Kinetics of C-14 Translocation in Soybean

II. KINETICS IN THE LEAF

DONALD B. FISHER
Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa

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ABSTRACT

The kinetics of 14C-assimilates in the soybean leaf were studied in pulse labeling and steady state labeling experiments. 14C-Sucrose apparently served as the ultimate source, at least, of translocated 14C-sucrose. However, since the specific activity of leaf sucrose reached a maximum within 5 minutes after pulse labeling, whereas that of exported sucrose did not reach a maximum until at least 20 minutes, it appeared that there were two sucrose compartments in the leaf. A possible physical basis for the two compartments may be the mesophyll (a photosynthetic compartment) and a specialized "paraveinal mesophyll" (a nonphotosynthetic compartment), through which photosynthesize must pass on its way to the veins.

The 14C kinetics of starch glcose, and probably esterified sterol glucoside, were similar to those for 14C-sucrose export. Starch glucoside was labeled only in its glucose moiety and was the only stem lipid which became strongly labeled during 14C-sucrose translocation. These sterile derivatives may act as membrane carriers of sucrose between the translocation stream and surrounding cells.

The kinetics of 14C-sucrose and its movement to the veins are discussed with reference to compartmentation within the leaf and metabolic exchange with other compounds, particularly with starch. Although a simple compartmental model gave a fairly accurate description of 14C-sucrose kinetics, an entirely accurate model could not be provided, primarily because of loss of 14C from sucrose, at an unknown rate, to starch.

The transport kinetics of 14C-photosynthesize have frequently been interpreted on the basis of mechanisms thought to operate in the stem which could account for the kinetics observed there. However, most available evidence indicates that the major features of the stem 14C kinetics are determined by factors operating in the leaf (7). This is especially true of the initial phase of translocation, during which an exponential translocation profile is often observed. Unfortunately, there are few data on 14C kinetics in the leaf during the initial period of one to several hours when most kinetic changes occur. A significant exception is provided by the experiments of Geiger and Swanson (10), which showed that the rate of 14C-sucrose export from sugar beet leaves was proportional to the specific activity of sucrose in the leaf.

The present experiments were conducted to determine the 14C kinetics in soybean leaves under conditions similar to those used for translocation studies (7). From the results it was hoped to explain the kinetics of 14C-photosynthesize translocation in soybean and possibly to identify any compounds that might be uniquely associated with the source pool for translocated sucrose.

METHODS

Experimental procedures for growing the soybean plants, and for extraction, chromatography, combustion, radioactivity determinations, and quantitative analysis were described in the previous paper (7).

Feeding and Sampling Procedures. Soybean plants were prepared for labeling as described earlier (7). Most leaf samples were taken with a paper punch and had an area of 0.334 cm2. Punches were taken from the leaf at random, except that none were taken "upstream" from previous punches, as indicated by the leaf venation. After extraction of the samples with 80% ethanol, lipids and water-soluble compounds were chromatographed, starch was determined by hydrolysis to glucose (see "Starch Hydrolysis"), and residual activity was determined by combustion to 14CO2.

The feeding chamber shown in Figure 1 allowed a soybean leaf to be sampled repeatedly during steady state labeling with 14CO2. A punch in the chamber could be manipulated through the plastic bag attached to the front. A sliding block in the bottom allowed removal of the sample with minimal loss of 14CO2. The hole in the block was aligned with the hole in the top of the block casing and the punch was dropped into the hole. By sliding the block forward, the punch could be removed through the hole in the bottom of the casing. A rubber "O" ring around the upper hole increased the effectiveness of the seal between the sliding block and its casing.

During steady state labeling, a constant level of 14CO2 was maintained by including an 85-liter Plexiglass box in the circulating atmosphere. The atmosphere was monitored with a thin end window Geiger tube connected with a rate meter and recorder (2). 14CO2 was generated before the experiment and circulated through the system, excluding the feeding chamber, for at least 30 min. To initiate the labeling period, the feeding chamber was spliced into the system. After the labeling period, the feeding chamber was replaced by a CO2 trap containing CO2-free 1 N NaOH, and the atmosphere was kept circulating for several hours until remaining activity had been absorbed. Its specific activity was determined by conversion to Ba14CO3, a sample of which was weighed and its activity determined by liquid scintillation counting of its regenerated 14CO2 (14).

For pulse labeling experiments, the circulating atmosphere included only a feeding chamber of two glass hemispheres (2), the
\[14^\text{C}O_2\] generator, and the finger pump. The labeling period was terminated by opening the chamber in a hood with a strong forced draft.

**Starch Hydrolysis.** Starch was determined by grinding the sample with sand and water, autoclaving at 112° for 15 min to swell the starch grains, and enzymatically hydrolyzing the starch to glucose. Enzymatic hydrolysis was carried out first by treatment of the autoclaved sample for 24 hr at 37° with an equal volume of crude salivary amylase (prepared by removing mucopolysaccharides from saliva with calcium acetate) to solubilize the starch. The resulting starch hydrolysate was converted entirely to glucose by treatment for 24 hr at 55° with 0.33 ml of a solution of amyloglucosidase (1 mg of Agidex 3000 per ml of 0.25 M sodium acetate buffer, pH 4.5; Glaxo Laboratories, Ltd., Middlesex, England) per 4 ml of solution to be hydrolyzed. This preparation of amyloglucosidase hydrolyzes \(a-1,6\)- as well as \(a-1,4\)-linked glucose units (1). Incubations were carried out in the presence of a few grains of thymol to prevent bacterial development. An aliquot was chromatographed and glucose was determined by Wager's procedure (18). Complete hydrolysis to glucose also allowed an estimate of nonstarch \(14^\text{C}\) released by the procedure. This amounted to 7 to 8% of the \(14^\text{C}\)-starch but was fairly constant in all samples.

**RESULTS**

**Pulse Labeling.** After labeling a leaf for 3 min with \(14^\text{C}O_2\), single-punch samples were taken at intervals during the following 3 hr. Although the \(14^\text{C}\) kinetics of virtually all labeled compounds were followed, only those aspects relevant to sucrose transport will be reported.

Data for some sugars are shown in Figure 2. Sucrose accounted for the bulk of soluble activity at all times, and its \(14^\text{C}\) kinetics indicated that, for such a large pool, it was turning over quite rapidly. The "peak" of activity at 20 min is misleading, since it was not duplicated in the data for specific activity (Fig. 3). In a similar experiment, run expressly to clarify this point, three punches were taken at each time, and the experiment was terminated after 20 min. Both the total and specific radioactivities of sucrose reached a maximum 5 min after removal of the leaf from \(14^\text{C}O_2\).

Labeled sugar phosphates almost disappeared within 15 min (Fig. 2), but traces remained even after 100 min. Although a detailed study of these compounds is desirable because of their possible role in translocation (15), this was beyond the scope of these experiments.

Starch accounted for most of the activity fixed and showed a slow, linear increase from about \(2.4 \times 10^7\) dpm per sample to about \(2.7 \times 10^6\) dpm (Fig. 4). A considerable amount of malto-oligosaccharides was solubilized by autoclaving the samples prior to enzymatic hydrolysis. However, the kinetics of their total activity were not appreciably different from those of the starch fraction, and the two are included together as "starch." Smaller amounts of activity remained insoluble after starch hydrolysis and increased linearly from \(7 \times 10^4\) dpm to \(1.4 \times 10^5\) dpm.

Of all compounds examined, only sterol glucoside and esteri-
fied sterol glucoside exhibited kinetics which resembled those of sucrose. Data for sterol glucoside are given in Figure 5. Except for the initial lag in labeling, its kinetics strongly resembled those of sucrose. Although esterified sterol glucoside did not separate adequately from other 14C-lipids for separate quantification, it was apparent from the autoradiograms that its behavior was quite similar to that of sterol glucoside. That the 14C kinetics of the latter compound were due entirely to its glucose moiety was shown by acid hydrolysis of sterol glucoside from a leaf which had photosynthesized 14CO2 at a steady state for 3 hr. When the products were partitioned in the solvents described by Dawson (4), 98% of the activity appeared in the aqueous phase. All of this activity cochromatographed with glucose. Although apparently no other compounds examined were directly concerned with sucrose translocation, they might nevertheless affect the kinetics of 14C-sucrose by exchange labeling. The kinetics of 80% ethanol-soluble compounds other than sucrose are shown in Figure 6. Little net change occurred in this group of compounds after the first sample time. The initial decrease was due largely to loss of activity from serine, alanine, and the sugar phosphates, which accounted for large, and roughly equal, amounts of activity in the first sample.

The data for total activity per sample are plotted in Figure 7. A decrease in activity is indicated, but the data cannot be said to demonstrate this unequivocally. If the decrease occurs as shown, it amounts to about 10%, or somewhat more than half that expected on the basis of experiments with intact plants (7). Translocation from the leaf did occur, since scanning the stem with a Geiger tube after the experiment showed a considerable amount of activity there.

The foregoing data were derived from multiple sampling of a single attached leaf. Figure 8 allows some comparisons with

![Fig. 5. Carbon-14 kinetics of sterol glucoside after pulse labeling with 14CO2.](image)

![Fig. 6. Carbon-14 kinetics of soluble non-sucrose activity after pulse labeling with 14CO2.](image)

![Fig. 7. Total 14C per sample after pulse labeling with 14CO2.](image)

![Fig. 8. Carbon-14 kinetics of some sugars from whole leaves after pulse labeling with 14CO2.](image)

![Fig. 9. Specific radioactivity of leaf sucrose during steady state labeling with 14CO2. The "theoretical upper limit" was calculated from the specific radioactivity of Ba14CO3, collected from the atmosphere after the labeling period.](image)

similar data taken from intact leaves of six plants that had been pulse labeled for 3 min. (Other aspects of this experiment were summarized in the previous paper [7].) The kinetic behavior of these compounds, as well as almost all others, was similar to that of the same compounds in samples from the single leaf. Where differences were quantitative rather than qualitative, it seemed plausible to attribute them to variations in pool sizes. However, this explanation was questionable in a few cases. It was nevertheless apparent that multiple sampling did not cause drastic changes in the metabolism of 14C-photosynthate.
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Fig. 10. Carbon-14 kinetics of sterol glucoside during steady state labeling with \(^{14}\)CO\(_2\).

**Steady State Labeling.** If the experiments were comparable in other respects, the curves obtained from this type of experiment should be the integral curves of those obtained in the pulse labeling experiment (13, 16). Except for a few ambiguous, but minor, cases, this relationship seemed to be a valid one, and only limited data will be presented for steady state labeling.

Although the data for sucrose specific activity (Fig. 9) seem to indicate that it was approaching isotopic equilibrium with the \(^{14}\)CO\(_2\), it is believed that a value which was only 62% of that expected from the specific activity of the Ba\(^{14}\)CO\(_2\) precipitated at the end of the experiment. Similar data were obtained from an earlier steady state labeling experiment (not reported here).

The curve for sterol glucoside (Fig. 10) was fairly similar to that expected on the basis of pulse labeling data (Fig. 5) and, again, resembled that for \(^{14}\)C-sucrose except during the shorter time intervals.

**DISCUSSION**

If the major features of \(^{14}\)C kinetics in the stem result from factors operating in the leaf, perhaps their most important effect will be on the \(^{14}\)C kinetics in the source pool for translocated sucrose. (Another effect of the leaf is due to its size in relation to the translocation velocity [8].) Aside from the rate of sucrose turnover, factors which might affect the source pool kinetics include sucrose compartmentation, exchange of label between sucrose and other compounds, and the rate of sucrose movement from the photosynthetic parenchyma to the veins. The model and other phases of transport could involve forms other than sucrose itself but, since sucrose is the only major labeled pool with kinetics resembling those for \(^{14}\)C export, the amount of such hypothetical forms must be small.

One obvious possibility for compartmentation exists in the soybean leaf in the form of a specialized paraxial mesophyll (6). Because of its position and relatively undeveloped chloroplasts, it apparently interposes an essentially nonphotosynthetic compartment between the veins and the major sites of \(^{14}\)CO\(_2\) fixation. On the basis of this consideration alone, it is possible to construct a two-compartment model which fairly accurately describes the kinetics of \(^{14}\)C-sucrose export (8). The model assumes that \(^{14}\)C-sucrose is produced in one pool and migrates through a smaller sucrose pool to the veins. Relative pool sizes were based on the relative volumes (7/1) of photosynthetic and paraxial mesophyll. The model differs from that of Geiger and Swanson for sugar beet (11), which assumes the presence of only one sucrose pool.

The \(^{14}\)C kinetics exhibited by sterol glucoside (and apparently by esterified sterol glucoside) after pulse labeling (Fig. 5) were almost identical to the kinetics of \(^{14}\)C-sucrose export from a pulse labeled leaf (Reference 7, Fig. 12). Apparently the metabolism of these compounds was in some way associated with the appearance of \(^{14}\)C-sucrose in the translocation stream. This interpretation is strengthened by the fact that sterol glucoside was the only stem lipid which became strongly labeled during \(^{14}\)C-sucrose translocation (7). The behavior of these two lipids provides tangible support for the hypothetical presence of a minor sucrose pool which exhibited \(^{14}\)C kinetics slightly different from those for sucrose as a whole. The relation of these sterol derivatives to translocated sucrose is conjectural but, as lipids, it is reasonable to expect them to be membrane constituents, possibly acting as carriers of sucrose between the translocation stream and surrounding cells.

A second type of sucrose compartmentation was suggested by the fact that sucrose specific radioactivity during steady state labeling reached only 62% of that expected from the specific radioactivity of \(^{14}\)CO\(_2\). Again, the leaf anatomy provides a possible explanation. Epidermal tissues occupy approximately 30% of the volume in a soybean leaf. Sugar produced in the mesophyll probably moves directly to the veins without passing through epidermal tissues (12). If their sucrose content can be judged from their relative volume, the epidermis might contain about one-third of the leaf’s sucrose in a pool which did not rapidly equilibrate with newly synthesized sucrose. Any estimate of the specific radioactivity of exported sucrose would have to be corrected to account for this. This applies, of course, to calculations made in the previous paper (7) for the rate of sucrose efflux from the soybean leaf.

It is also possible that the low sucrose specific radioactivity could have been caused by the commonly observed isotope effect in photosynthesis in combination with refixation of respired \(^{12}\)CO\(_2\). Isotope effects of up to 17% have been recorded for CO\(_2\) fixation by higher plants (19), and the same paper demonstrated that nonradioactive substrates may be used for respiration for quite long periods after a leaf is placed in \(^{14}\)CO\(_2\). Forrester et al. (9) have reported a respiratory rate of about 6 \times 10\(^{-3}\) mm carbon hr\(^{-1}\) for soybean leaf. The photosynthetic fixation rate, calculated from data provided by the steady state labeling experiment, was 7 times this value. If all the respiratory \(^{14}\)CO\(_2\) were reoxidized, and there was in addition an isotope effect of about 17%, this could result in a sucrose specific radioactivity close to the observed value.

To describe fully the \(^{14}\)C kinetics of sucrose, metabolic reactions leading to a net loss or gain of activity must be considered in addition to its loss by translocation. The relation of sucrose to starch is particularly important, since Chan and Bird (3) have shown that carbon exchange can occur between sucrose and starch in tobacco leaves even during periods of net starch synthesis. In the pulse labeling experiment, activity in soluble non-\(^{14}\)C sucrose compounds remained fairly constant (Fig. 6) while that in starch showed a definite increase (Fig. 4). The ultimate source of this activity, at least, must have been sucrose, and the loss accounted for roughly one-third of its initial activity. Apparently the remaining two-thirds was lost by translocation. Since sucrose specific radioactivity decreased exponentially, while \(^{14}\)C-starch increased linearly, the kinetic relation between the two was apparently a complex one. It is therefore impossible, on the basis of the data obtained, to provide a complete kinetic analysis of the behavior of \(^{14}\)C-sucrose. However, from the point of view of translocation, the variation in the specific radioactivity of the sucrose pool is of primary importance and can be accurately described by a simple compartmental model. This approach will be taken in the following paper (8) but, for the reasons just stated, the assumptions made can be regarded only as approximations of the actual mechanisms. They nevertheless lead to an accurate description of the kinetics of sucrose specific radioactivity.

Some approximate, but useful, calculations of turnover times for leaf sucrose can be obtained from several experiments. Since the bulk of sucrose is apparently in a single pool, the over-all kinetics of leaf sucrose will be due mostly to one pool. As an approximation, the turnover time can be calculated on the basis...
of that expected from a single compartment in which sucrose is synthesized and removed at equal rates. The data, in fact, fit this assumption fairly well. The turnover times calculated on this basis are as follows. For the pulse labeling experiment with sampling by punches, 90 min; for the pulse labeling experiment where intact plants were harvested, 70 to 90 min; and for the steady state labeling experiment, 45 min. Another estimate of the turnover time may be made from the calculated rate of sucrose efflux from the leaf (7). Since the average sucrose content of a leaf was 984 µg and the efflux rate was 22 µg min⁻¹, the turnover time (considering only translocation) would be about 46 min. The latter value seems somewhat low since, as discussed above, some of the sucrose utilization implied by the tracer experiments was due to metabolic conversion in addition to translocation. However, it is difficult to assess whether this discrepancy is real or whether it might be attributed to variation between plants or to a cumulative effect of approximations made in the calculations.

The rate of movement of translocate from its sites of synthesis to the veins might also affect the source pool kinetics. Evans et al. (5) have suggested that the initial exponential rise in the translocation profile in soybean during steady state labeling is due to varying diffusion times of sucrose to the sieve tubes. Vernon and Aronoff (17) had previously pointed out that an exponential curve might arise if diffusion played a limiting role in the overall transport of sucrose from the leaf. However, the proposed limiting diffusion rate must be sufficiently large to account for the total rate of sucrose efflux from the leaf. It is difficult to conceive of a diffusion-limiting step which, on one hand, would allow the necessary sucrose efflux but which, on the other hand, could also cause a 20-min delay in the movement of ¹⁴C-sucrose from a pulse labeled soybean leaf.

Of the factors discussed, the apparent physical necessity that newly synthesized sucrose must move through essentially non-photosynthetic cells on its way to the veins would seem to be the most likely to have an effect on source pool kinetics. Although the presence of a specialized paravascular mesophyll makes this particularly obvious in soybean, the bundle sheath and phloem parenchyma must also be included. It seems probable that the same consideration would apply to other species, although to a lesser extent.

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LITERATURE CITED