Plant Physiol. (1967) 42, 1463–1464

Short Communication

High Activity of the Glycolic Acid Oxidase System in Tobacco Leaves

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Received June 29, 1967.

Glycolic acid has been suggested as a major substrate for photorespiration. According to Zelitch (5, 6), respiration in the light is several times greater than dark respiration because many plants in the light synthesize large quantities of glycolic acid which is then oxidized, in part at least, to CO_2. Yet little glycolic acid is ever found in normal illuminated leaves. If any significant amount of CO_2 is released within leaves due to glycolic acid metabolism, then the glycolic acid oxidase enzymes must have high activity, for this significant rate of photorespiration must occur despite very low substrate concentrations. In fact many leaves do, indeed, seem to metabolize glycolic acid very rapidly. Maize, on the other hand, produces little CO_2 from glycolic acid, and its photorespiration is low (1, 2, 3, 4, 6). The following experiment demonstrates the rapidity of glycolic acid oxidation to CO_2 in tobacco and shows the contrastingly slow rate at which maize produces CO_2 from glycolic acid.

Leaves of maize (*Zea mays* L.) and tobacco (*Nicotiana tabacum* L.) were excised and recut beneath air-free water and sealed in a double-walled Plexiglas chamber with the cut end or the petiole extending from the chamber into a beaker. At first the beaker was filled with water, and transpiration and CO_2 evolution were measured. Then the water was replaced with a 0.1 M solution of glucose, sodium acetate or glycolic acid, and the new rate of CO_2 evolution and transpiration were measured. The evolution of CO_2 from the leaves was measured with an infrared analyzer by determining the change in CO_2 concentration of the air that passed over the leaf. Similarly, transpiration was measured by observing the change in water content of the air with an Aminco hygrometer. Thus, the number of moles of substrate taken up in the transpiration stream and the number of moles of CO_2 evolving from the leaf per unit time due to the added substrate could be calculated. The results are shown in table I.

The striking difference in activity of the oxidative enzymes for glycolate compared to either acetate or glucose in tobacco leaves is clearly seen. Respiration of leaves in darkness with their petioles in glycolate solution was 4 times as rapid as that of leaves with their petioles in water. Also, for each mole of glycolate taken up, approximately 1 mole of CO_2 was evolved. In contrast, sodium acetate and glucose had only a slight stimulating effect on CO_2 evolution and only about 1 mole of CO_2 was evolved for each 20 moles of substrate taken up. The percentage of carbon appearing as CO_2, therefore, was 50 times greater for glycolate than for glucose.

Another striking contrast was the low rate of glycolic acid metabolism to CO_2 in maize compared to tobacco. In maize, glycolate, as a respiratory substrate, was no different from glucose or sodium acetate and all resulted in about 1 mole of CO_2 per 20 moles of substrate taken up.

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Table I. *The Effect of CO_2 Evolution in Darkness of Feeding Substrate Solution Through the Petiole or Cut End of a Leaf*

Plant	Substrate	Replicates	Respiration ratio* substrate/water	Molar ratio** CO_2/substrate
Tobacco	Glycolate, 0.1 M	3	3.9	0.88
	Sodium acetate, 0.1 M	3	1.2	0.07
	Glucose, 0.1 M	3	1.1	0.05
Maize	Glycolate, 0.1 M	2	1.3	0.05

* Rate of CO_2 evolution with the petiole in substrate divided by the rate of evolution with the petiole in water.
** Molar ratio per unit time of the additional increase in CO_2 evolution due to substrate divided by the number of moles of substrate taken up during that time.

These results indicate that glycolic acid is metabolized very rapidly in tobacco leaves. Thus, the low concentration of glycolate found in illuminated leaves (5) does not indicate that little is synthesized. These results also support the hypothesis that one of the striking differences in CO_2 metabolism between tobacco and maize is in the amount of internal CO_2 liberated through glycolate metabolism.

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