Sucrose as a Product of Photosynthesis in Isolated Spinach Chloroplasts

R. Garth Everson, William Cockburn, and Martin Gibbs
Department of Biology, Brandeis University, Waltham, Massachusetts 02154

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Summary. Sucrose has been detected as a seasonal photosynthetic product in spinach chloroplast preparations. Sucrose when present accounted for up to 30% of the CO₂ fixed. Experiments in which sucrose was formed have been compared with experiments in which it was not formed, and a possible control mechanism for sucrose synthesis is discussed.

Chloroplasts are known to be capable of polysaccharide synthesis, both within intact cells and when isolated in an artificial medium (1). Sucrose, however, although an early and abundant product of photosynthesis in leaves is usually not formed to any extent in preparations of isolated chloroplasts (2, 3). In view of the reported presence of the necessary enzymes for sucrose synthesis in chloroplasts (4, 5) absence of sucrose as a photosynthetic product of isolated chloroplasts is somewhat surprising, unless critical enzymes are routinely lost during isolation of the chloroplasts.

We now have evidence that spinach chloroplasts can form sucrose from ¹⁴C₂O₃ although this capacity appears to be seasonal.

Methods

Spinach obtained from the Community Produce Company of Boston was stored in the cold room at 4°. Prior to use mature leaves were washed, placed in water at 20° and exposed to light, about 1000 ft-sec for 2 hours.

Chloroplasts were prepared as described by Gillis and Calo (6) in tris buffer (pH 7.6) with 0.16 M sucrose replacing 0.35 M NaCl in all solutions. Ascorbate was not used.

The reactions were in 1.3 × 10 cm tubes illuminated at 2000 ft-sec at 15°. Nitrogen was slowly bubbled throughout the reaction mixture throughout the experiment. Samples were taken at intervals and treated with formic acid (final concentration 4%). Radioactivity in the sample was measured in an aliquot placed on a planchet with a piece of lens tissue before Geiger counting (7). Counts were corrected by an empirical factor for self absorption. Further aliquots were immediately pipetted onto Whatman No. 541 acid-washed paper for 2-dimensional descending chromatography. For identification of the products non-radioactive markers were added to the origin of replicate chromatograms. Solvents were either n-propyl alcohol-ammonia-water or phenol-water in the first direction, tertiary amyl alcohol formic acid water in the second direction. Chromatograms were autoradiographed with Kodak No Screen X-ray film. After development and identification of spots, radioactivity was measured with a Geiger-Müller tube attached to a ratemeter.

Sucrose was located by spraying with aniline diphenylamine, phosphoric acid reagent (7). The eluate from this area was cochromatographed with authentic sucrose in butanol acetic acid water, eluted and treated with invertase (Sigma Grade VI). Upon further chromatography the only radioactive compound found were glucose and fructose with traces of sucrose.

Results

Sucrose was formed in considerable amounts in 5 experiments carried out during February and March 1966; it could not be detected as a product of chloroplasts in spinach obtained after the end of March. An autoradiograph from an experiment in which sucrose was produced, experiment 3S, is shown in figure 1. The sucrose was found to be comprised of glucose and fructose equally labelled. Figure 1 can be compared with figure 2 taken from experiment 9X carried out over a month later which like many other similar experiments yielded no sucrose.

Kinetics of CO₂ fixation are shown in figure 3. Apart from the short lag seen in experiment 9X (lower curve), which did not yield sucrose, there is no significant difference in the rates of photosynthesis in the 2 experiments. There is clearly a difference in the distribution of the photosynthetically fixed carbon. In figure 4 the course of appearance of ³¹C in photosynthetic products from experiment 3S is graphed and may

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2 Permanent address: David North Research Centre, Indooroopilly, Queensland, Brisbane, Australia
3 Permanent address: Imperial College, Botany Department, Prince Consort Road, London, S. W. 7 England.
Fig. 1. Chromatogram showing $^{14}$C-labeled products of 32 minutes photosynthesis by spinach chloroplasts, experiment 3S. Reaction mixture contained the following (mM): tris (pH 7.6), 6.8; sucrose, 480; MnCl$_2$, 0.6; Na$_2$HPO$_4$, 0.2; NaHCO$_3$, 1.25. 50 μ. Chlorophyll was 126 μg/ml.
Fig. 2. Chromatogram showing $^{14}$C-labeled products of 20 minutes photosynthesis by spinach chloroplasts, experiment 9N. Reaction mixture as in figure 1. Chlorophyll was 112 µg/ml.
be compared with a similar graph figure 5 relating to experiment 9N. When sucrose is a major product of photosynthesis (fig 4) it is accompanied by alanine which is virtually absent from the preparation that did not synthesize sucrose. Dihydroxyacetone-P, on the other hand, is in evidence only when sucrose is not found.

Apart from the apparent change in rate of sucrose formation after 16 minutes of photosynthesis the pattern of distribution of $^{14}$C among products in figure 4 is representative of the spinach obtained during February and March.

Many of the enzymes known to participate in sucrose synthesis have been reported to be present in chloroplasts. Sucrose-P synthetase and sucrose synthetase have been found in chloroplasts from sugar cane leaves (4) and in addition to these, sucrose 6-P phosphatase, UDP-glucose pyrophosphorylase, glucose-1-P mutase and glucose 6-P isomerase have been found in tobacco chloroplasts (5). Sucrose synthesis in tobacco chloroplasts is believed by way of sucrose phosphate synthetase, and sucrose cleavage by way of sucrose synthetase, invertase being absent. In our experiments the 2 monosaccharide moieties of the sucrose are equally labelled despite the abundance of sucrose in the reaction mixture which indicates net synthesis of sucrose rather than labelling of sucrose via an exchange reaction of sucrose synthetase. Sucrose phosphate could not be detected among the products of photosynthesis.

Comparison of the distribution of $^{14}$C among the products suggests that an enzyme between triose phosphate and fructose 6-P may determine whether or not sucrose is formed as a major product of photosynthesis in chloroplasts. Fructose 1,6-diphosphatase has been shown to be rate-limiting concentration in spinach chloroplasts (8). If diphosphatase activity is much less than fructose 1,6-diP aldolase, $^{14}$C would tend to accumulate in compounds in equilibrium with triose phosphates while high diphosphatase activity would favor the formation of fructose-6-P, a substrate for sucrose phosphate synthetase. Investigation of fructose 1,6-diphosphatase levels in chloroplasts making sucrose would appear to be worth while. The appearance of alanine accompanying sucrose synthesis in our preparations suggests that more than 1 enzyme may undergo a seasonal fluctuation in spinach chloroplasts.
Literature Cited


