
<td>297.3</td>
</tr>
<tr>
<td>pH 8.0</td>
<td>9.8</td>
<td>0.20</td>
<td>295.6</td>
</tr>
<tr>
<td>pH 9.0</td>
<td>11.1</td>
<td>0.02</td>
<td>280.9</td>
</tr>
</tbody>
</table>

**Figure 1.** Effect of exogenous CA (0.17 mg/ml) on the photosynthetic response of high C₄ cells of *Synechococcus* PCC7942 to C₄ (pH 8.0). Cells were suspended at a Chl density of 18 μg/ml.

**Figure 2.** Effect of 200 μM EZ on the photosynthetic response of high C₄ cells of *Synechococcus* PCC7942 to exogenous C₄ (pH 8.0). Responses are shown on an extended scale (A, log plot) and over a 1 mM C₄ range (B, linear plot).

Further evidence that high C₄ cells are dependent on CO₂ species for photosynthesis comes from examining the photosynthetic affinity for C₄ under conditions where the rate of CO₂ supply is limiting—such as when cell densities are high. In the absence of CA and at a Chl density of 18 μg/mL, high
C, cells exhibited a lower than normal affinity for C, with a $K_{0.5}$ (C,) of 960 $\mu$M (Fig. 1). Addition of CA increased the photosynthetic affinity for C, more than 4-fold to a $K_{0.5}$ (C,) of 212 $\mu$M (Fig. 1). $P_{\text{max}}$ (maximum photosynthetic rate) was unchanged. Since the addition of CA at this pH (pH 8) would have caused a large increase in the rate of CO$_2$ supply but little change in HCO$_3$ supply, it must be concluded that photosynthesis in high C, cells is strongly CO$_2$ dependent.

**Effect of EZ on the Photosynthetic Affinity for C,**

The photosynthetic response of high C, grown cells to C, (pH 8) was dramatically inhibited in the presence of 200 $\mu$M EZ (Fig. 2). The addition of EZ caused the rate at 1 mM C, to drop by 83% (Fig. 2B), even though this C, concentration was normally saturating for photosynthesis in high C, cells (Fig. 2B). EZ also increased the $K_{0.5}$ (CO$_2$) by over 33-fold, from 4.6 to 155 $\mu$M (Fig. 2A; assuming $P_{\text{max}}$ in the presence of EZ was attained at 100 mM C,). Photosynthesis in EZ-inhibited cells could be restored to better than 85% of the control level by progressively elevating exogenous C, up to 100 mM (Fig. 2A), presumably in response to free diffusion of CO$_2$ into the cell. Alleviation of EZ inhibition by high levels of C, indicates that photosynthesis, per se, was largely unaffected by EZ. This is consistent with the response expected if a CO$_2$ accumulating mechanism were largely inoperative such that photosynthetic affinity reflected the affinity of Rubisco for CO$_2$. Cyanobacterial Rubisco has a $K_{0.5}$ (CO$_2$) of 150 to 200 $\mu$M (3, 4).

EZ was less effective in inhibiting the photosynthetic response to C, in low C, grown cells (Fig. 3). The addition of 200 $\mu$M EZ caused a 44% reduction in the photosynthetic rate at 100 $\mu$M C, (Fig. 3B), a level of C, that was normally saturating for low C, cells assayed at pH 8 (Fig. 3A). The addition of 400 $\mu$M EZ had a larger inhibitory effect than 200 $\mu$M EZ. With either concentration the level of inhibition at 100 $\mu$M C, was similar to that at 1 mM C,. Again, in the presence of EZ it was possible to completely restore photosynthesis by progressively increasing C, up to 100 mM (Fig. 3A). Curiously, much of the response of low C, cells to limiting C, concentrations (0–100 $\mu$M C,) was insensitive to EZ (Fig. 3B).

**Effect of EZ at Different pH**

Figure 4 displays the photosynthetic response of high C, cells to C, at pH 7, 8, and 9 in the presence and absence of 200 $\mu$M EZ. As described above, photosynthesis in high C, cells is CO$_2$-dependent (Table 1) such that $K_{0.5}$ (C,) rises approximately 10-fold for each 1 unit rise in pH over the pH range 7 to 9. It is apparent that 200 $\mu$M EZ had a greater inhibitory effect on high C, cells at pH 7 than at pH 8 or 9, with inhibition decreasing with increasing pH (Fig. 4). This can be seen by comparing degrees of inhibition at the $K_{0.5}$ (C,) value in each case. At pH 7, 8, and 9 the degree of inhibition due to 200 $\mu$M EZ was 87%, 71%, and 39%, respectively. This may indicate that the inhibitory effect of EZ is dependent on the uncharged (unprotonated) form of the inhibitor. EZ is a lipid soluble CA inhibitor with a $K_{0.5}$ of 8.1 (14). This pH dependence of EZ inhibition suggests that the inhibitor may need access to the cytoplasm or the interior of the plasma membrane. At pH 8 and 9, higher concentrations of EZ were capable of producing greater levels of inhibition (Fig. 4, B and C).

**Concentration Dependence of EZ Inhibition**

The effect of various concentrations of EZ on photosynthesis in 1% CO$_2$ grown and air grown cells (high C,) and 30 $\mu$L/L grown cells (low C,) is shown in Figure 5. Photosynthetic rate was measured in the presence of 1 mM C, (pH 8), a C, concentration that was saturating for all three cell types in the absence of EZ. Inhibition by EZ in high C, cells (1% CO$_2$ or air grown) was close to maximal at 200 to 300 $\mu$M EZ (Fig. 5). As noted above (Fig. 3), low C, cells were less sensitive to EZ than high C, cells with the effect in low C, cells being maximal at 400 to 500 $\mu$M EZ (Fig. 5). A component of the photosynthetic response of low C, cells was unaffected by up to 600 $\mu$M EZ. The addition of less than 50 $\mu$M EZ had little effect on either high C, or low C, cells. In experiments similar to those in Figure 4, it was found that the concentration dependence of EZ inhibition at limiting C, levels (25 $\mu$M C,) was similar to that at saturating C, levels (data not shown).

Levels of DMSO above 0.2% had a small effect on photosynthetic rate (Fig. 5, control). Although EZ dissolved in DMSO has the advantage of not introducing C, into the O$_2$.

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**Figure 3.** Effect of 200 and 400 $\mu$M EZ on the photosynthetic response of low C, grown *Synechococcus* PCC7942 cells to exogenous C, Data are presented over an extended C, range (A) and a 0 to 1 mM C, range (B).
Electrode, as do hydroxide dissolved stocks, suitable controls need to be included when using more than 0.2% DMSO.

Effect of Other Carbonic Anhydrase Inhibitors

In addition to EZ, three other CA inhibitors, namely acetazolamide (Diamox), methazolamide, and chlorazolamide (Cl 36.580), were evaluated for their ability to inhibit the photosynthetic response of high C₄ cells (Fig. 4). Of the four inhibitors, EZ was the most effective, followed by chlorazolamide (Fig. 6). Methazolamide had a weak inhibitory effect, whereas acetazolamide had no apparent effect. The effectiveness of these inhibitors was roughly proportional to their lipid solubility (14). This is a further indication that the site(s) of inhibition may be within the plasma membrane or within the cytoplasmic compartment.

Effect of EZ on PSII Electron Transport

Although most CA inhibitors are quite specific, it has been shown in chloroplasts that some CA inhibitors, in addition to inhibiting CA activity, can cause a small decrease in the rate of PSII electron transport (22). Both PSI and PSII appear to be required for C₄ uptake in cyanobacteria (11, 18). The possibility that EZ inhibits PSII activity was checked by measuring PSII activity in the presence of 1 mM DMQ (18). DMQ accepts electrons from plastquinone allowing PSII activity to be measured as O₂ evolution. The addition of 200 μM EZ decreased PSII activity by 12.4%, and even at 600 μM EZ, PSII activity was reduced by only 18.2% (Table II). These levels of inhibition of PSII activity by EZ are relatively low and certainly do not explain the dramatic inhibition of photosynthesis seen in high C₄ cells with 200 μM EZ, especially since much of this effect can be alleviated by the use of very high C₄ levels (Fig. 2). EZ inhibition of PSII electron transport may, however, affect the maximum photosynthetic rate attained at 100 mM C₄ (Fig. 2).

Reversibility of EZ Inhibition

Inhibition of photosynthesis by EZ in high C₄ cells was readily reversed by either dilution or washing (Table III). Cells were preincubated with 400 μM EZ, an EZ concentration which typically inhibited photosynthesis by greater than 85%
Table II. Effect of EZ on PSII Activity

Table shows PSII activity in high C, grown cells of Synechococcus PCC7942 as O₂ evolution in the presence of 1 mM DMQ. Data presented as a percentage decrease against the control rate of photosynthesis (absence of DMSO and EZ). Control rate was 470.1 ± 4.9 μmol O₂·mg Chl⁻¹·h⁻¹ (so; n = 7). CO₂-saturated photosynthesis (no DMQ) was 543.7 ± 8.0 μmol O₂·mg Chl⁻¹·h⁻¹ (so; n = 7). "EZ only" data (column 4) calculated as "EZ + DMSO" minus "DMSO only."

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage Decrease against Control rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DMSO only</td>
</tr>
<tr>
<td>100 μM EZ (+0.1% DMSO)</td>
<td>0.5</td>
</tr>
<tr>
<td>200 μM EZ (+0.2% DMSO)</td>
<td>1.9</td>
</tr>
<tr>
<td>300 μM EZ (+0.3% DMSO)</td>
<td>2.3</td>
</tr>
<tr>
<td>400 μM EZ (+0.4% DMSO)</td>
<td>3.3</td>
</tr>
<tr>
<td>600 μM EZ (+0.6% DMSO)</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Table III. Reversibility of EZ Inhibition

O₂ evolution in high C, cells of Synechococcus PCC7942 in the presence of 1 mM C, (pH 8.0). Diluted cells and washed cells were resuspended to a Chl concentration of 2 μg/ml (±50 μM EZ), being initially 16 μg/ml (±400 μM EZ). Data in parentheses represented as percentage of control rate.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Photosynthetic Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control + preincubated with 400 μM EZ</td>
</tr>
<tr>
<td></td>
<td>μmol O₂·mg chl⁻¹·h⁻¹</td>
</tr>
<tr>
<td>Cells diluted 8-fold</td>
<td>303.6 (100)</td>
</tr>
<tr>
<td>+50 μM EZ</td>
<td>227.2 (74.8)</td>
</tr>
<tr>
<td>+200 μM EZ</td>
<td>48.2 (15.9)</td>
</tr>
<tr>
<td>Cells centrifuged and resuspended</td>
<td>301.6 (100)</td>
</tr>
<tr>
<td>+200 μM EZ</td>
<td>46.8 (15.5)</td>
</tr>
</tbody>
</table>

(Fig. 4B). When the cells were diluted 8-fold, the level of inhibition was equivalent to that seen in control cells with 50 μM EZ present (Table III). Also, cells preincubated with 400 μM EZ, then centrifuged and resuspended in fresh medium, showed no inhibition (Table III). These cells were inhibited normally when 200 μM EZ was added. Such results show that, in Synechococcus, EZ is a reversible inhibitor of possible CA-like function(s) in the cell.

Effect of EZ in Other Cyanobacterial Strains

Figure 7 depicts the photosynthetic response to C, in high C, grown cells of Anabaena variabilis M3 in the presence and absence of 200 μM EZ. The effect of EZ and its concentration dependence (Fig. 7, inset) was similar to that seen in high C, cells of Synechococcus PCC7942 (Fig. 2) with inhibition being maximal around 1 mM C, and relieved by high C, (Fig. 7). EZ also inhibited high C, cells of Synechocystis PCC6803 (Fig. 8), although the level of inhibition at limiting C, and the concentration dependence was less pronounced than in Synechococcus or Anabaena. EZ inhibition of high C, cells may be a common feature in many strains of cyanobacteria and may indicate that CO₂-dependent photosynthesis in cyanobacteria requires the operation of similar CA-like components.

DISCUSSION

There is now evidence that cyanobacteria possess a constitutive CO₂ transport capacity in addition to the better characterized, high affinity and inducible HCO₃⁻ transport/utilization system (1, 5–7, 24). Recent evidence has revealed that high C, grown cells of Synechococcus PCC6301 retain the ability to transport and accumulate CO₂ but lose the ability for HCO₃⁻ uptake, whereas low C, cells transport both CO₂ and HCO₃⁻, although CO₂ is still transported with higher efficiency (7). It now seems likely that a CO₂ pump that uses CO₂ as substrate forms the key component of the CCM and that HCO₃⁻ usage requires front-end conversion of HCO₃⁻ to CO₂ (5, 7).
This paper presents further evidence that high C, cells utilize only CO₂ species. High C, cells show the same photosynthetic affinity for CO₂ (i.e. K₀.₅ (CO₂) of 3.4–5.3 μM) over the pH range 7 to 9 (Table 1). This result indicates that these cells utilize CO₂ only. Miller and Canvin (16) have recently reported similar data for Synechococcus UTEX625 in that over the pH range 6 to 9 K₀.₅ (CO₂) was similar, ranging from 7 to 20 μM. Low C, cells of Synechococcus PCC7942 had the same K₀.₅ (C) of 10 to 11 μM over the pH range 7 to 9 (Table 1), indicating the ability for CO₂ and HCO₃⁻ utilization. Finally, in dense cell suspensions of high C, cells the photosynthetic affinity for CO₂ was low, due to the CO₂ supply rate lagging behind demand, but the normal high affinity could be restored by the addition of exogenous CA (Fig. 1). Such an effect is consistent with high C, cells being highly dependent on an external CO₂ supply for photosynthesis.

The addition of the CA inhibitor, EZ, to high C, grown cells of Synechococcus PCC7942 had the effect of decreasing photosynthetic affinity for CO₂. This produced a result that might be expected were a CO₂ accumulating system largely inoperative, i.e. K₀.₅ (CO₂) increased toward the K₀.₅ (CO₂) of Rubisco (Figs. 2 and 4). The addition of 200 μM EZ to high C, cells increased K₀.₅ (CO₂) from around 5 μM to greater than 155 μM (Fig. 2). Pₘ₅ was largely restored by the addition of very high C, levels, presumably in response to the diffusion of free CO₂ into the cells (Figs. 2 and 4). Although EZ caused a small decrease in PSII activity (Table II) the drop was too small to explain the strong inhibition of CO₂ usage caused by EZ. EZ inhibition was found to be reversible by wash or dilution (Table III). Lipophilic CA inhibitors such as EZ and chlorozolamide were found to most effectively inhibit CO₂ usage (Fig. 6). Furthermore, the pH dependence of EZ inhibition suggests that the uncharged form of EZ was most inhibitory (Fig. 4). The results above indicate that a CA-like step may be involved in photosynthetic utilization of CO₂ and that CA inhibitors may need to gain access to the cytoplasmic compartment or the interior of the cytoplasmic membrane. This is the first report that CO₂ utilization in Synechococcus PCC7942 can be inhibited by EZ. Synechococcus spp. had previously been thought to be unaffected by EZ, but earlier attempts had employed lower concentrations (10–100 μM) of the inhibitor (9). The effect of EZ on high C, cells may be a common feature of cyanobacterial photosynthesis since, in addition to Synechococcus PCC7942, high C, cells of Anabaena variabilis M3 and Synechocystis PCC6803 (Figs. 7 and 8) were sensitive to EZ.

In low C, grown cells of Synechococcus, 200 μM EZ had a smaller inhibitory effect than on high C, cells (cf. Figs. 2 and 3; Fig. 5) with K₀.₅ (C) increased from 10 to 50 μM, although 400 μM EZ increased K₀.₅ (C) to around 10 mM (Fig. 3). High concentrations of EZ were required to substantially inhibit low C, cells (Fig. 5). It is not clear why low C, cells should be less sensitive to EZ, but reduced access of EZ to the cell interior due to cell coat modification is a possibility. Alternatively, a HCO₃⁻–CO₂ front-end mechanism in low C, cells may alter the effect of EZ on the CO₂ uptake mechanism.

The inhibitory effect of EZ on the photosynthetic affinity of high C, cells might occur at one or both of two key CA-involving steps: i.e. inhibition of the CO₂ utilizing C, pump on the plasma membrane or inhibition of internal CO₂/HCO₃⁻ equilibria such that the rate of supply of CO₂ to Rubisco is impeded. From evidence presented in this paper, and from other current evidence, it seems reasonable to conclude that high C, grown Synechococcus spp. (6, 7, 16) and Anabaena spp. (1) possess a C, pump that uses CO₂ as substrate. Current evidence, however, suggests that transported C, arrives inside the cell as HCO₃⁻ (2, 24). This raises the possibility that the C, pump has a CA-like activity (CO₂ + OH⁻ → HCO₃⁻) associated with it, allowing exogenous CO₂ to be accumulated as HCO₃⁻. A demonstrated effect of the CA inhibitor, EZ, on the pump would be consistent with this view, especially since the sulfonamide group of a CA inhibitor appears to bind at the HCO₃⁻ site of the carbonic anhydrase enzyme (14).

The assertion that HCO₃⁻ is the species delivered inside the cell is quite critical to the proposal that the C, pump has CA-like activity. It also has bearing on the prospect that EZ might, as an alternative, inhibit cytoplasmic CA activity resulting in a drop in CO₂ supply to Rubisco. Volokita et al. (24) have shown through active species experiments, in Anabaena, that when either CO₂ or HCO₃⁻ are presented in disequilibrium a constant linear relationship exists between internal C, pool size and photosynthetic rate. This is interpreted to mean that a single species of C, is delivered to the cell interior whether CO₂ or HCO₃⁻ is supplied externally. A more compelling argument suggesting that HCO₃⁻ arrives internally, comes from the analysis of mutants of Synechococcus that exhibit normal C, uptake and accumulate high levels of C, but are unable to photosynthesize normally unless ambient CO₂ is raised to the 1.0% or 5.0% level (12, 19) (GD Price, MR Badger, unpublished data). Such mutants would appear to lack internal CA activity and their inability to utilize the internal C, pool suggests that HCO₃⁻ rather than CO₂ enters the cell. An essential role for internal CA in cyanobacterial photosynthesis has been identified and modeled, although the amounts required may be quite low (9). In a companion study (8) we report that low levels of CA activity can be detected in cells of Synechococcus PCC7942 using an ¹⁸O exchange method. Internal CA activity has been detected in several other cyanobacteria (9, 23, 26).

Thus schemes whereby EZ either inhibits a CO₂ utilizing C, pump with CA-like activity or inhibits an internal CA can be envisaged. An effect on either process would lead to inhibition of CO₂-dependent photosynthesis in high C, cells of Synechococcus PCC7942 (or Anabaena variabilis M3 and Synechocystis PCC6803). The situation in low C, cells may be more complicated than in high C, cells. For instance, EZ might inhibit a front-end mechanism for converting HCO₃⁻ to CO₂: (5, 7) for the C, pumps or it could inhibit a separate HCO₃⁻ pump. The most straightforward explanation, however, would be that EZ inhibits either the CO₂ utilizing C, pump or an internal CA activity. In a companion study (20), however, we show that EZ does inhibit the C, pump in high C, cells (and low C, cells) of Synechococcus PCC7942, and that EZ does not appear to inhibit internal CA in intact cells.

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LITERATURE CITED


