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Electrophoretic Comparison of Polypeptides from Enriched Plasma Membrane Fractions from Developing Soybean Roots[1]

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ABSTRACT

The polypeptide complement of enriched soybean (*Glycine max* [L.] Merr. cult. wells) root plasma membrane fractions was studied by two-dimensional gel electrophoresis. Good resolution was obtained when polypeptides were solubilized in sodium dodecyl sulfate and when butylated hydroxytoluene was included in the vesicle isolation and solubilization media. The pattern obtained on the two-dimensional slab gel for root plasma membrane was characteristic for that membrane. The polypeptide complements from mitochondrial membranes and from enriched fractions of three other endomembrane components were solubilized and electrophoresed for comparison. Each membrane preparation was identifiable on the basis of its characteristic electrophoretogram. Electrophoresis of protein solubilized from plasma membrane fractions isolated from meristematic and mature root tissue revealed both qualitative and quantitative differences in the respective protein complements.

The polypeptide composition of the plasma membrane has been widely studied in animals and lower organisms. Electrophoretic analyses of protein solubilized from isolated membranes have shown variation in the polypeptide turnover rates (16, 25), variation in complements from organ to organ (20), and changes with developmental age (22). In contrast, very little information is available on the electrophoretic characteristics of plant plasma membrane proteins (7, 9, 26).

A major difficulty in studying plasma membrane proteins from higher plants has been the inability to prepare membrane vesicles in a relatively pure state. Purity levels for plasma membrane preparations typically range from 60 to 80% (11, 19). Contamination is generally attributed to mitochondria (intact or broken) and various endomembrane components. Electrophoretic analyses of membrane preparations from the lower end of this purity range would be difficult to interpret because of the high level of contaminating membrane protein. Conversely, electrophoretic analyses of membrane preparations nearer the high end of this range should provide useful data on the nature of plasma membrane proteins. Absolute characterization of plasma membrane proteins, however, must await further improvements in membrane purification.

In an earlier report (23), we documented the combined use of phosphotungstic acid-chromic acid procedure staining and concanavalin A-ferritin binding in assessing the purity of enriched fractions of soybean root plasma membrane. Results suggest that the percentage of plasma membrane in fractions prepared from 4-day-old soybean roots approaches the higher end of the purity range. An electrophoretic analysis of the protein complement of the plasma membrane, made possible by the development of isolation and solubilization procedures for the electrophoresis of plant plasma membrane vesicles, extends and complements this work and is reported here.

Initially, technical difficulties were incurred (*e.g.*, streaking and smearing of the spots on two-dimensional gels, and the retention of large amounts of Coomassie staining material on both origins). These were greatly reduced by including the protective agent BHT[2] in the vesicle isolation and solubilization procedures.

A two-dimensional electrophoretic comparison of plasma membrane proteins from two developmentally distinct tissues, the meristematic root tip and the mature region tissue, suggests that each membrane type is identifiable on the basis of its characteristic electrophoretic pattern. The results must be considered preliminary, however, until remaining questions of membrane purity can be resolved.

MATERIALS AND METHODS

Chemicals. All electrophoresis chemicals were electrophoresis-purity-grade from Bio-Rad. Tris/Hepes buffers were Ultrol-grade from Calbiochem. All other chemicals were standard reagent-grade laboratory chemicals. The Nonidet P-40 (Bethesda Research Labs, Rockville, Md.) was a gift from R. W. Breidenbach.

Plant Material. Soybean seeds [*Glycine max* (L.) Merr. cult. Wells] were germinated in the dark in moist vermiculite at 30 C for 4 days. Meristematic tissue (terminal 3 to 4 mm) and mature-region tissue (section 1.5 to 4 cm behind meristematic zone) were excised below the region of lateral root development as described previously (24).

Isolation of Plasma Membrane Vesicles. The following modifications of previously published vesicle isolation procedures (10, 24) were adopted to improve the electrophoretic resolution of the solubilized plasma membrane polypeptides. About 40 g mature-region tissue or 2 to 4 g root meristematic tissue were ground with a mortar and pestle for 3 to 4 min with 10% (w/w roots) insoluble PVP in 1 volume GM consisting of 25 mM Tris-Hepes (pH 7.2), 3 mM EDTA, 2.5 mM DTT, 0.3 M sucrose, and 100 μg/ml BHT. The BHT, dissolved to 50 mg/ml in isopropyl alcohol, was added to the full volume of GM with rapid stirring. This resulted in a fine suspension of BHT and a concentration of alcohol in the GM of 0.5%.

The homogenate was diluted with another 2 volumes GM before being filtered through Miracloth. Mitochondria, other organelles, and debris were removed by centrifugation for 15 min at 15,000*g*. The resulting supernatant was centrifuged for 35 min at 80,000*g* (Spinco SW 27 rotor) to yield a total-membrane pellet. Membrane material was washed by resuspension with a glass

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[2] Abbreviations: BHT, butylated hydroxytoluene; GM, grinding medium; IEF, isoelectric focusing.

stirring rod into fresh GM and repelleted for 35 min at 80,000*g* (Spinco T-65 rotor). The pellet was resuspended in resuspension buffer consisting of 20% sucrose (w/w), 1 mM Tris/Hepes (pH 7.2), 1 mM EDTA, 1 mM DTT, and 100 μg/ml BHT, and layered on a simplified discontinuous sucrose density gradient with one interface at 34 to 45% sucrose as described previously (24) or onto a complex discontinuous sucrose density gradient consisting of 10 ml 45% sucrose, 6.8 ml 34% sucrose, and 6.4 ml each of 30, 25, and 20% sucrose (11, 14). All sucrose solutions were 1 mM in Tris/Hepes (pH 7.2), 1 mM in EDTA, 1 mM in DTT, and 10 μg/ml in BHT. The gradients were centrifuged for 135 min at 80,000*g* (Spinco SW 27 rotor). Vesicles were removed from the gradient interfaces with a Pasteur pipette, washed in 10 mM Tris buffer (pH 7.0), 1 mM EDTA, 1 mM DTT, 100 μg/ml BHT, and 200 mM KCl or NaCl, and pelleted for 30 min at 100,000*g* (Spinco T-65 rotor). This salt buffer wash, by eliminating the peripheral membrane proteins and any adhering cytoplasmic contaminants, reduced smearing and streaking significantly and eliminated much of the variability in faintly stained spots on the gels. The resulting pellet was used for solubilization.

Preparation of Mitochondria. Mitochondria were isolated by a modification of the procedure of Bonner (6). The pellet from the first centrifugation (15,000*g*) was resuspended in GM and centrifuged for 15 min at 1,000*g*. The resulting supernatant was centrifuged for 15 min at 10,000*g*. The pellet was resuspended in fresh GM and centrifuged for 15 min at 6,000*g*. The supernatant was discarded. The pellet was resuspended in resuspension buffer and layered on a simplified 34 to 45% discontinuous sucrose density gradient, and the gradient was centrifuged for 135 min at 80,000*g* (Spinco SW 27 rotor). Mitochondria were removed from the interface with a Pasteur pipette, washed with the same wash buffer as the plasma membrane vesicles, and pelleted for 30 min at 100,000*g* (T-65 rotor). This centrifugation scheme greatly reduced plasma membrane contamination in the mitochondria, so that the two-dimensional gels could be compared to determine the purity of the preparations.

Solubilization of Membrane Preparations. The membrane pellet was stirred with a glass rod in 25 to 100 μl solubilization buffer until no particles of membrane material remained visible. The solubilization buffer was 2% SDS, 0.375 M Tris (pH 8.9), 1 mM EDTA, 1 mM DTT, and 10 μg/ml BHT. The cloudy preparation was transferred to a thick-walled polyallomer tube, sealed with Parafilm to prevent evaporation of the small volume and held in boiling water for 2 min. Membrane residue was removed by centrifugation for 30 min at 100,000*g* (Spinco SW 60 rotor). The clear supernatant, containing the solubilized proteins, was removed to another tube preparatory to isoelectric focusing.

IEF. The basic procedure was a combination of the methods of O'Farrell (21) and Ames and Nikaido (1), with some modifications. Preliminary experiments revealed a large number of bands in the acid region of the focusing gel, so the per cent composition of ampholyte was chosen to expand the acid region (Fig. 1). IEF tube gels (3 mm × 110 mm) were poured according to the following protocol and overlayered with 8 M urea. Usually 8 to 10 gels were cast. The 15-ml final volume of gel solution consisted of 8.25 g urea, 2.0 ml 30% acrylamide monomer-bis stock (30:0.8), 3.0 ml 10% Nonidet P-40 detergent solution, 2.95 ml deionized H_2O, 0.45 ml ampholytes (pH range 3 to 10; Bio-Rad 40% solution), 0.3 ml ampholytes (pH range 4 to 6; Bio-Rad 40% solution), 10.5 μl *N,N,N',N'*-tetramethylethylenediamine, and 50 μl 10% ammonium persulfate made fresh daily. The solution was thoroughly degassed in a 125-ml vacuum flask, and the ammonium persulfate was added just before the gels were poured. After polymerization, the surfaces of the gels were rinsed with deionized H_2O. The gels were not prerun. To an aliquot of SDS-solubilized membrane protein was added a double volume of dilution buffer (1) (0.285 g urea dissolved in 0.4 ml 10% Nonidet P-40 plus 25 μl

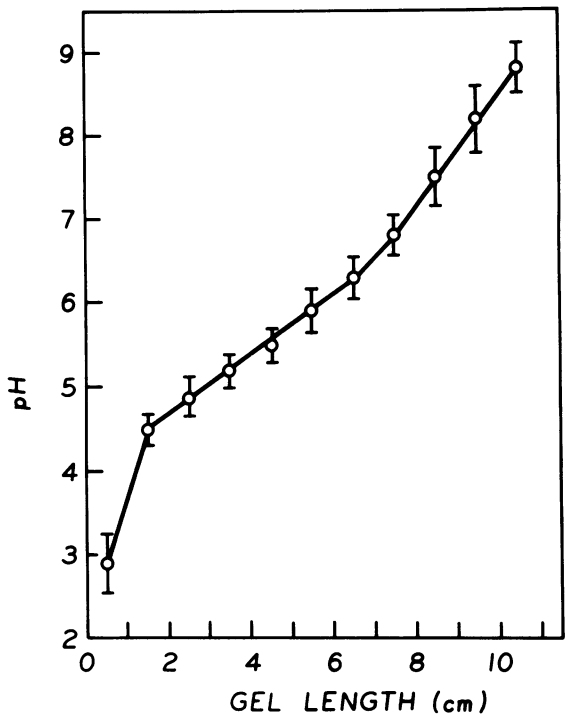

FIG. 1. Isoelectric focusing gradient as described under "Materials and Methods." Averages were plotted from 12 different pH gradients representing three experiments. Each focusing gradient received a different membrane polypeptide sample, an aliquot of low mol wt protein standard (Bio-Rad), or an aliquot of the dilution buffer (a blank).

β-mercaptoethanol and 25 μl ampholytes, (pH range 3 to 10)). Bromphenol blue in glycerol was added to the sample as an electrofocusing marker. The pI of this dye is acidic, so that when the blue dye had traveled the length of the gel and turned yellow, the focusing was complete. The sample, containing 50 to 150 μg membrane protein, was layered on the tube gels and overlayered with 4 M urea. The tubes were filled to the top with a 0.04 N NaOH-0.02 N $Ca(OH)_2$ solution and the upper reservoir was filled with the base. The lower reservoir solution was 0.06 N H_2SO_4. The electrofocusing was performed overnight at 300 v. It was found that raising the voltage to 400 v for 1 to 2 h at the end of the electrophoresis increased resolution significantly. The gels were removed from the tubes by water pressure from a H_2O-filled syringe attached directly to the gel tube with tygon tubing. One set of gels was immediately placed in 10% trichloroacetic acid for later staining with Coomassie brilliant blue R-250, a second set was placed in 2% SDS-equilibration buffer (the same composition as the solubilization buffer, but pH 6.8), and a third was cut into 1-cm slices for verification of the pH gradient. The pH gradient was measured on a Beckman model 4500 digital pH meter, equipped with a Beckman Futura electrode, after the gel slices had soaked in 1.0 ml 50 mM KCl under vacuum for at least 5 h.

SDS Slab Gel Electrophoresis. The second-dimension SDS gels were run with the Tris-Tris/glycine buffer system of Laemmli and Favre (12). A 1-mm thick 10% polyacrylamide slab gel with a 4.7% polyacrylamide stacking gel, both 0.4% in SDS, was poured and run in a vertical slab system similar to that of Ames and Nikaido (1).

The IEF gels to be run in the second dimension were equilibrated in the 2% SDS-equilibration buffer for 2 h to remove most of the ampholytes, the urea, and the Nonidet P-40. The tube gels were drained onto a piece of Parafilm on a paper towel, the 2% SDS-equilibration buffer/1% agarose sealer was pipetted into the trough, and the gel was quickly laid on top of the hot agarose solution (21). Any air trapped under the gel was removed after

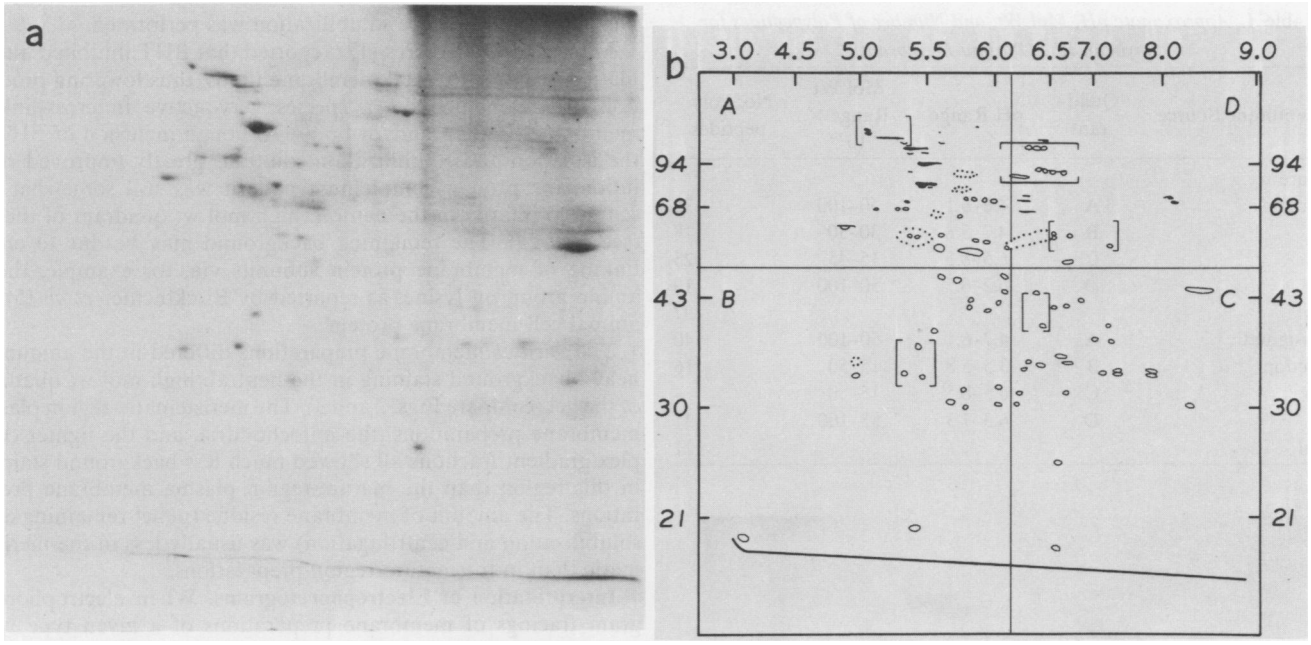

FIG. 2. Two-dimensional electrophoretogram of plasma membrane polypeptides from the mature region of the root. a, photograph of gel; b, tracing of same gel. About 95 spots were resolved. Measured pH range is noted at top of gel tracing. Mol wt $\times 10^{-3}$ is noted on sides of gel tracing. Especially heavily stained spots are filled in solid, medium-stained spots are outlined, and very faint smudges of Coomassie staining material are outlined with a dotted line. For ease of analysis, the gel is divided into quadrants in the tracing (see text for details). Factors affecting interpretation of the gel are discussed in the text.

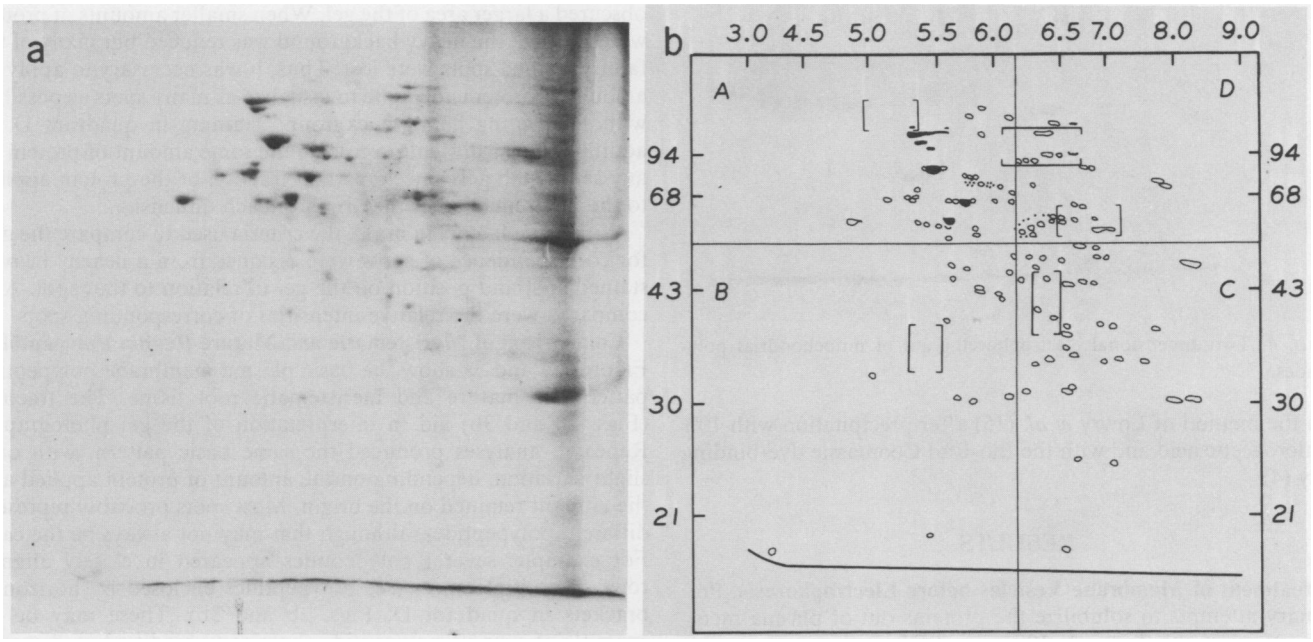

FIG. 3. Two-dimensional electrophoretogram of plasma membrane polypeptides from the meristematic region of the root. a, photograph of the gel; b, tracing of the same gel. About 121 spots were resolved. See legend to Fig. 2 for explanation of tracing.

the slab was sealed in place in the slab apparatus with 1% agarose solution and the buffer (0.1% in SDS) was in the upper reservoir. The front marker dye, bromphenol blue in glycerol, was layered on the inside edge of the tube gel either before or after the buffer was added to the upper chamber. Bubbles were removed from under the bottom edge of the gel with a curved Pasteur pipette. The slab gels were usually run in pairs. Electrophoresis was started with an initial voltage of 70 v, which gave a current of 20 mamp. It was found that streaking and smearing of the plasma membrane polypeptides was reduced if the current was held constant at 20 mamp. When the dye front had migrated to within 1 cm of the bottom edge of the gel, the gels were immediately removed from the slab plates and fixed overnight in 500 ml of a solution of 25% isopropanol-10% acetic acid. They were then stained and destained according to Fairbanks *et al.* (8), skipping the optional step. To speed the process, the times of staining were shortened (1). After complete destaining with several changes of 10% acetic acid, the slab gels were dried onto Whatman 3MM chromatography paper for examination.

Protein Determination. Membrane protein was determined both

Table I. *Approximate pH, Mol Wt, and Number of Polypeptides for Soybean Root Plasma Membrane*

Membrane Source	Quadrant	pH Range	Mol Wt Range × 10^{-3}	No. Polypeptides
Mature section	A	4.6–6.1	50–100	35
	B	4.7–5.8	30–50	18
	C	6.5–8.5	15–45	25
	D	6.2–6.5	50–100	19
Total				97
Meristematic section	A	4.7–6.1	50–100	40
	B	3.5–5.8	15–50	16
	C	6.5–8.5	15–50	34
	D	6.3–7.3	55–100	31
Total				121

FIG. 4. Two-dimensional electrophoretogram of mitochondrial polypeptides.

with the method of Lowry *et al.* (15) after precipitation with 10% trichloroacetic acid and with the Bio-Rad Coomassie dye-binding assay (4).

RESULTS

Treatment of Membrane Vesicles before Electrophoresis. Preliminary attempts to solubilize the proteins out of plasma membrane vesicles with Triton X-100, urea, SDS, and various combinations of these agents resulted in gels with the polypeptide pattern obscured by heavy horizontal streaking and vertical background smearing. The results suggested that the gel pores were partially blocked, perhaps by protein still associated with membrane lipids or by aggregation or cross-linking, causing the protein to "bleed in" during the course of the electrophoresis, with resulting blurring of the pattern. The critical factor appeared to be the degree of aggregation or cross-linking of the membrane proteins when electrophoresis was attempted. Accordingly, the isolation and solubilization procedures outlined above were developed. BHT, DTT, and EDTA in all solutions plus insoluble PVP in the grinding medium were essential, as was a salt buffer wash to remove peripheral membrane proteins and contaminating cytoplasmic proteins before solubilization was performed.

Minssen and Munkres (17) reported that BHT inhibited autoxidation of mitochondrial membrane lipids, thus lowering production of malonaldehyde, a species very active in cross-linking membrane proteins and/or lipids. Although inclusion of BHT in the isolation and solubilization solutions greatly improved resolution, the protein complement pattern was still somewhat obscured, primarily in the neutral, high mol wt quadrant of the gel (see Fig. 2a). The remaining background may be due to cross-linking of membrane protein subunits via, for example, the ϵ-amino group on lysine, as reported by Birckbichler *et al.* (5) for animal cell-membrane protein.

The various membrane preparations differed in the amount of heavy background staining in the neutral, high mol wt quadrant of the gel (compare Figs. 2 and 3). The meristematic-region plasma membrane preparations, the mitochondria, and the lighter complex gradient fractions all showed much less background staining in this region than the mature-region plasma membrane preparations. The amount of membrane residue (pellet remaining after solubilization and centrifugation) was usually less in the meristematic than in the mature-region preparations.

Interpretation of Electrophoretograms. When electrophoretogram tracings of membrane preparations of a given type were compared from one isolation to the next, corresponding spots generally did not match exactly. Stretching and compression of the first-dimension tube gel as it was laid in the trough of the second dimension slab gel contributed to this. Some distortion may have occurred also during drying of the slab gel.

When larger amounts of protein were electrophoresed, the streaking and smearing in quadrant D increased in intensity and obscured a larger area of the gel. When smaller amounts of protein were applied, the heavy background was reduced but many of the faintly stained spots were lost. Thus, it was necessary to apply an amount of protein adequate to visualize as many spots as possible, without creating heavy background staining in quadrant D. In addition, it was difficult to resolve the same amount of protein on any set of slab gels since a certain fraction of the protein applied to the gels remained at the origin of each dimension.

With these factors in mind, the criteria used to compare the gels for correspondence of spots were distance from a nearby heavily stained spot and position on the gel in relation to that spot. Also compared were the relative intensities of corresponding spots.

Comparison of Meristematic and Mature Region Polypeptides. Figures 2a and 3a show the basic plasma membrane polypeptide pattern for mature and meristematic root tissue. The tracings (Figs. 2b and 3b) aid in interpretation of the gel photographs. Repeated analyses produced the same basic pattern, with only slight variation, depending on the amount of protein applied and the amount retained on the origin. Most spots probably represent different polypeptides, although that may not always be the case. For example, several polypeptides appeared in closely aligned rows of multiple spots (see polypeptides enclosed by horizontal brackets in quadrant D, Figs. 2b and 3b). These may be an example of a single polypeptide chemically modified *in vivo* or *in vitro* to cause focusing at more than one pI, without affecting the mol wt. Anderson and Anderson (2) reported that *in vivo* sialic acid glycosylation of human blood plasma proteins resulted in similar sets of spots on a two-dimensional gel.

To determine whether the plasma membrane of cells in a given stage of development could be characterized by a specific complement of polypeptides and to investigate any differences between the complements depending on cell age, plasma membrane polypeptides were compared from root sections representing primarily meristematic or mature tissue. The implications of comparing plasma membrane vesicles from these tissues have been discussed (23, 24).

Electrophoretograms of polypeptides solubilized from plasma

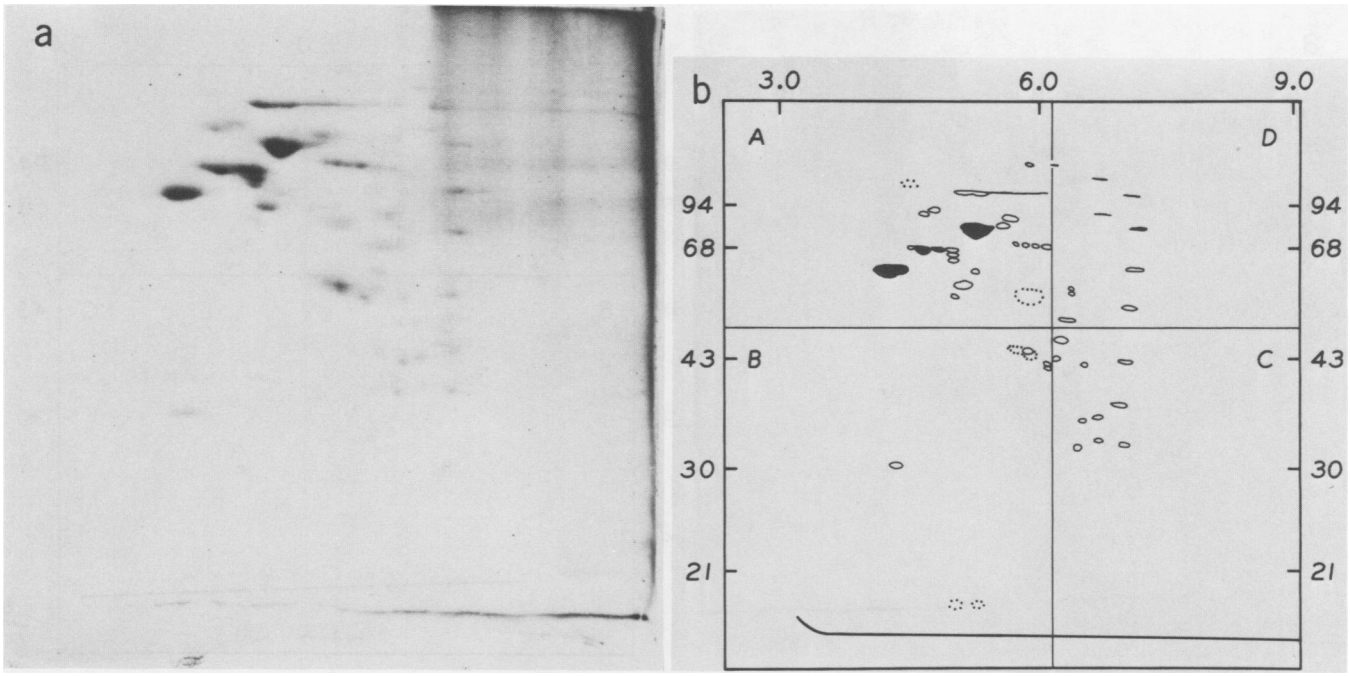

FIG. 5. Two-dimensional electrophoretogram of polypeptides solubilized from vesicles sedimenting at a 20 to 25% sucrose interface on a complex discontinuous sucrose density gradient. a, photograph of the gel; b, tracing of the gel. See legend to Fig. 2 for explanation of tracing.

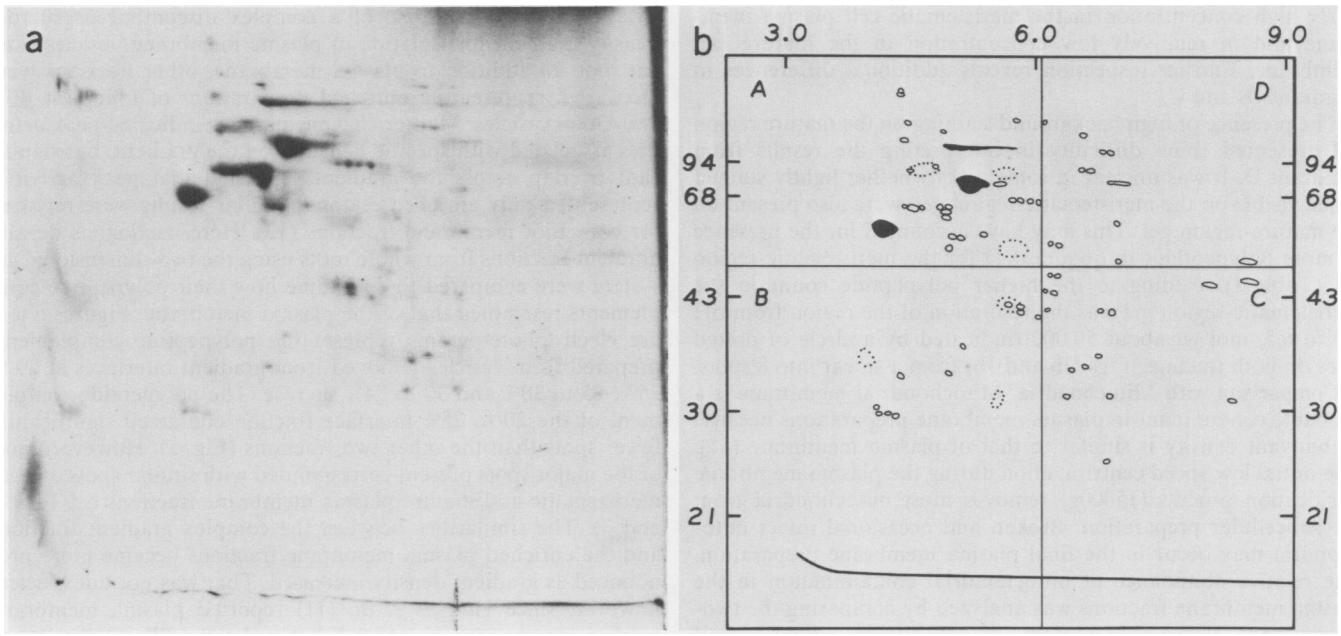

FIG. 6. Two-dimensional electrophoretogram of polypeptides from vesicles sedimenting at a 25 to 30% sucrose interface on a complex discontinuous sucrose density gradient. a, photograph of the gel; b, tracing of the gel. See legend to Fig. 2 for explanation of tracing.

membrane vesicles of mature and meristematic root tissue revealed significant and characteristic differences in the total number of polypeptides resolved from the two preparations (Figures 2 and 3). About 97 polypeptides were resolved in the mature preparation, compared with about 121 in the meristematic preparation. Polypeptides are organized by gel quadrant in Table I. Also given are pH range, approximate mol wt, and total number of spots in each quadrant.

Comparison of the electrophoretograms of plasma membrane proteins prepared from mature and meristematic tissue also revealed qualitative differences between the polypeptide complements. Small regions of each quadrant (noted by vertical brackets, Figs. 2b and 3b) are compared. For example, quadrant A of the mature gel contained four polypeptides of mol wt greater than 94,000, pI 5.0 to 5.3 (brackets), only one of which was common to the meristematic gel. Close inspection of the gel photographs and tracings reveals several other differences in both the composition of the polypeptide complement and the relative intensity of staining of specific polypeptides within quadrant A. Several polypeptides present in quadrant A of the meristematic gel were absent from the mature gel, noted by the dotted ovals on the mature gel tracing. The two polypeptides at pI 5.5 to 5.7 and mol wt about 55,000 and 65,000 stained very intensely on the meristematic region gel and very lightly on the mature region gel (compare

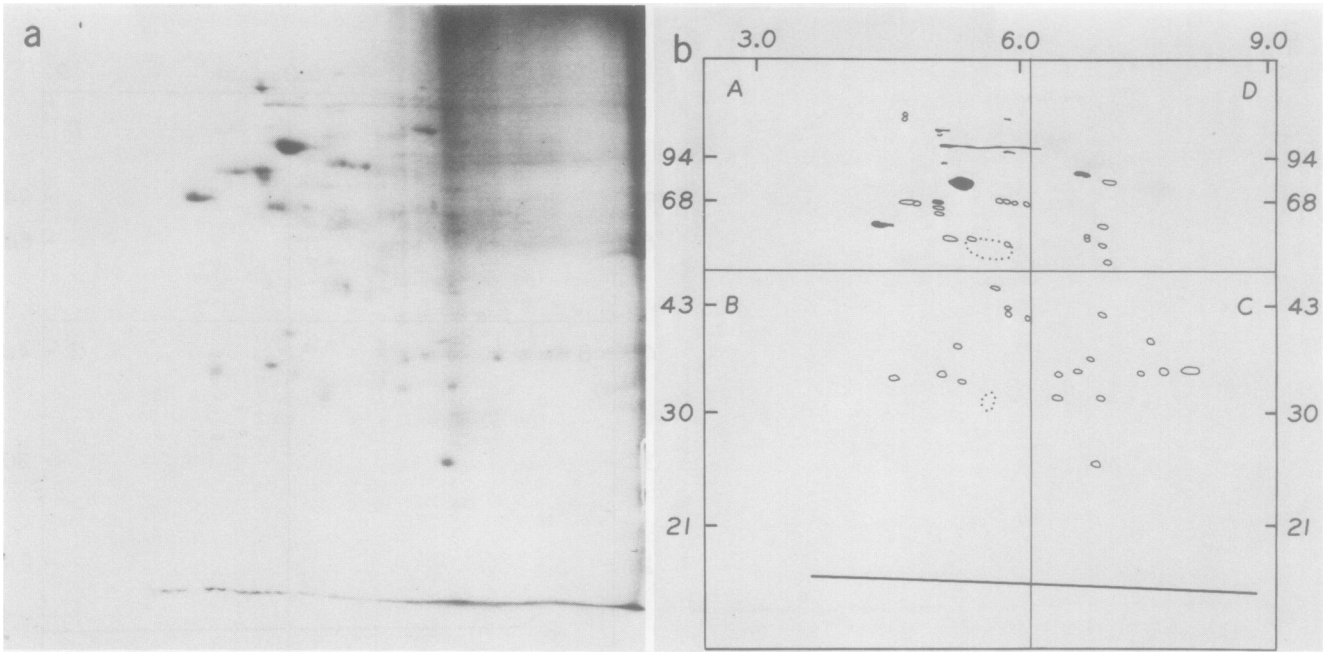

FIG. 7. Two-dimensional electrophoretogram of polypeptides from vesicles sedimenting at a 30 to 34% sucrose interface on a complex discontinuoussucrose density gradient. a, photograph of the gel; b, tracing of the gel. See legend to Fig. 2 for explanation of tracing.

Figs. 2a 3a, 2b, and 3b) and may represent proteins that occur in fairly high concentration in the meristematic cell plasma membrane and in relatively low concentration in the mature cell membrane. Further inspection reveals additional differences in quadrants B and C.

The presence of high background staining on the mature region gel presented some difficulty in interpreting the results from quadrant D. It was unclear in some cases whether lightly stained spots, visible on the meristematic-region gel, were also present on the mature-region gel. This may have accounted for the presence of more polypeptides in quadrant D for the meristematic-region gel (Table I). Adding to the higher polypeptide count in the meristematic-region gel was the resolution of the region from pH 6.0 to 6.5, mol wt about 50,000 (indicated by a circle of dotted lines on both tracings, Figs. 2b and 3b) from a smear into 8 spots.

Comparison with Mitochondria. Mitochondrial membrane is a probable contaminant in plasma membrane preparations because its buoyant density is similar to that of plasma membrane (11). The initial low speed centrifugation during the plasma membrane purification process (15000*g*) removes most mitochondria from the subcellular preparation. Broken and occasional intact mitochondria may occur in the final plasma membrane preparation. The relative abundance of mitochondrial contamination in the plasma membrane fractions was analyzed by comparing the two-dimensional electrophoretogram of solubilized mitochondrial polypeptides with the plasma membrane gels (Fig. 4 *versus* Figs. 2a and 3a). The comparison revealed little or no correspondence of major spots for the two membrane types. For example, the mitochondrial gel contained a large group of spots in the neutral pH, mid mol wt region of the gel, which stained as intensely as any other spots on the gel, yet no such spots of similar intensity were visible on either of the plasma membrane gels. If there were significant contamination by mitochondrial membranes in the plasma membrane fractions, then such spots should stain more intensely on the plasma membrane gels. Some of the faintly stained spots in this region, or elsewhere on the gels, may represent proteins from contaminating membranes. The results corroborate previously published electron microscope studies (3, 23) showing a relatively low degree of mitochondrial contamination of plasma membrane preparations isolated by the procedure used.

Comparison of Complex Gradient Fractions. Hodges *et al.* (11, 14) first reported the use of a complex discontinuous sucrose density gradient for isolation of plasma membrane vesicles from oat root. In addition to plasma membrane, other fractions were recovered, representing enriched preparations of tonoplast, ER, and Golgi vesicles. Marker enzyme analyses indicated peak activities associated with specific densities on the gradient, but significant overlap across the gradient suggested that peak activities represented only enriched regions. Similar results were reported for corn root membrane fractions (13). Here, analogous density gradient fractions from whole roots using the two-dimensional gel system were compared to determine how their polypeptide complements resembled that of the plasma membrane. Figures 5 to 7 are electrophoretograms representing polypeptide complements prepared from vesicles removed from gradient interfaces at 20 to 25%, 25 to 30% and 30 to 34% sucrose. The polypeptide complement of the 20 to 25% interface fraction contained significantly fewer spots than the other two fractions (Fig. 5). However, most of the major spots present corresponded with similar spots of both meristematic and mature plasma membrane fractions (*cf.* Figs. 2 and 3). The similarities between the complex gradient fractions and the enriched plasma membrane fractions became more pronounced as gradient density increased. That was not unexpected, however, since Hodges *et al.* (11) reported plasma membrane marker enzyme activity (pH 6, K^+-stimulated ATPase) distributed broadly across the gradient, with peak activity at higher density interfaces. Thus, the assessment that the fractions recovered from the lower density regions of the complex gradient represent fractions enriched for the various membrane types is quite reasonable. It appears that a major contaminant in those fractions was plasma membrane.

The dynamics of cell development suggest that there should be some correspondence between electrophoretograms of even highly purified membranes. Intermediates in the pathway of synthesis of plasma membrane may include nuclear, ER, and dictyosome components of the endomembrane system (18). This phenomenon may account for some of the correspondence of polypeptides on the complex gradient gels with plasma membrane gels. There are also characteristic differences between the density gradient interface electrophoretograms and plasma membrane electrophoreto-

grams. For example, one polypeptide at about pH 4.8, mol wt 55,000, stained very heavily in the lightest density-gradient fraction, and then, as the fractions increased in density, staining intensity decreased. Since plasma membrane is resolved at higher densities, one would expect an increase in the intensity of this spot if it represented a plasma membrane protein.

DISCUSSION

Electrophoretic analyses of the polypeptides solubilized from the plasma membrane of developing soybean roots revealed differences in both the total number of polypeptides resolved and the relative concentration of certain polypeptides. Some of these differences undoubtedly relate to the differential effect of the solubilization and electrophoresis procedures. Also, factors such as chemical modification (*e.g.* glycosylation), high background staining in quadrant D of the mature membrane gel and difficulty in resolving equivalent levels of protein on compared gels may have affected the total number of polypeptides resolved.

The potential for the contribution of polypeptides to the plasma membrane complement by contaminating membrane fractions cannot be overlooked. However, we believe this is not a major factor in interpreting the data. Results reported here and in a previous communication (23) suggest a relatively low level of mitochondrial contamination. Even so, other components of the cellular membrane complex are undoubtedly present as contaminating material. Polypeptides representing contaminating membranes likely consist of two major types, those which are common to most or all membrane components and those which are unique to a given membrane. The presence of common polypeptides, even from contaminating membranes, would not affect quantitative comparisons. The most likely effect would be a slight increase in intensity of staining of specific spots. The presence of proteins unique to membranes other than the plasma membrane would affect qualitative comparison; however, with plasma membrane purity levels approaching 80% (23), the intensity of spots representing such polypeptides would be greatly reduced. Thus, it is likely that some of the observed differences in protein complements of enriched plasma membrane fractions from meristematic and mature root tissue reflect developmental changes. The disappearance of certain proteins during development, with the subsequent appearance of others may reflect a changing pattern of physiological activity as the cell matures.

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LITERATURE CITED

1. AMES GF-L, K NIKAIDO 1976 Two-dimensional gel electrophoresis of membrane proteins. Biochemistry 15: 616–623
2. ANDERSON L, NG ANDERSON 1977 High resolution two-dimensional electrophoresis of human plasma proteins. Proc Natl Acad Sci USA 74: 5421–5425
3. BERKOWITZ RL, RL TRAVIS 1979 An electron microscope comparison of plasma membrane vesicles from meristematic and mature soybean root tissue. Plant Physiol 63: 1191–1197
4. Bio-Rad Laboratories Technical Bulletin 105 1977 Bio-Rad Protein Assay. Bio-Rad Laboratories, Richmond, Calif
5. BIRCKBICHLER PJ, RM DOWBEN, S MATACIC, AG LOEWY 1973 Isopeptide bands in membrane proteins from eukaryotic cells. Biochim Biophys Acta 291: 149–155
6. BONNER WD 1967 A general method for the preparation of plant mitochondria. Methods Enzymol 10: 126–133
7. CRIBBS DA, DA STELZIG 1978 Labeling, isolation and characterization of plasma membrane proteins from potato leaf protoplasts. Plant Physiol 61: S–113
8. FAIRBANKS G, TL STECK, DFH WALLACH 1971 Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry 10: 2606–2617
9. HALL JL, RM ROBERTS 1975 Biochemical characterization of membrane fractions isolated from maize (*Zea mays* L.) roots. Ann Bot 39: 983–993
10. HODGES TK, RT LEONARD 1974 Purification of a plasma membrane bound ATPase from plant roots. Methods Enzymol 32: 392–406
11. HODGES TK, RT LEONARD, CE BRACKER, TW KEENAN 1972 Purification of an ion-stimulated adenosine triphosphatase from plant roots: association of plasma membranes. Proc Natl Acad Sci USA 69: 3307–3311
12. LAEMMLI UK, M FAVRE 1973 Maturation of the head of bacteriophage T4. I. DNA packaging events. J Mol Biol 80: 575–599
13. LEONARD RJ, WJ VANDERWOUDE 1976 Isolation of plasma membranes from corn roots by sucrose density gradient centrifugation. An anomolous effect of Ficoll. Plant Physiol 57: 105–114
14. LEONARD RT, D HANSEN, TK HODGES 1973 Membrane-bound adenosine triphosphatase activities of oat roots. Plant Physiol 51: 749–754
15. LOWRY OH, NJ ROSEBROUGH, AL FARR, RJ RANDALL 1951 Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275
16. MATHEWS RA, TC JOHNSON, JE HUDSON 1976 Synthesis and turnover of plasma membrane proteins and glycoproteins in a neuroblastoma cell line. Biochem J 154: 57–64
17. MINSSEN M, KD MUNKRES 1973 Preparation of mitochondrial membrane proteins from *Neurospora crassa*: prevention of lipid autoxidation damage by an antioxidant. Biochim Biophys Acta 291: 398–410
18. MORRE DJ, HH MOLLENHAUER 1974 The endomembrane concept: a functional integration of endoplasmic reticulum and Golgi apparatus. *In* AW Robards, ed, Dynamic Aspects of Plant Ultrastructure. McGraw-Hill, New York, pp 84–137
19. NAGAHASHI G, RT LEONARD, WW THOMSON 1978 Purification of plasma membranes from roots of barley. Specificity of the phosphotungstic acid-chromic acid stain. Plant Physiol 61: 993–999
20. NEVILLE DM, H GLOSSMANN 1971 Plasma membrane protein subunit composition: a comparative study by discontinuous electrophoresis in sodium dodecyl sulfate. J Biol chem 246: 6335–6338
21. O'FARRELL PH 1975 High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250: 4007–4021
22. SIU CH, RA LERNER, WF LOOMIS JR 1977 Rapid accumulation and disappearance of plasma membrane proteins during development of wild-type and mutant strains of *Dictyostelium discoideum*. J Mol Biol 116: 469–488
23. TRAVIS RL, RL BERKOWITZ 1980 Characterization of soybean root plasma membrane during development: free sterol composition and concanavalin A binding studies. Plant Physiol 65: 871–879
24. TRAVIS RL, ML BOOZ 1979 Partial characterization of a potassium stimulated adenosine triphosphatase from the plasma membrane of meristematic and mature soybean root tissues. Plant Physiol 63: 573–577
25. TWETO J, D DOYLE 1976 Turnover of the plasma membrane proteins of hepatoma tissue culture cells. J Biol Chem 251: 872–882
26. YUNGHANS WN, DJ MOORE, JH CHERRY 1973 Separation of plant membrane proteins by ion exchange chromatography. Proc Indiana Acad Sci 82: 134–136