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A TECHNIQUE FOR RAPID IDENTIFICATION OF INITIAL PHOTOSYNTHETIC PRODUCTS

G. J. Williams III and J. L. Markley

Department of Botany and Program in General Biology
Washington State University
Pullman, Washington

and

Department of Botany
The University of Texas
Austin, Texas

GRASSLAND BIOME

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ABSTRACT

A technique for rapid identification of initial products of photosynthesis is described. Plants exposed to $^{14}CO_2$ after a steady-state photosynthesis condition was achieved are placed in boiling solutions of varied ratios of ethanol and water. The labeled products of photosynthesis were extracted, identified, and quantified using one-dimensional thin-layer chromatography and scintillation counting. A system of dyes and R_f values allowed for precise location of products of photosynthesis. Ratios of radioactive 3-phosphoglycerate-hexose phosphate to malate-aspartate were obtained to classify a plant species as C_3 or C_4. The technique can be carried out in 24 hours and the information can be used to rapidly identify the type of photosynthesis involved.

INTRODUCTION

The current interest in C_3 and C_4 photosynthetic pathways makes a rapid technique for distinguishing type of pathway a desirable tool. Although many methods of classification are available to the plant scientist (leaf anatomy, chloroplast structure, carbon dioxide compensation point, etc.), one of the most reliable is identification of the initial carboxylation product or products. Many techniques have been used to identify initial photosynthetic products, but they require either specialized equipment (radio-scanning densitometer) or lengthy, time-consuming procedures.

The purpose of this paper is to describe a very rapid and reliable technique for identifying and quantifying the major initial carboxylation products of photosynthesis using equipment which is generally available to a plant scientist.

MATERIALS AND METHODS

To determine photosynthetically produced products that result from initial carboxylation reactions, a system was devised to expose plant material to $^{14}CO_2$ for short-term intervals.

Plant Pretreatment and Exposure to $^{14}CO_2$

Potted plant material (grasses, forbs, etc.) was used by securing polyethylene bags over the tops of the pots containing the plants. These were placed into an airtight radioisotope glove box and were exposed to full sunlight or an artificial source (three General Electric 400 w bulbs, lucalux, metal halide, and deluxe white mercury) for a 10 to 30 min irradiation treatment prior to exposure to $^{14}CO_2$. The soil in the pots was wetted to field capacity to reduce soil-water stress.

After a 10 to 30 min preillumination equilibration period, $^{14}CO_2$ was introduced into the tent from a gas generator, and the plant material was exposed to labeled CO_2 for 10 sec. For each exposure of a plant sample, 0.5 mCi of $Ba^{14}CO_3$ (New England Nuclear Corporation, of specific activity of 0.5 mCi/mM) and 3 g $BaCO_3$ were placed in a gas generator. The $^{14}CO_2$ was released by addition of 6M HCl to the $Ba^{14}CO_3/BaCO_3$ mixture.

Product Extraction

After the 10-sec exposure to the $^{14}CO_2$ the plant material was clipped above the potting soil and immediately plunged into boiling 80% ethanol (v/v) for 10 min. The material was then transferred to boiling deionized water for 10 min. The liquids containing extracted initial products from each of the three above treatments were than combined and mixed with animal charcoal to decolorize them. This mixture was then vacuum filtered through Celite using a scintered glass funnel (known compounds were given this treatment, and only 0.5% loss resulted). The volume of the resulting colorless filtrate was then reduced by rotary evaporation to about 1 ml. From this amount aliquots of concentrated plant extract (10-15 µl) were spotted onto Eastman silica gel pre-coated chromatography sheets (without fluorescence indicator). Approximately 30 spots were applied to the origin of each 20 x 20-cm chromatography sheet. After the spottings were dried, the sheets were chromatographed by the ascending method using an acetone/methanol (7:3 v/v) solvent system. The front was allowed to advance 10 cm, and then the sheets were dried. Those sections of the silica gel containing the products of interest were scraped from the sheets, and the radioactivity was determined.

Product Location

In order to remove sections of silica gel containing products of interest, known samples of 3-phosphoglycerate, L-malate, L-aspartate, fructose-6-phosphate, fructose-1, 6-diphosphate, glucose-6-phosphate, and glycolate (Sigma Chemical Company) were chromatographed using the above-described procedure to obtain R_f values. A dye system for malate, aspartate, 3-phosphyglycerate, and glycolate, using a bromcresol green reagent (1 g bromcresol green in 100 ml ethanol adjusted to a blue color with 10% sodium hydroxide) allowed the acids to be located as colored spots as well as by R_f value. By spraying the bromcresol reagent onto the sheets, the acids were located as yellow spots on a blue background. Sugar phosphates were also located by a dye. Thin-layer chromatography sheets were sprayed with an alpha-naphthol solution (2 g alpha-naphthol in 100 ml 100% ethanol and 15 ml of concentrated sulfuric acid) and placed in a drying oven at 90°C for 5 min. The sugar phosphates were located as brown spots on a purple background. The combination of R_f values and the two dye techniques allowed precise determination of the location of products to be removed from silica gel sheets for counting radioactivity.

From each 20 x 20-cm sheet 2 of the 30 tracks of chromatographed materials were cut and one was sprayed with bromcresol green solution and the other with the alpha-naphthol solution to exactly locate compounds of interest. Therefore, R_f values could be used as relative indicators of a compound's position on the TCL sheet and the exact location could be fixed with the dyes.

Product Quantitation

After the products were located on unsprayed portions of the silica gel sheets (by comparison to sprayed ones), the gel was scraped off the sheets into glass vials. To these vials 0.5 ml of hydroxide of hyamine 10x was added and allowed to stand at room temperature for about 30 min. After this, 10 ml of PPO-POPOP in toluene (16 g PPO and 0.8 g dimethyl POPOP per gallon of toluene) were added. The vials were capped and placed into a refrigerated liquid scintillation spectrometer (Packard Tricarb Model 3375) for at least 12 hours of dark refrigeration prior to counting. Each vial was counted twice over a 10-min counting period. All the counts were combined, averaged, and the background subtracted.

Technique Validation

In order to validate the above techniques, actively growing plants of *Nicotiana tabacum* and *Zea mays* were tested as representative C_3 and C_4 species.

RESULTS

Using the acetone/methanol (7:3) solvent system, a front ascended 10 cm within 45 min to an hour. Using the above-described system, C_3 products of 3-phosphoglycerate, fructose-6-phosphate, fructose-1, 6-diphosphate, and glucose-6-phosphate had R_f values of 0.0 and thus could always be located on the origin of the chromatograph. The C_4 products of malate and aspartate had R_f values of 6.4 and 1.5, respectively. Glycolate was found to have an R_f value of 7.0.

The ratios of 3-phosphoglycerate-hexose phosphate to malate-aspartate in test plants of *Zea mays* and *Nicotiana tabacum* were 1:3 and 3:1,

respectively. Counts of radioactivity in the portions of the silica gel strips, remaining after the origin spot, the malate-aspartate spots, and the glycolate spot had been removed, were always less than 1% of the total radioactivity.

DISCUSSION

The reported technique allows for a rapid and accurate method of identifying initial products of photosynthesis. The results from labeling initial products demonstrate that the control plants, *Nicotiana tabacum* (C_3) and *Zea mays* (C_4) compare favorably with results reported by Hatch, Slack, and Johnson (1967).

The described techniques represent a series of steps which would enable an investigator to determine the pathway type (C_3 or C_4) of a species within a 24-hour period.

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