Short Communication

Translocation

EFFLUX OF SUGARS ACROSS THE PLASMALEMMA OF MESOPHYLL PROTOPLASTS

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STEVEN C. HUBER AND DONALD E. MORELAND
United States Department of Agriculture, Science and Education Administration, Agricultural Research, Departments of Crop Science and Botany, North Carolina State University, Raleigh, North Carolina 27650

ABSTRACT

During photosynthesis by mesophyll protoplasts of wheat and tobacco, a linear efflux of sucrose and hexoses to the medium was observed, with the size of the intraprotoplast sugar pools remaining constant. Efflux of metabolites labeled by 14CO2 fixation was initially low because of dilution by internal pools, but increased exponentially with time. The results have significance both in terms of the mechanism of translocation and the use of isolated protoplasts in photosynthetic studies.

How photosynthesize moves from the site of formation in leaf mesophyll cells to the sieve elements of the phloem has not been established. Two routes have been proposed. The symplastic postulate (1, 12) states that sugars move intercellularly via plasmodesmatal connections and never enter the cell-free space. In contrast, the apoplastic postulate (6, 10) describes an efflux of sugars from the mesophyll cell to the free space, followed by uptake into the phloem. The apoplastic model has received considerable support by the recent demonstration of active uptake of sugars from the free space into the phloem (4, 5). There is little direct evidence for an efflux of sugars, especially sucrose, from mesophyll cells to the free space.

We have used isolated wheat and tobacco mesophyll protoplasts to study the transport of sugars across the plasma membrane. Protoplasts and cells isolated from a variety of species, including wheat and tobacco, typically incorporate from 40 to 60% of assimilated 14CO2 into soluble sugars (the neutral fraction derived from ion exchange chromatography), principally sucrose (2). The release of acid-stable radioactivity to the medium, however, has been reported to be very low (2, 11), implying that the metabolic products of photosynthesis are retained within the protoplast. However, with both wheat and tobacco, we have observed a significant efflux of unlabeled sucrose and hexoses to the medium even though the release of labeled metabolites, derived from 14CO2 assimilation, was initially low.

MATERIALS AND METHODS

Protoplast Isolation. Protoplasts were isolated from fully expanded leaves of 11 to 20-day-old wheat (Triticum aestivum L) and 4- to 6-week-old tobacco (Nicotiana tabacum L.) plants. Wheat protoplasts were isolated as previously described (3). Tobacco leaf segments were incubated in a mixture containing 2% Cellulysin (Calbiochem), 1% Driselase (Kyowa Hakko, New York), 0.4% Macerozyme (Yakult Biochemicals, Nishinomiya, Japan), 0.4% PVP-40, 0.05% BSA, 0.5 m sorbitol, and 1 m CaCl2 (pH 5.5). Tobacco protoplasts were purified using the aqueous polyethylene glycol-dextran system of Kanai and Edwards (9).

14CO2 Fixation. Protoplasts (50–75 µg Chl/ml) were incubated at 25°C with or without illumination (60 nE/cm2·s) in a reaction mixture containing 0.5 m sorbitol, 7.5 mM NaH14CO3 (5 µCi/µmol), 50 mM Hepes-NaOH (pH 8.0), and 0.2 mM CaCl2. At various times, aliquots of the reaction mixtures were terminated in acid to measure total 14CO2 fixed or centrifuged at 200g for 20 s to pellet the protoplasts. Acid-stable radioactivity in the clear supernatant was then determined.

Sugar Efflux and Assays. Protoplasts were incubated in the 14CO2 fixation reaction mixture. At various times (usually 0, 10, 20, and 30 min), 0.1-ml aliquots were centrifuged to pellet the protoplasts. The supernatant was mixed with an equal volume of 0.04 N KOH and the pellet was resuspended in 0.04 N HCl. Sugars were enzymically assayed following the method of Jones et al. (8).

For sucrose determinations, 160-µl samples were added to 1 ml of a mixture containing 0.1 M Hepes-NaOH (pH 7.0), 0.4 mM NADP, 1 mM ATP, 5 mM MgCl2, 0.5 mM DTT, 16 units/ml invertase, 0.5 units/ml hexokinase, 2 units/ml phosphoglucoisomerase, and 6 units/ml glucose-6-P dehydrogenase. Absorbance at 340 nm was measured after 30 min at 25°C. For hexose (glucose + fructose) assays, samples were treated similarly, except that invertase was omitted, the mixture was buffered at pH 8.0, and the length of incubation was reduced to 10 min. Hexose-P was measured in certain experiments by eliminating invertase, hexokinase, and ATP from the assay mixture. Sugars present in the supernatant of the zero time sample were subtracted from subsequent samples.

The data are expressed as µatoms of C (in sugars) to facilitate comparisons with results from 14CO2 fixation experiments.

RESULTS AND DISCUSSION

Typical results obtained with wheat protoplasts showing the kinetics of 14CO2 assimilation and efflux of labeled photosynthesize to the medium are shown in Figure 1A. Total fixation of 14CO2 was linear after an initial lag phase of several min. In contrast, the release of acid-stable radioactivity to the medium was initially low, but increased exponentially with time. The relatively low


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The sugar efflux documented in Figure 1B was significant in comparison to the rate of photosynthetic sugar formation. Assuming that 50% of the $^{14}$CO$_2$ fixed was incorporated into sugars (2), the rate of sugar formation in the experiment of Figure 1 was approximately 34 $\mu$mol C into sugars/mg Chl-h. This value is very close to the observed rate of total sugar efflux (about 30 $\mu$mol C/mg Chl-h; Fig. 1B).

Typical results showing the time course of appearance of sugars in the reaction medium during photosynthesis by tobacco protoplasts are shown in Figure 2A. During the first 30 min of illumination, there was a relatively linear release of total sugars and (as observed with wheat) the relative proportions of sucrose versus hexoses varied with time. On a carbon equivalent basis, sucrose was the predominant sugar released during the first 20 min, after which hexoses began to appear. The accumulation of hexoses was probably not caused by inversion of sucrose after release to the medium because no significant increase in hexoses was observed when protoplasts were incubated with 5 mM sucrose. The intraprotoplasmic pools of sucrose and hexoses remained relatively constant during the 30-min period (Fig. 2B). In other experiments,
the total protoplast sugar pool (sucrose + hexoses) varied between 60 and 150 μatoms C/mg Chl (about 10–25 μmol hexose equivalents/mg Chl). Also, the sucrose to hexose ratio was variable. In the experiment of Figure 2B, the hexose pool was seven times larger, on a molar basis, than the sucrose pool. In other experiments with tobacco, and in all experiments with wheat protoplasts, pools were often nearly equal in size. The basis for the variation is not known. However, regardless of the sugar pool composition, internal pool size remained constant (Fig. 2B). Age-dependent changes in both the size and composition of the sugar pool in tobacco leaves have been reported (7). The limited efflux of sugars labeled by 14CO2 assimilation may be caused by dilution with the relatively large unlabeled sugar pool(s) within the protoplast. The sugar efflux observed with tobacco protoplasts (Fig. 2A) was significant on a quantitative basis. Based on the CO2 fixation rate of 85 μmol CO2 fixed/mg Chl·h and assuming that 50% was incorporated into sugars, the rate of photosynthetic sugar production in the experiment of Figure 2 was about 40 μatoms C/mg Chl·h. Hence, the rate of total sugar efflux (about 54 μatoms C/mg Chl·h; Fig. 2A) nearly equaled the rate of photosynthetic sugar formation, an observation consistent with the constancy of the internal protoplast sugar pools.

In other experiments, the rate of sugar efflux in the light averaged 22 and 61 μatoms C/mg Chl·h for wheat and tobacco, respectively (average of at least six separate experiments). With wheat, the efflux of sugars observed was usually similar to or slightly less than the calculated rate of photosynthetic sugar formation, whereas the observed efflux with tobacco was usually somewhat higher than the amount formed from carbon assimilation. Presumably, the sugars effluxed in excess of that formed photosynthetically, as well as the efflux observed in the dark, may be attributed to mobilization of reserves such as starch.

Several lines of evidence suggest that the sugar efflux observed in these experiments was not simply leakage from damaged protoplasts: (a) hexose phosphates were never detected in the medium; (b) the intraprotoplast sugar pools remained relatively constant; (c) the efflux was observed during the first 30 min of photosynthesis when rates of CO2 assimilation were linear; and (d) light microscopy failed to detect an appreciable population of ruptured protoplasts over the periods tested.

The results provide evidence for the transport of sucrose and hexoses across the plasmalemma of isolated leaf protoplasts and, hence, provide direct support for the apoplastic mechanism of translocation. The results also have significance for various photosynthetic studies using isolated mesophyll protoplasts. It is clear that with protoplasts, the external medium constitutes a large “sink” for sugars formed during photosynthesis in vitro. The relative constancy of the internal sugar pools (Fig. 2B) suggested that a significant fraction of the protoplasts in the population had the capacity to export photosynthetically formed sugars. Although experimental proof is lacking, it is thought that in vivo sugar transport between mesophyll cells distal from the vascular tissue may occur through plasmodesmata. Only in the region of the phloem, where plasmodesmata are lacking, are sugars effluxed to the apoplast (4). Our results may suggest that all mesophyll cells have the capacity to transport sucrose across the plasmalemma, but that mechanism may only be utilized when symplastic sugar movement is no longer possible.

The sucrose and hexose efflux observed in vitro may reflect the type of in vivo transport that occurs in the region of the vascular tissue. Studies to be reported elsewhere with various inhibitors and ionophores suggest that an energized membrane and a sugar-K+ symport are involved in the movement of sucrose and hexoses across the plasmalemma (Huber and Moreland, in preparation).

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LITERATURE CITED