Bull. Univ. Osaka Pref., Ser, B, Vol. 42,
大阪府立大学紀要，農学・生物学，1990

Effect of High Concentration of Carbon Dioxide in the Atmosphere on Carbonic Anhydrase Activity in Plants

Masayuki KATAYAMA

Laboratory of Biological Chemistry, Department of Agricultural Chemistry,
College of Agriculture

(Received October 31, 1989)

Abstract

Lettuce, *Lactuca sativa* cv. Wayahead, and cucumber, *Cucumis sativa* cv. Nagafushinari No. 4, plants were transferred to cabinets maintained at 27°C, continuous irradiation of 2×10^4 lux, 70% relative humidity, and 300 ppm or 5,500 ppm of atmospheric CO_2. Changes of carbonic anhydrase activity and chlorophyll contents in the leaves were observed for several days. Lettuce leaves at the high CO_2 concentration showed enhanced carbonic anhydrase activity within 1 day which then decreased gradually and a lower chlorophyll content than the control. Cucumber leaves at the high CO_2 concentration showed a similar trend of carbonic anhydrase activity to the lettuce leaves, and gradual decrement of the chlorophyll content. After 126 hours under the high CO_2 concentration, carbonic anhydrase activity levels in leaves of cucumber at the middle position was maximum, when expressed in units per g fresh weight of leaves, and those at the upper position was higher, when expressed in units per mg of chlorophyll content.

Introduction

Modern horticulture in which plants are grown under high CO_2 concentrations demands knowledge on the effects of CO_2 concentration on enzyme systems related to biomass production. CO_2 concentration in the atmosphere is increasing according to observations at the Mauna Loa Observatory, Hawaii, which makes it of interest to know the effect of increased CO_2 concentration on plant carbonic anhydrase.

This paper presents a study on the effect of a high concentration of CO_2 on the chlorophyll contents and the activity of plant carbonic anhydrase.

Experimentals

Plant materials: Lettuce, *Lactuca sativa* cv. Wayahead, and cucumber, *Cucumis sativa* cv. Nagafushinari No. 4, were used. Lettuce stocks with several leaves were selected for the experiments. Cucumber stocks were grown until 12 to 13 leaves had developed.

Growth condition: Plant seeds were sown on sand and grown on small pebbles in bisque pots by hydroponic culture (Hoagland II medium) in a greenhouse. For the experiment under continuous light irradiation, the plants were transferred to cabinets inside a house. Light intensity, humidity and temperature were controlled.

When the cabinet was opened to remove sample pots, the interior conditions were recovered within 15 minutes.

Condition of exposure to high CO_2: Plants grown in pots were placed in a cabinet inside the house. Two cabinets were regulated to maintain the same conditions. The CO_2 concentration was maintained at two levels: One cabinet was used for the control experiment with 300 ppm of the CO_2 in the air and the other for the high CO_2 experiment with 5,500 ppm of CO_2 in the air. Plants were irradiated continuously at 27°C under 2×10^4 lux. The relative humidity was maintained at 70%.

Preparation of enzyme solution: Leaves were sampled and washed thoroughly with cold distilled water containing a small amout of 2-mercaptoethanol. After the excess water had been blotted, the leaves were homogenized with 100 mM Tris-SO_4 buffer solution (pH 8.4), containing 10 mM 2-mercaptoethanol and 1 mM EDTA. The homogenate was squeezed between eight sheets of gauze and a portion of it was removed to determine the chlorophyll content. The other portion was centrifuged at 1.7×10^4 Xg for 5 min at 4°C. Part of the supernatant was stored at −20°C for the determination of protein content and another portion was used for gel filtration. The supernatant was passed through a Sephadex G25 column to replace the buffer with 25 mM veronal buffer (pH 8.2), and used for the determination of carbonic anhydrase activity.

Determination of enzyme activity: Different volumes of enzyme solution dissolved in 1.0 ml of water were assayed in 2.0 ml of 25 mM veronal buffer (pH 8.2) and 2.0 ml of CO_2-saturated solution. The time in seconds of the pH change from pH 8.2 to pH 6.2 was measured at 2°C using the indicator, bromophenolblue (BTB). The enzyme activity was expressed as follows[1], using boiled enzyme solution for the time of non-engyme.:

$$\text{Units} = \frac{\text{Time (non-enzyme)} - \text{Time (enzyme)}}{\text{Time (enzyme)}} \times 10$$

Determination of chlorophyll: The sample dispersed in 80% acetone solution was sent through a small tube with a cotton wad as a filter, and the OD was measured at 645 nm and 663 nm. The contents of chlorophyll a and b were calculated according to the factors of MacKinney[2].

Determination of protein: Protein was determined by the method by Lowry *et al.*[3]

Results

Chlorophyll content in lettuce leaves: At the beginning of the experiment, lettuce leaves contained 1.25 mg of chlorophyll a and b per 1 g of fresh weight of leaves. The content decreased to below that of the control on exposure to the high CO_2 concentration for 24 hours (Fig. 1-A). The ratio of chlorophyll a to chlorophyll b, about 2.5 at the beginning, became 3.4 after 24 hours, then reached 3.0. The ratio in the younger leaves showed a value closer to that in the older leaves under 5,500 ppm of CO_2 (Fig. 1-B).

Carbonic anhydrase activity in lettuce leaves: Under the high concentration of CO_2 (5,500 ppm), the carbonic anhydrase activity (units per g of leaf fresh weight) in the leaves decreased gradually after enhancement of the activity over 12 hours (Fig. 2). This phenomenon was similar to that observed in an experiment on day-and-night cycles[5].

Chlorophyll content in cucumber leaves: Cucumber leaves contained about 1.8 mg of the chlorophylls per g of fresh weight of leaves at the beginning, and the amount decreased

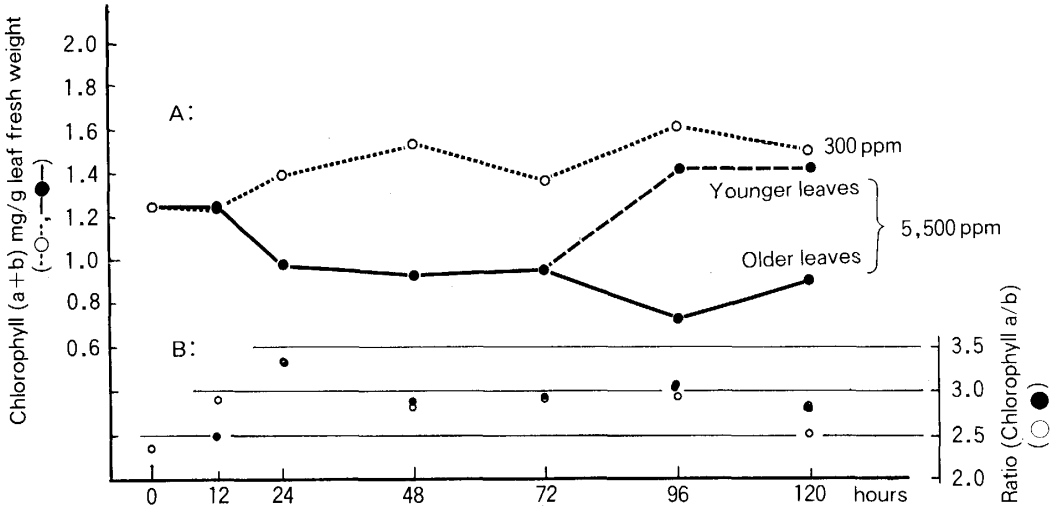

Fig. 1 Time course changes of chlorophyll contents in lettuce leaves kept under normal (300 ppm; open circles) and high (5,500 ppm; closed circles) concentrations of atmospheric carbon dioxide.
A: Chlorophyll contents (a + b) expressed as mg per g fresh weight of leaves.
B: Ratio of chlorophyll a to chlorophyll b.
Lettuce plants were placed in two cabinets which had different CO_2 levels and were regulated at 27°C and 70% of relative humidity. Light irradiation at 2×10^4 lux was continuously provided.

Fig. 2 Time course changes of carbonic anhydrase activity in the lettuce leaves kept under a high concentration (5,500 ppm) of atmospheric carbon dioxide.
The relative enzyme activity was expressed as the ratio of the activity in the leaves of high CO_2 concentration (5,500 ppm) to that of normal CO_2 concentration (300 ppm). The carbonic anhydrase activity was expressed in units per g fresh weight of the leaves. The cabinet was regulated as described in Fig. 1.

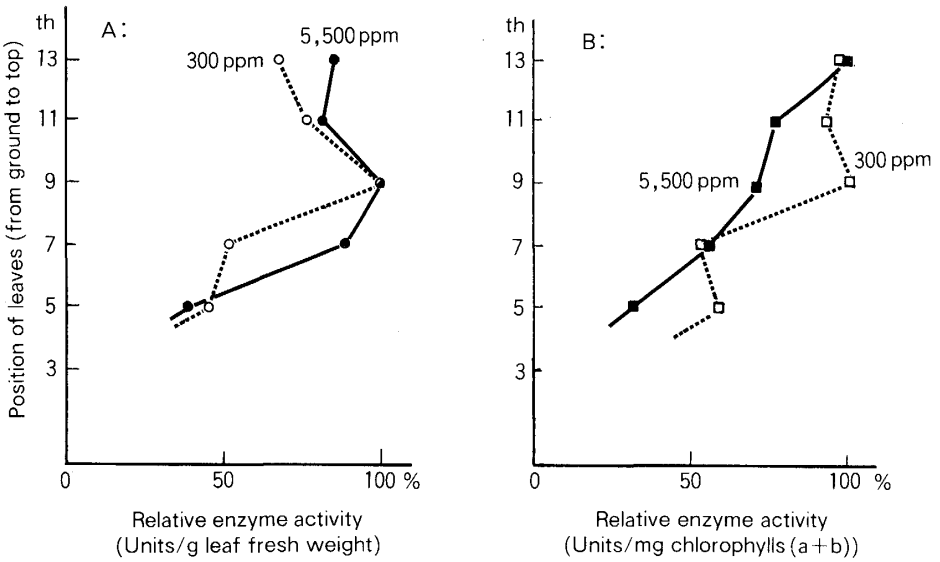

Fig. 3 Distribution of carbonic anhydrase activity in cucumber leaves kept at normal (300 ppm; open symbols) and high (5,500 ppm; closed symbols) concentrations of atmospheric carbon dioxide.
The relative carbonic anhydrase activity was expressed as the percentage to the maximum activity. The enzyme activity was expressed in A as the units per g fresh weight of leaves and in B as the units per mg chlorophyll content. The cabinet conditions were as described in Fig. 1.

gradually during the experiment under 5,500 ppm of CO_2. After 24 hours, 1.4 mg of the chlorophylls was detected, after 72 hours, 1.25 mg and after 120 hours, 0.65 mg (9th leaf) to 0.95 mg (6th leaf). The ratio of chlorophyll a to b was 2.7 at first and rose to 3.1 after 120 hours.

Carbonic anhydrase activity in cucumber leaves: In general, the activity of carbonic anhydrase increased over in 24 hours and then decreased to a lower level. The control (300 ppm) sample showed some enhancement as well.

After 126 hours, the carbonic anhydrase activity levels in various leaves were determined (Fig. 3). At the high CO_2 concentration (5,500 ppm), the distribution of carbonic anhydrase activity levels in these leaves differed from those in the control (300 ppm of CO_2). The leaves at the middle position showed maximum activity, when expressed in units per g fresh weight of leaves (Fig. 3-A). The activity was distributed more uniformly in the leaves kept under 5,500 ppm CO_2.

The relative enzyme activity expressed in units per mg chlorophyll content (Fig. 3-B) showed a different pattern from that of Fig. 3-A. The plants at 5,500 ppm CO_2 showed higher activity in the upper leaves.

Discussion

Leaf adaptation to a high CO_2 concentration is suggested by the decrease in the chlorophyll content after 24 hours under 5,500 ppm CO_2. But it is unknown how much the differences of the cabinets affect the chlorophyll contents in the fresh weight of leaves. The higher ratio of chlorophyll a to b at 24 hours means that chlorophyll b is

either catabolized faster or synthesized slower than chlorophyll a during adaptation to the new environment.

In order to eliminate the effect of pH in the vacuole and cytosol, the crude enzyme solution was replaced with veronal buffer by gel filtration. Thus, the lower activity of carbonic anhydrase under the high concentration of carbon dioxide (CO_2) is not an artifact, i.e., it is not due to the acidity of the vacuole or cytosol.

The enhancement and subsequent decrement of the carbonic anhydrase activity under the high CO_2 concentration has also been observed in an experiment on day-and-night cycles[5]. These findings suggest that leaves modified their metabolic regulation as though indicating that a lower enzyme activity is enough for the CO_2 demand by the chloroplasts.

In order to maintain the high level of carbonic anhydrase activity as well as photosynthetic activity under a high CO_2 concentration, a discontinuous supply of CO_2 might be effective.

Acknowledgment

The author expresses his appreciation to Prof. K. Yabuki, Department of Agricultural Engineering, for the usage of the growth cabinets.

References

1) RICKLI, E.E., GHAZANFAR, S.A.A., GEFFONS, B.H. and EDSALL, J.T. (1964) Carbonic anhydrase from human erythrocytes. *J. Biol. Chem.*, **239**, 1065–1078.
2) MACKINNEY, G. (1941) Absorption of light by chlorophyll solutions. *J. Biol. Chem.*, **140**, 315–322.
3) LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. and RANDALL, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**, 265–275.
4) KATAYAMA, M. (1976) Electrophoresis of plant carbonic anhydrase. *Jpn. J. Agr. Chem. Soc.*, **50**, 621–623.
5) KATAYAMA, M. in preparation.