Sucrose Compartmentation in the Palisade Parenchyma of Vicia faba L.¹

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Intracellular sucrose compartmentation in the palisade parenchyma of Vicia faba L. leaves was investigated by comparing the specific radioactivity of photosynthetically labeled [14C]sucrose in samples enriched in vacuole to that in samples enriched in cytoplasm. Brief centrifugation of leaflet punches was used to sediment most of the palisade parenchyma cytoplasm in the adaxial ends of the cells. The punches were quick-frozen, freeze-substituted, and embedded in methacrylate. Samples enriched in cytoplasm or in vacuoles were obtained from paradermal sections. After pulse-labeling, the sucrose specific radioactivity in vacuole-enriched samples was fairly constant. Sucrose specific radioactivity in cytoplasm-enriched samples was about 2.5 times that in vacuole-enriched samples initially and declined thereafter. Earlier interpretation of intracellular sucrose compartmentation (Plant Physiol. 1975 55:704–711) had predicted larger specific activity differences (up to 20 times) between the cytoplasm and vacuole. The difference between the actual and predicted behavior is ascribed to the observed extent of cross-contamination in samples and, more importantly, to the confinement of sucrose to extrachloroplastic regions of the cytoplasm.

In a recent investigation of sucrose compartmentation in Vicia faba L. leaves, Outlaw et al. (4) demonstrated that the specific radioactivity of [14C]sucrose after a 14CO₂ pulse was higher in veins than in mesophyll tissues. This observation indicated the occurrence of two kinetically distinct pools of sucrose in mesophyll cells. The cytoplasm and vacuole were presumed to be the most likely possibilities for the location of the more readily translocated and less readily transported pools, respectively. We have directly demonstrated that this is the case by following the kinetics of [14C] sucrose specific radioactivity in samples containing mostly cytoplasm or mostly vacuole.

MATERIALS AND METHODS

Growth and Labeling of Plants. V. faba L. seeds were planted in a Vermiculite-soil mixture and grown in a greenhouse for 3 to 5 weeks. Five days before the experiment, a plant was transferred to a growth chamber under a 14-h photoperiod at 21 C during the day and 15 C during the night. PAR at the plant level was 170 µE m⁻² s⁻¹ (about 900 ft-c) provided by a mixture of incandescent and fluorescent lamps.

Two h before labeling, the experimental leaf on the intact plant was sealed into an open Plexiglas chamber in a fume hood. Illumination of 170 µE m⁻² s⁻¹ was provided by two incandescent lamps. Just before labeling, the chamber was closed and 0.5 mCi of 14CO₂ was circulated through the chamber for 4 min. The chamber was then opened in the draft of the fume hood and subsequent photosynthesis took place in ambient 14CO₂.

Sampling and Specific Radioactivity Determination. At various times after labeling, leaf samples were taken with a number 3 cork borer. All samples were taken from the same leaflet. Immediately after sampling, the leaf punch was centrifuged for 3 min at 1,200g, upper epidermis down, to collect most of the palisade cell cytoplasm in the centrifugal (adaxial) ends of the cells. The punch was quick-frozen in a mixture of isopentane-methylcyclohexane (9:1, v/v) cooled to about −170 C with liquid N₂. The time elapsed between sampling and freezing was about 3.5 min.

The frozen punches were transferred into propylene oxide for freeze substitution at −65 C. After 10 days, they were warmed to room temperature and transferred into dry toluene in three steps. Working in a dry box, the edges of the punch were trimmed away and suitable-sized squares were cut from the central portion of the punch for embedding in methacrylate. These procedures for freeze substitution and methacrylate embedment have been demonstrated to retain the common water-soluble metabolites in plant tissues (1) and were used in our earlier work (4).

After polymerization, the blocks were trimmed and oriented on an ultramicrotome so as to give sections which were as close to paradermal as possible. Serial sections 4 µm in thickness were cut from the palisade parenchyma on a dry glass knife and transferred, in order, to a dry microscope slide. Areas of sections containing mostly cytoplasm or vacuole were cut out with a razor blade. The total volume of a "cytoplasmic" sample was about 4,000 µm³ of leaf tissue; that of a "vacuolar" sample was about 8,000 µm³. (The samples will be referred to in quotation marks, since "cytoplasmic" samples contained some vacuole, and vice versa.) Samples were extracted three times with water on a Teflon sheet for 10 min at 100 C. The extracts were combined in a small test tube and dried in an air stream.

For sucrose analysis, a convenient volume of 0.02 N NaOH was added to the dried extract. Aliquots were assayed by the method of Jones et al. (3). Assays were run in triplicate and required less than 10% of the total extract. Thirty µg of carrier sucrose were added to the remaining extract, which was then chromatographed descendingly on Whatman No. 4 filter paper in butanol-propionic acid-water (23:12:15, v/v). Sucrose was localized and assayed for radioactivity as before (4).

Microscopic Observations. The dried section fragments of each
sample were transferred from the Teflon sheet to a microscope slide and stained with 1% Aniline blue black in 7% acetic acid. The sections were examined by bright field microscopy and a visual estimate was made of the relative amount (i.e., relative to the actual cellular volume) of vacuole in "cytoplasmic" samples and of cytoplasm in "vacuolar" samples. The total volume of each sample was determined by measuring the areas of the section fragments and multiplying by the section thickness (4 μm).

Centrifuged leaf samples were also prepared for electron microscopy by conventional fixation procedures. A centrifuged leaf disc was cut into small squares and fixed for 75 min in 2% depolymerized paraformaldehyde-3% glutaraldehyde in 50 mM cacodylate buffer at pH 6.8. The tissue was postfixed in 1% OsO₄, dehydrated in acetone, and embedded in Spurr's epoxy mixture (5). Thin sections were stained with uranyl acetate and lead citrate and examined in an electron microscope.

Estimates of the relative volumes occupied by various cell components in "cytoplasmic" and "vacuolar" samples were obtained from 24 electron micrographs (12 each) of the cytoplasmic and vacuolar regions of the cell. For "cytoplasmic" samples, areas were determined by cutting out the various regions from the electron micrographs and weighing them. The cytoplasmic area in "vacuolar" samples was calculated as the product of the mean thickness of the cytoplasmic layer and the cell circumference.

RESULTS AND DISCUSSION

Figure 1 illustrates the structural appearance of a Vicia leaflet cross-section after centrifugation, freeze substitution, and methacrylate embedment. The cytoplasm of palisade parenchyma cells formed a layer just below the upper (adaxial) epidermis. Owing to slight undulation of the leaf surface, only patches of palisade cells with this cytoplasmic layer appeared in any one section. Although the palisade cells appear to be somewhat bulbous as a result of centrifugation, this appearance is regarded as an artifact resulting from collapse of the less well-supported vacuolar end of the cells during methacrylate embedment. This interpretation is supported by the angular appearance of the walls and the fact that distinctively bulbous ends were not observed after centrifugation in hand sections of fresh material or in glutaraldehyde-fixed tissue. Quantitative measurements of cell areas in paradermal sections from the latter showed no significant difference in the ratio of intercellular to cellular volume (55:45) at the cytoplasmic versus vacuolar ends of palisade cells in centrifuged leaf discs. Since the ratio of sucrose concentrations in "cytoplasmic" and "vacuolar" samples, calculated on a section volume basis, was consistently less than

1.0 (0.75 ± 0.23 SD), the average sucrose concentration in the cytoplasmic ends of the cells was correspondingly less than that in the vacuolar end.

The kinetic behavior of sucrose specific radioactivity in "cytoplasmic" and "vacuolar" samples is shown in Figure 2. A visual estimate of the per cent cellular volume actually represented by cytoplasm of vacuole is given for each sample. "Vacuolar" samples contained very little cytoplasm but, even though only selected regions of sections were taken for "cytoplasmic" samples, the latter contained appreciable amounts of vacuole. Nevertheless, the data show well-defined and distinctly different kinetics of sucrose specific radioactivity in the vacuole and cytoplasm. The observations clearly support our earlier explanation for the relatively high sucrose specific radioactivity in minor veins shortly after labeling with 14CO₂: newly synthesized sucrose is preferentially exported from the leaf because of relatively slow exchange between vacuolar and cytoplasmic sucrose pools in photosynthetic tissues.

In quantitative terms, the kinetic behavior of cytoplasmic and vacuolar specific radioactivities that were predicted in our earlier model for compartmentation (4, replotted in Fig. 3) differs substantially from the experimental values shown in Figure 2. However, we believe that our earlier model is accurate, and attribute the quantitative differences to cross-contamination of our "cytoplasmic" and "vacuolar" samples. That such contamination might be a problem is suggested by the observation that the specific radioactivity of sucrose in "vacuolar" samples remained essentially constant throughout the experiment. Particularly in view of the changes shown by sucrose in "cytoplasmic" samples, this suggests a surprisingly rapid rate of movement from a specific pool of newly synthesized sucrose into the vacuole. However, reports that
sucrose is synthesized outside of chloroplasts (2) make an alternative explanation more plausible. If sucrose is excluded from chloroplasts the thin layer of cytoplasm surrounding the vacuole would be a much greater source of contamination in "vacuolar" samples than would be suggested by its volume alone. Also, the volume of the cytoplasmic sucrose pool would be much less than that suggested by the cytoplasmic volume, since the latter consisted mostly of chloroplasts.

To test the quantitative implications of this possibility, the relative volumes occupied by vacuole, chloroplasts, and nonchloroplastic protoplasm were estimated from electron micrographs of sections through centrifuged palisade parenchyma (Table I). Nonchloroplastic protoplasm accounted for an average of only 20% of the protoplasm. Although sucrose might be excluded from other organelles as well, for purposes of calculation we assumed that it occupied 20% of the protoplasm in the centrifugal end of the cell and all of the cytoplasmic volume around the vacuoles (no chloroplasts were present there). Using the average figures for the "purity" of "vacuolar" and "cytoplasmic" samples (Fig. 2A), the average ratio of sucrose concentrations in the two samples ("cytoplasmic" concentration/"vacuolar" concentration = 0.75; see above), and assuming that sucrose occupied only 20% of the centrifugal protoplasm, it can be calculated that the cytoplasmic sucrose pool was three times as concentrated as the vacuolar pool. With the same assumptions, the data of Outlaw et al. (Fig. 3) may be used to predict the kinetics that would be observed with the degree of cross-contamination shown in Figure 2A. These calculations are shown in Figure 4. The ratios of these calculated specific radioactivities are plotted in Figure 2B for comparison with the experimental ratios. The predicted values are in good agreement with experimental ones. We feel that this agreement provides further support for our original model of sucrose compartmentation in Vicia leaves (4).

**LITERATURE CITED**