

Short Communication**Calvin-type Carbon Dioxide Fixation in Sugarcane Stalk Parenchyma Tissue¹**

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HUGO P. KORTSCHAK AND LOUIS G. NICKELL

Physiology and Biochemistry Department, Experiment Station, Hawaiian Sugar Planters' Association, Honolulu, Hawaii 96822

Since the discovery of the 4-carbon dicarboxylic acid pathway of photosynthetic carbon dioxide fixation in sugarcane (11), this pathway has been found operative in many species of plants (3, 7, 9). So far, however, efforts to isolate chloroplasts from the leaves of sugarcane plants active in carbon dioxide fixation have not been successful, although they have been shown to reduce TPN (8). Therefore, we have looked for other photosynthetically active tissues from which functional chloroplast suspensions might be prepared.

When isolates are made from stalk parenchyma to establish permanent callus cultures (12) and kept under lights, the isolated tissue, whitish when excised, turns green rapidly and remains green for several months, unless blackened by oxidase action. We studied photosynthesis by such green tissues in the hope that they could be used to help clarify the 4-carbon dicarboxylic acid pathway (6). Callus tissues forming from such greened parenchyma are never themselves green. In fact, at no other time do we see green tissue in sugarcane cultures except at sites of organ (shoot) formation, an observation confirmed by Nitsch (13).

MATERIALS AND METHODS

The top 2 to 3 mm were sliced from separately grown isolates of sugarcane parenchyma tissue about 3 months after excision from the cane plant; each isolate was about 1 cm in diameter. (Other isolates made at the same time but not used in these experiments remained green for at least an additional 3 months—except where callus had formed.) These slices were placed in a 100-ml beaker with a few drops of water to prevent dehydration. After preillumination for 20 min with tungsten light, 2000 ft-c, the beaker was covered with cellophane and ¹⁴CO₂ was injected with a hypodermic needle. CO₂ concentration was 10% for the 3-sec test, 1% for the 10-sec test.

After either 3 or 10 sec, the samples were dropped into 100 ml of boiling 95% ethanol. Extraction and chromatographic analyses were carried out as with leaves (1).

RESULTS AND DISCUSSION

The high concentration of carbon dioxide used in the 3-sec test was required to obtain sufficient counts for analysis (11). The results of the chromatographic analyses are given in Table I.

The high percentage of the total radioactivity found in glycerate-3-P, the absence of malic acid activity, and the low or ab-

sent activity in aspartic acid are in striking contrast to what is found at similar exposure times with sugarcane leaves (5, 10, 11). On the contrary, this is the pattern commonly accepted as demonstrating the operation of the Calvin cycle, where the predominant carboxylation is that of ribulose diphosphate (1, 10).

Slack *et al.* found enzymes of the 4-carbon dicarboxylic acid pathway localized in the mesophyll chloroplasts of maize, whereas the parenchyma sheath chloroplasts contained a full complement of the enzymes of the reductive pentose pathway (14). Since our material was parenchyma tissue, there is a strong indication that this is also true for sugarcane.

Table I. *Distribution of Counts from ¹⁴CO₂ Fixed by Greened Parenchyma Tissue from Sugarcane Stalk*

| | Percentage of Total Counts | |
|---------------------------------|----------------------------|--------|
| | 3 sec | 10 sec |
| Malate | 0 | 0 |
| Aspartate | 6 | 0 |
| Glycerate-3-P | 34 | 13 |
| Glycerate | 12 | 0 |
| Glucose | 2 | 6 |
| Glucose-6-P | 18 | 21 |
| Fructose-diP | 0 | 13 |
| Pentose-P | 0 | 9 |
| Serine | 4 | 16 |
| Alanine | 6 | 0 |
| Total counts × 10 ⁻³ | 6.8 | 10.3 |

One deviation from the usual course of the later part of the Calvin pathway is the early appearance of activity in free glucose, but not in free fructose. We have found this consistently in experiments with this type of tissue. In leaves, independent of the type of carboxylation reaction, only phosphorylated hexoses become labeled at short exposure times (2, 11). In the present case the preponderance of glucose over fructose is also found on chemical analysis. Chromatographically separated sugars from a separate sample were glucose, 0.040%; fructose, 0.015%; sucrose, 0.531% of fresh weight. This is the opposite of what is found in sugarcane leaves, where fructose usually predominates (4).

The photosynthetic efficiency of these samples, based on area and corrected total counts, was 36 mg CO₂/dm²·hr for 10%

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CO₂, 20 mg for 1% CO₂, compared to 50 to 58 mg for cane leaves (11).

This is the first report of both pathways being separately operative in a single plant species.

SUMMARY

Freshly isolated sugarcane parenchyma tissue cultured *in vitro* produces chlorophyll prior to formation of callus tissue. In photosynthesis by these greened isolates, the first stable compound found is glycerate 3-phosphate, whereas sugarcane leaves use the 4-carbon dicarboxylic acid pathway.

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