Regulation of Chloroplastic Carbonic Anhydrase

EFFECT OF MAGNESIUM

Michael A. Porter and Bernard Grodzinski
Department of Horticulture Science, University of Guelph, Guelph, Ontario N1G 2W1 Canada

ABSTRACT

It was previously reported that magnesium ion inhibited carbonic anhydrase (Bamberger and Avron 1975 Plant Physiol 56: 481–485). Studies with partially purified carbonic anhydrase from spinach (Spinacia oleracea L.) chloroplasts show that the effect was the result of the chloride counterion and not the magnesium ion. Enzyme activity was reduced 50% upon addition of 3 to 10 millimolar MgCl₂ or KCl while all additions of MgSO₄ between 0.3 and 10 millimolar were mildly stimulatory.

The role of carbonic anhydrase (carbonate hydrolyase, EC 4.2.1.1) in plant cells is not well defined and debate continues about the importance of the enzyme (6, 10, 12). The fact that the enzyme interconverts the major forms of inorganic carbon and the fact that many workers locate the bulk of the enzyme in the chloroplast (1, 5, 6, 9, 13) tends to focus discussion on the role of carbonic anhydrase in photosynthesis. While many reports concentrate on the role of supplying CO₂ to carboxylating enzymes (10, 12, 13) in the alkaline medium of the illuminated chloroplast stroma (4, 6, 13), others point to the excess of the enzyme relative to the apparent need for CO₂ during maximal photosynthesis (6, 10). These authors point to a possible role of carbonic anhydrase in maintaining ionic balance within and between chloroplastic compartments by rapidly interconverting CO₂ and HCO₃⁻ (6, 10).

Disregarding which role is predominant, high rates of carbonic anhydrase activity would favor maximum photosynthesis, thus a previous report (1) that magnesium ion inhibited carbonic anhydrase is contrary to expectations. Magnesium ion concentration is known to increase in the stroma upon transition from dark to light (2, 4) and has been shown to activate a number of reductive pentose phosphate cycle enzymes (4). The inconsistency between the reported inhibition of carbonic anhydrase by magnesium ion and the general stimulatory role of magnesium during carbon fixation (2, 4, 7) is examined in this report.

MATERIALS AND METHODS

Freshly harvested spinach (Spinacia oleracea L. cv Vienna) was purchased locally. Approximately 900 g of washed, destemmed leaves were homogenized in 50-g lots with 2 volumes/g of 330 mM sorbitol, 50 mM Tricine-KOH (pH 7.6), 2 mM MgCl₂, 2 mM Na₂EDTA in 10-s bursts with a Polytron. After filtration through Miracloth, the chloroplasts were collected by differential centrifugation (8). The resulting pellet was suspended in homogenization buffer without sorbitol. After an incubation period to insure disruption, the suspension was centrifuged for 30 min at 20,000g and the membrane pellet was discarded.

The stromal protein was collected by precipitation with 60% saturated ammonium sulfate, dissolved in a minimal volume of 50 mM K-phosphate (pH 7.8), 1 mM dithioerythritol, and eluted from a 3-× 86-cm Sephacryl S-300 column using the same buffer. The UV-absorbance (280 nm) and carbonic anhydrase activity (see below) of the fractions was determined (Fig. 1). The major peak of carbonic anhydrase activity was pooled and stored as a suspension in 60% saturated ammonium sulfate, 30% v/v glycerol at −20°C until use. Specific activity of this preparation was approximately 500 to 600 units (see below) /mg protein (3). Approximately 10% of the stromal protein loaded onto the column was collected in this preparation. The preparation was contaminated by a number of proteins detectable by SDS gel electrophoresis.

Immediately before use, the suspension was dissolved in 10 mM sodium barbital (pH 8.05 with H₂SO₄), 1 mM dithioerythritol and desalted over a short column (1 × 9 cm) of Sephadex G-25 equilibrated with the same buffer. While thiol is reported to be unnecessary for carbonic anhydrase activity of spinach (10, 12), it was found that substantial activity was lost within 2 h in the absence of thiol in the dilute preparation.

The enzyme was assayed by a modification of the Wilbur-Anderson electrometric method (14) using 10 mM sodium barbital as the assay buffer. The enzyme extract and test solution were equilibrated for at least 2 min in 2 ml of assay buffer. The reaction was initiated by the addition of 0.5 ml of CO₂-saturated water (approximately 76 mM at 0°C). The time for the pH to decline from 8.0 to 7.5 was recorded. Boiled enzyme and no enzyme gave identical results. Additions of test solutions were made to both

![Graph](https://example.com/graph.png)

FIG. 1. Elution of chloroplast carbonic anhydrase from S-300. The plain line shows the A_280 profile of the elution while the line with the triangles (▲) shows the activity profile for carbonic anhydrase. Fractions were 3.0 ml. Enzyme units are defined in the text.

---

1 Supported by grants from the Ontario Ministry of Agriculture and Food and the Natural Science and Engineering Research Council, Canada, to B. G.

2 To whom correspondence may be addressed.
enzyme and blank assays. Enzyme units were calculated with the formula:

\[ \text{enzyme units} = \left( \frac{t_b}{t_c} - 1 \right) \]

where \( t_b \) is the time (in seconds) for the blank pH to change 0.5 pH units and \( t_c \) is the comparable time for the enzyme assay.

RESULTS AND DISCUSSION

The alkalinization of the stroma alters the equilibrium between CO\(_2\) and HCO\(_3^-\), favoring the ionic form. It is generally accepted that CO\(_2\), not HCO\(_3^-\), is the substrate for ribulose BisP carboxylase (7), thus the transition of the stroma from dark to light acts to reduce the substrate available for carbon fixation. One major role proposed for carbonic anhydrase in the chloroplast is maintenance of the CO\(_2\) concentration by rapidly converting HCO\(_3^-\) to CO\(_2\).

The previous report of magnesium inhibition of lettuce carbonic anhydrase emphasizes the similar response of light-induced CO\(_2\) fixation by intact chloroplasts and carbonic anhydrase activity to increasing MgCl\(_2\) (1). In addition, MgSO\(_4\), between 1 and 10 mM was reported to inhibit carbonic anhydrase by 50%. Both crude spinach extracts (data not shown) and a partially purified chloroplast carbonic anhydrase (Fig. 2A) showed a marked decrease in enzyme activity upon addition of MgCl\(_2\) above 1 mM, a result similar to that previously reported. However, addition of MgSO\(_4\) up to 10 mM did not inhibit enzyme activity but was mildly stimulatory (Fig. 2A).

Considerable literature has appeared for both plant (5, 10, 12) and animal (11) enzymes showing the inhibition of carbonic anhydrase by small monoanions. Figure 2B clearly shows that, at equivalent chloride concentrations, the response of carbonic anhydrase to KCl and MgCl\(_2\) is very similar. Potassium sulfate had no effect on enzyme activity. Taken together, the data in Figure 2, A and B indicate that the inhibition of carbonic anhydrase by MgCl\(_2\) was due to Cl\(^-\), not Mg\(^{2+}\).

Regulation of carbonic anhydrase in the chloroplast is uncertain because of ambiguities about the in vivo chloride concentration (2). These data suggest that Mg\(^{2+}\) fluxes in the stroma do not affect the activity of carbonic anhydrase in vivo and the possible compensatory role of carbonic anhydrase in the alkaline stroma in the light.

LITERATURE CITED