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Turnover of Soluble Proteins in the Wheat Sieve Tube

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ABSTRACT

Although the enucleate conducting cells of the phloem are incapable of protein synthesis, phloem exudates characteristically contain low concentrations of soluble proteins. The role of these proteins and their movement into and out of the sieve tubes poses important questions for phloem physiology and for cell-to-cell protein movement via plasmodesmata. The occurrence of protein turnover in sieve tubes was investigated by [^{35}S]methionine labeling and by the use of aphid stylets to sample the sieve tube contents at three points along a source-to-sink pathway (flag leaf to grains) in wheat plants (*Triticum aestivum* L.). Protein concentration and composition were similar at all sampling sites. The kinetics of ^{35}S-labeling of protein suggested a basically source-to-sink pattern of movement for many proteins. However, an appreciable amount of protein synthesis and, presumably, removal also occurred along the path. This movement appeared to be protein specific and not based on passive molecular sieving. The results have important implications for the transport capacities of plasmodesmata between sieve tubes and companion cells. The observations considerably expand the possible basis for ongoing sieve tube-companion cell interactions and, perhaps, interaction between sources and sinks.

Although the presence of proteins in sieve tube exudates has been recognized for many years, little is known about their function or metabolic stability. Most efforts to characterize their biochemical properties have concentrated on the structural proteins (P-proteins), which appear in fairly high concentrations (40–100 mg mL^{-1}) in phloem exudates from members of the Cucurbitaceae (6). Because of their high concentration, and because P-protein structures are evident before and after the final stages of sieve element differentiation (5), P-proteins do not appear to be mobile components of the translocation stream. Instead, their presence in exudate apparently results from the disruptive effects of sharply steepened pressure gradients in severed sieve tubes.

Soluble, and therefore presumably mobile, proteins nevertheless appear to be normal constituents of the translocation stream. Protein concentrations in exudates from non-cucurbit species are typically low (approximately 0.2–2 mg mL^{-1} [7, 14, 22, 24]) and, at least as evidenced by enzyme activities, may remain constant over many hours of exudation (2). Finally, proteins have been detected in aphid stylet exudate (1, 13), where normally existing pressure gradients would be altered only slightly by an exuding stylet.

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Because mature sieve elements lack both a nucleus and ribosomes (4), they are incapable of protein synthesis. Clearly, the ongoing presence of proteins in the translocation stream requires their continual replacement by movement from adjacent nucleate cells. Companion cells are the most likely origin of such proteins and characteristically possess an active-appearing cytoplasm, including abundant ribosomes. Recently, Nakamura et al. (16) strikingly demonstrated the appearance of newly synthesized protein in rice sieve tubes by separating more than 100 [^{3}H]leucine-labeled polypeptides in stylet exudate collected from rice plants. Some years earlier, Nuske and Eschrich (17) showed that even "structural" P-proteins are involved in rapid turnover and presented microautoradiographic evidence for their synthesis in companion cells.

The occurrence of protein turnover in sieve tubes raises a number of intriguing and physiologically significant questions. Movement of proteins into and out of the sieve tube presumably occurs via plasmodesmata, which, except for pathological conditions, appear to provide passageways too small for intercellular protein movement (21). Although their functions are unknown, the proteins presumably have some role in source-sink and/or sieve tube-companion cell relations. As Raven (20) recently emphasized, the striking longevity of sieve elements as enucleate cells poses ongoing maintenance problems that almost certainly require intercellular protein transport. Finally, the synthesis and movement of phloem proteins in healthy plants may provide insight into the replication and movement of phloem-limited viruses and *Mycoplasma*-like organisms.

The following experiments were undertaken to investigate some of the overall characteristics of soluble sieve tube proteins, especially their number and variability along the transport pathway, and their patterns of synthesis, transport, and turnover.

MATERIALS AND METHODS

Plant Material

Wheat plants (*Triticum aestivum* L. cv SUN 9E) were grown in a growth chamber as described previously (9). Experiments were performed with plants in the middle portion of the grain-filling stage (approximately 15–25 d after anthesis). Figure 1 illustrates the appearance of plants at this stage and summarizes the sites of exudate collection and [^{35}S]methionine application.

Collection of Sieve Tube Exudate

Caged aphid colonies (*Rhopalosiphum padi* L.) were placed on a plant in the evening, and their stylets were severed by

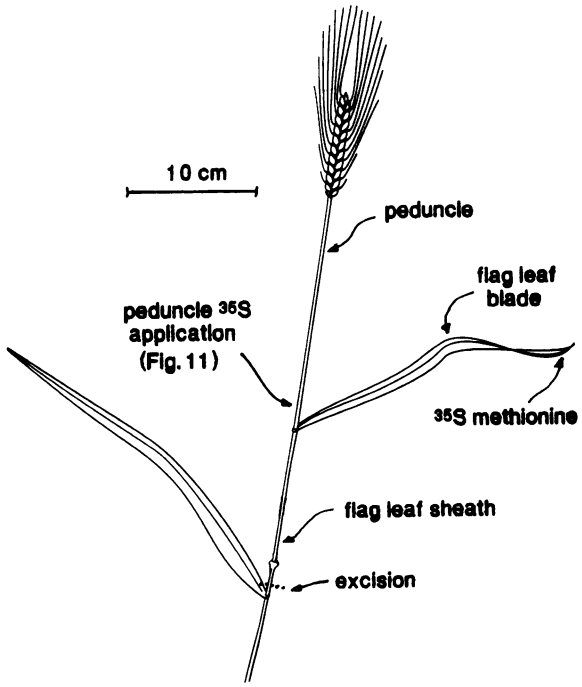

Figure 1. Sites of stylet exudate collection and [^{35}S]methionine application. At each site, stylets were restricted to a 2-cm segment of the path. "Excision" refers to part of a single experiment (Table I; Fig. 3A, lane d); plants were intact in all other experiments.

radiofrequency microcautery the following morning (10). The cages restricted aphids to a 2-cm segment of the selected site. After the stylets were cut, the plant surface was washed thoroughly in running water, and, subsequently, exudate was allowed to air dry as it accumulated. Typically, exudate was collected from about 15 to 25 stylets at each site. When samples were collected frequently, as in tracer kinetics experiments, accumulated exudate was dissolved by washing the plant surface with a 50-μL aliquot of 0.2% BSA. (Because of the low concentrations of exudate protein, BSA was included to reduce nonspecific protein loss and to serve as a carrier in subsequent TCA precipitations.) When larger quantities were collected during longer intervals, as for protein determinations, the semisolid exudate droplets were lifted from the plant surface with a fine needle and transferred to a microcentrifuge tube. Samples were stored at −80°C until analysis.

In one experiment, phloem exudate was collected without the use of aphid stylets by breaking grains from their pedicels just above the lodicules (11). Exudate usually flowed from the broken pedicel stumps for about 5 to 15 min, during which time it was collected with a fine-tipped disposable micropipet.

[^{35}S]Methionine-Labeling Experiments

The flag leaf was labeled by cutting off the tip under water and moving the cut end of the leaf into approximately 75 μL of a solution containing 0.1 to 1.0 mCi of [^{35}S]methionine (New England Nuclear; 1000 Ci/mmol). (In one experiment in which the objective was simply to achieve general labeling for autoradiographic purposes, a mixture of [^{35}S]methionine and cysteine was used.) The labeling solution was absorbed by the leaf in about 1 h and, 30 to 60 min later, an additional 2 cm of the flag leaf tip was removed. Although dipping the cut end of a leaf into a solution can, under some conditions, result in appreciable reverse flow of solute in the xylem, ^{35}S movement from the leaf blade occurred only via the phloem, probably because such small amounts of methionine were rapidly absorbed by the leaf tissue. Thus, the velocity of tracer movement, both within the flag leaf and along the rest of the phloem pathway, was similar to movement of ^{14}C-photosynthate and ^{32}P translocation (9), and microautoradiographs showed labeling only in the phloem (see below). Also, similar results, but lower amounts of radioactivity, were obtained when labeled methionine was applied to an abraded area of the leaf rather than to the cut end.

In several experiments, [^{35}S]methionine was introduced into a segment of the path (the peduncle; Fig. 1) rather than the flag leaf (source). In this case, a "window," approximately 5 mm long by 1 mm wide, was cut into one side of the hollow peduncle just above the flag leaf. To block downward wicking of solution inside the peduncle, a plug of modeling clay was inserted and sealed into place with cyanoacryalate adhesive. The window was then sealed with modeling clay. A 50-μL aliquot of [^{35}S]methionine (0.1 mCi) was injected into the center of the peduncle 2 cm above the window. The water potential of the labeling solution was adjusted to −5.3 bars with PEG 8000 (0.2 g/g of water) to minimize water movement from the solution into the plant (8).

Exudate ^{35}S-protein content was determined by overnight precipitation with 10% TCA of solutions containing 0.2% BSA.

Soluble ^{35}S compounds were separated by one-dimensional TLC on cellulose using *n*-propanol:formic acid:water (20:1:5, v/v) (23).

Protein Assay

Exudate protein content was assayed by a Coomassie blue dye-binding procedure (Pierce Chemical Co.). Volume was reduced to 220 μL (20 μL of sample plus 200 μL of reagent), giving a sensitivity of 0.17 *A* units per μg of protein. The semidry exudate samples were first diluted with water to an appropriate concentration (approximately 20–30 μg dry weight μL^{-1}). Other potentially sensitive assays tried (colloidal gold binding, bicinchoninic acid) showed strong interference from other solutes in the exudate (approximately 25% dry weight).

Electrophoresis, Silver Staining, and Gel Autoradiography

Phloem exudate protein composition was analyzed by one-dimensional SDS-PAGE or by electrofocusing under equilibrium or nonequilibrium conditions followed by SDS-PAGE in the second dimension (18). For SDS-PAGE, exudate was mixed with an equal volume of 2× SDS extraction buffer (50 mM Tris-HCl [pH 7.5], 2% SDS, 10% glycerol, 10% β-mercaptoethanol) and heated in boiling water for 2 min. Electrophoretic separation was performed in a 10 to 20% (w/v) linear gradient polyacrylamide gel containing 0.1% SDS,

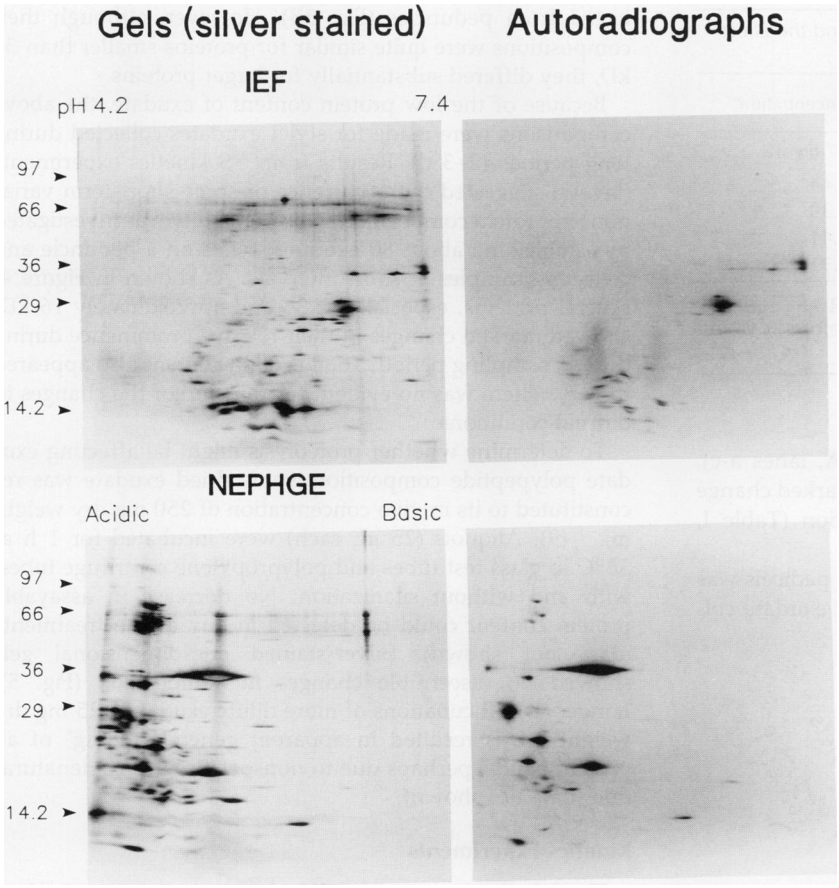

Figure 2. Two-dimensional separations of ^{35}S-proteins in peduncle phloem exudate. IEF was performed under equilibrium conditions (IEF, top gel) or NEPHGE conditions (bottom gel). To obtain enough protein for satisfactory staining, exudate was accumulated during a 5-d period; the plant was labeled with 5 mCi [^{35}S]-methionine/cysteine (77/18%) on the second day. Each gel was loaded with half of the combined exudate (125 mg dry weight per gel). Tissue samples for microautoradiographs (Fig. 12) were also taken from this plant. Arrows mark the positions of molecular mass standards (kD).

stabilized by a 5 to 17% linear sucrose gradient, and conducted at 25°C for 4 h using a constant current of 30 mA per gel. 3-[(3-(Cholamidopropyl)dimethylammonio]-1-propane sulfonate, instead of *N,N'*-methylene-bis-acrylamide, was used as a cross-linker. Polypeptides were visualized by silver staining (19).

For two-dimensional separation, exudate was mixed with an equal volume of the lysis buffer of O'Farrell et al. (18) containing 1.5% pH 5 to 7 and 0.5% pH 3 to 10 ampholines for IEF[2] or 3% pH 3 to 10 ampholine for NEPHGE. The IEF gels were prefocused first for a total of 400 V-h (15 min at 200 V, 30 min at 300 V, and 30 min at 400 V). IEF of proteins was run for 13.5 h at 400 V and 1 h at 800 V for a total of 6200 V-h. The pH gradient formed along the gel, measured with a micro pH electrode, was between 4.2 and 7.4. NEPHGE was run for 3.5 h at 400 V for a total of 1400 V-h, without prefocusing. The cylindrical gels were removed from tubes at the end of the run, equilibrated in SDS buffer, and run on the second-dimensional SDS-PAGE gels.

Gels containing ^{35}S-proteins were dried and exposed to x-ray film for autoradiographs.

Microautoradiography

Six hours after uptake of 5 mCi of [^{35}S]methionine by the flag leaf, small pieces of the peduncle were fixed in a solution of 4% paraformaldehyde and 2 mM $CaCl_2$ buffered at pH 7.0 with 40 mM Pipes. After dehydration and embedment in LR White resin, 1.5-μm cross- and longitudinal sections were stained by the periodic acid-Schiff reaction and autoradiographed by dipping in Ilford L4 nuclear track emulsion.

RESULTS

General Patterns and Comparisons of Composition

When proteins were separated by IEF over a pH range of 4.2 to 7.4 in the first dimension followed by SDS-PAGE in the second dimension, more than 100 polypeptides could be detected by silver staining of a two-dimensional gel (Fig. 2, top gel). However, comparison with a gel run under non-equilibrium pH conditions (Fig. 2, bottom gel) showed that a substantial number of proteins, including several prominent ones, had been lost during equilibrium focusing. Thus, their isoelectric points must be more basic than 7.4. In all, the total number of soluble proteins in wheat sieve tubes must approach 200.

Most exudate proteins appeared to be labeled by the [^{35}S]-methionine/cysteine mixture (77/18%) used in this experiment (Fig. 2). Several prominently staining proteins, however (e.g. some in the cluster of higher molecular weight proteins in the IEF gel), showed no detectable labeling.

Protein content and composition were similar in stylet exudate samples collected from widely separated points along the phloem pathway from source to sink (flag leaf blade, flag

[2] Abbreviations: IEF, isoelectric focusing; NEPHGE, nonequilibrium pH gradient electrophoresis.

Table I. *Protein Concentrations Along the Pathway and the Effect of Excision*

Collection Site	Collection Interval	Protein Concentration[a]
	h	*μg mg⁻¹ dry wt*
Flag leaf blade	70	0.44
Flag leaf sheath	70	0.39
Peduncle	70	0.41
Peduncle (excised)	44	0.31

[a] Typical exudate concentration is about 250 μg μL^{-1} (9); the protein concentration of sieve tube sap would therefore be about 0.1 μg μL^{-1}.

leaf sheath and peduncle; Table I and Fig. 3A, lanes a-c). Excision of the peduncle did not result in any marked change in exudate protein concentration or composition (Table I, Fig. 3A).

Phloem exudate collected from broken grain pedicels was similar in protein concentration to aphid stylet exudate collected from peduncles (Fig. 3B). However, although their compositions were quite similar for proteins smaller than 30 kD, they differed substantially for larger proteins.

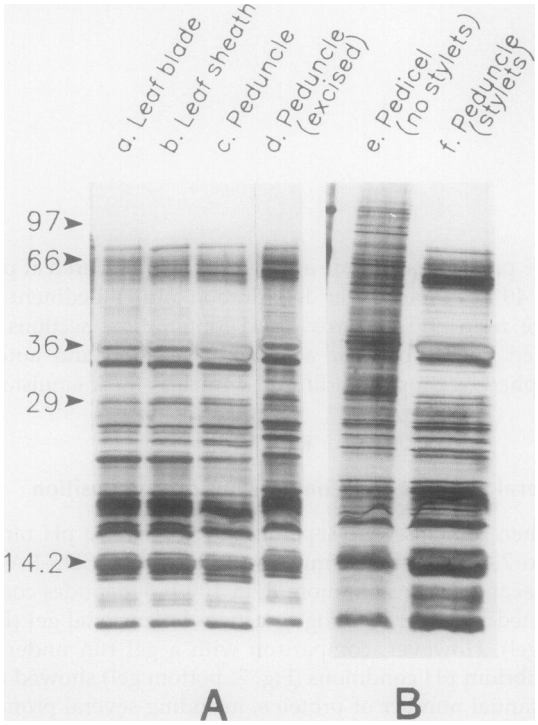

Figure 3. Silver-stained SDS/PAGE profiles of phloem exudate proteins. All lanes were loaded with 5 mg (dry weight) of phloem exudate (approximately 2 μg of protein), except for lane f, where 10 mg was applied. A, Aphid stylet exudates collected from the flag leaf blade, flag leaf sheath, and peduncle of the same plant during a 70-h period (lanes a–c). Lane d is an additional 44-h collection from the peduncle stylets after excision of the shoot just below the flag leaf node. The flag leaf and sheath were also removed. B, Grain pedicel exudate, collected without the use of aphid stylets (lane e), compared with peduncle stylet exudate collected later from the same plant (lane f). Arrows mark the positions of molecular mass standards (kD).

Because of the low protein content of exudate, the above comparisons were made for stylet exudates collected during long periods (2–3 d). Results from ^{35}S kinetics experiments (below) suggested the occurrence of some short-term variations in protein composition. This possibility was investigated by establishing about 80 exuding stylets on a peduncle and collecting samples at short intervals. As shown in Figure 4, several proteins, especially at 36 and approximately 16 kD, showed marked changes in their relative prominence during the 2-d sampling period. Total protein content also appeared to vary. There was no evident relationship of the changes to diurnal conditions.

To determine whether proteolysis might be affecting exudate polypeptide composition, some dried exudate was reconstituted to its in vivo concentration of 250 mg dry weight mL^{-1} (9). Aliquots (25 μL each) were incubated for 1 h at 38°C in glass test tubes and polypropylene centrifuge tubes, with and without silanization. No decrease in assayable protein content could be detected in any of the treatments (data not shown). Silver-stained one-dimensional gels showed no discernible changes in composition (Fig. 5). Longer (2 h) incubations of more dilute exudate (125 mg dry weight mL^{-1}) resulted in apparent general "fading" of all protein bands, perhaps due to nonspecific surface denaturation (data not shown).

Kinetics Experiments

The labeling protocol produced a distinct pulse of ^{35}S in the sieve tube contents (Fig. 6). Although the relative pro-

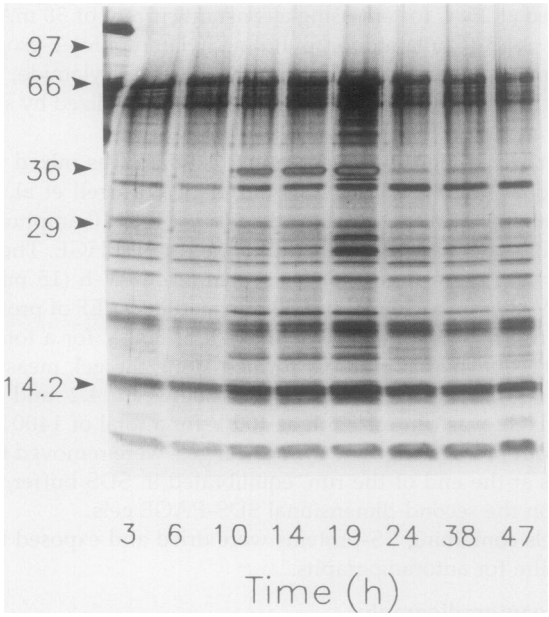

Figure 4. Silver-stained SDS/PAGE profiles of phloem exudate proteins collected at short intervals during 2 d. Each lane was loaded with 5 mg (dry weight) of exudate, except for the 6-h sample, which was inadvertently loaded with 3.5 mg. Arrows mark the positions of molecular mass standards (kD).

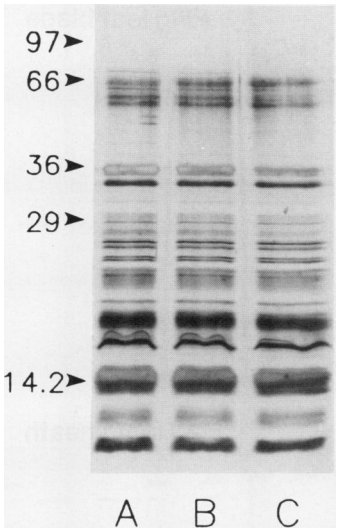

Figure 5. Effect of incubation for 1 h at 38°C on exudate protein composition (silver-stained SDS/PAGE profiles). Each lane was loaded with 25 μL of phloem exudate containing 0.25 mg dry weight μL^{-1}. Lane A, No incubation. Lane B, Incubated in silanized polypropylene centrifuge tubes. Lane C, Incubated in nonsilanized polypropylene centrifuge tubes. Arrows mark the positions of molecular mass standards (kD).

portion of soluble and protein ^{35}S changed somewhat with time, their kinetics were basically similar, with the pulse of ^{35}S-protein trailing soluble ^{35}S by about 0.5 to 1 h. Except initially, [^{35}S]methionine accounted for well less than half of the soluble activity (Fig. 7). Judging from the low R_F (Fig. 7, inset), most of the soluble ^{35}S may have been in the form of small methionine-containing peptides. The relative proportion of methionine and its putative peptide form changed substantially, in an inverse relationship, during movement of ^{35}S past the exuding stylets.

Although the possibility of overlapping bands complicates interpretation, the patterns of ^{35}S incorporation into soluble proteins appeared to fall into three basic categories (Fig. 8).

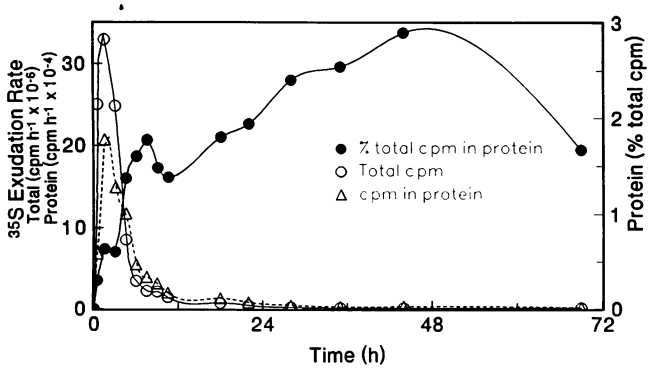

Figure 6. Distribution of ^{35}S between soluble and protein fractions of phloem exudate samples collected from aphid stylets on the peduncle after pulse labeling the flag leaf with [^{35}S]methionine. Time zero is the time of initial tracer appearance, about 1 h after labeling. Same experiment as Figures 7 and 8.

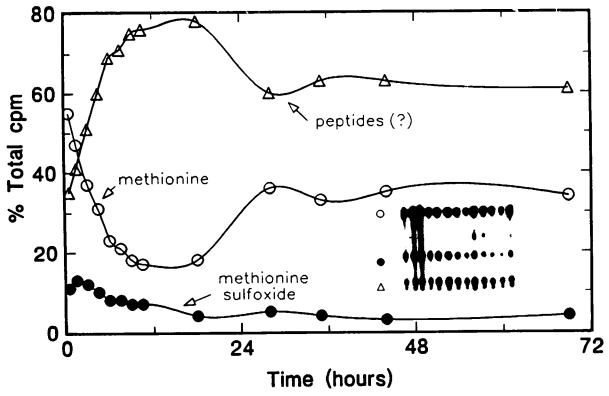

Figure 7. Distribution of ^{35}S among methionine, methionine sulfoxide, and "peptide" in phloem exudate collected from aphid stylets on the peduncle after pulse labeling the flag leaf with [^{35}S]-methionine. Same experiment as Figures 6 and 8. Inset, Autoradiograph of TLC-separated compounds.

Most were labeled promptly and continued to contain label throughout the experiment. (The discontinuity in labeling of the 36-kD band was presumably due to a temporary decrease in the amount of 36-kD protein [Fig. 4].) Several proteins showed little label initially but later increased in their relative proportion of labeling. A third group, exemplified by proteins larger than about 40 kD, remained virtually unlabeled.

When ^{35}S exudate was collected from three points along the phloem pathway from source to sink (flag leaf blade, flag leaf sheath, and peduncle), the time course for labeling was similar in virtually all respects at the three positions. This included the forms of soluble activity ("peptides" and methionine; data not shown) and the percentage of total activity as protein (Fig. 9). Unfortunately, only a few of the many severed stylets on the flag leaf blade continued exuding for more than 1 h, greatly reducing the amount of ^{35}S-protein collected at that site. Nonetheless, the autoradiographs

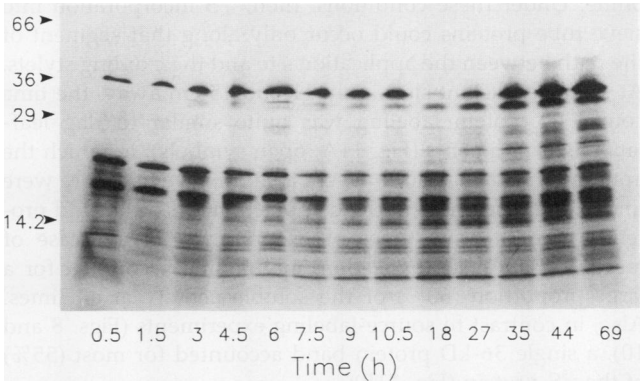

Figure 8. Autoradiograph of SDS/PAGE-separated proteins in phloem exudate collected from aphid stylets on the peduncle after pulse labeling the flag leaf with [^{35}S]methionine. Same experiment as Figures 6 and 7. Equal amounts of protein ^{35}S were loaded on each lane. Arrows mark the positions of molecular mass standards (kD).

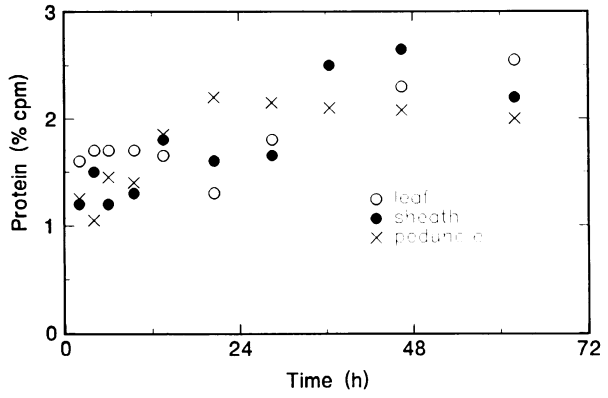

Figure 9. Protein labeling in aphid stylet exudates collected from three different positions on the same plant after pulse labeling the flag leaf with [^{35}S]methionine. Time zero is the time of tracer appearance at each site. Same experiment as Figure 10.

showed a pattern of protein labeling that was clearly similar in most major respects at all three sites (Fig. 10). In particular, this included the irregularities in labeling of the 36-kD protein, which were similar, although not identical, at all three positions (e.g. the 13-h sheath sample showed relatively stronger labeling than the leaf or peduncle samples.) Also, the nearby 34-kD band was clearly more weakly labeled at all times in leaf exudate.

When application of [^{35}S]methionine was restricted to a 2-cm length of the path (peduncle), there was a progressive increase in the extent of protein labeling with time and distance above (i.e. "downstream" from) the application site (Fig. 11A). Surface counts of the peduncle showed that very little ^{35}S had moved below (i.e. "upstream" from) the application site. In one experiment, no activity was detected further than 2 cm below the application site and, in another, the count rate decreased within 3 cm to about 10% of the count rate above the application site and was negligible 7 cm below (data not shown). This confirms the effectiveness of the measures taken to block reverse movement of [^{35}S]methionine. Under these conditions, then, ^{35}S incorporation into sieve tube proteins could occur only along that segment of the path between the application site and the exuding stylets. At the most distant sites, only 11 and 15 cm away, the time course of protein labeling was quite similar to flag leaf-labeling experiments (Fig. 11A, open symbols), in which the application site was 50 to 60 cm away. However, there were important differences in the composition of soluble and protein ^{35}S in the two types of experiments. In the case of localized peduncle application, methionine accounted for a large proportion (60%) of the soluble activity at all times. Also, in contrast to source-labeling experiments (Figs. 8 and 10), a single 36-kD protein band accounted for most (55%) of the ^{35}S-protein (Fig. 11B).

Microautoradiography

The movement of ^{35}S as a distinct pulse with nearly complete washout (Fig. 6) strongly suggested that radioactivity was confined to the phloem (3, 9). This was confirmed by

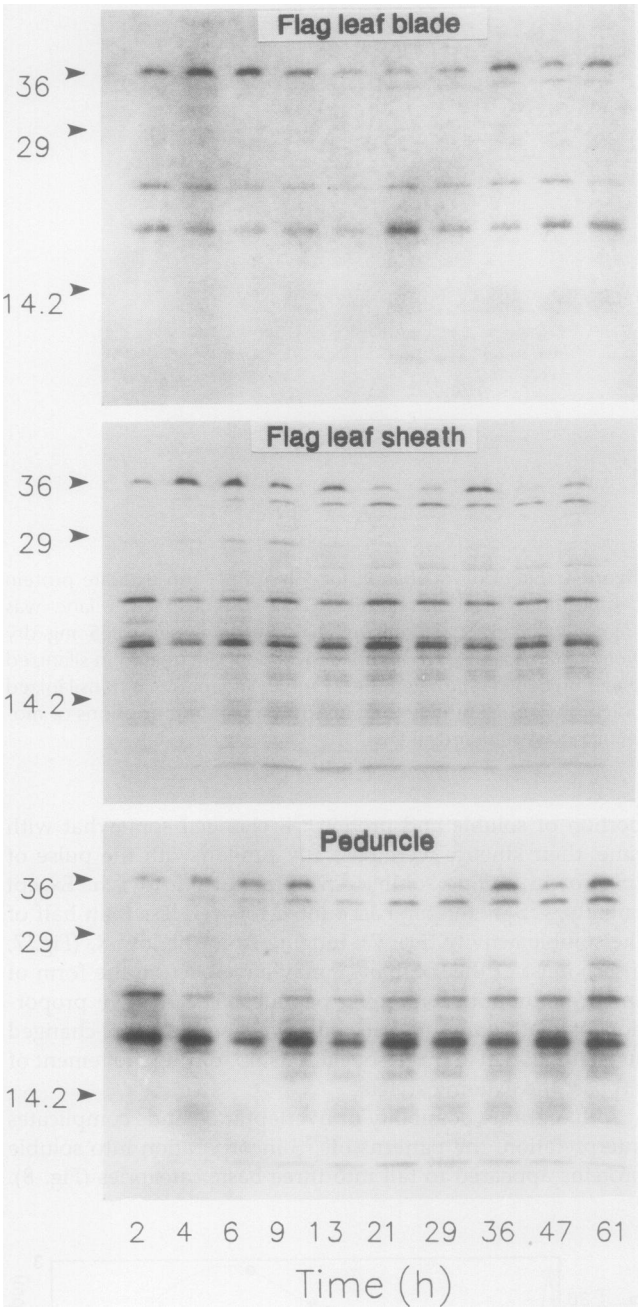

Figure 10. Autoradiographs of SDS/PAGE-separated proteins in aphid stylet exudates after pulse labeling the flag leaf tip with [^{35}S]methionine. Time zero is the time of tracer appearance at that site. In each series, equal amounts of protein ^{35}S were loaded on each lane. Same experiment as Figure 9. Distances of exuding stylets from the [^{35}S]methionine labeling site: Leaf blade, 5 cm; leaf sheath, 25 cm; peduncle, 55 cm. Arrows mark the positions of molecular mass standards (kD).

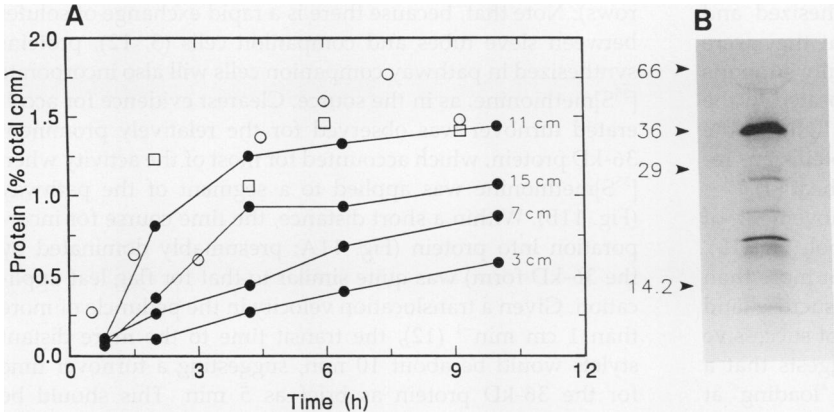

Figure 11. Incorporation of ^{35}S into phloem exudate proteins during spot application of [^{35}S]methionine to a 2-cm length of the pathway (peduncle). A, ^{35}S-Protein labeling at four sites downstream from the application site. Open symbols represent similar data from flag leaf-labeling experiments, approximately 60 cm away from the peduncle stylets. Time zero is the time of [^{35}S]methionine application. (Separate experiment from that shown in Fig. 11B.) B, Autoradiograph of SDS/PAGE-separated proteins in phloem exudate collected for 6 h from stylets 10 cm downstream from the application site. (Separate experiment from that shown in Fig. 11A.) Arrows mark the positions of molecular mass standards (kD).

the microautoradiographs, in which label was detected only in the sieve tubes and companion cells (Fig. 12). (Note that low molecular weight water-soluble compounds would be lost from the tissues by the aqueous fixation procedure used.) For the most part, no attempt was made to identify possible structural associations, because the fixation procedure was aimed at maximizing "surging" and the resultant filtering of sieve element contents on the sieve plates (4). Dense accumulations of silver grains were often associated with the sieve plates. However, labeling also occurred throughout the sieve tubes. Companion cells were labeled more heavily than sieve tubes, especially over their nuclei.

DISCUSSION

As Nakamura et al. (16) have shown for rice, well over 100 soluble proteins are present in the translocation stream of wheat plants. Although their molecular masses are generally low, there was no indication that these proteins might arise from proteolytic activity. This is evident from their stability on incubation of the exudate and from their characteristic composition during widely different collection times and conditions ranging from 30 min to 3 d, with and without inclusion of BSA. Exudates collected from different parts of the plant were likewise similar in composition. The only exception was broken grain pedicels, from which phloem exudate samples could be obtained without the use of aphid stylets. Again, however, the consistency of the protein composition under widely different conditions, including the number of aphids and time after their removal from the plant, makes it unlikely that the difference in pedicel exudate composition can be attributed to the use of aphids. Instead, particularly given the microautoradiographic observations, it seems more likely that the differences in the pedicel exudate sample were related to the sieve tube-plugging process and other responses to surging in severed sieve tubes.

The general labeling of most exudate proteins clearly in-

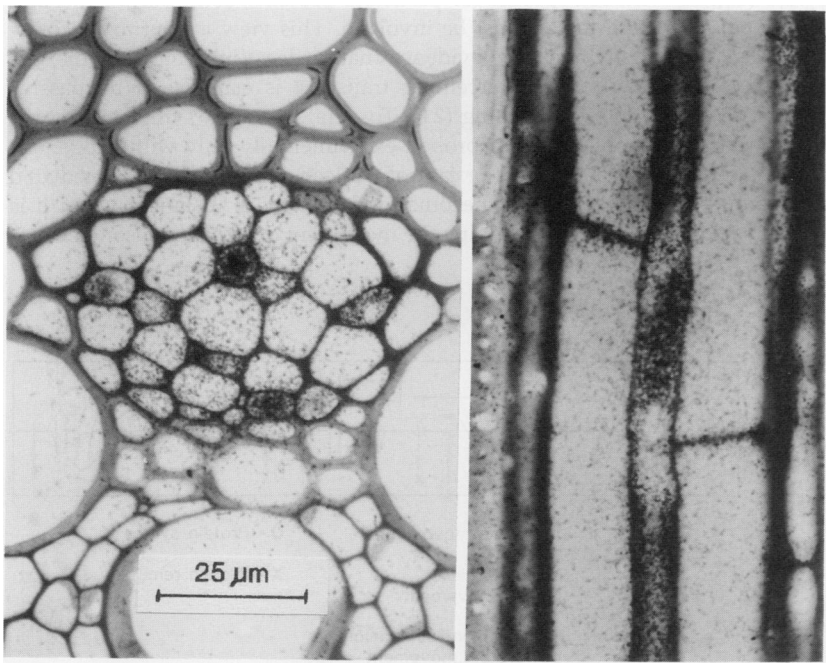

Figure 12. Microautoradiographs of peduncle Phloem 6 h after labeling the flag leaf with [^{35}S]methionine. Sections 1.5 µm thick were stained with the periodic acid-Schiff reaction. Two sieve plates are visible in the longitudinal section (right); in between is a companion cell and nucleus. Same plant as shown in Figure 2. A window of tissue, 1 × 5 mm, was removed from the peduncle on the side opposite to the exuding stylets. Both micrographs are printed at the same magnification.

dicates that they were continually being synthesized and removed from the soluble protein pool, i.e. that they were turning over. Thus, the evidence for turnover fully supports the interpretation that these proteins are translocated in the phloem. The observations appear to shed little light on the turnover of P-proteins, however, because P-proteins are reportedly absent in many monocots, including wheat (4).

Several patterns are conceivable for the movement of proteins into and out of the soluble protein pool (Fig. 13), and the variety of labeling kinetics indicates that more than one is involved. As with small solutes (e.g. sucrose and phosphate [3, 9]), the basic similarity in kinetics at successive points along the pathway (Figs. 9 and 10) suggests that a substantial extent of turnover occurs simply by "loading" at the source and "unloading" at the sink (Fig. 13, solid arrows), with tracer kinetics being driven by events at the source. However, these events appear to differ for different proteins. This is evident, for example, when comparing the 69-h sample shown in Figure 8 with the 0.5-h sample. Exudate in the 69-h sample accumulated during the previous 25 h under what amounted to steady-state labeling conditions (constant exudate [^{35}S]methionine level; Fig. 7A); therefore, the relative labeling of individual proteins should approximate their methionine content. Many proteins (e.g. approximately 10 kD and those just below the prominent 36- and 34-kD bands) showed little incorporation at first but increased in relative importance with time. Such differences may arise from different patterns of protein pooling and processing in source leaf companion cells. In this context, it is worth noting the much larger volume, suggesting larger protein pools, of companion cells in leaf minor veins.

Kinetic interpretations are also clouded by uncertain precursor-product relations. This includes the putative methionine-containing peptide, which appears to predominate over methionine in the translocation stream but has an unknown relationship to protein synthesis. Also, despite the apparent metabolic inertness of isolated exudate, some proteins (e.g. the 36-kD polypeptide) might undergo metabolically dependent conversions to other forms within the sieve tube itself. Thus, turnover of some proteins may not involve their physical movement into or out of the sieve tube.

In addition to the "flow-through" turnover resulting from source-to-sink movement, there is for some proteins an added component of turnover imposed by synthesis and, presumably, removal along the pathway itself (Fig. 13, dotted arrows). Note that, because there is a rapid exchange of solutes between sieve tubes and companion cells (3, 12), proteins synthesized in pathway companion cells will also incorporate [^{35}S]methionine, as in the source. Clearest evidence for accelerated turnover was observed for the relatively prominent 36-kD protein, which accounted for most of the activity when [^{35}S]methionine was applied to a segment of the pathway (Fig. 11B). Within a short distance, the time course for incorporation into protein (Fig. 11A; presumably dominated by the 36-kD form) was quite similar to that for flag leaf application. Given a translocation velocity in the peduncle of more than 1 cm min^{-1} (12), the transit time to the more distant stylets would be about 10 min, suggesting a turnover time for the 36-kD protein as brief as 5 min. This should be regarded as a lower limit, however, because comparison of incorporation based on soluble ^{35}S does not take into account the much greater proportion of free [^{35}S]methionine in peduncle- versus flag leaf-labeling experiments (60 versus about 20%).

In contrast to previous evidence concerning symplastic transport, our observations clearly implicate plasmodesmata as a pathway for the normal symplastic movement of macromolecules. Given the basically constant protein composition and concentration along the path (Table I, Fig. 3A), essentially all proteins must be "loaded" at the source (leaf) and "unloaded" in the sink (grains). In the source and sink, then, protein movement into and out of the sieve tubes appears to be unselective. Along the path, however, sieve tube-companion cell protein movement is evidently selective and not based simply on molecular sieving. There, most intercellular movement appears to involve a single medium-sized (36 kD) protein. Additionally, the concentration of proteins in the sieve tubes is so low that free movement of all molecules 36 kD and smaller would likely be incompatible with the functions of the companion cell ground cytoplasm.

If movement through plasmodesmata along the path is indeed specific for particular proteins, some sort of recognition factor must be involved. This view is contrary to presently available evidence that, at least in healthy plants, the control of intercellular transport is established by passive molecular sieving (21). To date, however, symplastic movement has been probed experimentally only with exogenous tracers. As in the case of nuclear pores (15), signal-mediated transport may become evident only in instances when it is possible to examine the movement of endogenous molecules.

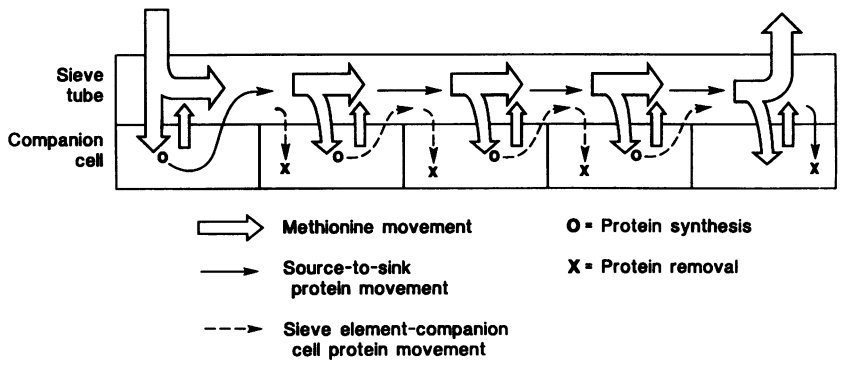

Figure 13. Diagrammatic representation of some processes presumed to contribute to the labeling pattern of soluble sieve tube proteins. (Direct movement of methionine into and out of the sieve tube in the source and sink is illustrated for convenience only.)

For the most part, the results provide little direct evidence concerning the function of soluble sieve tube proteins. Their large number and low concentrations argue against a role in nutrition or chelation. More probably, they are involved in sieve tube plugging, sieve tube maintenance (20), and, perhaps, some form of source-sink interaction. Most important, the observations emphasize the dynamic status of the soluble protein pool in sieve tubes. Clearly, the relationship between sieve tubes and companion cells is more complex than a simple exchange of metabolites.

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