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CARBONIC ANHYDRASE LEVELS IN *CHLAMYDOMONAS**

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Abstract—Levels of carbonic anhydrase in *Chlamydomonas reindhardtii* were 20-fold higher on both a protein and chlorophyll basis when algae were grown on 0·03 per cent CO_2 as compared to algae grown on 1 per cent CO_2. This result suggests a functional role for carbonic anhydrase in photosynthesis.

INTRODUCTION

RECENT work has indicated that levels of CO_2 in media for algae might regulate metabolic processes associated with their growth. The glycolate pathway in the green algae *Chlorella* and *Chlamydomonas* is repressed by an excess level of CO_2 during growth, leading to excretion of glycolate rather than metabolism.[1,2] The products and rates of CO_2 fixation in *Chlorella* have been shown by Graham and Whittingham[3] to be effected by CO_2 levels during growth. When rates of photosynthesis in low CO_2 (air) were compared, they found that prior growth of *Chlorella* in 5 per cent CO_2 resulted in a decreased rate of photosynthesis as compared to cells grown on air (0·03 per cent CO_2). Furthermore, after a long induction phase, the decreased rate of photosynthesis by *Chlorella* grown on 5% CO_2 was eliminated. This observation suggests that an enzyme might be limiting in algae grown on excess CO_2. Extending this work, Reed and Graham[4] suggested that carbonic anhydrase might be the limiting enzyme. We have examined the levels of carbonic anhydrase in *Chlamydomonas* to determine if they are altered by the amount of CO_2 available during growth.

RESULTS AND DISCUSSION

The relative levels of carbonic anhydrase found in *Chlamydomonas* grown on air was 10–20-fold greater than in cells grown on air supplemented with 1% CO_2 (Table 1). This increase in enzyme levels in air-grown cells was the same whether expressed on a chlorophyll or protein basis. It appears as though high CO_2 levels in the medium repressed the level of carbonic anhydrase. This observation, coupled with that of Graham and Whittingham[3] showing that *Chlorella* grown on 5% CO_2 have a decreased ability to photosynthetically fix CO_2, when placed in air, suggest that high levels of carbonic anhydrase might be involved with efficient photosynthesis. It is not certain whether the level of carbonic anhydrase is limiting photosynthesis by cells grown on high CO_2; however, carbonic anhydrase appears to be the only adaptive enzyme.[4]

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TABLE 1

Experiment number	Cell type	Assay	Units/mg protein	Units/mg chlorophyll
1	Air	A	57	—
	1% CO_2		5·6	—
2	Air	B	90	6840
	1% CO_2		4·4	372
3	Air	B	61	4970
	1% CO_2		2·4	251

Besides the evidence presented above there are two recent studies which also link carbonic anhydrase to photosynthesis. Everson and Slack[5] have found that carbonic anhydrase is located in the chloroplasts of plants that have the reductive photosynthetic carbon cycle (Calvin Cycle) for CO_2 fixation. Tobin[6] has recently shown that carbonic anhydrase accounts for 1 per cent of the soluble protein of parsley. The role of carbonic anhydrase in photosynthesis is suggested by its very substrate, CO_2. Its presence in large amounts in green tissue apparently associated with the chloroplast and its apparent involvement in CO_2 fixation ability in algae suggest even more strongly that its role in photosynthesis is a major one.

EXPERIMENTAL

Chlamydomonas reinhardtii was grown on either 1% CO_2 supplemented air or on air as previously described.[1] For enzyme assays the cells were harvested and washed once with distilled water. They were then resuspended to approximately 30% v/v in 0·1 M Tris–HCl, 0·001 M EDTA, and 0·01 M 2-mercaptoethanol, pH 8·3. This suspension was passed through a precooled French Press at 10,000 lb/in^2 and then centrifuged at 30,000 *g*. The supernatant was used for all assays. Protein was determined by Lowry's method[7] and chlorophyll by Arnon's method.[8]

Carbonic anhydrase [carbonate-hydro-lyase EC 4.2.1.1] was assayed by two methods which measured the length of time required for a fixed drop in pH to occur due to the hydration of CO_2. In assay A the length of time was recorded for the pH drop from 8·2 to 6·3 as measured by a pH electrode. Reagents were mixed exactly as described by Rickli *et al.*[9] and the time recorded with a stop-watch. All reagents were at 2 to 4° and the reaction was carried out at this temperature. Assay B involved a modification of the standard colorimetric technique.[9] In a 1 cm cuvette 2·5 ml Veronal buffer, 0·02 M, pH 8·3, containing 1 mg bromothymol blue per 100 ml was mixed with 0·5 ml of enzyme and the reaction was initiated by adding 1 ml of CO_2-saturated water. The cuvette was immediately placed in a spectrophotometer and the time necessary to reach a steady level of absorbance at 619 nm was recorded. Bromothymol blue absorption decreases at this wavelength with the pH drop.

A unit of activity of carbonic anhydrase is calculated by the following equation,[9]

$$\text{Unit} = 10\,\frac{[(T_b/T_c)-1]}{\text{mg protein or chlorophyll}}$$

where T_b is the time of the uncatalysed reaction and T_c is the time of the catalysed reaction.

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