Net photosynthetic O_2 evolution and calcium precipitation in *Chlamydomonas reinhardtii*

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Abstract

The relationship between carbonic anhydrase activity, net photosynthetic O_2 evolution rate and calcium precipitation in *Chlamydomonas reinhardtii* was studied. By adding bovine carbonic anhydrase and inhibitor of carbonic anhydrase-acetazolamide to SE medium to alter the activity of external carbonic anhydrase, the variation of net photosynthetic O_2 evolution rate and calcium precipitation in *Chlamydomonas reinhardtii* was determined. Calcium precipitation of *Chlamydomonas reinhardtii* was determined by the difference of algal calcium content during the culture period. The result shows that net photosynthetic O_2 evolution rate and calcium precipitation in *Chlamydomonas reinhardtii* were enhanced when bovine carbonic anhydrase was added to medium. Acetazolamide can inhibit calcium precipitation and photosynthetic O_2 evolution. Carbonic anhydrase may be an important factor to accelerate algal calcium precipitation. Moreover, the geological significance of carbonic anhydrase in algal calcium precipitations and the relationship between algal calcium precipitation and biomineralization of carbonate precipitation are discussed.
Keywords: calcium precipitation, carbonic anhydrase, Chlamydomonas reinhardtii, net photosynthetic O_2 evolution rate.

1 Introduction

The biogeochemical cycle, which regulates all the biochemical reactions, is the important "driving force" of the geo-environment. The carbon cycle is one of the


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most important element cycles. CO_2 is the important link and ligament of global carbon cycle [1]. The photosynthetic fixation of CO_2 and precipitation of $CaCO_3$ are closely connected both spatially and temporally. Photosynthetic carbon assimilation is commonly thought as the cause of calcium carbonate precipitation in algae [2]. External HCO_3^- is inorganic carbon resource of calcification in the algal cells [3, 4]. Carbonic anhydrase (Carbonic anhydrase, CA; carbonate hydrolyase, EC 4.2.1.1) is a ubiquitous enzyme catalyzing the reversible conversion of CO_2 to bicarbonate. The ability to use HCO_3^- for photosynthesis has been associated with the activity of external CA [5, 6]. Hence, there are the putative relationship between CA, the photosynthetic fixation of CO_2 and calcification in algae. This work is intended to investigate the effect on calcification and photosynthesis by CA, and the significance of CA in biomineralization.

2 Materials and methods

2.1 Culture growth

Chlamydomonas reinhardti, obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, was grown axenically in continuously stirred artificial freshwater medium SE, which was provided by the Freshwater Algae Culture Collection (http://www.ctcccas.ac.cn). The flasks were closed with bacteriological cotton plugs. Two-day preculture was conducted in medium SE free of calcium (NaCl 20 mgl^{-1} instead of 20 mgl^{-1} $CaCl_2 \cdot 2H_2O$) before treatment. It was harvested by means of centrifugation(1,700×g for 10 min); The pellet was suspended in medium SE. Growth of culture was initiated by introducing inoculum containing about 10^8 alga cells. Treatment 1: Cells were cultured in the medium SE containing an inhibitor of extracellular carbonic anhydrase--acetazolamide (AZ) (30 mM), at 25.0°C ±1.0°C, 150 μmol m^{-2} s^{-1} light on a 16-h/8-h day/night cycle. The control culture was in the same condition except that AZ was omitted and distilled water was used. Treatment 2: Cells were cultured in the medium SE containing bovine carbonic anhydrase (BCA) (10μg ml^{-1}) (Sigma C2522), at 25.0°C±1.0°C, 150 μmol m^{-2} s^{-1} light on a 16-h/8-h day/night cycle. The control culture was the same above. After 6, 12, and 24 hours, the packed cell volume, the algal content of calcium, and the net photosynthetic O_2 evolution rate were determined, respectively.

2.2 Determination of packed cell volume

Packed cell volume was determined by centrifugation of 5 mL of cell suspension in hematocrit tubes for 15 min at 2,200×g.

2.3 Assay for the algal content of calcium

100 ml algal solution is filtered through analytical filter paper (Whatman), the filter paper with algal cells is dried at 80°C. The dried sample is ashed at 500°C.

The ash is dissolved in a nitric acid solution (50 ml final volume), and analyzed on an ICP spectrometer for calcium.

2.4 Measurements of photosynthetic O_2 evolution

The net photosynthetic O_2 evolution rate was measured with a Clark-type oxygen electrode (YSI-5300, USA) under a photon flux density of 150μmol m^{-2} s^{-1} and a temperature of 25°C. The effect of acetazolamide on the cells O_2 evolution was estimated after 6, 12, and 24 hours' incubation in medium SE containing the acetazolamide (30 mM). The effect of bovine carbonic anhydrase (BCA) on the cells O_2 evolution was estimated after 6, 12,and 24 hours' incubation in medium SE containing the BCA (10μg ml^{-1}). Cells separated from the medium SE (by centrifugation at 5000 × g for 5 min), were re-suspended in 2 ml of 25 mM HEPES-KOH (pH 8.2) and transferred into an electrode chamber. Before determining the dissolved inorganic carbon-dependent O_2 evolution, cells were allowed to photosynthesize to deplete possible intracellular pool of "CO_2" until no net O_2 evolution was observed. Following the addition of the concentration of 2 mM bicarbonate (final concentration), or the concentration of 2 mM bicarbonate with 30 mM AZ (final concentration), or the concentration of 2 mM bicarbonate containing 10 μg ml^{-1} the BCA (final concentration), the rate of O_2 evolution was measured.

2.5 Statistics

Each treatment consisted of five replicates. The mean and standard deviation are calculated for each treatment. One-way ANOVA and LSD tests are conducted for each group.

3 Results

3.1 The effect on the algal content of calcium

After 6, 12, and 24 hours' incubation in medium SE containing BCA, the algal contents in calcium of *Chlamydomonas reinhardtii* are significantly higher than those of the control at the same period. After 24 hours' incubation in medium SE containing AZ, the algal contents in calcium of *Chlamydomonas reinhardtii* are significantly lower than those of the control at the same period (Table 1).

Table 1: The algal contents of calcium in *Chlamydomonas reinhardtii* with time. Values are means ± SD of five replicates.

Time Hours	Control mg ml^{-1} PCV	+BCA mg ml^{-1} PCV	+AZ mg ml^{-1} PCV
6	7.44 ±0.67	10.36±1.12*	6.54±0.63
12	8.72 ±0.76	12.85±1.03*	7.25±0.75
24	10.36 ±0.83	14.78±1.34*	7.72±0.81*

* The mean difference is significant between the culture containing BCA/AZ and the control culture at the same period ($P<0.05$).

3.2 The effect on the net photosynthetic O_2 evolution rate

After 6, 12 and 24 hours' incubation in medium SE containing BCA, the net photosynthetic O_2 evolution rate is significantly higher than that of the control at the same period (Table 2). After 12 and 24 hours' incubation in medium SE containing AZ, the net photosynthetic O_2 evolution rates are significantly lower than those of the control at the same period (Table 2).

Table 2: The net photosynthetic O_2 evolution rate of *Chlamydomonas reinhardtii* with time. Values are means ± SD of five replicates.

Time Hours	Control nmol O_2 s^{-1} ml^{-1} PCV	+BCA nmol O_2 s^{-1} ml^{-1} PCV	+AZ nmol O_2 s^{-1} ml^{-1} PCV
6	15.45±1.74	26.35±3.25*	12.82±1.73
12	18.56±1.69	24.70±2.33*	10.87±1.52*
24	16.48±1.95	25.26±2.20*	10.25±1.29*

* The mean difference is significant between the culture containing BCA/AZ and the control culture at the same period ($P < 0.05$).

3.3 The effect on the net photosynthetic O2 evolution rate

From tables 1 and 2, the ratio of the Ca precipitation quantity (CaPQ) to the net photosynthetic O_2 evolution rate (Pn) in *Chlamydomonas reinhardtii* can be calculated (Table 3). During 6-12 hours, the ratio of Pn/CaPQ is smaller than that during 12-24 hours. It shows that the Ca precipitation efficiency during 6-12 hours is greater than that during 12-24 hours.

Table 3: The ratio of the Ca precipitation quantity (CaPQ) to the net photosynthetic O_2 evolution rate (Pn) in *Chlamydomonas reinhartii.*

Time Spaces	Control Pn/ CaPQ	+BCA Pn/ CaPQ	+AZ Pn/ CaPQ
6-12 h	12.5:1	8.6:1	13.2:1
12-24 h	17.4;1	22.7:1	36.9:1

4 Discussion

Calcification is one of the most important biological processes in the living world. Although, it has been proved that the calcification of corals provides CO_2 source for its photosynthesis, the role of CA isn't confirmed yet [7]. This experiment confirmed the relation between the activity of CA and calcium precipitation.

The difference of the algal content of calcium in *Chlamydomonas reinhardtii* with time reflects the calcium precipitation and calcification. The inhibitor of

extracellular carbonic anhydrase—AZ decreases the calcium precipitation and the net photosynthetic O_2 evolution. Extraneous bovine carbonic anhydrase can accelerate the calcium precipitation and the net photosynthetic O_2 evolution. i.e. external CA facilitates the calcium precipitation and the net photosynthetic O_2 evolution.

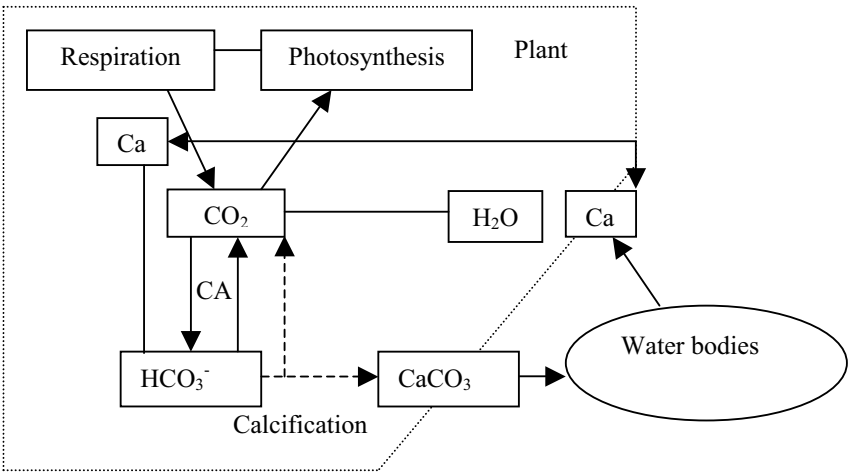

Figure 1: The sketch map regulation of photosynthesis and calcification by CA.

The role of CA on the calcium ion precipitation in cell membrane and carbonate transportation as well as biomineralization of calcium carbonate has a vital significance. The activity of CA expresses the ability to regulate the carbon cycle in the ecosystem. The carbon cycle in the ecosystem is related to the transportation of calcium ion and carbonate. The variation of the ratio of the calcium precipitation to the net photosynthetic O_2 evolution is great. i.e. the variation of rate of calcification is very great. It is similar to some other results [8, 9]. From this experiment, we can conclude that the calcium carbonate precipitation reacts rapidly at first, gradually slows down afterwards. It is because no calcium carbonate crystals such as coccoliths can be produced not to be capable of holding more calcium carbonate on the cell surface of *Chlamydomonas reinhardtii*. The rates of absorption and the precipitation on calcium were gradually decreased, even to zero. The calcium carbonate precipitation reaction is known to produce CO_2 according to the following equation: $Ca^{2+}+2HCO_3^- \rightarrow CaCO_3 + CO_2$. Therefore, algal calcification exerts a vital effect on calcium transportation and carbon cycle. CA influenced carbon cycle by regulating the reaction: $H_2O+CO_2 \leftrightarrow HCO_3^-+H^+$[10]. Algal calcification is also related to the concentration of HCO_3^-. Therefore, the relationship between algal calcification, the net photosynthetic O_2 evolution and the CA activity can be found in aquatic ecosystem (Figure 1). From Figure 1, Calcium transportation was led by carbon cycle; CO_2 is the core of carbon cycle. Some

biomineralization reaction was led by calcium transportation. CA regulates photosynthetic O_2 evolution and calcification through the influence on the concentration of inorganic carbon. The regulation of algal photosynthesis and calcification by CA is, therefore, an important factor in the global carbon cycle and biomineralization.

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