

## Photosynthesis is required for induction of the CO<sub>2</sub>-concentrating system in *Chlamydomonas reinhardtii*

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### 1. INTRODUCTION

*Chlamydomonas reinhardtii* cells grown photoautotrophically at air levels of CO<sub>2</sub> (0.03–0.04%) exhibit a much higher affinity for inorganic carbon in photosynthesis than do cells grown at elevated (1–5%) CO<sub>2</sub> concentrations [1]. This is also true of other unicellular green algae [2,3]. The apparent  $K_m$  (CO<sub>2</sub>) of air-adapted *C. reinhardtii* is much lower than that of RuBP carboxylase from the same cells [1]. Two explanations have been offered for this phenomenon, one being solely dependent on the activity of carbonic anhydrase [4,5] and the other based on a CO<sub>2</sub> concentrating mechanism [6,7]. Although not well characterized, the proposed CO<sub>2</sub> concentrating mechanism appears to involve active HCO<sub>3</sub><sup>−</sup> transport into the algal cells [6–8], raising the internal inorganic carbon concentration several-fold higher than that of the surrounding medium [6,8,9]. Carbonic anhydrase activity increases substantially in high CO<sub>2</sub>-adapted algae upon transfer to air levels of CO<sub>2</sub> [2,4,10], as does the apparent capacity for energy dependent

HCO<sub>3</sub><sup>−</sup> transport [6]. This suggests that carbonic anhydrase may play a specific role in the CO<sub>2</sub> concentrating mechanism of these algae. We have utilized photosynthesis-deficient mutants of *Chlamydomonas reinhardtii* and manipulation of growth conditions in wild-type *C. reinhardtii* to study regulation of carbonic anhydrase activity and the capacity for bicarbonate transport. We conclude that there is a coordinated regulation of carbonic anhydrase and bicarbonate transport in *Chlamydomonas* and that this regulation may be mediated by photosynthetic or photorespiratory metabolism.

### 2. MATERIALS AND METHODS

#### 2.1. Algal strains and culture conditions

*Chlamydomonas reinhardtii* Dangeard wild type strain 2137 *mt+* [11] and mutant strains were obtained from R.J. Spreitzer. Unless otherwise indicated, the wild-type strain was cultured photoautotrophically in liquid, minimal medium as described by Spreitzer and Mets [11] except that the Tris buffer was replaced by 50 mM MOPS (pH 7.0) and the micronutrients were as according to Allen [12]. The non-photoautotrophic mutant strains were grown in the same medium supplemented with 10 mM sodium acetate (acetate medium). Cells grown in light were exposed to a quantum flux density of approximately 150  $\mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  (9000 lux) from powergroove fluorescent lamps.

**Abbreviations:** MOPS, 3-(*N*-morpholino)propanesulfonic acid; DTT, dithiothreitol

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## 2.2. Reagents

$\text{NaH}^{14}\text{CO}_3$ ,  $[^{14}\text{C}]$ sorbitol, and  $^3\text{H}_2\text{O}$  were obtained from New England Nuclear Corporation. MOPS and DTT were obtained from Sigma Chemical Company. All other reagents were analytical grade or better.

## 2.3. Inorganic carbon accumulation

Uptake and accumulation of inorganic carbon were determined using silicone oil filtering centrifugation [6].

## 2.4. Carbonic anhydrase assays and chlorophyll determination

For assay of carbonic anhydrase (EC 4.2.1.1), the cells were harvested by centrifugation, resuspended in 25 mM barbital (pH 8.5) plus 1 mM DTT and sonicated five times for one minute each at full power with a Savant Instruments Insonator. Alternatively, the cells were resuspended in the same solution plus 0.01% (v/v) Triton X-114 and exposed to ten freeze-thaw cycles with liquid nitrogen. Assays were performed on the extracts without centrifugation. No significant difference in carbonic anhydrase activity was found between extracts prepared by sonication or by the freeze-thaw method.

Carbonic anhydrase assays were performed by monitoring pH change at  $2^\circ\text{C}$  in a barbital-buffered solution upon addition of  $\text{CO}_2$ . Barbital buffer (25 mM) and the extract (10 to 100  $\mu\text{l}$ ) were taken to a total volume of 3.0 ml and allowed to equilibrate to a constant pH. Then 2.0 ml of water saturated with  $\text{CO}_2$  at  $2^\circ\text{C}$  was added to initiate the reaction, and the pH change was monitored continuously with a glass combination pH electrode connected to a recorder. Enzyme units were calculated from the equation enzyme units =  $10(t_b/t_e - 1)$ , where  $t_b$  and  $t_e$  represent the time(s) needed for the pH change (8.0 to 7.0) with buffer alone ( $t_b$ ) and with extract ( $t_e$ ).

Chlorophyll was determined after extraction in 96% (v/v) ethanol [13].

## 3. RESULTS AND DISCUSSION

Growth of many algae at elevated  $\text{CO}_2$  concentrations (1–5%) results in significantly lower carbonic anhydrase activity relative to air-adapted algae [2,4,10]. As can be seen from the data in

Table 1

Effect of growth conditions on carbonic anhydrase activity in *Chlamydomonas reinhardtii*

Growth conditions	Units CA/mg Chl
Air	2357
5% $\text{CO}_2$ , 21% $\text{O}_2$	59
400 $\mu\text{l/l}$ $\text{CO}_2$ , no $\text{O}_2$	628
2 Days after transfer from 5% $\text{CO}_2$ to:	
air in light	921
air in dark	81
Acetate, light	501

tables 1 and 2, carbonic anhydrase activity was significantly reduced as well by low  $\text{O}_2$ , acetate and dark treatments, and in mutants defective in photosynthesis. Upon transfer from culture at 5%  $\text{CO}_2$  to air levels of  $\text{CO}_2$ , carbonic anhydrase activity increased markedly in *C. reinhardtii* (table 1). This increase was light dependent (tables 1 and 2), suggesting a role for photosynthetic metabolism in regulation of carbonic anhydrase level or activity. The involvement of photosynthetic metabolism in regulation of carbonic anhydrase was supported by the observation that mutants of *C. reinhardtii* defective in photosynthesis lacked substantial carbonic anhydrase activity ([14], table 2). The photosynthesis-deficient mutants were grown in acetate medium, which depressed the level of carbonic anhydrase activity in wild type (table 1). However, it is evident from the data in table 2 that, even when grown in acetate medium, wild type carbonic anhydrase activity was very much higher than that of any of the mutants.

Relative to air-adapted *C. reinhardtii*, carbonic anhydrase activity was lower in cells cultured at either reduced  $\text{O}_2$  concentration or elevated  $\text{CO}_2$  concentration (table 1). Since low  $\text{O}_2$  and elevated  $\text{CO}_2$  concentrations both classically suppress photorespiration, photorespiratory metabolites in particular might be involved in regulation of the carbonic anhydrase level in *C. reinhardtii*.

Time courses for the accumulation of inorganic carbon in *C. reinhardtii* photosynthesis-deficient mutants and wild type are illustrated in fig.1. As

Table 2

Carbonic anhydrase activity in photosynthesis-deficient mutants of *Chlamydomonas reinhardtii*

Strain <sup>a</sup>	Units carbonic anhydrase/mg Chl		
	Dark	Light ( $150 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )	Low light ( $75 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ )
Wild type (2137)	57	501	521
F-60 <sup>b</sup>	15	57	62
10-6C <sup>c</sup>	< 10	n.d. <sup>h</sup>	97
NLS 6-2A <sup>d</sup>	< 10	74	102
11-4D <sup>e</sup>	< 10	n.d. <sup>h</sup>	36
12-5A <sup>f</sup>	< 10	n.d. <sup>h</sup>	109
12-7 <sup>g</sup>	< 10	n.d. <sup>h</sup>	88

<sup>a</sup> All are strains of *Chlamydomonas reinhardtii* grown in acetate media under different light levels.

<sup>b</sup> Acetate requiring mutant defective in phosphoribulokinase [15].

<sup>c</sup> Light sensitive, acetate requiring mutant (*rcl-u-1* locus) defective in the large subunit of RuBP carboxylase/oxygenase [16].

<sup>d</sup> Same as (c) but with a mutation which suppresses light sensitivity [17].

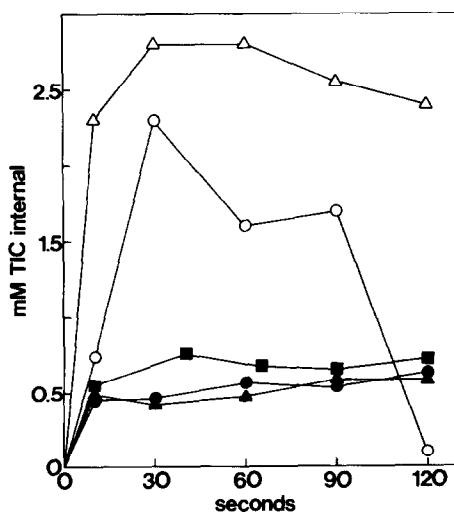
<sup>e</sup> Light sensitive, acetate requiring mutant deficient in photosystem II activity [11].

<sup>f</sup> Light sensitive, acetate requiring mutant deficient in photophosphorylation [11,18].

<sup>g</sup> Light sensitive, acetate requiring mutant deficient in photosystem I activity [11].

<sup>h</sup> Not determined. Strains 10-6C, 11-4D, 12-5A, and 12-7 cannot be cultured at  $150 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  (9000 lux) due to photosensitivity.

has been previously demonstrated [6], air-adapted, wild-type *C. reinhardtii* accumulated inorganic carbon internally to a level several-fold higher than



that of the external medium. This level eventually dropped due to depletion of external inorganic carbon (data not shown). Passive distribution of inorganic carbon was determined by measuring the accumulation in wild-type cells in the dark. It can be seen from fig.1 that accumulation of inorganic carbon in the photosynthesis deficient mutants F-60 and NLS-6-2A did not differ significantly from that of wild type in the dark. Since these two mutants were necessarily grown on ace-

Fig.1. Time course of internal inorganic carbon accumulation in *Chlamydomonas reinhardtii* wild type (○—○), acetate-grown wild type (△—△), wild type in the dark (●—●), and photosynthesis-deficient mutants F-60 (■—■) and NLS-6-2A (▲—▲). The initial external inorganic carbon concentration was 80  $\mu\text{M}$  and the pH 7.0. Chlorophyll concentrations were: wild type, 34  $\mu\text{g}/\text{ml}$ ; acetate-grown wild type, 33  $\mu\text{g}/\text{ml}$ ; F-60, 39  $\mu\text{g}/\text{ml}$ ; NLS-6-2A, 37  $\mu\text{g}/\text{ml}$ . TIC indicates total inorganic carbon.

tate-supplemented medium, inorganic carbon accumulation was also measured for wild type grown under identical conditions. Accumulation of inorganic carbon in acetate-grown wild type was greater than in air-adapted wild type. Therefore the lack of inorganic carbon accumulation in the two photosynthesis-deficient mutants was not a result of growth in acetate medium.

The data presented here demonstrate that photosynthesis-deficient mutants have reduced carbonic anhydrase activity and apparently lack the capacity for active accumulation of inorganic carbon. This is also true for 5% CO<sub>2</sub>-adapted *C. reinhardtii* ([6], Spalding unpublished observation). These observations suggest that the CO<sub>2</sub>-concentrating mechanism in *C. reinhardtii* involves a coordinated regulation of carbonic anhydrase and bicarbonate transport, and that this regulation is mediated in some way by photosynthetic or photorespiratory carbon metabolism.

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