EFFECT OF FLUORIDE ON CARBONIC ANHYDRASE ACTIVITY AND PHOTOSYNTHETIC OXYGEN EVOLUTION OF THE ALGAE CHLAMYDOMONAS REINHARDTI

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SUMMARY: Fluoride ion (F) has been found to have a significant effect on carbonic anhydrase (CA) activity and photosynthetic oxygen evolution of the algae Chlamydomonas reinhardtii. Below 2.0 mM F, there was a positive effect on CA activity and net O2 evolution. Above 2 mM, F inhibited CA activity and O2 release. Around 2.0 mM F, CA activity peaked at 13.58±0.72 Wilbur-Anderson units (WAU)/10^9 cells and net photosynthesis O2 evolution reached 84.48±6.31 nmol O2/hr/µg chlorophyll. In the absence of F (control), CA activity was significantly lower at 8.82±0.83 WAU/10^9 cells and net O2 evolution was only 57.28±8.50 nmol O2/hr/µg chlorophyll. Moreover, CA activity had a significant positive linear correlation with the net rate of O2 evolution, thereby indicating that the effect of F on CA activity directly affects photosynthesis O2 evolution in Chlamydomonas reinhardtii.

Keywords: Algae photosynthesis; Carbonic anhydrase activity; Chlamydomonas reinhardtii; Fluoride activation; Fluoride inhibition; Oxygen from photosynthesis.

INTRODUCTION

Fluoride ion (F) is known to affect ion absorption, photosynthesis, respiration, cell membrane permeability, and certain enzyme activity in microalgae, especially photosynthesis. Photosynthetic evolution of O2 is inhibited by reducing the Hill reaction and depleting cellular ATP. However, this inhibition is not found universally in all microalgae, since several algal forms such as Chlorella, Scenedesmus obliquus, Ankistrodesmus braunii, and Chlamydomonas moewusii show little or no F-induced reduction in photosynthetic O2 evolution. What is the reason for this seemingly paradoxical difference? Possibly, a key enzyme, carbonic anhydrase (CA, EC 4.2.1.1), which is related to photosynthesis, may be involved. The present research was therefore undertaken to investigate the impact of F on algal photosynthesis through F-induced effects on CA activity.

MATERIALS AND METHODS

Culture growth: Samples of Chlamydomonas reinhardtii were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences, and grown axenically in the artificial freshwater SE medium. Growth was conducted with inoculum containing about 10^9 alga cells in 250 mL of continuously stirred medium in 500-mL conical flasks closed with bacteriological cotton plugs. F concentrations from NaF in the different sets of SE media were fixed at 0.0 (control), 0.5, 2, 8, and 32 mM F. Culturing was conducted at 25.0°C ± 1.0°C, 150 µmol/m2 s light, and a 16-hr/8-hr day/night cycle. After 3 days, the number of cells was counted directly by microscopy; the chlorophyll a was

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extracted from the cells with 80% acetone and determined spectrophotometrically.

Measurement of photosynthetic \( \text{O}_2 \) evolution: The net photosynthetic \( \text{O}_2 \) evolution rate was measured with a Clark-type oxygen electrode (YSI-5300, USA) under a photon flux density of 150 \( \mu \text{mol/m}^2 \text{s} \) and a temperature of 25°C. The effect of \( \text{F} \) on \( \text{O}_2 \) evolution was estimated after 3 days of incubation at the above-mentioned \( \text{F} \) concentrations. Cells separated from the SE medium by centrifugation at 5000 \( \times g \) 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 \( \text{O}_2 \) evolution, cells were allowed to photosynthesize to deplete possible intracellular pool of “\( \text{CO}_2 \)” until no net \( \text{O}_2 \) evolution was observed. Following the addition of 2 mM sodium bicarbonate, the rate of \( \text{O}_2 \) evolution was measured.

Assay of carbonic anhydrase (CA) activity: Three days after addition of \( \text{F} \) to the SE medium the \emph{Chlamydomonas reinhardtii} algae were harvested by centrifugation at 1600 g for 10 min. These algae were suspended in 15 mL of ice-cold barbital buffer (pH 8.30) for one hr for extracellular CA (CAext) activity measurements. Ten mL of ice-cold \( \text{CO}_2 \)-saturated water was subsequently added to this buffer and the time (T) was recorded over which the pH decreased from 8.20 to 7.20. Measurements were recorded in units of Wilbur-Anderson (WA) enzymatic activity, defined as 25 \( ([T_0/T]-1) \), where \( T_0 \) and \( T \) are the times for the pH change to occur in the non-enzymatic and enzymatic reaction, respectively. Each treatment consisted of three replicates. The mean and standard deviation were calculated for each treatment. One-way ANOVA and LSD tests were conducted for each group.

RESULTS AND DISCUSSION

As seen in the Table, \( \text{F} \) had a significant effect on the extracellular activity of CA and the net photosynthetic \( \text{O}_2 \) evolution of \emph{Chlamydomonas reinhardtii}. Up to 2 mM, \( \text{F} \) had positive effect on the CA activity and net photosynthetic \( \text{O}_2 \) evolution compared to the control, but at 8 mM and above, \( \text{F} \) had negative effect. Maximum CA activity and net photosynthetic \( \text{O}_2 \) evolution occurred at 2 mM \( \text{F} \).

<table>
<thead>
<tr>
<th>F Concentration (mM)</th>
<th>CA Activity (WAU/10^9 cells)</th>
<th>Net photosynthetic ( \text{O}_2 ) evolution (mol ( \text{O}_2 )/hr µg chlorophyll)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>8.82±0.83</td>
<td>57.28±8.50</td>
</tr>
<tr>
<td>0.5</td>
<td>10.65±0.96c</td>
<td>71.08±6.14c</td>
</tr>
<tr>
<td>2.0</td>
<td>13.58±0.72d</td>
<td>84.48±6.31d</td>
</tr>
<tr>
<td>8.0</td>
<td>7.93±0.62d</td>
<td>45.17±2.31b</td>
</tr>
<tr>
<td>32.0</td>
<td>6.40±0.96a</td>
<td>34.25±4.55a</td>
</tr>
</tbody>
</table>

Note: Values followed by different letters are significantly different from each other in the same column (ANOVA, \( P<0.05 \)).

Carbonic anhydrase is a zinc-containing metalloenzyme that catalyzes the reversible conversion of \( \text{CO}_2 \) into bicarbonate. It is widely distributed in animals, plants, archea, and eubacteria, where it is involved in diverse physiological processes, such as ion exchange, acid-base balance, carboxylation/
decarboxylation reactions, and inorganic carbon diffusion between the cell and its environment as well as within the cell.\(^4\)-\(^6\) Inhibition of CA activity by F has been reported,\(^7\) but here lower concentrations of F increased CA activity in *Chlamydomonas reinhardtii*. A possible explanation may lie in osmotic and/or molecular cell membrane changes that under lower concentrations of F, permeability of inorganic carbon (bicarbonate ion) across the cell membrane became strong. Greater amounts of inorganic carbon crossing the cell membrane may cause the reduction of its concentration in the extracellular environment. Consequently, extracellular CA activity would increase. At higher F concentrations (noted at 8 mM and higher), cell membrane function was probably impaired: extracellular CA was damaged, and extracellular CA activity was decreased. This phenomenon of increased dose response to fluoride ion at low concentration followed by decreased effects at higher concentration occurs in many situations.\(^8\)-\(^10\) Other reasonable explanations for what is observed here are possible but need more evidence.

As seen in the Figure below, CA activity has a significant positive linear correlation with the net photosynthetic O\(_2\) evolution rate of *Chlamydomonas reinhardtii* (P<0.01). This result is similar to results found in such plants as tobacco and *Brassica juncea*.\(^11\),\(^12\) It thus appears to be another way for F to act on photosynthesis, not through reduction in the Hill reaction and depletion of cellular ATP, but by affecting CA activity.

![Figure](image-url)  
**Figure.** Relationship between CA activity and net photosynthetic O\(_2\) evolution rate of *Chlamydomonas reinhardtii* (data from the experiment values of 5 treatments, each treatment consisting of three replicates).

**ACKNOWLEDGEMENTS**

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