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SHORT COMMUNICATION

EFFECT OF CO_2 ON CARBONIC ANHYDRASE IN *AVENA SATIVA* AND *ZEA MAYS**

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Abstract—In leaves of *Zea mays* kept in air with reduced or increased CO_2, the level of carbonic anhydrase is reduced or increased respectively. In *Avena sativa* an opposite effect of pCO_2 is observed. In both cases the enzyme activity rapidly reached normal values when the plants were transferred back to normal atmosphere.

INTRODUCTION

RECENT work has shown that an increase in the concentration of CO_2 in algae culture medium inhibits carbonic anhydrase (CA) activity.[1,2]

It is also known that species fixing CO_2 via the C_4 dicarboxylic acid pathway, have CA in the cytoplasm, whereas species fixing CO_2 via the Calvin pathway have the enzyme in the chloroplasts. Furthermore, the activity of the enzyme is considerably lower in the first type of species.[3] It is also known that such species have negligible photorespiration and show a greater net photosynthesis than species with the Calvin pathway.[4]

Therefore, assuming that the CA activity might be controlled by CO_2 concentration in higher plants as in algae, the activity of this enzyme was measured in *Zea mays* and *Avena sativa,* which have the C_4 and the Calvin pathway respectively grown in varying CO_2 concentrations.

RESULTS

CA Activity in Zea mays *and* Avena sativa

In both species the CA activity, on a fresh weight basis reached a maximum value when the leaf length is about 60–70 mm and the activity then suddenly decreases; protein and chlorophyll contents continue to increase over the whole period. The data are present in Fig. 1.

Effects of CO_2 *Concentration on CA Activity*

A remarkable increase of CA activity is observed in *Avena* leaves when the CO_2 concentration is reduced from 300 ppm (normal air) to 80 ppm. On the other hand an increase in

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[1] M. L. REED and D. GRAHAM, *Plant Physiol.* **43,** S29 (1968).
[2] E. B. NELSON, A. CENEDELLA, N. E. TOLBERT, *Phytochem.* **8,** 2305 (1969).
[3] R. G. EVERSON and C. R. SLACK, *Phytochem.* **7,** 581 (1968).
[4] I. ZELITCH, *Plant Physiol.* **43,** 1829 (1968).

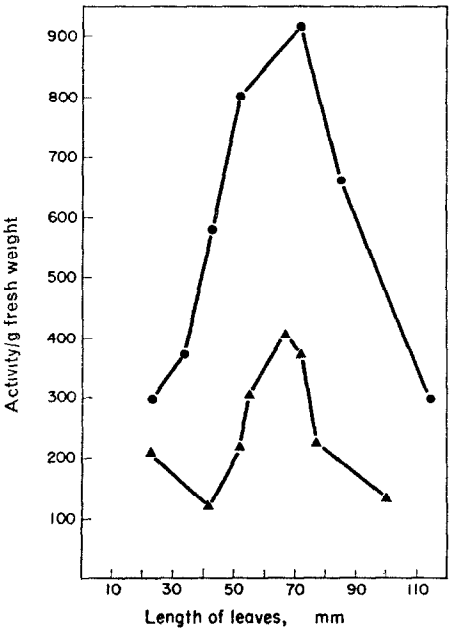

FIG. 1. CARBONIC ANHYDRASE IN *Avena sativa* (●) AND *Zea mays* (▲)

the CO_2 concentration to 600 ppm causes a sharp reduction in the CA activity after 24 hr. Later the activity increases but does not reach the control value (Table 1). In *Zea mays* a

TABLE 1. ACTIVITY OF CARBONIC ANHYDRASE IN LEAF EXTRACTS OF *Zea mays* AND *Avena sativa* GROWN IN DIFFERENT CO_2 CONCENTRATIONS

Species	CO_2 conc. (ppm)	Days	CA activity as % of control in air referred to Fresh wt.	Protein	Chlorophyll
Avena sativa		0	100	100	100
		1	170	165	170
	80	2	260	270	212
		3	169	153	230
		4	179	140	260
		0	100	100	100
		1	40	37	46
	600	2	81	74	92
		3	64	60	74
		4	73	71	79
Zea mays		0	100	100	100
		1	69	70	60
	80	2	51	56	54
		3	83	81	72
		4	89	95	85
		0	100	100	100
		1	158	112	140
	600	2	123	124	167
		3	132	110	145
		4	134	128	130

decrease in the CO_2 concentration causes a reduction, while an increase in the CO_2 concentration causes an increase in the enzyme activity.

Recovery After Treatments

Avena and *Zea* plants were kept for 12 hr in an atmosphere containing 600 ppm of CO_2. Subsequently, the plants were transferred to a normal atmosphere. The results are reported in Table 2. As already observed, in *Avena sativa* plants the enzyme activity in high CO_2 decreases. When brought back to normal conditions, it shows, during the first hour an

TABLE 2. CARBONIC ANHYDRASE ACTIVITY IN PLANTS TRANSFERRED FROM CO_2 ENRICHED (600 ppm) TO NORMAL AIR (300 ppm)

Time of recovery in normal air (hr)	Carbonic anhydrase activity as % of the control in normal air	
	Avena sativa	*Zea mays*
0	67	133
1	137	107
2	94	98
3	106	104
4	93	101
5	105	98

increase in the activity to a value greater than the control value. The activity later decreases to the control value. On the other hand, *Zea mays* plants were transferred to 300 ppm a sudden decrease in the enzyme activity occurs and the control values are reached during the first 2 hr after return to normal CO_2 concentration.

CONCLUSIONS

The results reported indicate that the CA activity in higher plants is modified by the atmospheric CO_2 concentration and that the induced changes are rapidly reversible.

From a physiological point of view the adaptive behaviour of the enzyme could be explained as follows. For plants which follow the Calvin photosynthetic pathway (*Avena*), the CA—which is very active and localized in the chloroplasts[3,5,6]—could regulate the CO_2 uptake in the chloroplast. When the CO_2 concentration decreases, CO_2 transport in the plant increases because of the higher CA activity and thus increases the availability of CO_2 for ribulose diphosphate carboxylase. When the CO_2 concentration is very high, an electrolytic and pH unbalance may occur in the chloroplast. In this case CO_2 transport is reduced by the decrease in CA activity.

For plants which follow the C_4 pathway, the low CA activity localized in the cytoplasm possibly indicates that the CA enzyme plays a secondary or indirect role in CO_2 absorption by the chloroplast.[3] Our results agree with this hypothesis. In fact, if the enzyme does not take part as a carrier in the transport of CO_2 to the chloroplast, it could be assumed that its function might consist in regulating the bicarbonate pool in the cytoplasm. If this is so, the CA activity will follow the variation of the external pressure, so that the CO_2 available for the chloroplast is maintained at an optimal value.

[5] A. GEREBTZOFF and J. L. RAMANT, *Physiol. Plantarum* **27**, 574 (1970).
[6] R. G. EVERSON, *Phytochem.* **9**, 25 (1970).

EXPERIMENTAL

Avena sativa and *Zea mays* were used. The seeds were germinated in peat or pots filled with sterilized sand. The pots were placed in plexiglass containers (22 cm dia. × 40 cm) which allowed circulation of the gas mixtures.

In the preliminary work (Fig. 1) plants were cultivated under normal condition for the duration of the experiments. For the experiments under controlled atmosphere, plants were maintained in normal air until they reached 50–60 mm height (maximum CA activity, see Fig. 1). At the beginning of the experiments the plexiglass containers were closed and the desired gas mixtures circulated. The flow rate was 500 ml/min giving replacement of the atmosphere every 30 min.

Germination and experiments were performed in controlled rooms with a light intensity (solar spectrum) of 9000 lx and an 8-hr day at 25° and a 16-hr dark at 15°. The relative humidity was 75%. All analyses were performed at 10.00 hr.

The extraction of the enzyme and the determination of its activity were performed by the method of Everson and Slack.[3] Chlorophyll was determined by Mackinney's method.[7,8] Protein was determined by Lowry's method.[9]

[7] G. MACKINNEY, *Z. Biol. Chem.* **140,** 315 (1951).
[8] J. BRUINSMA, *Biochim. Biophys. Acta* **52,** 576 (1961).
[9] O. H. LOWRY, N. J. ROSEBROUGH, A. FARR and R. J. RANDALL. *J. Biol. Chem.* **193,** 265 (1951).