Active transport of CO$_2$ and bicarbonate is induced in response to external CO$_2$ concentration in the green alga *Chlorella kessleri*

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**Abstract**

The time-course of induction of CO$_2$ and HCO$_3^-$ transport has been investigated during the acclimation of high CO$_2$-grown *Chlorella kessleri* cells to dissolved inorganic carbon (DIC)-limited conditions. The rate of photosynthesis of the cells in excess of the uncatalysed supply rate of CO$_2$ from HCO$_3^-$ was taken as an indicator of HCO$_3^-$ transport, while a stimulation of photosynthesis on the addition of bovine carbonic anhydrase was used as an indicator of CO$_2$ transport. The maximum rate of photosynthesis ($P_{max}$) was similar for high CO$_2$-grown and low CO$_2$-grown cells, but the apparent whole cell affinity for DIC and CO$_2$ of high CO$_2$-grown cells was found to be about 30-fold greater than in air-grown cells, which indicates a lower affinity for DIC and CO$_2$. It was found that HCO$_3^-$ and CO$_2$ transport were induced in 5.5 h in cells acclimating to air in the light and in the presence and absence of 21% O$_2$, which indicates that a change in the CO$_2$/O$_2$ ratio in the acclimating medium does not trigger induction of DIC transport. No active DIC transport was detected in high CO$_2$-grown cells maintained on high CO$_2$ for 5.5 h in the presence of 5 mM aminooxyacetate, an aminotransferase inhibitor. These results indicate no involvement of photorespiration in triggering induction. Active DIC transport induction was inhibited in cells treated with 5 μg ml$^{-1}$ cycloheximide, but was unaffected by chloramphenicol treatment, indicating that the induction process requires *de novo* cytoplasmic protein synthesis. The total DIC concentration eliciting the induction and repression of CO$_2$ and HCO$_3^-$ transport was higher at pH 7.5 than at pH 6.6. The concentrations of external CO$_2$ required for the induction and repression of DIC transport were 0 and 120 μM, respectively, and was independent of the pH of the acclimation medium. Prolonged exposure to a critical external CO$_2$ concentration elicits the induction high CO$_2$-grown *Chlorella kessleri* cells to dissolved inorganic carbon (DIC)-limited conditions. The rate of photosynthesis of the cells in excess of the uncatalysed supply rate of CO$_2$ from HCO$_3^-$ was taken as an indicator of HCO$_3^-$ transport, while a stimulation of photosynthesis on the addition of bovine carbonic anhydrase was used as an indicator of CO$_2$ transport. Introduction

Microscopic algae and cyanobacteria respond to limitations in extracellular dissolved inorganic carbon (DIC) by the induction of a high DIC-affinity photosynthesis, described as a carbon concentrating mechanism (CCM). This results from the induction of active CO$_2$ and/or active bicarbonate transport systems and, in some microalgae, the induction of an extracellular carbonic anhydrase (CA) (Badger *et al*., 1980; Kaplan *et al*., 1994; Matsuda and Colman, 1995a, b; Matsuda *et al*., 1998; Beardall *et al*., 1998; Sültemeyer *et al*., 1998a, b; Moroney and Somanchi, 1999). The acclimation of cells to DIC-limited conditions is characterized by an increased capacity to accumulate intracellular DIC against a concentration gradient which elevates the CO$_2$ concentration around the main carboxylating enzyme, Rubisco, and thus reduces the inhibition of CO$_2$ fixation by O$_2$. During acclimation to low CO$_2$, there is also a marked decrease in the whole cell $K_{1/2}$ for DIC and CO$_2$ and a decrease in the CO$_2$-compensation point of the cells (Matsuda and Colman, 1995a, b; Matsuda *et al*., 1998; Colman *et al*., 1998; Palmqvist *et al*., 1988). *De novo* protein synthesis of cytoplasmic proteins encoded by the phototroph’s}

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nuclear genome is thought to arise during the induction of the CCM (Shiraiwa and Miyachi, 1985; Palmqvist et al., 1988; Matsuda and Colman, 1995a; Matsuda et al., 1998). The regulation of de novo protein synthesis in the CCM is important in understanding the acclimation response to low CO₂. Although the signalling pathway which initiates induction in green algae and cyanobacteria is not known, it has been proposed that the induction of high affinity photosynthesis may be in response to a build-up of photorespiratory pathway intermediates within the cell (Marcus et al., 1983). The photorespiratory signal model had been proposed because there is an intracellular accumulation and release of glycolate into the external medium during the acclimation of Chlamydomonas reinhardtii to ambient CO₂ levels. This is thought to be the result of a decrease in the CO₂/O₂ ratio in the growth medium, which would stimulate the oxygenase activity of Rubisco, and cause an increase in photorespiratory pathway intermediates. The trigger for induction is therefore light- and O₂-dependent. The requirement for light has also been reported in regulating the activity of external carbonic anhydrase, an enzyme which is thought to play a role in the CCM of C. reinhardtii (Spalding and Ogren, 1982). For example, it was found that an increase in CA mRNA occurred in C. reinhardtii within 2 h of acclimation to low-CO₂ in the light; but remained unchanged when cells were acclimated in darkness (Dionisio-Sese et al., 1990). However, it was also demonstrated that periplasmic CA transcript was made in the dark after a lag period (Rawat and Moroney, 1995).

There is increasing evidence to suggest that cells do not respond to an internal metabolic signal, but to a critical concentration of dissolved CO₂ in the external growth medium (Matsuda and Colman, 1995b; Matsuda et al., 1998). In the unicellular green alga, Chlorella ellipsoidea, the induction of the CCM occurs when cells are acclimated to low CO₂ in darkness (Matsuda and Colman, 1995b) and similarly, a decrease in the K₁/₂ CO₂ for Chlorella regularis was reported during acclimation to low CO₂, which was independent of photosynthesis (Umino et al., 1991). These results cannot be explained by the photorespiratory signal model. A model was proposed in which the concentration of dissolved CO₂ molecules occupying a CO₂ sensor at the cell surface dictates the magnitude of active DIC transport: the response is graded as opposed to being an all-or-nothing type of response (Matsuda and Colman, 1996a).

In this report, the induction of active DIC uptake in the unicellular green alga Chlorella kessleri, during the acclimation of high-CO₂ grown cells to low-CO₂ conditions, is described. The effect of dissolved O₂ concentration on the induction process was also investigated and the critical concentrations of total DIC and CO₂, eliciting the induction of active DIC transport in C. kessleri were determined.

Materials and methods

An axenic culture of Chlorella kessleri (Fott et Nováková, UTEX 1808) was obtained from the University of Texas Culture Collection. Cells were grown axenically in batch culture, in Bold’s basal medium as described previously (Gehl et al., 1990), under a constant light fluence (100 μmol m⁻² s⁻¹). Cultures were aerated with 5% CO₂ (high-CO₂) or with air (0.035% CO₂); zero CO₂-grown cells were aerated with CO₂-free air or at a low aeration rate (0.01 l min⁻¹) with an ambient air stream, to ensure that cells were maintained in suspension, both of which gave a DIC concentration in the medium of approximately zero.

The physiological characteristics of cells grown under the various CO₂ concentrations were assessed after they had been harvested at mid-log growth phase (A₅₇₀ 0.4–0.5) by centrifugation at 4500 g for 3 min at room temperature. Cells were washed twice with N₃-equilibrated, 50 mM Na⁺/K⁺-phosphate buffer (pH 7.8), containing less than 5 μM DIC, and resuspended in the same buffer. Photosynthetic oxygen evolution rates at various DIC concentrations were measured in a Clark-type O₂ electrode as described previously (Gehl and Colman, 1985) with a light fluence of 400 μmol m⁻² s⁻¹. The apparent whole cell affinity (K₁/₂) for DIC and CO₂ was determined according to the method of Rotatore and Colman, with and without the addition of bovine CA (Rotatore and Colman, 1991). The CO₂-compensation point of the cells was measured by gas chromatography (Birmingham and Colman, 1979).

Physiological changes in high CO₂-grown cells were determined during periods of acclimation to air. High CO₂-grown cells were harvested at mid-log phase, resuspended in Bold’s basal medium (pH 6.6), and allowed to acclimate to air for 24 h. The DIC concentration in the medium was monitored periodically. During the acclimation process, cells were harvested periodically and the capacity of the cells to actively take up HCO₃⁻ was assessed by comparing the O₂ evolution rate at 50 μM DIC, pH 7.8 and 25 °C, with the spontaneous rate of CO₂ formation from HCO₃⁻ in the medium, calculated according to the method of Miller and Colman (Miller and Colman, 1980). Stimulation of the O₂ evolution rate at a DIC concentration of 50 μM upon the addition of bovine CA (10 μg ml⁻¹), was used as a measure of active CO₂ uptake. The effect of O₂ concentration in the medium during acclimation was examined by transferring high CO₂-grown cells to Bold’s basal medium (pH 6.6), aerated with O₂-free N₂, enriched with 0.035% CO₂.

The critical DIC concentrations corresponding to the induction of active CO₂ and HCO₃⁻ transport were determined by the procedure of Matsuda and Colman (Matsuda and Colman, 1995b). High CO₂-grown cells were harvested at mid-log growth phase (A₅₇₀ 0.4), and resuspended in Bold’s basal medium (phosphate-buffered at pH 6.6 or 7.5). The cell suspensions were axenically transferred to 0.5 l cylindrical culture vessels equipped with a sampling port plugged with a rubber serum stopper, and aerated with defined CO₂ concentrations, in the range of zero to 0.42%. A constant dissolved CO₂ concentration in the medium was maintained by adjusting the pH to ±0.1 units, by injections of 2.0 M HCl or 2.0 M NaOH and by controlling the inflow CO₂ concentration. Inflow CO₂ concentrations and the DIC concentration of the medium were measured by gas chromatography. Equilibrium conditions between HCO₃⁻ and CO₂ in the culture medium were verified by comparing the calculated concentrations of DIC at each pH and inflow CO₂ concentration (Buch, 1960; Stumm and Morgan, 1981) with the measured concentration of DIC in the medium. Cells were harvested after 5.5 h of acclimation to the
defined CO₂ concentration and rates of photosynthetic oxygen evolution at 50 μM DIC, pH 7.8 and 25 °C were determined, with and without bovine CA.

The effect of protein synthesis inhibitors on the acclimation of high-CO₂-grown cells to low-CO₂ was determined by the method of Matsuda and Colman (Matsuda and Colman, 1995a). High CO₂-grown cells were harvested and resuspended in Bold’s basal medium containing 5 μg ml⁻¹ cycloheximide or 400 μg ml⁻¹ chloramphenicol and aerated with 0.035% CO₂ for 5.5 h. Cells were assayed for active DIC transport after the acclimation period following the method described above. The effect of 5 mM aminooxyacetate, an aminotransferase inhibitor, on high CO₂-grown cells maintained on high CO₂ for 5.5 h was also examined.

Results

Photosynthetic affinity

C. kessleri cells were grown under 5% CO₂, 0.035% CO₂ and 0% CO₂ conditions, and photosynthetic oxygen evolution rates measured at various DIC concentrations at pH 7.8, once the CO₂ compensation point of the cell suspension had been reached. C. kessleri cells grown under DIC-limited conditions demonstrated a high photosynthetic affinity (K₁/₂) for DIC and CO₂ (Table 1) in comparison to 5% CO₂-grown cells. The K₁/₂ DIC was lowest in cells grown in CO₂-free medium (Table 1). When bovine CA was added to algal cell suspensions during the assay there was a further decrease in the K₁/₂ DIC and K₁/₂ CO₂ values under all growth conditions (Table 1). The maximum rate of photosynthetic oxygen evolution (P_max) was similar for cells grown in CO₂-enriched and CO₂-limited media (Table 1). The CO₂ compensation point was also found to decrease when the CO₂-level in the growth medium was reduced (Table 1).

The time-course of acclimation

Suspensions of high CO₂-grown cells were allowed to acclimate to air or to O₂-free nitrogen supplemented with 0.035% CO₂ for 24 h. The DIC concentration in the medium was initially about 5.0 mM at pH 6.6 and decreased to 30 μM at pH 6.6 after 2 h of acclimation.

Samples of the cell suspension were taken at intervals over the 24 h period for the determination of photosynthetic rates.

Photosynthetic O₂ evolution rates for cells acclimating to 0.035% CO₂ in the presence and absence of 21% dissolved O₂ were measured at 50 μM DIC, pH 7.8 and 25 °C. Under these conditions, the calculated maximum dehydrogenation rate of HCO₃⁻ is 5.39 nmol CO₂ ml⁻¹ min⁻¹, following the method of Miller and Colman (Miller and Colman, 1980). Within 2 h of acclimation, in the presence and absence of O₂, O₂ evolution rates measured in the absence of CA were significantly greater than the calculated maximum rate of CO₂ supply and, assuming a photosynthetic quotient of unity, this indicates that active HCO₃⁻ transport was induced in cells within 2 h. The addition of CA during this measurement stimulated the O₂ evolution rate 1.5-fold (Fig. 1), indicating active CO₂ transport, since the addition of excess CA maintains the CO₂ supply available to the cells. In both O₂-free acclimated and air-acclimated cells, O₂ evolution rates of cells acclimating for 6 h and measured without added CA, were 1.5-fold greater than those of cells harvested at 2 h. The O₂ evolution rates measured with added CA were approximately 2-fold greater than cells harvested at 2 h. Active HCO₃⁻ and CO₂ transport were fully induced after 6 h of acclimation to air, regardless of the concentration of dissolved O₂ in the medium (Fig. 1).

Critical external DIC concentration during acclimation

Cell suspensions were aerated with various inflow CO₂ concentrations, in the range of zero to 0.42%, at pH 6.6 and pH 7.5, and allowed to acclimate for a 5.5 h period. The rate of HCO₃⁻ and CO₂ transport at 50 μM DIC, pH 7.8 and 25 °C was assayed at the end of the acclimation period. At pH 6.6, O₂ evolution rates measured in the absence of CA, indicated that HCO₃⁻ transport was repressed at approximately 240 μM DIC (Fig. 2), when the O₂ evolution rate was compared to the maximum rate of uncatalysed CO₂ supply, whereas at pH 7.5, the DIC concentration in the external medium that repressed DIC transport induction in Chlorella

Table 1. Photosynthetic characteristics of Chlorella kessleri cells grown in high CO₂ or low CO₂ conditions

Values were determined using cell suspensions of 40 μg Chl ml⁻¹ at pH 7.8 and 25 °C, with and without added CA.

<table>
<thead>
<tr>
<th>Growth type</th>
<th>CP⁻</th>
<th>K₁/₂</th>
<th>P_max</th>
<th>K₁/₂</th>
<th>CP⁻</th>
<th>P_max</th>
<th>K₁/₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂-free</td>
<td>0.0</td>
<td>102.5 ± 4.3</td>
<td>16.4 ± 4.3</td>
<td>0.6 ± 0.1</td>
<td>0.0</td>
<td>109.6 ± 7.5</td>
<td>20.8 ± 8.3</td>
</tr>
<tr>
<td>Air</td>
<td>12.38 ± 6.9</td>
<td>111.8 ± 6.8</td>
<td>51.9 ± 14.5</td>
<td>1.8 ± 0.5</td>
<td>13.1 ± 5.7</td>
<td>107.9 ± 6.3</td>
<td>35.5 ± 10</td>
</tr>
<tr>
<td>High CO₂</td>
<td>48.45 ± 3.1</td>
<td>138.7 ± 15</td>
<td>1887 ± 452</td>
<td>64.2 ± 15</td>
<td>32.5 ± 10</td>
<td>117.4 ± 14</td>
<td>850 ± 294</td>
</tr>
</tbody>
</table>

*The CO₂-compensation point concentration (μM) is the mean ± SD of five separate experiments.
*The maximum rate of photosynthesis (μmol O₂ mg⁻¹ Chl h⁻¹) is the mean ± SD of five separate experiments.
*The apparent whole cells affinity for DIC, K₁/₂ [DIC] in μM, was measured according to the method of Rotatore and Colman (Rotatore and Colman, 1991). Values are the means ± SD of five separate experiments.
*K₁/₂ [CO₂] is reported in μM, and was calculated based on the premise that DIC in the O₂ evolution rate experiments is in equilibrium. At pH 7.8, 34% of the total DIC is dissolved CO₂. Values are means ± SD of five separate experiments.
Fig. 1. Changes in the photosynthetic $O_2$ evolution rate in high $CO_2$-grown cells of *Chlorella kessleri* during acclimation to air and $O_2$-free air. $O_2$ evolution rates were measured at 50 $\mu$M DIC, pH 7.8 and 25 °C, at approximately 40 $\mu$g Chl ml$^{-1}$. $O_2$ evolution rates in cells acclimating to air assayed with (□) and without (○) added CA; and acclimating to $O_2$-free $N_2$ supplemented with 0.035% $CO_2$ assayed with (●) and without (▲) added CA. The dashed line represents the rate at which the spontaneous formation of $CO_2$ is maximum at 50 $\mu$M DIC and pH 7.8. Values are the means ± SE of three separate experiments.

Fig. 2. Acclimation of high $CO_2$-grown *Chlorella kessleri* cells to various external concentrations of DIC and $CO_2$ (inset) at pH 6.6 for 5.5 h. $O_2$ evolution rates were determined at 50 $\mu$M DIC, pH 7.8 and 25 °C with (□) and without (○) added CA. The dashed line represents the calculated maximum rate of $CO_2$ formation from 50 $\mu$M HCO$_3^-$ at pH 7.8 and 25 °C.

Fig. 3. Acclimation of high $CO_2$-grown *Chlorella kessleri* cells to various external concentrations of DIC and $CO_2$ (inset) at pH 7.5 for 5.5 h. $O_2$ evolution rates were determined at 50 $\mu$M DIC, pH 7.8 and 25 °C with (□) and without (●) added CA. The dashed line represents the calculated maximum rate of $CO_2$ formation from 50 $\mu$M HCO$_3^-$ at pH 7.8 and 25 °C.

active HCO$_3^-$ transport was 1300 $\mu$M (Fig. 3). At both pH 6.6 and 7.5 during the 5.5 h acclimation period, the dissolved $CO_2$ concentration eliciting the fully-repressed responses were similar, being 86.4 and 86.0 $\mu$M, respectively.

The DIC concentration corresponding to the $O_2$ evolution rate at which half the maximum HCO$_3^-$ transport is induced was greater in cells acclimating at pH 7.5 than in cells adapting at pH 6.6 (Table 2). The dissolved external $CO_2$ concentration corresponding to the induction of the half maximum HCO$_3^-$ transport was approximately 10 $\mu$M at both pH values (Table 2). The total DIC concentration in the external medium corresponding to repression of active DIC transport in *C. kessleri* cells was approximately 5.5-fold greater in cells acclimating at pH 7.5, in comparison to cells acclimating at pH 6.6; whereas the external $CO_2$ concentration eliciting the same response was 120 $\mu$M at both pH values. The presence of CA in the measurement of $O_2$ evolution rates in *C.
kessleri cells acclimating to defined external CO₂ concentrations indicated that at 120 μM CO₂, the rate of photosynthetic O₂ evolution was comparable to rates measured in 5% CO₂-grown cells (Figs 2, 3). High CO₂-grown C. kessleri cells acclimating to external CO₂ concentrations greater than 120 μM, showed no significant difference in O₂ evolution rates measured at 50 μM DIC, pH 7.8 and 25°C with and without added CA (data not shown) indicating that the cells had a greatly reduced capacity to transport CO₂ when acclimated at CO₂ concentrations greater than 120 μM.

Effect of metabolic inhibitors

High CO₂-grown cells were allowed to acclimate to 0.035% CO₂ at pH 6.6 for 5.5 h in the presence of protein synthesis inhibitors. Treatment with a cytoplasmic protein synthesis inhibitor, cycloheximide (5 μg ml⁻¹), inhibited the induction of active HCO₃⁻ and CO₂ transport, the activities of which remained comparable to those of cells maintained on 5% CO₂ for 5.5 h. Treatment with the chloroplastic protein synthesis inhibitor, chloramphenicol (400 μg ml⁻¹), did not inhibit the induction of active DIC transport in C. kessleri cells acclimating to low CO₂, and the O₂ evolution rates measured in the presence and absence of CA were similar to cells acclimating to low CO₂ with no inhibitor (Fig. 4).

It has been suggested that the accumulation of intermediates of the photosynthetic pathway, possibly phosphoglycolate (Marcus et al., 1983; Suzuki et al., 1990) or glycocolate could act as triggers for the induction of the CCM in algae. In order to test this hypothesis, high CO₂-grown cells, maintained on high CO₂, were treated with the photosynthetic pathway inhibitor, 5 mM AOA and 10 mM isonicotinyl hydrazide for 5.5 h. Neither AOA (Fig. 4) or INH (data not shown) had a stimulatory effect on the induction of active DIC transport of C. kessleri cells.

Discussion

C. kessleri cells induce a CCM during acclimation to low CO₂ in response to a critical dissolved CO₂ concentration in the external medium during the acclimation process. Cells grown under a 5% CO₂ aeration, exhibit a low affinity for DIC and for CO₂ in comparison to low CO₂-grown cells, although there is no difference in the maximum rate of photosynthesis between the two growth conditions (Table 1). High affinity photosynthesis in low CO₂-grown cells was similar to that in other green alga (Matsuda and Colman, 1995a; Sultemeyer et al., 1991; Mayo et al., 1986; Badger et al., 1980) and to cases where the CCM is constitutively expressed under all CO₂ concentrations as in Chlorella saccharophila and CO₂-insensitive C. ellipsoidea (Matsuda and Colman, 1996a, b). Air-grown C. kessleri cells have a high affinity for dissolved CO₂ (Table 1) and there was an increase in affinity for DIC in CO₂-free-grown cells, indicating that a fully inducible CCM may be responding to an external CO₂ concentration between ambient and CO₂-free conditions.

Air-grown cells had a high affinity for CO₂ as indicated by the low Kᵣ/₂ CO₂ in the presence of bovine CA which would maintain a constant CO₂ concentration in the medium (Table 1). The CO₂ affinity of air-grown and CO₂-free-grown cells is high, but the increase in CO₂ affinity of high-CO₂ cells on the addition of CA indicates the presence of some CO₂ transport activity in these cells (Table 1). In all growth conditions the cells display a higher affinity for CO₂ than for HCO₃⁻ (Table 1). It has been reported that air-grown C. ellipsoidea and C. saccharophila had higher affinities for CO₂ in comparison to that for HCO₃⁻ (Matsuda et al., 1999). C. kessleri showed the same phenomenon, but had affinities that were signi-
significantly higher for both DIC species (Matsuda et al., 1999). It appears that C. kessleri utilizes available inorganic carbon better than other Chlorella spp. (Matsuda et al., 1999).

C. kessleri cells fully induce and repress active DIC transport at critical dissolved CO₂ concentrations which are pH independent (Figs 2, 3). After 5.5 h, DIC transport is fully induced at about 120 µM dissolved CO₂ and induction reached its maximum at about 0 µM CO₂. The inflow CO₂ concentrations required to achieve these critical CO₂ concentrations were 0.4% and 0% CO₂, respectively. Active CO₂ transport in C. kessleri does not seem to be completely repressed under high CO₂ conditions and is similar to the low affinity CO₂ uptake that has been shown to occur in high CO₂-grown C. reinhardtii cells (Stueltemeyer et al., 1989). The low affinity CO₂ transport system is not a characteristic of all green algae, however; for example, a basal level of CO₂ uptake is absent in high CO₂-grown C. ellipsoidea cells (Matsuda and Colman, 1995a).

There is a marked difference in the CO₂ concentration triggering the induction of the two uptake systems. In C. kessleri, bicarbonate transport is induced at a lower CO₂ concentration in comparison to active CO₂ transport. Although high affinity CO₂ transport is induced at 120 µM CO₂, active bicarbonate uptake remains repressed (Figs 2, 3). In C. kessleri cells acclimating to an inflow CO₂ concentration of 0.2% CO₂, which corresponds to a dissolved CO₂ concentration of 60 µM, active bicarbonate transport is first observed (Table 2). At this external CO₂ concentration, active CO₂ transport is 30% induced. The derepression of the bicarbonate transport system occurred at a similar external CO₂ concentration in C. ellipsoidea cells (Matsuda and Colman, 1995b). In comparing the induction of active DIC transport in C. ellipsoidea with that in C. kessleri cells, active DIC transport is fully induced at 35 µM CO₂ in C. ellipsoidea, and this critical CO₂ concentration corresponds to 50% of fully induced active DIC transport in C. kessleri (Figs 2, 3). The CO₂ concentration range corresponding to the full repression to the full induction of active DIC transport is wider in low CO₂-acclimating C. kessleri, than in C. ellipsoidea cells (Matsuda and Colman, 1995b).

There is also a temporal separation in the induction of the two active uptake systems. High CO₂-grown cells transferred rapidly to a medium with a low partial CO₂ pressure derepress active CO₂ transport prior to derepression of active HCO₃⁻ transport; high CO₂-grown C. kessleri cells acclimating to air-induced active CO₂ transport within 1 h, whereas active bicarbonate transport remained repressed (Fig. 1). A similar phenomenon has been reported in high CO₂-grown C. ellipsoidea cells acclimating to air, where active CO₂ transport was induced within 2 h, and active HCO₃⁻ transport remained repressed (Matsuda and Colman, 1995b).

It has been postulated that the induction of active DIC transport in microalgae and cyanobacteria is triggered by the intracellular accumulation of an intermediate of the photorespiratory pathway (Marcus et al., 1983; Suzuki et al., 1990; Coleman, 1991; Marek and Spalding, 1991; Kaplan et al., 1994). This theory is consistent with the large decrease in CO₂ concentration and increase in O₂ concentration which occur when high-CO₂-grown cells are transferred to air. However, if this mechanism were pivotal to the induction of active DIC transport during acclimation to low CO₂, the induction of the CCM in C. kessleri should only occur in the light. The induction of active HCO₃⁻ and CO₂ transport was apparent during acclimation to 0.035% CO₂ in darkness (Matsuda et al., 1998; Colman et al., 1998), although the maximum O₂ evolution rate in the bicarbonate and CO₂ transport assays was higher in cells adapting in the presence of light. The difference in the degree of active transport between acclimation in the light and in darkness, may be a modification of the energy-coupled reactions, that might accompany active DIC transport.

The change in the CO₂/O₂ concentration ratio in the external medium might also provide a signal for CCM induction, since more substrate will be available for the oxygenase activity of Rubisco, and hence there will be an increase in phosphoglycolate and other photorespiratory pathway intermediates (Marcus et al., 1983). In C. kessleri cells adapting to 0.035% CO₂, in the absence of O₂, there is no effect on the degree and rate of induction of CO₂ or HCO₃⁻ transport (Fig. 1), which suggests that a change in the CO₂/O₂ ratio in the external medium does not play a role in induction of the CCM. The lack of induction by AOA and INH under high-CO₂ conditions, which allow for an intracellular increase in photorespiratory pathway intermediates, also demonstrated that a build-up of phosphoglycolate does not serve as the inducing signal in C. kessleri (Fig. 4). The rationale for using AOA during acclimation to high CO₂-grown conditions, is that the inhibition of aminotransferase activity results in an increase in phosphoglycolate, and this inhibition is independent of the external CO₂ concentration for growth. The induction of an alanine-ketoglutarate aminotransferase in C. reinhardtii cells during acclimation to low CO₂ was reported, but the inhibition of this protein by AOA did not change the whole cell affinity for CO₂ (Chen et al., 1996).

The induction of CCM in high CO₂-grown C. kessleri cells acclimating to low CO₂ in the presence of protein synthesis inhibitors, demonstrates that new cytoplasmic proteins are made during the induction of active DIC transport (Fig. 4). Lack of inhibition by chloramphenicol, a chloroplastic protein synthesis inhibitor revealed that the induction of a CCM is not chloroplast based. Some researchers have pointed to protein modification playing a pivotal role in CCM induction.
The inhibition of protein kinases reported during the acclimation of cyanobacteria to low CO₂ prevented a rapid induction phenomenon (Sültemeyer et al., 1998a, b).

The level of active DIC transport is directly related to the concentration of external CO₂, and there is a gradient of active transport activity from fully repressed to fully induced when comparing cells acclimated to various external CO₂ concentrations (Figs 2, 3). The induction of DIC transport is therefore not an all-or-nothing response as would occur in the case of an internal metabolic signal, but an induction of transport activity proportional to an external signal. A continuous exposure to such a signal is also required to accomplish full induction of the CCM since it has been reported that DIC transport is repressed in C. ellipsoidea cells acclimating to low CO₂ when they are transferred back to 5% CO₂ conditions (Matsuda and Colman, 1995b).

The induction of the CCM in C. kessleri appears to occur in response to external CO₂ concentration, and fits the model proposed previously (Matsuda and Colman, 1996a), in which a CO₂ sensor at the Chlorella cell surface surrounded by a high concentration of CO₂, transduces a signal to repress active DIC transport; and during acclimation to low CO₂, there is a signal to derepress active CO₂ and DIC transport. The CO₂ sensor is thought to be absent from C. saccharophila UTEX 2469 and some mutants of C. ellipsoidea (Matsuda and Colman, 1996a), since they express a constitutive CCM, irrespective of the dissolved CO₂ concentration in the growth medium (Matsuda and Colman, 1996b).

The presence of a CO₂ sensor on the plasma membrane is not without precedent. A number of haem-based sensors have been identified in bacteria, which regulate physiological processes in response to the presence of an extracellular inorganic compound. For example, in Bradyrhizobium japonicum, FixL proteins not bound by O₂ at the haem-binding domain promote kinase activity during acclimation to hypoxic conditions (Gong et al., 1998). A similar phenomenon has also been characterized in the photosynthetic bacterium, Rhodospirillum rubrum, which involves binding of CO to the haem-domain of CooA protein, promoting oxidation of CO (Shelver et al., 1997). It is possible that a similar sensor may exist in Chlorella spp, but bind CO₂ rather than O₂. It is apparent, however, that C. ellipsoidea and C. kessleri cells, limited by the amount of dissolved CO₂ in the external growth medium, fully induce a CCM in response to a critical concentration of CO₂, independent of pH, which requires de novo cytoplasmic protein synthesis.

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References


