THE REGULATION BY CARBON DIOXIDE OF PROTEIN SYNTHESIS IN TOMATO LEAVES[1]

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Received June 23,1977

SUMMARY: The presence of atmospheric carbon dioxide supresses the light-dependent synthesis of proteinase Inhibitor I in leaves of intact, wounded tomato plants and in detached leaves induced with the proteinase inhibitor inducing factor, PIIF. In carbon dioxide-free air, or in 100% oxygen, the rate of synthesis of Inhibitor I is over two times the rate in the presence of normal air. The results suggest that either CO_2 directly or through a product or products of photorespiration is regulating protein synthesis in tomato leaves.

INTRODUCTION

Two proteinase inhibitors, called Inhibitors I (1) and II (2) accumulate in leaves of intact tomato plants in response to wounding (3,4), or in excised leaves in response to the proteinase inhibitor inducing factor (PIIF) isolated from tomato leaves (5). The accumulation of these inhibitors is light-dependent (2) and their synthesis results from new mRNA that competes for the cytoplasmic pool of amino acid (6). Unlike most other cellular proteins the inhibitors are not degraded but are stored in the leaf vacuoles for long periods of time, probably as a defense mechanism against attacking pests. Thus, during PIIF-induced synthesis and accumulation the inhibitors can act as a reporter in monitoring the rates of protein synthesis in PIIF-induced tomato leaves.

We recently noted that excised, PIIF-induced leaves incubated in an air-tight chamber accumulated Inhibitor I at a much accelerated rate than when

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1. Scientific paper No.4848, Project 1791, College of Agriculture Research Center. This work was supported in part by a grant from NSF, PCM-75-23629, and United States Department of Agriculture, CSRS Grant 316-15-60.

ISSN 0006-291X

incubated in chambers open to air. An examination of this phenomenon led us to find that carbon dioxide plays an important role in regulating the light-dependent synthesis of inhibitor protein. In this communication the initial data concerning these observations is presented.

METHODS AND MATERIALS

Tomato plants were grown in peat pots in growth chambers under 1000 ft-c of light with 18 hr days at 31°C and 6 hr nights at 21°C. The plants were approximately 5-6 cm in height when excised near the soil level for experiments described herein. The plants had two lower expanding leaves and a smaller apical leaf. For experiments involving intact plants with wounded leaves, plants 6-8 cm in height, having three expanding leaves and a small apical leaf were employed. The plants were wounded on the two lower leaves by crushing them in several locations between a rubber stopper (size 00) and a flat file.

Proteinase Inhibitor I was assayed immunologically with the radial diffusion assay as previously described (7). Carbon dioxide free air was prepared by purging compressed air through a 1 liter cylinder of 10 N KOH, followed by a water vapor trap and then through a 30 x 4 cm ascarite trap. To ensure carbon dioxide free air in the appropriate experiments, CO_2 traps, consisting of 10 N KOH in 13 x 23 cm trays, were placed in the incubation chambers during the experiments. A mixture of 5% CO_2 and 95% compressed air was purchased from Liquid Air Inc., San Francisco, California.

In typical experiments excised plants were supplied with a solution containing 2 mg/ml of crude PIIF (5) dissolved in water through the cut petiole for 30 min. The petioles were rinsed with distilled water and immersed in water in small vials. The plants were incubated for 24 hr under 1000 ftc of light at 31°. The incubation of either excised plants or whole plants was performed in 4000 cc capacity polyethylene trays covered with plexiglass (0.5 cm thickness) and clamped tightly to minimize air exchange. In some experiments the atmospheric gases in the trays were controlled through inlet and outlet tubes. The rate of gas flow into the trays was about 60 cc/min, resulting in a complete turnover of the atmosphere about every 70 min.

RESULTS AND DISCUSSION

The effects of varying the concentration of atmospheric gases within incubation chambers during PIIF-induced accumulation of proteinase Inhibitor I is shown in Table I. The presence of a CO_2 trap in a sealed chamber, with no replenishment of air, caused almost a doubling of the rate of accumulation of the inhibitor protein over the rate in chambers open to air. The introduction of a continuous supply of CO_2-free air, along with the CO_2 trap, increased the accumulation of Inhibitor I even more. The introduction of 5% CO_2 in the atmosphere of the PIIF induced leaves during incubation depressed the rate of accumulation to one-fourth of the levels in the CO_2-free air. The presence of

TABLE I. Effects of Varying Levels of Atmospheric Gases on the Accumulation of Proteinase Inhibitor I in PIIF-Induced Excised Tomato Leaves.

Incubation Atmosphere*	Inhibitor I Accumulated in 24 hr after PIIF-induction (μg/g tissue)
Air	105
Air + CO_2 trap	203
CO_2-Free air + CO_2 trap	253
5% CO_2, 95% air	62
100% O_2 + CO_2 trap	164
100% N_2 + CO_2 trap	0

*1000 ft-c, 31°C.

100% oxygen in the presence of a CO_2 trap also increased the rate of accumulatio of Inhibitor I but the rate did not achieve the maximum rates in CO_2-free air. No accumulation took place under a 100% N_2 atmosphere.

The time course of accumulation of Inhibitor I in excised, PIIF-induced and uninduced leaves in CO_2-free air and normal air is shown in Figure 1. The increased rate of accumulation due to CO_2-free air continues for many hours, but slows down at about the fourth day. The leaves at that time were quite yellowed and apparently were in a late senescent condition. The leaves in air were still green and were continuing to accumulate inhibitors at a linear rate. Uninduced leaves acquired inhibitors, probably due to cutting the petioles, but those incubated in CO_2-free air consistantly accumulated less Inhibitor I than when incubated in normal air.

The effects of the atmospheric gas composition on the accumulation of Inhibitor I in excised leaves was also observed with leaves of intact wounded plants. Table II shows that the wound-induced Inhibitor I accumulation is greatly enhanced when the plants are incubated in CO_2-free air instead of normal

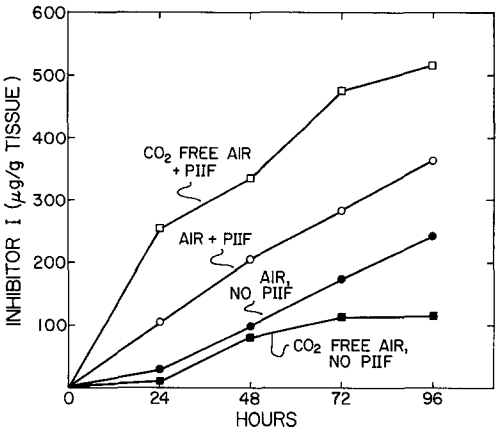

Figure 1. Time course of the accumulation of proteinase Inhibitor I in PIIF-induced and control leaves of excised tomato leaves under the atmospheric conditions indicated in the figure.

TABLE II. Effects of CO_2-Free Air on the Accumulation of Proteinase Inhibitor I in Non-wounded Leaves of Young Tomato Plants Wounded on the Lower Leaf

Incubation Atmosphere*	Inhibitor I Accumulated in 24 hr after wounding (μg/g tissue)
Air	186
CO_2-Free air, CO_2 trap	297
5% CO_2, 95% air	101

*1000 ft-c, 31°C.

air. Thus, in these experiments, senescent effects imposed by excising the plants were avoided. Our earlier evidence indicated that the PIIF-induced accumulation of inhibitors was due to the synthesis of new mRNA that competed with ongoing cellular protein synthesis (6). The accumulation of newly synthesized inhibitors ensues because the inhibitors are not degraded. Thus, the rate of accumulation of Inhibitor I can be used as a direct monitor of its rate of synthesis. Since the PIIF-induced synthesis of inhibitor has a light

requirement of at least 800 ft (4), the significant increase in the rate of synthesis when CO_2 was removed from the atmosphere strongly suggests that increased photorespiration is somehow stimulating this rate. In an earlier communication we demonstrated an unusual Q_{10} between 25°C and 30°C of about 4.0 for PIIF-induced Inhibitor I accumulation (4). It has been reported that Q_{10} for CO_2 output in tobacco leaves is about 3.7 (8). The similarities in the unusually high Q_{10}'s of these two processes further indicates that Inhibitor I synthesis may be monitoring a photorespiration effect.

Widholm and Ogren (9) have reported that a light-induced senescence of soybean plants at low CO_2 concentrations can be ascribed to photorespiration. Our results with tomato plants also indicate that both increased inhibitor synthesis and senescence are enhanced by low CO_2 concentrations and we suggest that both phenomena may be a result of a general increased turnover of proteins (both synthesis and degradation) at lowered CO_2 concentrations. The nature of the regulation of synthesis of Inhibitor I by CO_2 is under further investigation.

ACKNOWLEDGMENTS

The author thanks Mr. Joel Bryant for his excellent assistance and to Mr. Charles Oldenburg for growing the plants used in this study.

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