Planta (1993) 191:207–213

Planta
© Springer-Verlag 1993

Specific proteins in the sieve-tube exudate of *Ricinus communis* L. seedlings: separation, characterization and in-vivo labelling

Thomas Sakuth, Christian Schobert, Attila Pecsvaradi*, Arnulf Eichholz, Ewald Komor, Gabriele Orlich

Pflanzenphysiologie, Universität Bayreuth, Postfach 101251, D-95440 Bayreuth, Germany

Received: 4 February 1993 / Accepted: 5 March 1993

Abstract. *Ricinus communis* L. seedlings exuded pure phloem sap from the cut hypocotyl for several hours. Throughout the entire exudation period proteins were present in the phloem exudate at a constant concentration ranging from 0.11 to 0.41 $mg \cdot ml^{-1}$ depending on the culture conditions and the age of the seedlings. Manipulation of the nutrient supply at the cotyledons after removal of the endosperm did not change the protein concentration in the exudate. Comparison of sieve-tube exudate proteins (STEPs) with soluble proteins extracted from the hypocotyl and the cotyledons showed a unique abundance of small proteins in the exudate, with molecular weights ranging from 10 to 25 kDa. Bands at 18, 19 and 20 kDa were especially dominant. The proteins found transiently in the xylem exudate, which might represent proteins secreted at the wound surface, were different in pattern. Two-dimensional separation of STEPs revealed that more than 100 distinct polypeptides occurred in the sieve-tube exudate, most of them slightly acidic with isoelectric points ranging from 4 to 6 and a few basic ones around 8. [^{35}S]Methionine fed to the cotyledons led to labelling of STEPs, demonstrating their rapid synthesis. It is concluded that there is a continuous synthesis and translocation of specific sieve-tube proteins, whose function is unknown.

Key words: Euphorbiaceae – Phloem protein – *Ricinus* – [^{35}S]Methionine labelling – Sieve tube exudate – Xylem exudate

Introduction

In plants, nutrients and chemical signals are transported from source to sink tissues in the sieve tubes. These highly specialized cells, which accumulate sucrose and other nutrients (Geiger 1975), lack nuclei, ribosomes, tonoplast and dictyosomes (Parthasarathy 1975). Nevertheless, there are proteins in the sieve tubes, some bound to membranes and others in the sieve-tube sap.

Electron microscopy has indicated that proteins and protein aggregates termed P-proteins are present in developing and functional sieve tubes (Cronshaw 1975; for review: Cronshaw and Sabnis 1990). In the case of the Cucurbitaceae, a family in which phloem exudate is readily available, careful biochemical examination of P-proteins has been performed (Weber et al. 1974), including extensive characterization of a dominant phloem lectin (e.g. Sabnis and Hart 1978; Read and Northcote 1983; Sham and Northcote 1987; Smith et al. 1987), following an early report on lectin activity of proteins found in the sieve-tube sap of *Robinia pseudoacacia* (Kauss and Ziegler 1974). The phloem sap of members of the Cucurbitaceae is special in the sense that it has an unusually high protein content (10–60 $mg \cdot ml^{-1}$). Analysis of the protein pattern of phloem sap from different species of the Cucurbitaceae has revealed a heterogeneity which indicates a species-specific composition of proteins in the phloem sap (Sloan et al. 1976).

Ricinus communis is one of the few plants besides cucurbits from which sieve-tube sap can be obtained in amounts sufficient for quantitative analysis of transported nutrients and proteins (Hall and Baker 1972; Smith and Milburn 1980). Recently, seedlings of *Ricinus communis* have been used for research on phloem loading of nutrients (Kallarackal et al. 1989; Kallarackal and Komor 1989; Schobert and Komor 1989; Orlich and Komor 1992) because of their ability to absorb nutrients from defined solutions via their cotyledons and to exude sieve-tube sap for several hours from the cut hypocotyl.

This plant system has now been used to study and characterize in detail the protein composition of the

* *Present address*: Department of Plant Physiology, Attila Jozsef University, P.O. Box 654, H-6701 Szeged, Hungary

Abbreviations: IEF = isoelectric focussing; pI = isoelectric point; STEP = sieve-tube exudate protein; SDS-PAGE = sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TCA = trichloroacetic acid

Correspondence to: G. Orlich; FAX: 49(921)552642

phloem exudate and to demonstrate rapid synthesis of these proteins.

Materials and methods

Plant material. Seeds of *Ricinus communis* L. cv. Carmencita were purchased from Jelitto Staudensamen, Hamburg, FRG, germinated and grown either in vermiculite, or in liquid culture as described earlier (Kallarackal et al. 1989).

Phloem and xylem exudation. Five- to six-day-old seedlings were used in the experiments (the exact age of the seedlings is indicated in the figures and tables).

Phloem exudate from seedlings with cotyledons embedded in the endosperm, or from seedlings with cotyledons incubated in buffer (the respective compositions are given in the legends to figures and tables) was obtained after cutting the hypocotyl at the hook (Kallarackal et al. 1989). Exudate from the hypocotyl stump was collected into graded microcapillaries. The cut seedlings were incubated at 27° C in a closed water bath with high air humidity (80%).

Xylem exudate driven by root pressure was collected after cutting the hypocotyl 1 cm above the root or at the hook, and treating the cut stump with 10 µl of 0.1 M $CaCl_2$ to prevent phloem exudation (Schobert and Komor 1990). With the root immersed in 1 mM KNO_3, 0.5 mM $CaCl_2$, 5 mM K-phosphate, pH 6.0, exudate was collected from the cut hypocotyl stump in the same way as described for phloem exudation.

Protein extraction, determination, separation and labelling. Plant tissue was frozen in liquid nitrogen, powdered in a mortar and mixed vigorously with extraction buffer [25 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (Hepes)/bis-tris-propane (BTP), pH 7.4; 3 mM EGTA; 1 mM dithiothreitol (DTT); 1 mM benzamidine; 1 mM 6-aminocaproic acid; 0.2 mM phenylmethylsulfonyl fluoride (PMSF)]. The supernatant was analysed after centrifugation for 5 min in an Eppendorf centrifuge (Eppendorf, Hamburg, FRG). The protein was quantified using a micro protein assay (Bio-Rad, München, FRG) with bovine serum albumin as standard.

Proteins were separated by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) according to the procedure of Schägger and v. Jagow (1987) with 10% and 4% acrylamide in the separating and stacking gels, respectively, in a Bio-Rad minigel system, or in a horizontal Multiphor II setup (Pharmacia, Freiburg, FRG) for two-dimensional separation after native isoelectric focussing (IEF), using Servalyte 4–6 (Serva, Heidelberg, FRG) in 5% acrylamide (Robertson et al. 1987) which was run in a Bio-Rad minigel system.

When necessary, sieve-tube exudate proteins (STEPs) were concentrated by centrifugation (Centricon 3; Amicon, Beverly, Mass., USA) at 5000 · *g* overnight.

Radioactivity incorporated into proteins was determined by scintillation counting after trichloroacetic acid (TCA) precipitation (Mans and Novelli 1961) on filter papers (Schleicher and Schüll, Dassel, FRG). After separation by SDS-PAGE, labelled proteins were electroblotted to Immobilon polyvinylidenedifluoride membrane (Millipore, Bedford, Mass., USA) using an LKB (Gräfelfing, FRG) Novablot apparatus, following the protocol of the manufacturer. After drying, the blots were exposed to Kodak X-Omat AR (Eastman Kodak, Rochester, N.Y., USA) film.

[^{35}S]L-Methionine was purchased from NEN-Dupont, Bad Homburg, FRG, or from Hartmann Analytical, Braunschweig, FRG.

Table 1. Protein concentration in the sieve-tube exudate of *Ricinus* seedlings in relation to the culture conditions and the developmental state. The cotyledons of the seedlings were embedded in the endosperm. The exudation time was 1 h when grown in vermiculite culture and 2 h when grown in hydroponic culture. The first 5-µl sample to be exuded after cutting was discarded. Data are means ± SD; number of seedlings in parentheses

	Fresh weight of cotyledons (mg)	Exudation rate (µl · h^{-1})	Protein concentration (µg · ml^{-1})	Protein exuded (µg · h^{-1})
Grown in vermiculite				
for 5 d (5)	97 ± 12	6.9 ± 3.4	312 ± 37	2.2 ± 0.9
for 6 d (3)	202 ± 13	7.3 ± 4	410 ± 153	2.7 ± 1.4
Grown in liquid culture				
for 5 d (22)	79 ± 11	21 ± 1	112 ± 9	2.4 ± 0.3
for 6 d (15)	127 ± 29	18 ± 5	132 ± 37	2.4 ± 0.8

Table 2. The influence of nutrient supply on the protein concentration in the phloem sap of *Ricinus* seedlings. Phloem exudate was collected from 6-d-old seedlings which were preincubated for 2 h either in buffer alone (5 mM 2-(N-morpholino) ethanesulfonic acid-KOH, pH 5.5), or in buffer including 100 mM sucrose, or in buffer including 85 mM sucrose, 5 mM K-phosphate and 10 mM amino acids [amino-acid composition analogous to that found in the apoplast between cotyledon and endosperm according Schobert and Komor (1989; Table 2)]. The first 5-µl sample exuded after cutting was discarded. Data are means ± SD; number of seedlings in parentheses

Incubation condition	Exudation rate (µl · h^{-1})	Protein concentration (µg · ml^{-1})	Protein exuded (µg · h^{-1})
in buffer (6)	8 ± 2	100 ± 33	0.8 ± 0.1
+ sucrose (7)	10 ± 4	122 ± 21	1.0 ± 0.3
+ sucrose, amino acids and phosphate (5)	5 ± 2	89 ± 19	0.44 ± 0.15

Results

Proteins are continuously present in pure sieve-tube exudate. Sieve-tube exudate obtained in pure form from 5- to 6-d-old *Ricinus* seedlings (Kallarackal et al. 1989) contains low-molecular-weight substances, especially sucrose (300–400 mM), amino acids (100–200 mM), potassium ions (30 mM), some other ions (below 10 mM, Komor et al. 1989), glycolysis metabolites (Geigenberger et al. 1993) and plant hormones (data not shown). In addition, sieve-tube sap contains protein (Kallarackal et al. 1989), in contrast to the root-pressure exudate of *Ricinus* seedlings (Schobert and Komor 1990).

The protein concentration in the sieve-tube sap was dependent on the culture conditions and the developmental state of the seedlings (Table 1). The exudate from seedlings grown in vermiculite had the highest protein concentrations, namely 312 µg · ml^{-1} at day 5 and up to 410 µg · ml^{-1} at day 6 after germination. The amount of exuded protein did not depend on the weight of the cotyledons. In the sieve-tube sap of seedlings grown in liquid culture the protein concentration was 110–

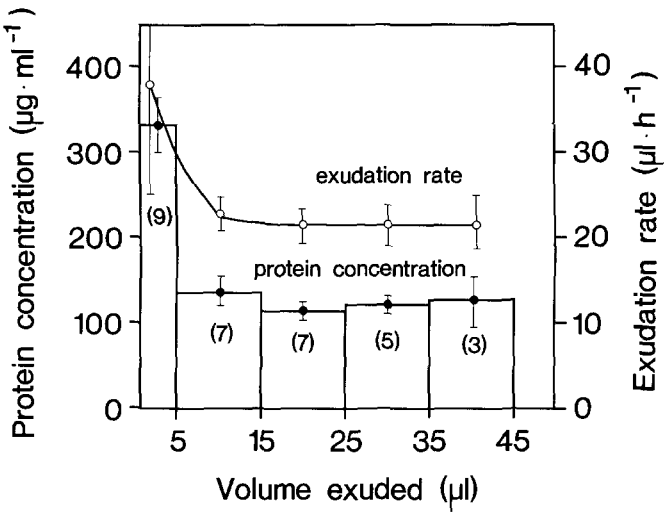

Fig. 1. The protein concentration in the sieve-tube exudate of *Ricinus* seedlings and the exudation rate during 2 h of exudation. The phloem exudate was collected in graded microcapillaries from individual 6-d-old seedlings. Protein concentration and exudation rate were determined in the first 5-µl sample after cutting and in the subsequent 10-µl samples from the individual seedlings. Data are means ± SD; number of seedlings in parentheses

130 µg · ml^{-1}, but the exudation rate was more than twice that of seedlings grown in vermiculite, so that the amount of protein exuded by the seedling per hour was the same. Because seedlings grown in vermiculite stopped exudation after 1 h, seedlings grown in liquid culture were used for further analysis.

Removal of the endosperm and incubation of the cotyledons in buffer lowered the amino-acid concentration in the sieve-tube exudate from 160 mM to 16 mM (Schobert and Komor 1989). In contrast, the protein concentration in the exudate did not change very much when the endosperm was removed (Table 2; compare with Table 1). Feeding of sucrose either alone or in combination with amino acids and phosphate did not significantly affect the protein concentration in the sieve-tube sap, whereas the exudation rates were reduced and, consequently, the amount of protein exuded was also less (Table 2). This was especially true for the incubation with phosphate.

When the protein concentration was determined in subsequent samples of phloem sap collected from individual seedlings over an extended exudation period, the protein concentration stayed constant for the entire exudation time (Fig. 1). In the first 5 µl of exudate, however, a three fold-higher protein concentration was usually found due to contamination by protein from the cut parenchyma cells of the hypocotyl.

Proteins in sieve-tube exudate show a unique pattern. Separation by SDS-PAGE showed that the protein pattern of sieve-tube exudate was different from that of tissue extracts obtained either from the hypocotyl hook or from the cotyledons (Fig. 2). When proteins present in the sieve-tube exudate (lane 4) were compared with identical amounts of protein extracted from the hypocotyl (lane 5) or the cotyledons (lane 3), it was found that many proteins from tissue extracts had molecular weights higher than 30 kDa, whereas smaller polypeptides dominated in the sieve-tube exudate. (In this respect it is important to note, that no protease activity could be detected in the exudate.) Three major bands at 18, 19 and 20 kDa were especially prominent. Whether these proteins were really exclusive to the exudate could not be decided, because when high amounts of proteins from hypocotyl or cotyledons were analysed on SDS-PAGE (lane 2, 6) some bands with similar molecular weights showed up.

When the sieve-tube exudate was obtained at different sites along the hypocotyl axis, no substantial difference in the protein pattern was observed (Fig. 3).

The phloem proteins exuded by members of the Cucurbitaceae gel when exposed to air and can be kept

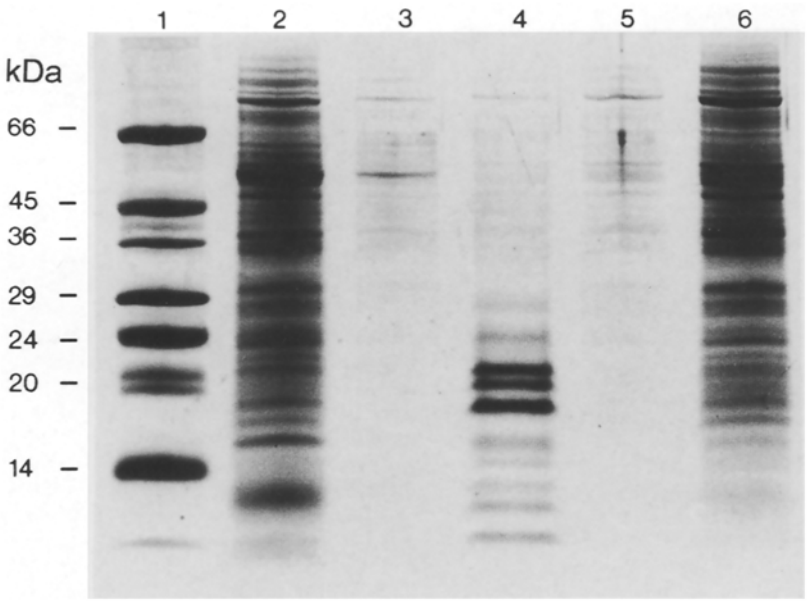

Fig. 2. Comparison of proteins present in the cotyledons, the hypocotyl and the sieve-tube exudate of *Ricinus communis* seedlings. Phloem exudate and extracts from hypocotyls and cotyledons were obtained from 5-d-old seedlings. Proteins were separated by SDS-PAGE. *Lane 1*, molecular-weight marker (Dalton Mark VII-L, Sigma Deisenhofen, FRG); *lane 2*, cotyledon extract (30 µg protein); *lane 3*, cotyledon extract (3 µg protein); *lane 4*, sieve-tube exudate proteins (3 µg protein); *lane 5*, hypocotyl extract (3 µg protein), *lane 6*, hypocotyl extract (30 µg protein)

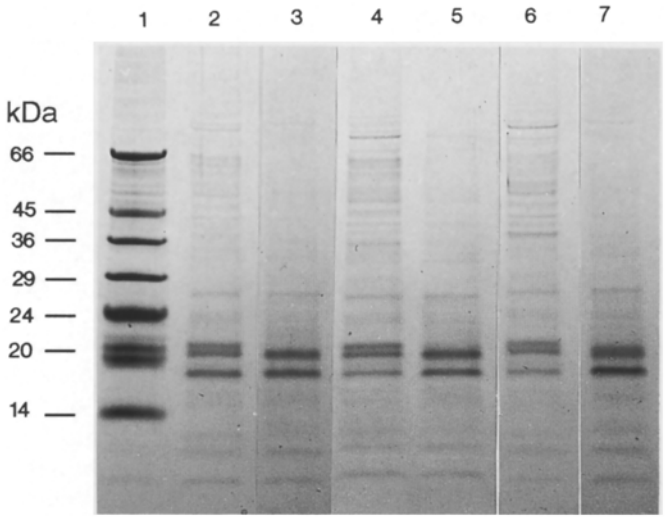

Fig. 3. Comparison of STEPs collected at various positions along the hypocotyl axis of *Ricinus* seedlings, and their sensitivity to sulfhydryl reduction. Phloem-exudate samples (10 µl) were harvested from 6-d-old seedlings and separated by SDS-PAGE, either with mercaptoethanol present in the sample buffer *(lane 2, 4, 6)* or without mercaptoethanol *(lane 3, 5, 7)*; phloem exudate was harvested after cutting the hypocotyl either 0.5 cm away from the cotyledons *(lanes 2, 3)*, or at the hypocotyl hook *(lanes 4, 5)*, or 1 cm above the root *(lanes 6, 7)*. *Lane 1*, molecular-weight markers

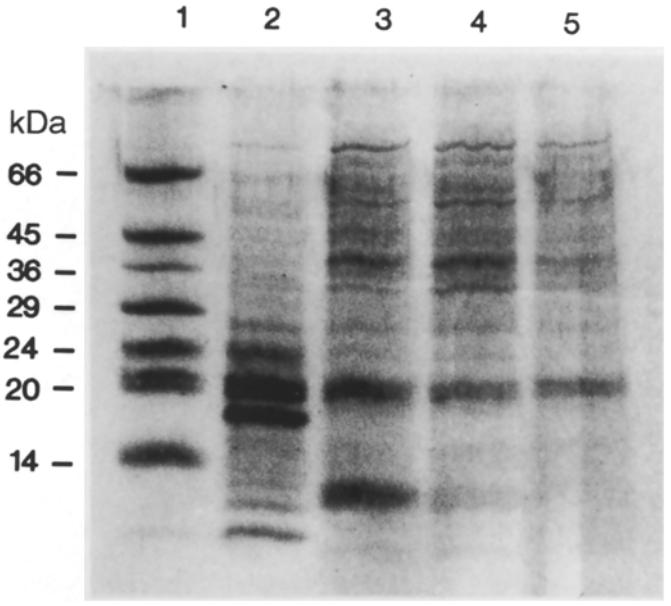

Fig. 4. Comparison of STEPs and proteins present in xylem exudate. Samples of xylem and phloem exudate were obtained from 5-d-old *Ricinus* seedlings. Proteins were separated by SDS-PAGE. *Lane 1*, molecular-weight marker (Dalton Mark VII-L, Sigma); *lane 2*, sieve-tube exudate, collected at the hypocotyl hook, (3 µg protein); *lane 3*, xylem exudate, collected at the hypocotyl hook, first sample of 18 µl (3 µg protein); *lane 4*, xylem exudate, collected at the hypocotyl 1 cm above the root, first sample of 15 µl (3 µg protein); *lane 5*, xylem exudate (25 µl) collected after the sample shown in lane 4 (1.8 µg protein)

solubilized by sulfhydryl reagents (Walker and Thaine 1971). In contrast, STEPs from *Ricinus* seedlings could be kept in solution without sulfhydryl reagents. The SDS-PAGE analysis of STEPs in the presence or absence of mercaptoethanol revealed no disulfide-dependent polymerization (Fig. 3), in contrast to phloem proteins from *Cucurbita* maxima (Sloan et al. 1976). In the absence of mercaptoethanol, however, the band at 20 kDa merged with the band at 19 kDa, which may indicate the presence of an intramolecular disulfide bridge.

To test whether some of the proteins in the sieve-tube exudate were extracellular polypeptides secreted by parenchyma cells of the cut hypocotyl surface, root-pressure exudate was analysed from the opposite surface of the cut hypocotyl. In these xylem exudate samples (Schobert and Komor 1990) protein was detectable only in the first 5–20 µl, at a concentration around 250 µg · ml^{-1} when harvested next to the root, and up to 450 µg · ml^{-1} when harvested at the hypocotyl hook. Thereafter, the protein concentration in the xylem exudate decreased to less than 20 µg · ml^{-1}. Analysis by

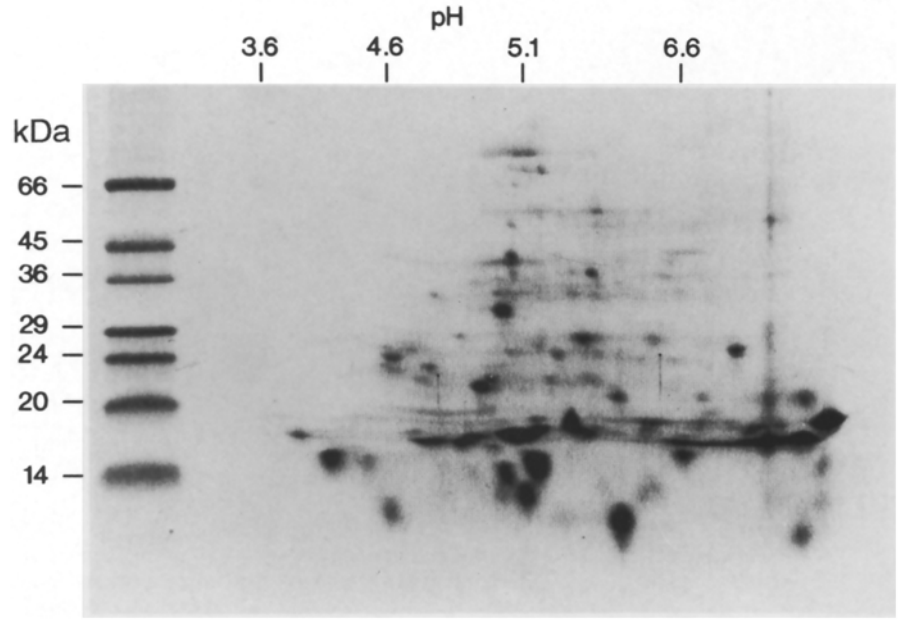

Fig. 5. Two-dimensional separation of STEPs. Sieve-tube exudate (70 µg protein) obtained from 5-d-old *Ricinus* seedlings was separated in the first dimension by native IEF in parallel with pI marker proteins (pI 3.6: amyloglucosidase; pI 4.6: trypsin inhibitor; pI 5.1: β-lactoglobulin A; pI 6.6: carbonic anhydrase I). Note that there is a steep pH gradient above pH 6.6. The lane containing sieve-tube protein was cut out, equilibrated in SDS-sample buffer and subsequently run on horizontal SDS-polyacrylamide gels

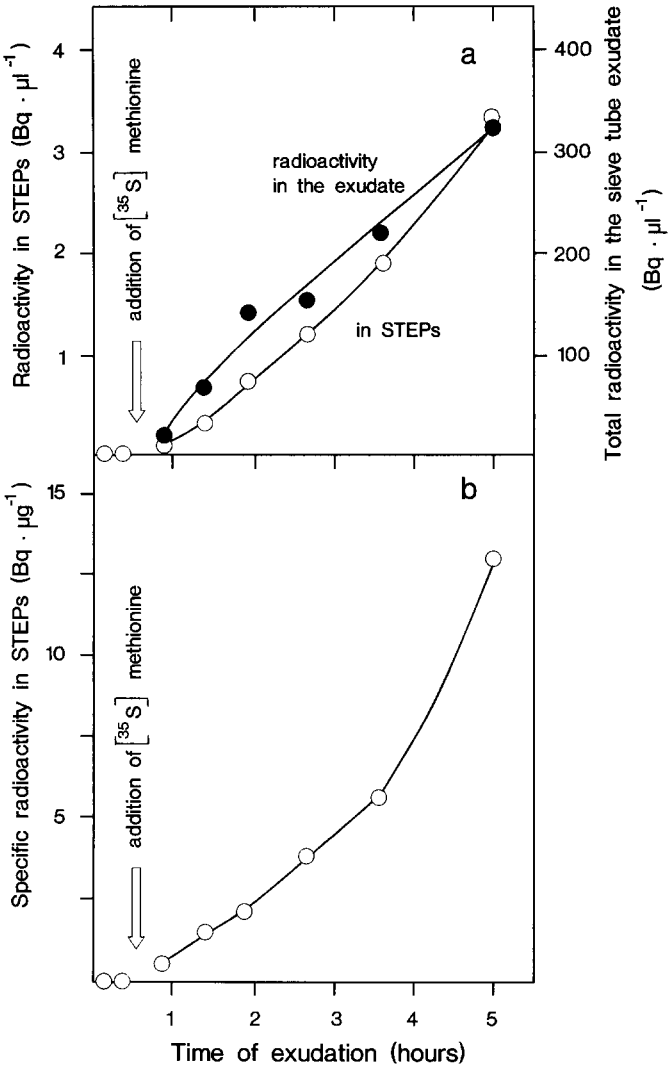

Fig. 6a, b. Time course of protein labelling in the sieve-tube exudate. The cotyledons of seven intact 5-d-old *Ricinus* seedlings were preincubated for 1 h in 50 ml 10 mM sucrose, 1 mM L-glutamine and 5 mM K-phosphate. The hypocotyl was cut and exudate was collected for 30 min. Afterwards, [^{35}S]methionine was added to the medium (37 Bq · µl^{-1}, 37 kBq · pmol^{-1}), and exudate was collected for another 270 min. The [^{35}S]methionine which was withdrawn from the medium by the cotyledons was replaced every hour by addition of 8 Bq · µl^{-1}

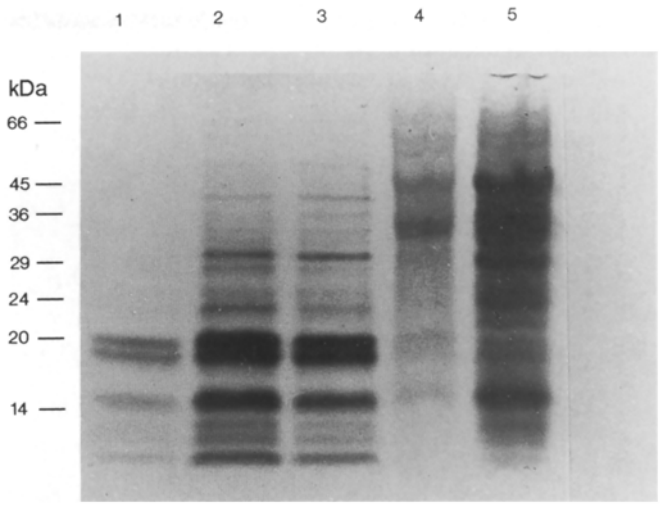

Fig. 7. Autoradiogram of SDS-PAGE-separated ^{35}S-labelled proteins from the sieve-tube exudate, the hypocotyl and the cotyledons of *Ricinus* seedlings. Proteins were labelled by preincubating cotyledons of intact 5-d-old seedlings for 2 h in [^{35}S]methionine (10 nM, 44 MBq · nmol^{-1}). The hypocotyl was then cut, the first 2 µl of exudate discarded and the exudate collected for 4 h. At the end of the experiment, cotyledons and hypocotyl were extracted. The proteins were separated by SDS-PAGE, blotted to Immobilon membrane and exposed to X-ray film. *Lanes 1–3,* STEPs (3 µg per lane) collected within the first 30 min after cutting the hypocotyl *(lane 1)*, collected within the next 50 min *(lane 2)* and the following hour *(lane 3)*; *lane 4,* 60 µg protein extracted from the hypocotyl; *lane 5,* 60 µg protein extracted from the cotyledons

SDS-PAGE revealed that xylem exudate samples showed a very different protein pattern (Fig. 4, lanes 3–5) compared with that in the sieve-tube exudate (Fig. 4, lane 2). Because of the specific pattern of the proteins in the sieve-tube exudate and because of the constant exudation of these proteins over more than 8 h, it was concluded that the exuded proteins are components of the sieve-tube sap in vivo.

Further analysis of STEPs was made by 2-dimensional electrophoresis (IEF followed by SDS-PAGE). In total, up to 115 polypeptides could be detected on the gels (Fig. 5). Especially abundant in number and quantity were weakly acidic proteins with an isoelectric point (pI) around 5. Only small amounts of protein with a pI more basic than 8 were noticed (data not shown). The three major bands resolved by SDS-PAGE alone obviously consist of more than 20 polypeptides.

Labelling of proteins in the sieve-tube sap by [^{35}S]methionine. To examine whether the continuous flow of proteins in the sieve-tube sap results from the breakdown of a large pool of proteins during the exudation or whether the proteins are synthesized continuously, [^{35}S]methionine was supplied to the cotyledons of *Ricinus* seedlings and the radioactivity in the sieve-tube sap was analysed. A time course of such an experiment demonstrated that both soluble and TCA-insoluble radioactivity showed up in the first exudate sample and increased throughout the entire exudation period, indicating that [^{35}S]methionine was rapidly incorporated into the protein fraction (Fig. 6a). The specific radioactivity of the proteins did not reach a steady-state level during the labelling period of 4.5 h (Fig. 6b). Within the first 2 h of exudation about 0.5% of the total radioactivity in the sieve-tube sap was measured as TCA-insoluble material; thereafter, the proportion increased to 1%. In the cotyledons, the percentage of [^{35}S]methionine incorporated into the protein fraction amounted to about 40% of the total radioactivity (data not shown) which reflects the higher ratio of protein to amino acid within the cotyledons.

The autoradiogram of ^{35}S-labelled proteins of the sieve-tube sap separated by SDS-PAGE revealed that protein bands of 16, 19 and 20 kDa were the first to become labelled within 2.5 h (Fig. 7, lane 1); the labelling pattern of exudate samples taken thereafter (Fig. 7, lanes

2 and 3) very much resembled the protein pattern after Coomassie-blue staining (compare with Figs. 2 and 3). However, the prominent protein band at 18 kDa was not labelled by [^{35}S]methionine. This may indicate either that these proteins do not contain methionine or that they are synthesized at a much lower rate.

Proteins from the hypocotyl stump (Fig. 7, lane 4) and the cotyledons (lane 5) displayed a very different labelling pattern. It should be noted that these proteins were loaded onto the gel in amounts 20 times higher than STEPs to compensate for the 7–20 times lower specific radioactivity in proteins from the cotyledons and the hypocotyl, respectively.

Incorporation of label into STEPs was also achieved by incubating the cotyledons in [^{14}C]glutamine and [^{14}C]arginine (data not shown). Since these radioactive compounds were, however, not available at the high specific radioactivity of [^{35}S]methionine, the incorporation of label into the STEPs was consequently lower.

In summary, the rapid labelling of STEPs shows that the proteins translocated in the sieve-tube sap do not derive from the breakdown or mobilisation of stored proteins in the cotyledons but are the result of a rapid and continuous protein synthesis.

Discussion

The proteins in the phloem exudate of *Ricinus* seedlings are definitely sieve-tube proteins, characteristic of the sieve-tube exudate and not of the xylem exudate. At least some of the low-molecular-weight proteins seem to be exclusive to the sieve tubes, as judged by comparing the protein pattern of the exudate with those of the cotyledons and the hypocotyl. The proteins are produced continuously and are carried along by the mass flow in the sieve tubes. The concentration of STEPs of *Ricinus* seedlings (0.1–0.2 mg · ml^{-1}) is similar to that found in the phloem exudate of *Oryza* (0.15–0.2 mg · ml^{-1}; M. Chino, Department of Agricultural Chemistry, University of Tokyo, Tokyo, Japan, personal communication), and *Tilia* (0.3 mg · ml^{-1}; Kennecke et al. 1971), but very different from that of *Cucurbita* (10 mg · ml^{-1}; Kollmann et al. 1970).

Rapid labelling of STEPs in *Ricinus* seedlings rules out that they are derived from stored proteins in protein bodies. Their origin as breakdown products arising from the differentiation of sieve tubes in the cotyledons seems to be excluded by the following calculation. The cotyledons of 5- to 7 d-old seedlings grow at a rate of 2 mg FW · h^{-1} · seedling^{-1}; since sieve tubes comprise 1.2% of the cotyledon's volume (Köhler et al. 1991) they differentiate at a rate of 25 µg FW · h^{-1} · seedling^{-1}. The protein content of cotyledons is 20 mg · g^{-1} FW; 25 µg FW therefore corresponds to 0.5 µg protein. This is the maximal amount of protein that can be liberated per hour and seedling from differentiation of cotyledonary cells into sieve tubes, a number which is tenfold lower than the actual protein flow in the sieve tube sap (5 µg protein · h^{-1} · seedling^{-1}).

An efficient wound-response mechanism exists in sieve tubes of most plant species, so that severed sieve tubes are plugged immediately. So-called P-proteins (phloem proteins), which have been identified by electron microscopy in many plants, were proposed to be involved in sieve-tube sealing, because they readily form filaments under aerobic conditions (Kleinig et al. 1975). In the phloem sap of *Ricinus* seedlings less than 5% of STEPs form high-molecular-weight aggregates under non-reducing conditions (data not shown), so that most of the STEPs are not directly involved – if at all – in sieve-tube sealing. Although there are several enzymic functions that have to be fulfilled in the sieve tubes, e.g. the breakdown of callose (Aloni et al. 1991) and sieve-tube maintenance (Raven 1991), no correlation between any of the protein bands of the phloem sap and an enzymatic activity has so far been found. Enzymatic activities were demonstrated in phloem exudates a long time ago (Eschrich and Heyser 1975), though contamination by cut parenchyma cells could not be fully excluded at that time (Ziegler 1975). Geigenberger et al. (1993) measured the activity of enzymes of glycolysis and sucrose breakdown in the sieve-tube exudate of *Ricinus* seedlings. Since the rates were below 1% of those from the hypocotyl extract, they concluded that these enzymes are not translocated in vivo but may be either tightly bound to cellular structures in the sieve tubes or located in the companion cells.

At present, it can only be speculated about the site of synthesis of those proteins which are moving with the mass flow in mature sieve elements. Earlier results are consistent with the view that synthesis of sieve tube proteins proceeds in the companion cells: Gietl and Ziegler (1979) did not detect mRNA in the phloem exudate of different plant species. Labelling studies with *Vicia faba* had already shown that newly synthesized proteins were present in the sieve tubes without concomitant RNA synthesis (Neumann and Wollgiehn 1964). Microautoradiographic studies on the synthesis of P-proteins in *Cucurbita maxima* demonstrated preferential labelling of proteins in the companion cells (Nuske and Eschrich 1976). If the proteins present within the sieve tubes are synthesized in the companion cells, a mechanism must exist for the transfer of proteins into the sieve tubes. A potential path for the transfer is via the plasmodesmata connecting the two cell types. Given the size exclusion limit of plasmodesmata of about 1 kDa (Wolf et al. 1991), transient structural modifications of the proteins and-or the plasmodesmata must be considered to accomplish selective protein transfer.

Because of the relative ease in obtaining sufficient amounts of sieve-tube sap, the STEPs from *Ricinus communis* may allow isolation of individual sieve-tube proteins and cloning of the corresponding genes to gain further knowledge of the synthesis and function of the up-to-now enigmatic sieve-tube proteins.

During revision of this manuscript Fisher et al. reported on the soluble proteins in the aphid stylet exudate of wheat (Fisher et al. 1992), which are strikingly similar to STEPs from *Ricinus* seedlings with respect to concentration, size and synthesis. Furthermore, Bostwick et al.

(1992) showed that the genes for a phloem lectin of pumpkin are specifically expressed in companion cells.

We wish to thank Pia Großmann and Libuše Badewitz for technical help.

References

Bostwick, D.E., Dannenhoffer, J.M., Skaggs, M.I., Lister, R.M., Larkins, B.A., Thompson, G.A. (1992) Pumpkin phloem lectin genes are specifically expressed in companion cells. Plant Cell **4,** 1539–1548

Aloni, R., Raviv, A., Peterson, C. (1991) The role of auxin in the removal of dormancy callose and resumption of phloem activity in *Vitis vinifera.* Can. J. Bot. **69,** 1825–1832

Cronshaw, J. (1975) P-proteins. In: Phloem transport, pp. 79–115, Aronoff, S., Dainty, J., Gorham, P.R., Swanson, C.A., eds. Plenum, New York London

Cronshaw, J., Sabnis, D.D. (1990) Phloem proteins. In: Sieve elements, pp. 257–284, Behnke, H.-D., Sjolund R.D., eds. Springer, Berlin Heidelberg New York

Eschrich, W., Heyser, W. (1975) Biochemistry of phloem constituents. In: Encyclopedia of plant physiology, N.S., vol. 1: Transport in plants I. Phloem transport, pp. 101–136, Zimmermann, M.H., Milburn, J.D., eds. Springer, Berlin Heidelberg New York

Fisher, D.B., Wu, Y., Ku, M.S.B. (1992) Turnover of soluble proteins in the wheat sieve tube. Plant Physiol. **100,** 1433–1441

Geigenberger, P., Langenberger, S., Wilke, J., Heineke, D., Heldt, H.W., Stitt, M. (1993) Sucrose is metabolized by sucrose synthase and glycolysis within the phloem complex of *Ricinus communis* L. seedlings. Planta **190,** 446–453

Geiger, D.R. (1975) Phloem loading. In: Encyclopedia of plant physiology, N.S., vol. 1: Transport in plants I. Phloem transport, pp. 395–431, Zimmermann, M.H., Milburn, J.D., eds. Springer, Berlin Heidelberg New York

Gietl, C., Ziegler, H. (1979) mRNA in phloem exudates? Naturwissenschaften **66,** 523–524

Hall, S.M., Baker, D.A. (1972) The chemical composition of *Ricinus* phloem exudate. Planta **106,** 131–140

Kallarackal, J., Orlich, G., Schobert, C., Komor, E. (1989) Sucrose transport into the phloem of *Ricinus* seedlings as measured by sieve-tube sap analysis. Planta **177,** 327–335

Kallarackal, J., Komor, E. (1989) The transport of hexoses by the phloem of *Ricinus* seedlings. Planta **177,** 336–341

Kauss, H., Ziegler, H. (1974) Carbohydrate-binding proteins from the sieve tube sap of *Robinia pseudoacacia.* Planta **121,** 197–200

Kennecke, M., Ziegler, H., De Fekete, M.A.R. (1971) Enzymaktivitäten im Siebröhrensaft von *Robinia pseudoacacia* L. und anderer Baumarten. Planta **98,** 330–356

Kleinig, H., Thönes, J., Dörr, I., Kollmann, R. (1975) Filament formation in vitro of a sieve tube protein from *Cucurbita maxima* and *Cucurbita pepo.* Planta **127,** 163–170

Köhler, J., Fritz, E., Orlich, G., Komor, E. (1991) Microautoradiographic studies of the role of mesophyll and bundle tissues of the *Ricinus* cotyledon in sucrose uptake. Planta **183,** 251–257

Kollmann, R., Dörr, I., Kleinig, H. (1970) Protein filaments – structural components of the phloem exudate. I. Observations with *Cucurbita* and *Nicotiana.* Planta **95,** 86–94

Komor, E., Kallarackal, J., Schobert, C., Orlich, G. (1989) Comparison of solute transport in the phloem of the *Ricinus communis* seedling and the adult plant. Plant Physiol. Biochem. **27,** 545–550

Mans, R.J., Novelli, G.D. (1961) Measurement of the incorporation of radioactive amino acids into protein by a filter-paper disk method. Arch. Biochem. Biophys. **94,** 48–53

Neumann, S., Wollgiehn, R. (1964) Über die Beziehungen der RNS- und Proteinsynthese zum Zellkern in Siebzellen von *Vicia faba.* Z. Naturforsch. **19b,** 1066–1071

Nuske, J., Eschrich, W. (1976) Synthesis of P-protein in mature phloem of *Cucurbita maxima.* Planta **132,** 109–118

Orlich, G., Komor, E. (1992) Phloem loading in *Ricinus* cotyledons: sucrose pathways via the mesophyll and the apoplasm. Planta **187,** 460–474

Parthasarathy, M.V. (1975) Sieve element structure. In: Encyclopedia of plant physiology, N.S., vol. 1: Transport in plants I. Phloem transport, pp. 3–56, Zimmermann, M.H., Milburn, J.D., eds. Springer, Berlin Heidelberg New York

Raven, J.A. (1991) Long-term functioning of enucleate sieve elements: possible mechanisms of damage avoidance and damage repair. Plant Cell Environ. **14,** 139–146

Read, S.M., Northcote, D.H. (1983) Chemical and immunological similarities between the phloem proteins of three genera of the Cucurbitaceae. Planta **158,** 119–127

Robertson, E.F., Dannelly, H.K., Malloy, P.J., Reeves, H.C. (1987) Rapid isoelectric focussing in a vertical polyacrylamide minigel system. Anal. Biochem. **167,** 290–294

Sabnis, D.D., Hart, J.W. (1978) The isolation and some properties of a lectin (haemagglutinin) from *Cucurbita* phloem exudate. Planta **142,** 97–101

Schägger, H., v. Jagow, G. (1987) Tricine sodium dodecyl sulfate polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal. Biochem. **166,** 368–377

Schobert, C., Komor, E. (1989) The differential transport of amino acids into the phloem of *Ricinus* seedlings as revealed by sieve-tube sap analysis. Planta **177,** 342–349

Schobert, C., Komor, E. (1990) Transfer of amino acids and nitrate from the roots into the xylem of *Ricinus communis* seedlings. Planta **181,** 85–90

Sham, M.H., Northcote, D.H. (1987) Transcription and translation of phloem protein (PP2) during phloem differentiation in *Cucurbita maxima.* Planta **170,** 392–399

Sloan, R.T., Sabnis, D.D., Hart, J.W. (1976) The heterogeneity of phloem exudate proteins from different plants: A comparative survey of ten plants using polyacrylamide gel electrophoresis. Planta **132,** 97–102

Smith, J.A.C., Milburn, J.A. (1980) Osmoregulation and the control of phloem sap composition in *Ricinus communis* L. Planta **148,** 28–34

Smith, L.M., Sabnis, D.D., Johnson R.P.C. (1987) Immunocytochemical localisation of phloem lectin from *Cucurbita maxima* using peroxidase and colloidal gold labels. Planta **170,** 461–470

Walker, T.S., Thaine, R. (1971) Proteins and fine structural components in exudate from sieve tubes in *Cucurbita pepo* stems. Ann. Bot. **35,** 773–790

Weber, C., Franke, W.W., Kartenbeck, J. (1974) Structure and biochemistry of phloem-proteins isolated from *Cucurbita maxima.* Exp. Cell Res. **87,** 79–106

Wolf, S., Deom, C.M., Beachy, R., Lucas, W.J. (1989) Movement protein of tobacco mosaic virus modifies plasmodesmatal size exclusion limit. Science **246,** 377–379

Ziegler, H. (1975) Nature of transported substances. In: Encyclopedia of plant physiology, N.S., vol. 1: Transport in plants I. Phloem transport, pp. 59–100, Zimmermann, M.H., Milburn, J.D., eds. Springer, Berlin Heidelberg New York