Planta (Berl.) 132, 109–118 (1976)

Planta

Synthesis of P-protein in Mature Phloem of *Cucurbita maxima**

Joachim Nuske and Walter Eschrich

Forstbotanisches Institut, Universität Göttingen, Büsgenweg 2, D-3400 Göttingen-Weende, Federal Republic of Germany

Summary. Cotyledons of *Cucurbita maxima* Duch. seedlings were provided with ^{14}C-labeled amino acids for 12 h. Besides the bulk of labeled amino acids the sieve-tube exudate also carried labeled proteins. 80% of the incorporated radioactivity was found in the P-protein, 20% in a neutral protein, and traces were found in acidic proteins after fractionation on diethyl-aminoethyl cellulose columns. The radioactive elutes were characterized by autoradiographs of both disc- and sodium dodecyl sulfate-gelelectropherograms, and by isoelectric focusing. The P-protein fraction appeared with the void volume from the diethylaminoethyl-cellulose column. Obviously, this is the protein that gels when oxidized and that is reversibly precipitable giving rise to filaments when processed for electron microscopy. Its main component has a molecular weight of 115,000 Dalton. By isoelectric focusing this fraction separated into 3 proteins with isoelectric points of 9.8, 9.4, and 9.2. The isoelectric point 9.2-protein probably is identical with an oligomer of a 30,000 Dalton protein with neutral isoelectric point, which keeps 20% of the incorporated label. Microautoradiographs suggest that the labeled proteins were synthesized in companion cells. The results indicate that P-protein of *Cucurbita maxima* is synthesized continuously in mature phloem. It can be assumed that P-protein has a relatively high turnover rate. Therefore it seems unlikely that P-protein is a "structural" protein.

Introduction

The term phloem-protein or P-protein was proposed by Esau and Cronshaw (1967) to replace the inappropriate term "slime". Since then, many students of phloem physiology, dissatisfied with the pressure-flow theory of translocation, have suggested that P-protein somehow participates in assimilate transport. The reason for it is that fixed P-protein can be resolved under the electron microscope as filamentous material, which sometimes occludes the sieve pores. Thus, P-protein has become a name for protein that appears filamentous and sometimes occupies the lumen of sieve pores when processed for electron microscopy. Its occurrence seems to be restricted to angiosperms.

Biochemical investigations of P-protein were initiated on *Cucurbita* species (Walker and Thaine, 1971; Kleinig et al., 1971; Weber and Kleinig, 1971), where exudate from severed organs has been shown to consist entirely of sap from mature sieve tubes (Eschrich et al., 1971). This exudate contains P-protein, which under certain conditions can be precipitated. Since precipitation by potassium chloride is followed by dissolution during dialysis, it was conjectured that the filaments could be a contractile protein similar to actin (Kleinig et al., 1971; Ilker and Currier, 1974). However, recent investigations have failed to confirm this hypothesis (Kleinig et al., 1975; Palevitz and Hepler, 1975).

Sieve tube exudate of *Cucurbita* gels when exposed to air (Nägeli, 1861). This is due to the presence of a basic protein, which becomes oxidized (Walker, 1972). Analytical procedures therefore have to be carried out under reducing conditions. Agreement exists that the P-protein of *Cucurbita* is composed of two major fractions, a 116,000 and a 30,000 Dalton fraction. The latter can di- or polymerize and then appear as a 60,000 Dalton protein or as part of the 116,000 fraction. Recently, Kleinig et al. (1975) demonstrated that only the 116,000 Dalton fraction forms filaments when precipitated and processed for electron microscopy. This is the P-protein and presumably it is a "structural" protein.

Our results are in partial agreement with those

* Supported by Deutsche Forschungsgemeinschaft.

Abbreviations: DEAE = diethylaminoethyl; SDS = sodium dodecyl sulfate; pI = isoelectric point

of Kleinig et al. (1975). However, in this communication we show that P-protein is composed of more than one protein, and that the P-proteins can be synthesized anew in mature phloem.

Material and Methods

Cucurbita maxima Duch. cv. Gelber Zentner was grown at 25° C ± 2° C in the greenhouse. In winter additional light was provided by several Osram HQLS 400 Watt lamps to give 22,000 Lux at the level of the plants from 7 am to 7 pm. Sieve-tube exudate was tapped from cut hypocotyls of seedlings as described previously (Eschrich et al., 1971; Heyser et al., 1974). Exudate of 30 seedlings was collected in 1 ml 50 mM Tris/HCl buffer, pH 7.8, with 10 mM 2-mercaptoethanol and 0.5 mM dithiothreitol.

Exudate-proteins were labeled by applying 1.5 µCi of a mixture of [^{14}C]amino acids (protein hydrolysate from *Chlorella*; Amersham-Buchler, Braunschweig) in small droplets to each cotyledon of 14-day-old seedlings, which 3 days earlier had been transferred to aerated Hoagland's solution. The surface of the cotyledons had been roughened with carborundum powder. Plants were incubated with [^{14}C]amino acids for 12 h before sieve-tube exudate was collected.

Protein precipitation for liquid scintillation counting was carried out with an equal volume of 10% trichloroacetic acid and 1% silicotungstic acid in acetone. Liquid scintillation counting was performed either with a Tracerlab (ICN) Corumatic-200 or a Hewlett-Packard Tricarb III in either Bray's solution, Aquasol (NEN) or Unisolve 1 (Zinsser, Frankfurt).

Sieve-tube exudate was cleared of small molecules in a 15-ml column of Sephadex G-25 at 4° C eluted with the collecting buffer. Small peptides were removed by this procedure. The reunited protein fractions then were led through a 30 cm column of DEAE cellulose SS (Serva, Heidelberg), and fractions were collected as described previously (Heyser et al., 1974).

Disc-gelelectrophoresis was carried out as described previously (Eschrich et al., 1971).

SDS-gelelectrophoresis was performed according to Shapiro et al. (1967) and Dunker and Rueckert (1969). Ferritin, catalase, aldolase, egg-albumin, bovine serum albumin, chymotrypsinogen A and cytochrom c were used as marker substances with known molecular weights. Electrophoresis was performed at room temperature with 7 mA/gel for 5 to 6 h. The gels then were forced out with compressed air, kept in 20% sulfosalicylic acid for 24 h in the dark, stained for 12 h with 0.02% Coomassie brilliant blue R 250 (ICI), dissolved in 12.5% trichloroacetic acid, and destained with 10% trichloroacetic acid in the dark for 2 to 3 days.

For preparative isoelectric focusing according to Haglund (1967), 110 ml columns from LKB were loaded as suggested by the manufacturer. Electrophoresis was started with 300 V. After 20 h the current was adjusted to 700 V. Electrophoresis was terminated after the current strength had been constant for 12 h. The sucrose gradient was made up with 6.5 M urea solution. 10 ml of 1% aspartic acid were layered between the sucrose and the anode liquid. These precautions were necessary to prevent precipitation of acidic proteins. 20-drop fractions were collected automatically (LKB 7000) and their protein content was recorded at 280 nm (PMQ II, Zeiss). The pH was taken from elutes of every second fraction. For measurement of radioactivity by liquid scintillation counting, fractions were filled up to 4 ml with distilled water, and 10 ml of either Aquasol or Unisolve was added.

Gross autoradiographs of freeze-dried seedlings were prepared according to Eschrich (1966).

Microautoradiographs of phloem tissue were made as follows: Seedlings, labeled with 30 µCi of [^{14}C]protein hydrolysate for 12 h were plunged intact into liquid nitrogen. Three min later the hypocotyls, broken off the plants, were transferred to an ethanol/acetic acid mixture (3:1) made very cold with lumps of dry ice. After 12 h freeze-substitution in a deep-freeze, the fixative was allowed to warm up to room temperature. Pieces of vascular bundles were cut out, postfixed in buffered OsO_4, and run through a graded series of ethanol and propylene oxide. Embedment was in Epon 605 at 60° C. 1 µm-sections were taken on an LKB-ultratome III with glass knives. The sections were stretched and dried on glass slides. Pieces of Kodak AR 10 stripping-film, floating on double distilled water with 2% sucrose and 0.001% KBr were picked up with submerged slides. After drying on silica gel, the slides were kept for 3 to 4 weeks at 4° C in a dry box. Microautoradiographs were developed with Kodak D 19b developer for 5 min (20° C), fixed, washed, dried, and enclosed in gum arabic/potassium acetate-syrup (Eschrich and Currier, 1964). Photomicrographs were taken with phasecontrast illumination.

For autoradiographic recording of labeled proteins, 1 mm slices of the polyacrylamide gels were cut longitudinally as suggested by Fairbanks et al. (1965). The wet slices were arranged on a filter paper which covered a porous clay plate tightly fixed to an evacuation box made of plexiglas. Gel slices and filter paper sheets were covered smoothly with plastic foil. Using a water-jet vacuum pump to reduce the pressure below the clay plate, the gel slices were firmly appressed to the filter paper, and kept flat during the drying time of 2 to 3 h, shortened by use of a warm lamp. X-ray film (Agfa-Gevaert Curix RP 1) was used for autoradiographs.

Results

Movement and Incorporation of ^{14}C-labeled Amino Acids

When seedlings of *Cucurbita maxima*, growing in aerated Hoagland's solution, were provided with 1.5 µCi/cotyledon of a mixture of ^{14}C-labeled amino acids, gross autoradiographs of the plants showed a distribution of the tracer as indicated in Figure 1. Within 5 h of incubation, radioactivity had been exported from both cotyledons, reaching down to the roots and up to the shoot tip. The young primary leaf in Figure 1 imported labeled material mainly into the lower part of the leaf blade, which obviously was not yet fully differentiated (Turgeon and Webb, 1975).

When such a seedling is quick-killed by freezing in liquid nitrogen, microautoradiographs of the vascular bundles of the hypocotyl can be obtained, in which only radioactivity incorporated in insoluble material is recorded. This is because all soluble tracers have been washed out. The quick-killing procedure is necessary to keep the radioactive material in place. Severing of the bundles before killing allows displacement of sieve-tube contents, including the result of sudden turgor release in the sieve tubes. It has been demonstrated histochemically that surging also causes desplacement of enzymes from companion cells into the sieve tubes (Lehmann, 1973).

Figure 2 shows the distribution of label in a cross section of the internal phloem of a hypocotyl bundle. Silver grains are concentrated at sites of companion

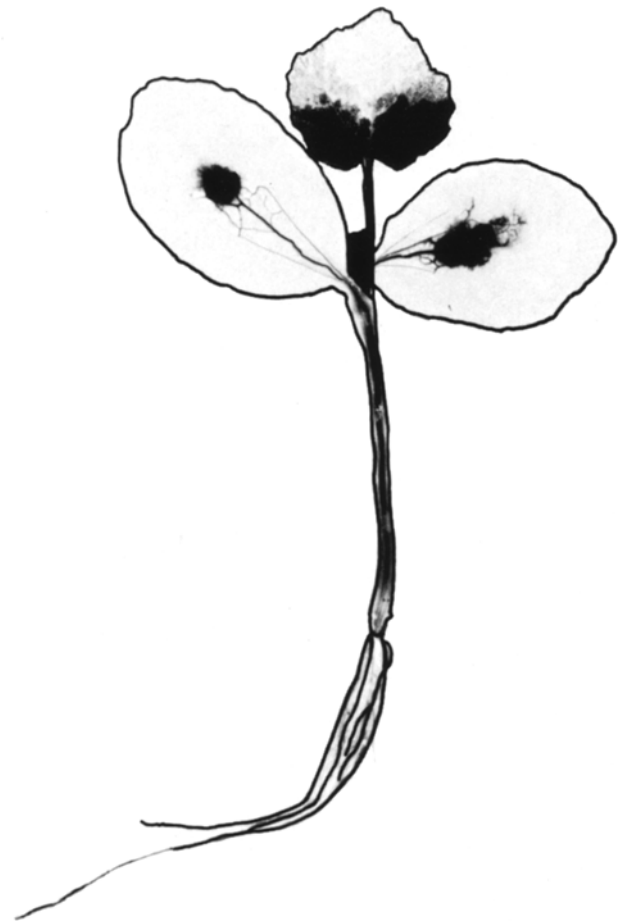

Fig. 1. Autoradiograph of a *Cucurbita* seedling treated for 5 h with 1.5 µCi L-[^{14}C]leucine/cotyledon. Borders of the freeze-dried plant are outlined in black

cells. The same distribution was found in the external phloem. Figure 3 shows microautoradiographs of two serial radial longitudinal sections of the external phloem. The cambial layer is diffusely labeled. The prominent secondary sieve tube with the torn inner network probably was immature. It contains only unlabeled material, as do the parenchyma cells adjacent to the right. However, companion cells of the late metaphloem sieve tubes are covered by dense accumulations of silver grains.

It can be concluded that ^{14}C-labeled amino acids applied to the cotyledons were exported via the metaphloem and that, in the mature metaphloem, some were utilized for synthesis of insoluble material in the companion cells.

Identification of the Insoluble Radioactive Material as Protein

The sieve-tube exudate of seedlings treated with [^{14}C]amino acids for 12 h was highly radioactive. However, most of the radioactivity was from the transported labeled amino acids.

When the exudate was treated with trichloroacetic acid and silicotungstic acid, macromolecular material was precipitated and could be washed free of soluble radioactive compounds. This precipitate, taken up with 0.4 N NaOH, was radioactive also. In another experiment this precipitate was dialyzed and incubated with pronase P, a mixture of proteolytic enzymes from *Streptococcus griseus*. Subsequent precipitation with 10% trichloroacetic acid and 1% silicotungstic acid and centrifugation produced radioactive material in the supernatant only; the pellet was free of radioactive compounds.

Thus, besides the bulk of radioactivity represented by labeled amino acids, the sieve-tube exudate contained a small amount of labeled proteins or acid-precipitable peptides. The specific activity of this fraction was low, 5 to 6 dpm/µg protein.

Fractionation of Labeled Proteins

Fractionation of sieve-tube exudate on a column of DEAE cellulose was found by Heyser et al. (1974) to be advantageous, because the gelling components are eluted first, obviously with the void volume. Thus, several protein fractions, including some with enzymic activities, can be eluted subsequently by applying a linear gradient of NaCl or KCl to the same column. The solid-lined curve in Figure 4 shows peaks 1 to 15, which correspond with the numbering introduced by Heyser et al. (1974, Fig. 5). Peaks 8 to 15 are eluted by the NaCl-gradient.

The pattern changes, when the sieve-tube exudate is cleared from substances of low molecular weight prior to fractionation. Best results were obtained by passing the sieve-tube exudate through a small column of Sephadex G-25. The dotted-lined curve in Figure 4 shows the peaks eluted from a DEAE-cellulose column, which had been loaded with sieve-tube exudate cleared from small molecules by Sephadex G-25. It is evident that the former peaks 5, 6 and 7 are now missing. We assume that they had been produced earlier by peptides of low molecular weights.

All fractions of DEAE-cellulose columns were collected and separately subjected to liquid scintillation counting. Table 1 gives the absolute and the specific activities of each peak.

Comparing these data with Figure 4, it can be seen that the shaded sections of the solid curve indicate labeled protein peaks.

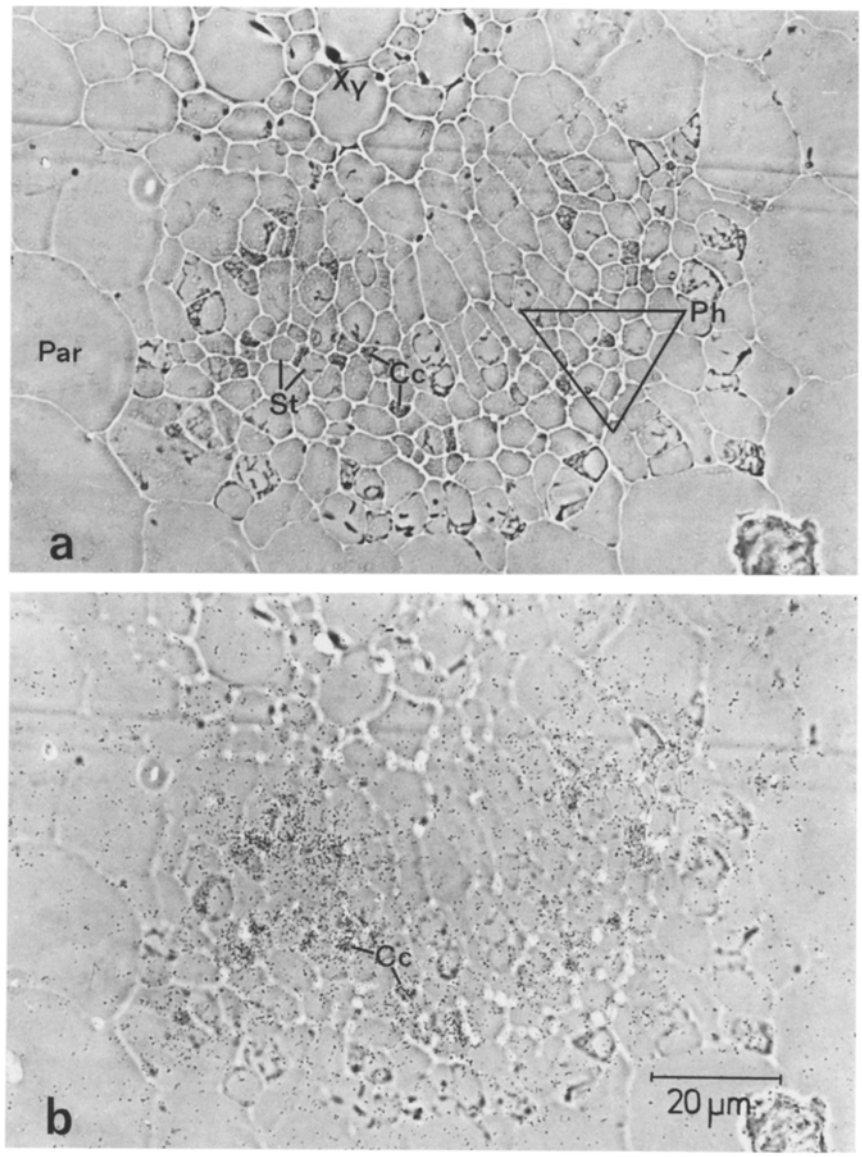

Fig. 2a and b. Microautoradiograph of internal phloem of a hypocotyl bundle. **a** Focused on section showing xylem (*Xy*), metaphloem (*Ph*) and parenchyma (*Par*); *St* sieve tubes; *Cc* companion cells. **b** Focused on photographic emulsion showing dense accumulations of silver grains at sites of companion cells (*Cc*)

Fig. 3A and B. Microautoradiographs of external phloem of a hypocotyl bundle. **A** and **B** Two serial radial longitudinal sections. *a* focused on sections, *b* focused on photographic emulsion. *Ca* cambial layer; *Cc* companion cells; *Par* parenchyma; *St* sieve tubes of the metaphloem; *sSt* immature secondary sieve tube; *sXy* secondary xylem. Arrows in *b* point to identical sites in both sections

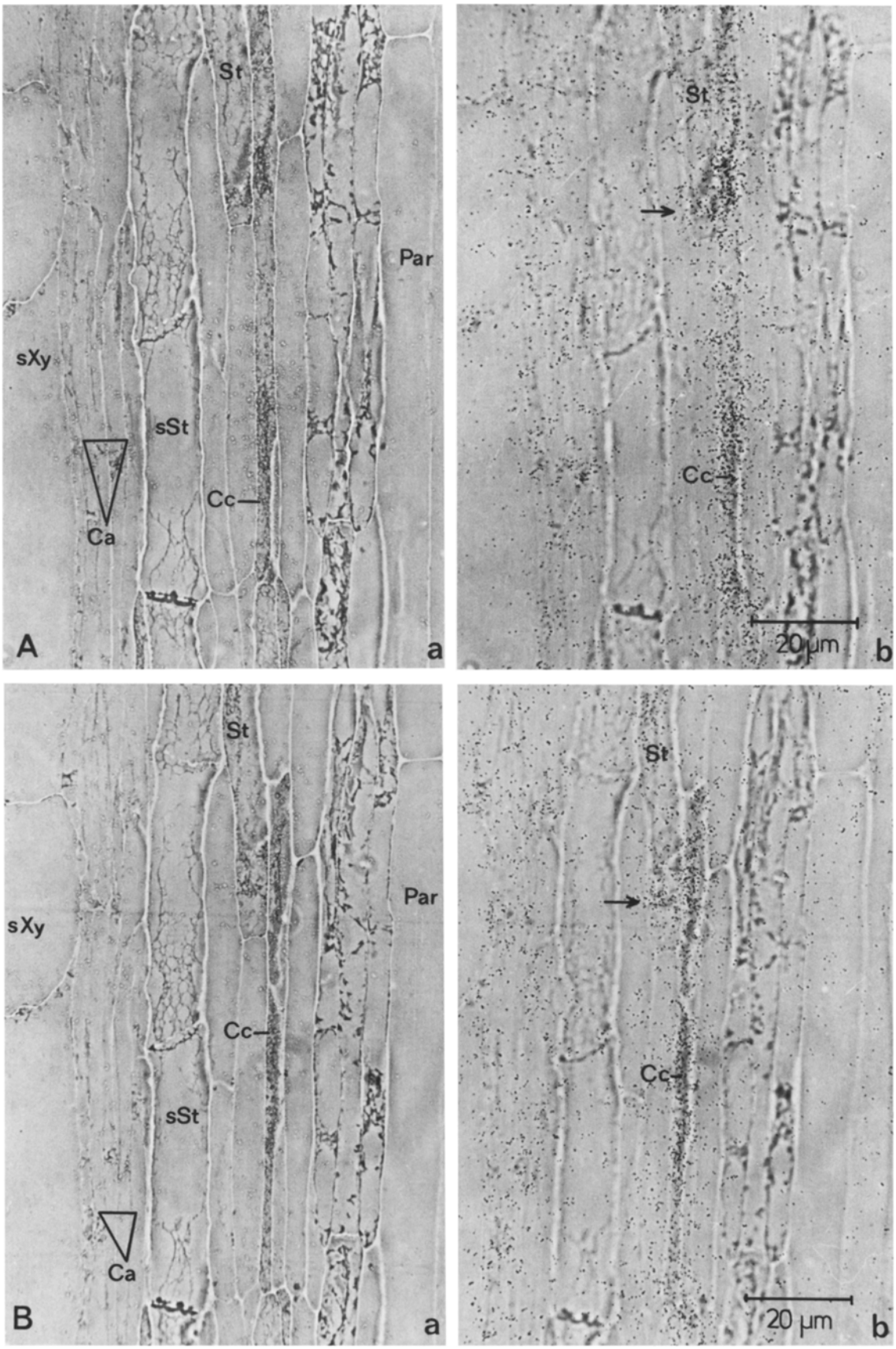
St
Par
sXy
sSt
Cc
Ca
A
a
St
Cc
20 µm
b
St
Par
sXy
Cc
sSt
Ca
B
a
St
Cc
20 µm
b

Fig. 3A and B

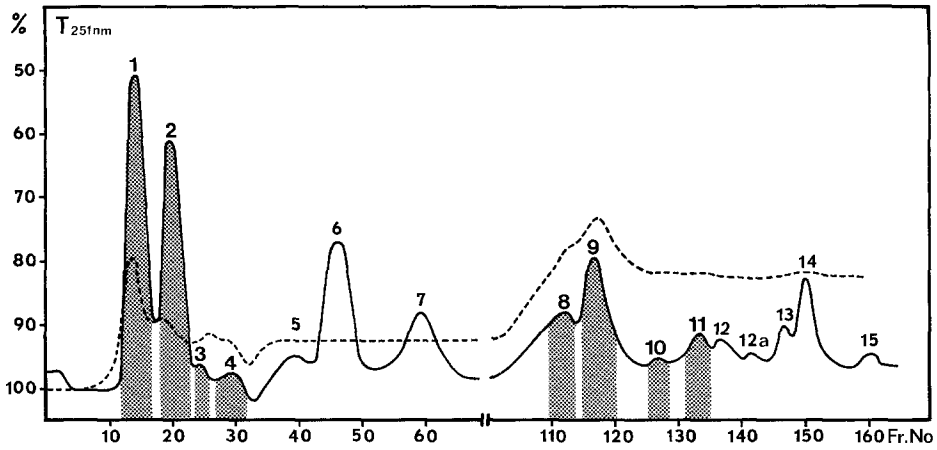

Fig. 4. Separation of sieve-tube exudate on a DEAE-cellulose column. *Solid line*: Transmission at 251 nm of elute from exudate from 60 seedlings of *Cucurbita maxima*, collected in 50 mM Tris buffer, pH 7.8, with 0.5 mM dithiothreitol and 10 mM 2-mercaptoethanol. Peaks 1–7 were eluted with 50 mM Tris buffer, pH 7.8, with 0.5 mM dithiothreitol. Peaks 8–15 were eluted with a linear gradient of 0 to 500 mM NaCl in the same buffer. *Dotted line*: Exudate was cleared of low molecular weight substances with a 15 ml column of Sephadex G-25 prior to fractionation on DEAE cellulose. Peaks 5, 6 and 7 are now missing. They may have been consisted of low molecular weight peptides. Shaded peaks are those, which contain radioactive proteins when the cotyledons of the seedlings had been provided with ^{14}C-amino acids (compare Table 1)

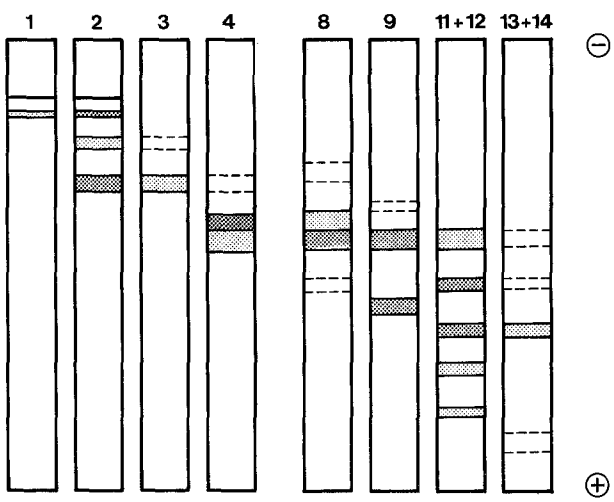

Fig. 5. Patterns of protein bands appearing when single peaks from DEAE-cellulose elutes were subjected to discelectrophoresis on 7.1% polyacrylamide, pH 8.9, and stained with Amidoblack 10B

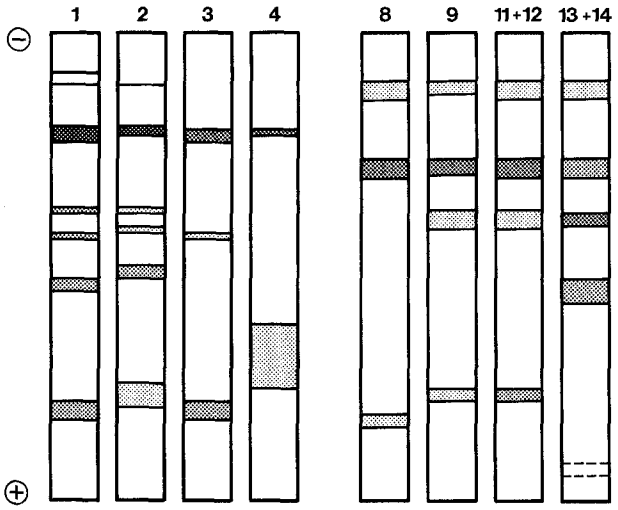

Fig. 6. Elutes of peaks 1–14 from the DEAE-cellulose column were denatured with 4 M urea and 1% SDS, and subjected to gelelectrophoresis by employing reducing conditions with 2% 2-mercaptoethanol and with 0.1% SDS. Staining with Coomassie brilliant blue reveals patterns of distribution according to molecular weights (compare Table 2)

Table 1. Distribution of radioactivity of protein fractions after fractionation on DEAE cellulose (dpm)

Peak-No.	1	2	3	4	5	6	7	8+9	10	11	12	13	14	15
Abs. activity	11,864	225	107	1,177	132	–	–	240	215	161	–	–	–	–
Spec. activity	6.0	–	–	10–250	–	–	–	6.0	6.0	6.0	–	–	–	–

Characterization of Labeled Proteins

The fractions obtained from DEAE-cellulose columns were combined to give elutes of peaks 1, 2, 3, 4, 8, 9, 11+12, and 13+14, as numbered in Figure 4.

Subjecting these elutes to disc-electrophoresis on polyacrylamide and staining with Amidoblack 10 B (Merck) shows that each peak contains several proteins, and that some of the same proteins obviously occur in more than one gel. Figure 5 shows schematically the distribution of the main bands of proteins.

When the same elutes of peaks 1–4 and 8–14 of the DEAE-cellulose column are subjected to SDS-gel electrophoresis, proteins are separated according to their molecular weights (Fig. 6). It seems noteworthy that peaks 1–3 in Figure 6 show more bands than in the disc-pherogram of Figure 5. As will be shown later, this relates to highly basic proteins in peaks 1–3 that have not entered the disc gel, which was run with buffer of pH 8.9.

By comparison with marker proteins of known molecular weights, the molecular weights of proteins in SDS gels can be estimated as indicated in Table 2.

In order to get some idea as to which of the proteins in the gels was radioactive, we tried methods of gel-autoradiography, gel dividing, and counting of 1 mm slices of the gels. Eventually, it turned out that the method involving exposure of longitudinally-sliced gel sections to X-ray film gave reproducible results.

Figure 7A shows an autoradiograph of a gel slice taken from a SDS-electropherogram including all proteins of the exudate. Two bands are labeled heavily, and two others lightly with radioactivity. The drawing to the left illustrates the bands of an SDS gel stained with Coomassie brilliant blue. The labeled bands are indicated by dots. They correspond to estimated molecular weights of 115,000, 60,000, 30,000, and 21,000 Dalton.

Figure 7B shows an autoradiograph of a slice of disc gel. Much less radioactivity is recorded here than in Figure 7A. As mentioned earlier, and to be shown later on, this is because basic proteins with a pI greater than the pH 8.9 of the electrode buffer are not included. Comparison of the two autoradiographs, gives one the impression that the bulk of the labeled proteins is in the SDS-gel–that is, in the gel containing the basic proteins.

Table 2. Molecular weights ($\times 10^3$) of proteins in SDS gels. Gel numbers correspond to peak numbers in Figure 4

Peak No.	1	2	3	4	8	9	11+12	13+14
Molecular	170	–	–	–	–	–	–	–
weights	155	155	–	–	150	150	150	150
	115	115	115	115	–	–	–	–
	–	–	–	–	95	95	95	95
	75	75	–	–	–	70	70	70
	60	65	60	–	–	–	–	–
	45	50	–	–	–	–	–	–
	–	–	–	30	–	30	30	–
	22	24	22	–	22	–	–	15

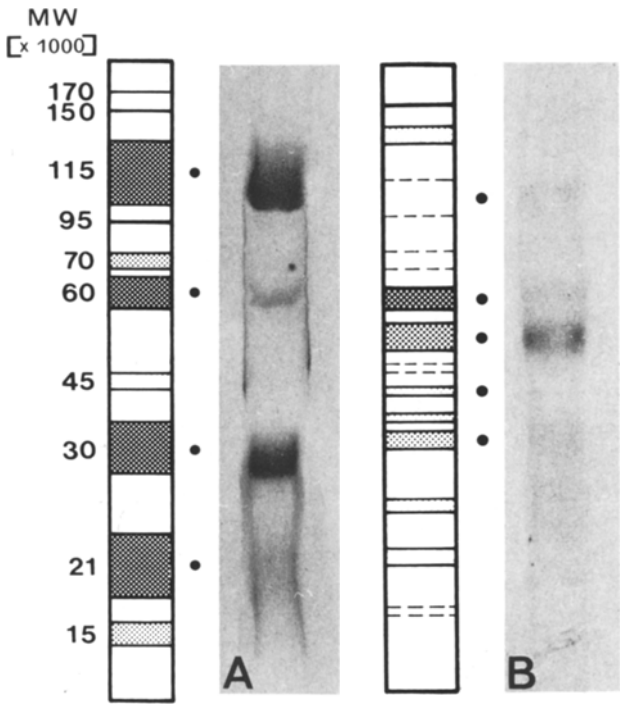

Fig. 7. Autoradiographs of gel slices from SDS-**A** and disc-**B** electropherogram, each aligned to drawings with the stained protein bands. Black dots indicate radioactivity of the band, even though not always apparent in photograph, because of very low specific activity

The radioactivity of some bands indicated by black dots is very low. It must be remembered, however, that the amount of protein administered to a gel was small. Commonly, a single gel was loaded with a protein solution of only 500 to 1,000 dpm. This handicap of low specific activity prohibits the autoradiography of single peaks obtained from DEAE-cellulose columns. An increase of specific activities may be possible when plants are exposed to the ^{14}C-labeled amino acids for a longer period. However, experiments of this kind indicated that the labeled proteins of the sieve-tube exudate are subject to protein degradation.

Fractionation by Isoelectric Focusing

Beyenbach et al. (1974) investigated both a protein fraction I with a molecular weight of 115,000 Dalton

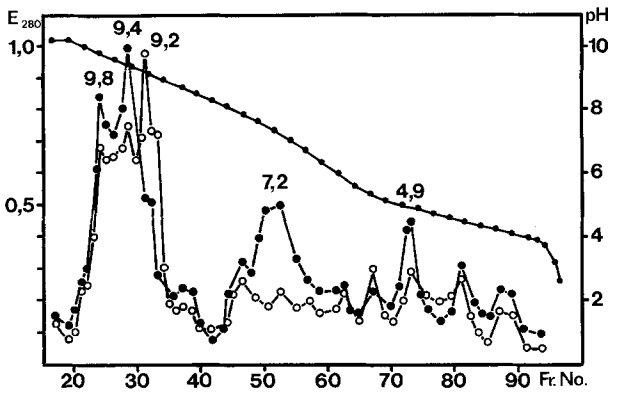

Fig. 8. Isoelectric focusing of sieve-tube exudate from 100 seedlings of *Cucurbita maxima*. Curve with black dots as measuring points was obtained from exudate kept under strong reducing conditions with 25 mM dithiothreitol. Curve with open circles as measuring points was obtained from exudate kept under relatively weak reducing conditions with 0.5 mM dithiothreitol. Dotted line represents pH values of the fractions

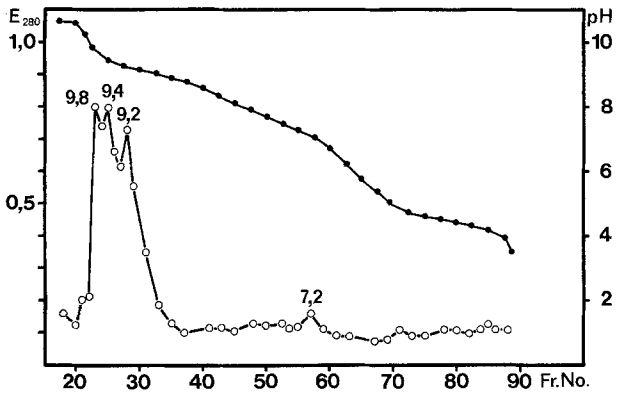

Fig. 9. Isoelectric focusing pf peak 1 of the DEAE-cellulose elute. Note that this basic protein fraction gives rise to a small peak with pI 7.2, while the peak pI 9.2 is slightly reduced compared with that in Fig. 8

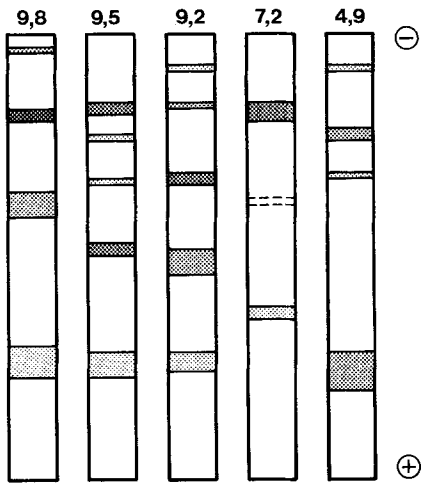

Fig. 10. SDS-electropherograms of peak fractions obtained by isoelectric focusing. Bands came into view by staining with Coomassie brilliant blue. For scaling of approximate molecular weights compare scale in Fig. 7A

and a protein fraction II with a molecular weight of 30,000 Dalton by electrophoresis on cellulose acetate. They concluded from their results that the isoelectric points of both kinds of proteins must be higher than pH 9.5.

Since recent results of Kleinig et al. (1975) clearly show that these basic proteins include the P-protein, which appears filamentous when processed for electron microscopy, the results we obtained with isoelectric focusing of sieve-tube exudate should be of special interest.

Unfortunately, this method has its upper limit at pH 10. However, by adding 6.5 M urea to the sucrose gradient and positioning a cushion of 1% aspartic acid between the sucrose gradient and the anode liquid, we were able to obtain reproducible curves with 3 different peaks of basic proteins. Figure 8 shows such a curve with open circles as measuring points. The isoelectric points of the proteins were estimated with 9.8, 9.4, and 9.2. However, this type of curve was obtained only when weak reducing conditions were employed, with 0.5 mM of dithiothreitol in the collecting buffer.

When the amount of the reducing agent was increased to 25 mM, there was a drastic change, as indicated by the curve in Figure 8 with black dots as measuring points. The basic peak with pI 9.2 disappeared and instead a new peak with pI 7.2, a neutral protein, appeared.

The 3 basic proteins (pI 9.8, 9.4, 9.2) belong to peak 1 of the DEAE-cellulose elute. In Figure 9, only this fraction was subjected to isoelectric focusing. One can see that in addition to the 3 basic proteins part of the protein with pI 7.2 is included. This suggests that the neutral protein is a derivative of the basic proteins. Since strong reducing conditions promote the formation of the pI 7.2 protein and the concomitant disappearance of pI 9.2, we can conclude that the neutral protein is a reduction product of the basic protein pI 9.2.

Figure 8 shows some protein peaks with pI 4.9 and lower. These acid proteins are those eluted from the DEAE cellulose by a linear NaCl-gradient and numbered as peaks 8–15.

In another experiment the peak fractions obtained by isoelectric focusing were subjected to SDS gelelectrophoresis. Results are shown in Figure 10. Surprisingly, a considerable number of proteins with identical pIs, but with different molecular weights appeared. Although some of these bands may be smaller units of one and the same protein, it seems evident

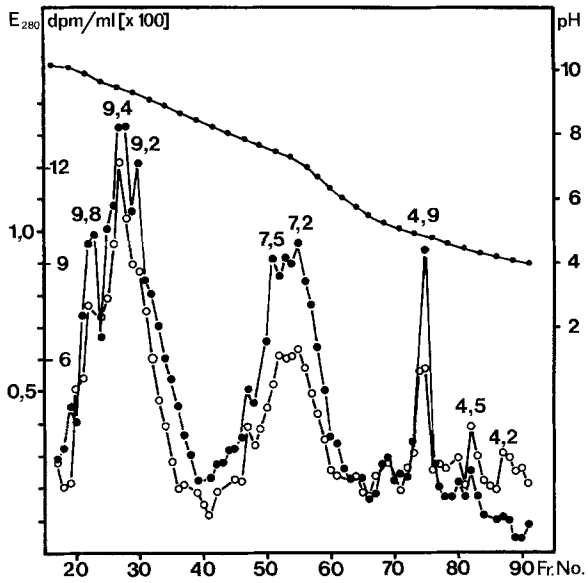

Fig. 11. Isoelectric focusing of labeled exudate cleared of low molecular weight material by Sephadex G-25. Curve with open circles as measuring points gives optical density (280 nm) of fractions. Curve with black dots as measuring points shows radioactivity of fractions

that (1) in addition to the 3 basic P-proteins (pI 9.8, 9.4, 9.2) there exist more than 1 protein with the same pI; and (2) pI 7.2 is the only fraction that contains the 30,000 Dalton protein designated as the monomer of P-protein fraction II by both Beyenbach et al. (1974) and Kleinig et al. (1975).

In order to determine which of the proteins was radioactive, exudates of 20 labeled and 80 unlabeled plants were subjected to isoelectric focusing after being cleared of low-molecular weight material by Sephadex G-25 (Fig. 11). The curve with the open circles as measuring points gives optical densities at 280 nm; the curve with the black dots as measuring points indicates the radioactivites of the fractions expressed in dpm/ml ($\times 100$).

All data show that the mature phloem of *Cucurbita maxima* is able to synthesize continuously 3 groups of proteins, which are available in the exudate of the sieve tubes (Table 3):

Table 3. Characterization of radioactively labeled proteins from sieve-tube exudate of *Cucurbita maxima*. Peak numbers correspond with those of Figure 4

Group	Peak No.	pI	Molecular weights ($\times 10^3$)	% of radioactivity incorporated in proteins
1	1–3	9.8; 9.4; 9.2	20 60 115	ca. 80%
2	4	7.5–7.2	30	ca. 20%
3	8–11	4.9	20	traces

Discussion

According to Kleinig et al. (1975), the P-protein of *Cucurbita maxima* is a proteinaceous material of the sieve-tube exudate that forms filaments when precipitated and processed for electron microscopy. Unquestionably, these filaments are identical with those found in sectioned sieve elements. This filament-forming material has been characterized by Beyenbach et al. (1974): it is highly basic; its molecular weight is about 116,000 Dalton; it cannot be separated into smaller units; and, in the absence of SH-protecting agents it forms a gel.

Similar results have been obtained by Weber et al. (1974). They found P-protein to be pleomorphic, i.e., appearing in different forms when precipitated and processed for electron microscopy. Filaments occurred when exudate or a mixture of all basic proteins of the exudate were precipitated. Weber et al. (1974) also checked P-protein from *Cucumis sativus* and found "virtually the same pattern of bands in polyacrylamide electrophoresis in SDS or acidic urea solutions as well as similar IEPs and elution patterns from Sephadex and DEAE cellulose columns". Thus, it can be surmised that differing data obtained with *Cucurbita pepo* P-protein (Walker and Thaine, 1971) are due to differing qualities of P-protein fractions.

The most striking aspect of our results is that 80% of the incorporated radioactivity is represented by the P-protein. This P-protein is exactly the same biochemically as that which Beyenbach et al. (1974), and Weber et al. (1974) designated as the 116,000 Dalton protein, and which appears filamentous in the electron microscope (Kleinig et al., 1975). Isoelectric focusing has revealed, however, that this P-protein can be separated into 3 proteins with slightly differing pIs. One of them (pI 9.2) seems to be a mixture of natural oligomers. Under strong reducing conditions, it disaggregates into smaller units, mainly a 30,000 Dalton unit, and shows a shift to pIs between 7.5 and 7.2. The 2 other P-proteins with pI 9.4 and 9.8 also are separated into several "units" with different molecular weights by SDS gelelectrophoresis (Fig. 10). However, components with molecular weights of 115,000 and 110,000 Dalton can be found in all fractions of the labeled P-protein.

Upon examination of Table 1, it becomes apparent that the acidic proteins, which give only trace amounts of total protein radioactivity, have the same specific activity as peak-1 P-protein of the DEAE-cellulose elute. Thus, we can assume that synthesis of the gelling P-protein proceeds at least as rapidly as synthesis of enzyme proteins. However, the neutral peak-4 protein of the DEAE-cellulose column, presumably derived from basic oligomers with pI 9.2,

exhibits higher specific activities, ranging from 10 to 250 dpm/μg protein.

Our results do not support the suggestion that P-protein is derived from the ribosomes of differentiating sieve elements (Weber et al., 1974). Rather it seems to be synthesized anew and continuously in the companion cells.

We believe that our results are sufficient to conclude that P-protein is not a "structural" protein capable of contraction and relaxation like muscle protein, as some workers speculated. When a protein has a high rate of synthesis, it is difficult to imagine that its turnover would be as slow as, for example, myosin with a half-life of 6 months.

We greatfully acknowledge critical examination of the manuscript and valuable suggestions by Dr. Ray F. Evert, Madison.

References

Beyenbach, J., Weber, C., Kleinig, H.: Sieve tube proteins from *Cucurbita maxima*. Planta (Berl.) **119**, 113–124 (1974)

Dunker, A.K., Rueckert, R.R.: Observations on molecular weight determinations on polyacrylamide gel. J. Biol. Chem. **244**, 5074–5080 (1969)

Esau, K., Cronshaw, J.: Tubular components in cells of healthy and tobacco mosaic virus-infected *Nicotiana*. Virology **33**, 26–35 (1967)

Eschrich, W.: Translokation ^{14}C-markierter Assimilate im Licht und im Dunkeln bei *Vicia faba*. Planta (Berl.) **70**, 99–124 (1966)

Eschrich, W., Currier, H.B.: Identification of callose by its diachrome and fluorochrome reactions. Stain Technol. **39**, 303–307 (1964)

Eschrich, W., Evert, R.F., Heyser, W.: Proteins of the sieve-tube exudate of *Cucurbita maxima*. Planta (Berl.) **100**, 208–221 (1971)

Fairbanks, G.Jr., Levinthal, C., Reeder, R.H.: Analysis of ^{14}C-labeled proteins by disc-electrophoresis. Biochem. Biophys. Res. Commun. **20**, 393–399 (1965)

Haglund, H.: Isoelectric focusing in pH gradients-A technique for fractionation and characterization of ampholytes. In: Methods of biochemical analysis. Vol. 19. pp. 1–104. Ed.: Glick, D. New York, London, Sydney, Toronto: Interscience Publ. 1967

Heyser, W., Eschrich, W., Hüttermann, A., Evert, R.F., Burchardt, R., Fritz, E., Heyser, R.: Phosphodiesterase in sieve-tube exudate of *Cucurbita maxima*. Z. Pflanzenphysiol. **71**, 413–423 (1974)

Ilker, R., Currier, H.B.: Heavy meromyosin complexing filaments in the phloem of *Vicia faba* and *Xylosma congestum*. Planta (Berl.) **120**, 311–316 (1974)

Kleinig, H., Dörr, I., Weber, C., Kollmann, R.: Filamentous proteins from plant sieve tubes. Nature New Biol. **229**, 152–153 (1971)

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

Lehmann, J.: Zur Lokalisation von Dehydrogenasen des Energiestoffwechsels von *Cucurbita pepo* L. Planta (Berl.) **111**, 187–198 (1973)

Nägeli, C.: Über die Siebröhren von *Cucurbita*. Sitzber. Königl. Bayer. Akad. Wiss. **1**, 212–238 (1861)

Palevitz, B.A., Hepler, P.K.: Is P-protein actin-like?-Not yet. Planta (Berl.) **125**, 261–271 (1975)

Shapiro, A.L., Vinuela, E., Maizel, J.V.: Molecular weight estimation of polypeptide chains by electrophoresis in SDS polyacrylamide gels. Biochem. Biophys. Res. Commun. **28**, 815–820 (1967)

Turgeon, R., Webb, J.A.: Physiological and structural ontogeny of the source leaf. In: Phloem Transport. pp. 297–313. Eds.: Aronoff, S. et al. New York, London: Plenum Press 1975

Walker, T.S.: The purification and some properties of a protein causing gelling in phloem sieve tube exudate from *Cucurbita pepo*. Biochim. Biophys. Acta **257**, 433–444 (1972)

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

Weber, C., Kleinig, H.: Molecular weights of *Cucurbita* sieve tube proteins. Planta (Berl.) **99**, 179–182 (1971)

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

Received 9 April; accepted 7 May 1976