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TWO-DIMENSIONAL ELECTROPHORESIS OF SOYBEAN ROOT PLASMA MEMBRANE PROTEINS SOLUBILIZED BY SDS AND OTHER DETERGENTS

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Key Word Index—*Glycine max*; Leguminosae; soybean; plasma membrane proteins; two-dimensional electrophoresis; membrane protein solubilization; selected detergents.

Abstract—Proteins solubilized from enriched soybean root plasma membrane with sodium dodecyl sulphate (SDS) and selected non-denaturing detergents (octyl-β-D-glucopyranoside, Zwittergent 312, Zwittergent 314, Zonyl FSK, and Nonidet P-40) were electrophoresed in two-dimensions by standard procedures. The basic electrophoretogram 'fingerprint' was similar for all detergents tested. However, differences in the total number of polypeptides resolved and the presence or absence of certain polypeptides on specific two-dimensional gels indicated some selectivity. Of all detergents tested, SDS solubilized the most polypeptides (*ca* 95) and provided the best resolution. The other detergents solubilized 50–80 polypeptides with varying resolution. Of those tested, octyl-β-D-glucopyranoside consistently provided the best balance between the number of polypeptides resolved (*ca* 70) and the level of resolution. The results suggest that selected detergents may prove useful in plant plasma membrane studies which require non-denaturing conditions.

INTRODUCTION

Despite considerable study on the nature of the plasma membrane of higher plants, little information is available regarding the molecular bases of the numerous physiological and/or biochemical functions of this important organelle. The nature of plasma membrane proteins has contributed to that situation. One reason for this is the intractable nature of plant plasma membrane proteins which makes them unamenable to analysis by standard biochemical techniques. Solubilization with various detergents, including SDS* [1–4], combined with one-dimensional [5–7] or two-dimensional polyacrylamide gel electrophoresis [8–10] has proved useful in a wide variety of microbial and mammalian studies; however, until recently [11], those techniques have generally not been successful with higher plant plasma membrane proteins.

Initial attempts at resolving SDS-solubilized soybean plasma membrane proteins by standard two-dimensional gel electrophoretic methods were hampered by considerable streaking and smearing and loss of resolution over the entire second dimension gel [11]. The lack of available information on plant plasma membrane proteins makes it difficult to assess why that problem occurred. However, the extensive mammalian literature does provide insight into some possible difficulties. For example, the high insolubility of some SDS-solubilized proteins may result in aggregation and precipitation during electrophoresis [10]. The tendency of membrane components to autoxidize, with resultant crosslinking [12] may also affect electrophoretic resolution. Also, specific proteins may undergo chemical modification during solubilization [13, 14]. While there is no evidence to support the existence of these problems in higher plant plasma membrane studies, their occurrence is certainly a possibility. Many of the difficulties associated with solubilization and electrophoresis were overcome in the soybean root plasma membrane study by maintaining high ionic strength and incorporating appropriate protective agents in the vesicle isolation, solubilization and electrophoresis buffers [11].

Although SDS can effectively solubilize plasma membrane proteins ([15], and as demonstrated in the soybean root system [11]), it has the disadvantage of denaturing most membrane protein activities. Accordingly, the effectiveness of alternative, non-denaturing detergents was compared. All of the detergents selected (Fig. 1) carry no net charge, as compared to the anionic nature of SDS, but they differ either in the chemical nature of the head group or in the nature of the non-polar detergent 'tail'. Thus, the potential for selective solubilization (e.g. the number of polypeptides solubilized, and/or whether certain polypeptides may have a chemical affinity for a particular detergent head group) was tested. Selective solubilization has been widely applied in the analysis of microbial and animal membrane proteins [16–21], but it has been used only for the analysis of chlorophyll–protein complexes from photosynthetic lamellae in plants [22–25].

* Abbreviations: SDS, sodium dodecyl sulfate; MES, 2-(*N*-morpholino)ethanesulfonic acid; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; EGTA, ethyleneglycol-bis-(β-aminoethyl ether)*N*,*N*′-tetraacetic acid; CMC, critical micelle concentration.

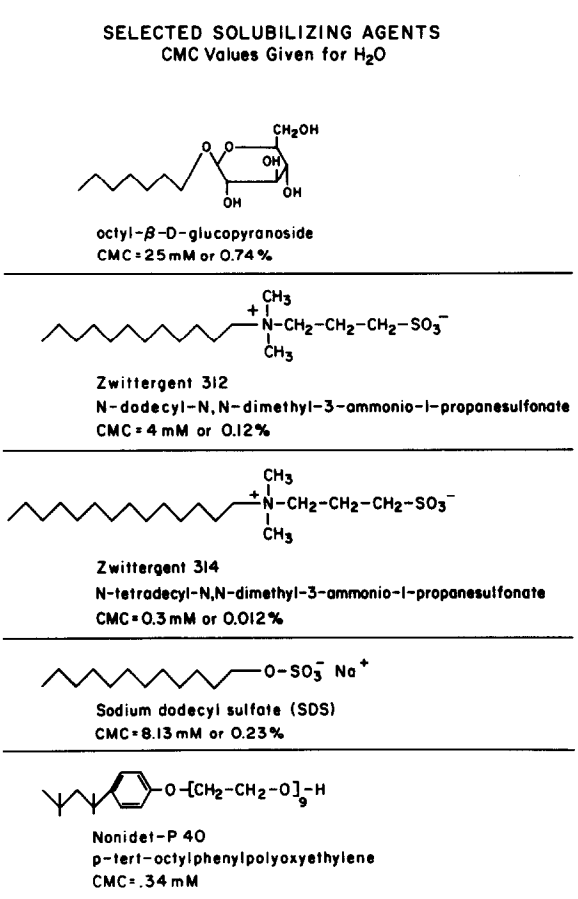

Fig. 1. Detergents used to solubilize proteins of soybean root plasma membrane vesicles. The detergent head groups are of three types: anionic, zwitterionic and non-ionic. The zwitterionic detergent head groups are of two different types, as are the non-ionic detergent head groups. The non-polar regions of the detergents also differ considerably. The CMCs in water are noted below each detergent. Structure for Zonyl FSK not available.

We report here the solubilization with selected non-denaturing detergents and subsequent resolution by two-dimensional gel electrophoresis of *ca* 50–80 polypeptides from enriched plasma membrane vesicles isolated from whole roots. The resolution of *ca* 95 polypeptides solubilized with SDS is included for comparison. The basic pattern or 'fingerprint' of the two-dimensional gels was similar for all detergents, including SDS. Variation in the total number of polypeptides solubilized and minor differences in the two-dimensional electrophoretograms indicated some selectivity in solubilization among the detergents. A preliminary account of this work has been presented [26].

RESULTS

Solubilization with SDS followed by two-dimensional gel electrophoresis resolved *ca* 95 polypeptides from an enriched preparation of soybean whole root plasma membrane vesicles (Fig. 2a). To facilitate interpretation the gel was traced and divided into quadrants (Fig. 2b). The distribution pattern or 'fingerprint' of the major polypeptides resolved was characteristic, with minor variations of SDS and also of all other detergents tested (cf. Figs. 2a, 3c and 4c). That similarity indicated that SDS did not remain bound to the solubilized polypeptides in any significant quantity during isoelectric focusing and did not alter the pattern obtained on the second dimension gel.

Polypeptides were solubilized with several detergents to test for selective solubilization and to determine whether a non-denaturing alternative to SDS would show improved resolution and/or reduction in background staining. Concentrations of two detergents, octyl-β-D-glucopyranoside and Zwittergent 312, were varied from below the CMC (0.05%, Figs. 3a and 4a) to far in excess of it (1.0%, Figs. 3c and 4c). As the detergent concentration was increased, more protein was solubilized from equal amounts of membrane vesicles (350 μg membrane protein), but the basic electrophoretic pattern obtained was independent of detergent concentration. Although increasing the detergent concentration beyond 1% solubilized more total protein, no additional polypeptides were observed (electrophoretograms not shown). Table 1 compares the number of polypeptides solubilized by each concentration up to 1% of these two detergents.

Octyl-β-D-glucopyranoside, in addition to solubilizing more polypeptides, gave better resolution than Zwittergent 312 (compare Figs. 3a–c with 4a–c). For both detergents, one group of acidic, high MW polypeptides (shown in Figs. 3d and 4d) was characterized by relatively intense staining at all detergent concentrations. These appear to be the same polypeptides as many of those noted in quadrant 1 of the SDS gel (Figs. 2a, b). They were solubilized with relative ease, and relatively large amounts were removed from the membrane vesicles at low detergent concentrations.

Figs. 3f and 4f, the tracings of the 1% octyl-β-D-glucopyranoside and Zwittergent 312 gels, point out three polypeptides which were more difficult to extract (vertical brackets). With octyl-β-D-glucopyranoside these polypeptides were not solubilized until the detergent concentration approached the CMC (cf. Figs. 3a and 3b). However, they were faintly visible in the 0.05% Zwittergent 312 gel (Fig. 4a). These results suggest that some degree of selectivity is shown between these detergents. Additional evidence of selectivity is provided by careful analysis of the gels. For example, one polypeptide, which was soluble in SDS and octyl-β-D-glucopyranoside, was only sparingly soluble in Zwittergent 312 (enclosed in dashed circle on the gel tracings, Figs. 2b, 3f and 4f). Similarly, several polypeptides, evident at pH range, 5.8–7.0 and MW range 35 000–75 000 (Figs. 3c and 3f) were solubilized by octyl-β-D-glucopyranoside, but did not appear in the Zwittergent 312 gel (Figs. 4c and 4f). They are noted in the large horizontal brackets on the gel tracings. Other polypeptides appear in this region on the Zwittergent 312 gel, but they are very faint and obscured by the background staining.

Compared to SDS, which solubilized *ca* 95 polypeptides from whole root plasma membrane vesicles, octyl-β-D-glucopyranoside and Zwittergent 312 solubilized *ca* 71 and 50 polypeptides, respectively. The major difference between SDS and those detergents appeared to be in the larger number of polypeptides present in quadrant 3 of the SDS gel (Fig. 2a vs 3c and 4c). However, solubilization with SDS also provided the best resolution on the two-dimensional slab gels. Three other detergents, Zwittergent 314 (83 polypeptides resolved), Nonidet P-40

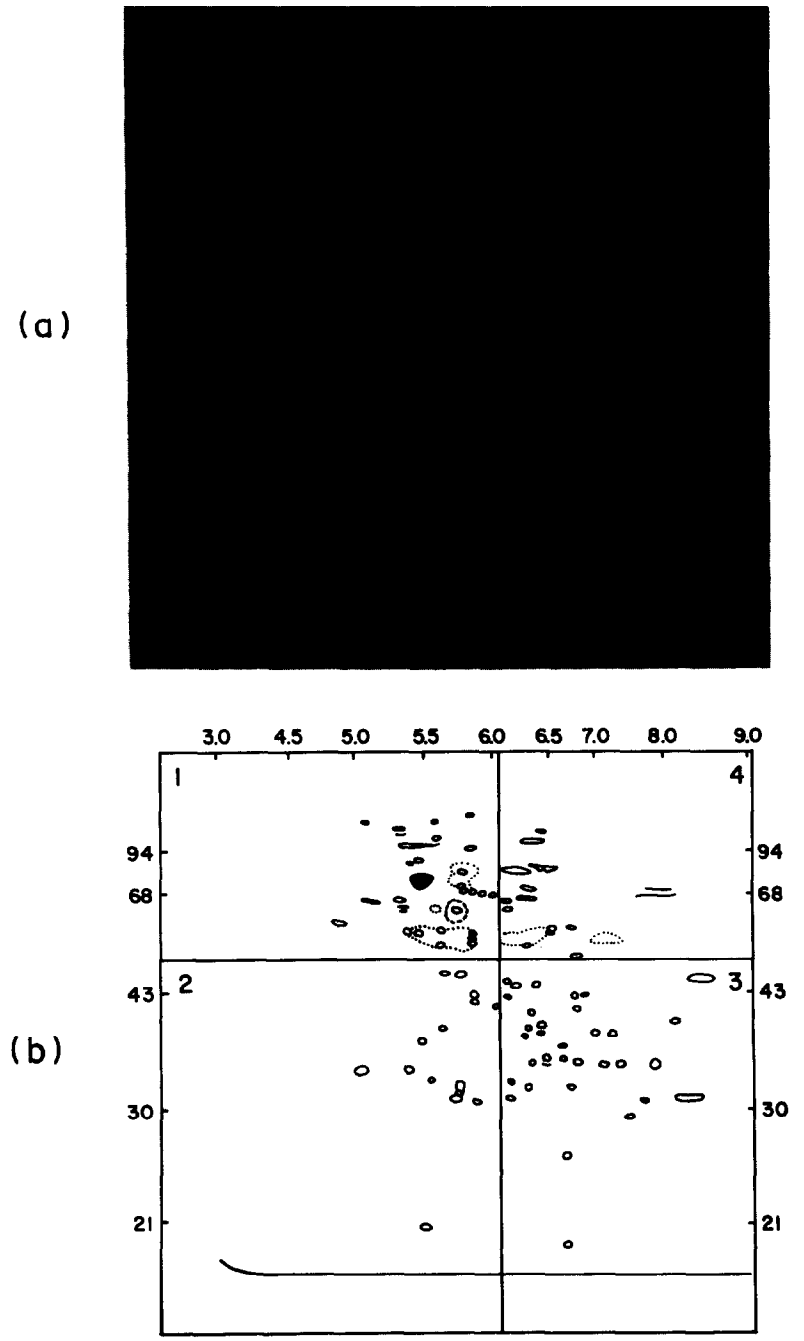

Fig. 2. Two-dimensional electrophoretogram of polypeptides solubilized by SDS from soybean root plasma membrane vesicles. Solubilized polypeptides were subjected to isoelectric focusing in the first dimension followed by SDS-polyacrylamide gel electrophoresis in the second dimension. (a) Photograph of the gel; (b) tracing of the gel, divided into quadrants, with the measured pH gradient noted on the horizontal axis and the MW values $\times 10^{-3}$ on the vertical axis. About 95 polypeptides were resolved. The dashed circle outlines a polypeptide soluble in SDS and octyl-β-D-glucopyranoside (cf. Fig. 3c) but only sparingly soluble in Zwittergent 312 (cf. Fig. 4c).

(81 polypeptides) and Zonyl FSK (52 polypeptides), were tested for comparison (data not shown). The basic electrophoretogram pattern for each detergent was similar, with only minor variations, to those reported here. Of all non-denaturing detergents tested, octyl-β-D-glucopyranoside consistently provided the best balance between the number of polypeptides resolved and the level of background staining in the neutral pH, high MW region of the slab gel.

DISCUSSION

Mammalian membrane proteins have been selectively solubilized by varying detergent type and concentration

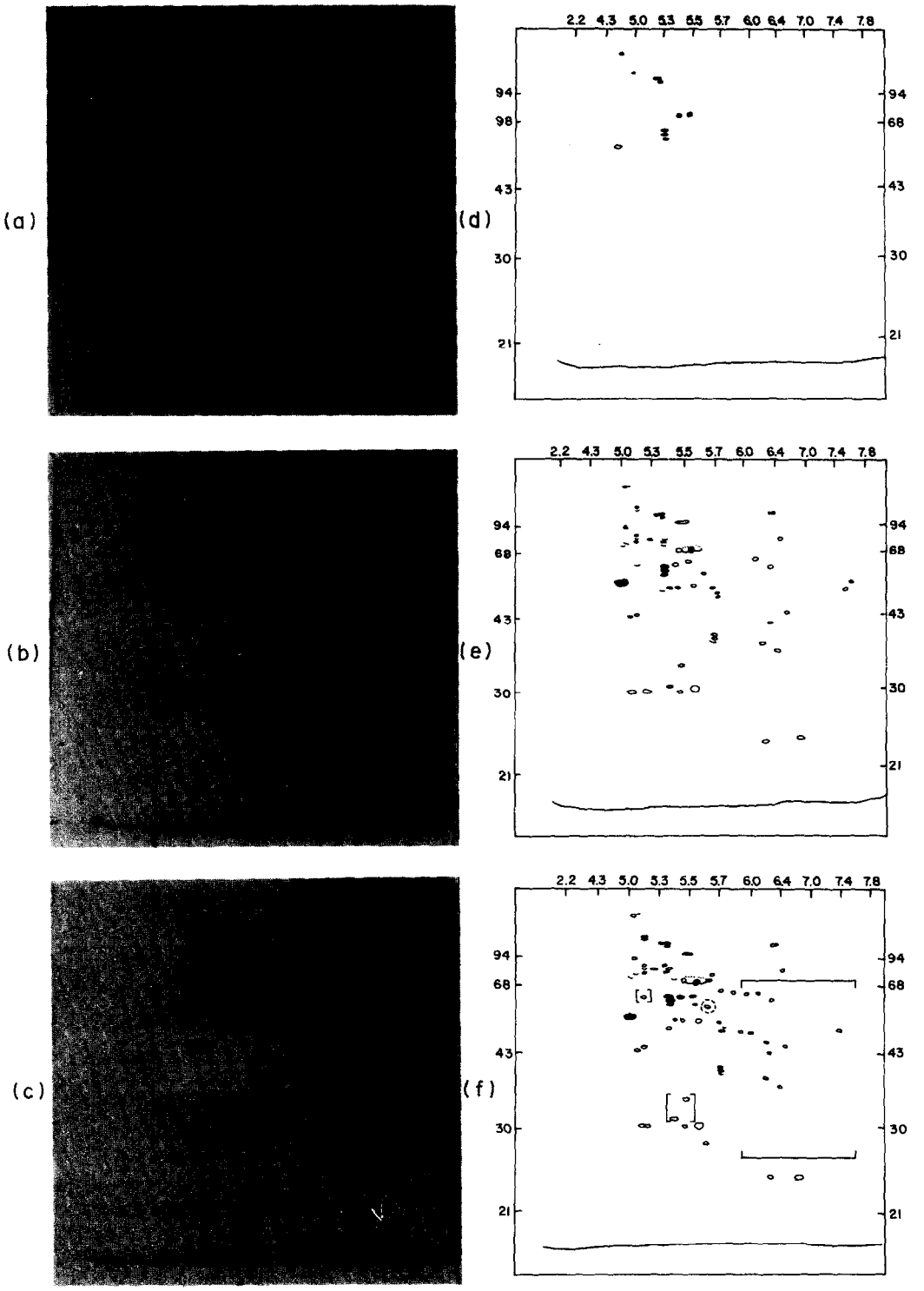

Fig. 3. Whole root plasma membrane polypeptides solubilized by three concentrations of octyl-β-D-glucopyranoside. Solubilized polypeptides were subjected to isoelectric focusing in the first dimension followed by SDS-polyacrylamide gel electrophoresis in the second dimension. (a) 0.05% gel, below the CMC; (b) 0.5% gel, at about the CMC; (c) 1.0% gel, far above the CMC; (d) tracing of the 0.05% gel, only the easily solubilized polypeptides are shown; (e) tracing of the 0.5% gel; (f) tracing of the 1.0% gel. The measured pH gradients are noted on the horizontal axis and the approximate polypeptide MWs × 10^{-3} are on the vertical axis. In (f) the vertical brackets indicate polypeptides which were solubilized only at high detergent concentrations. The dashed circle denotes a polypeptide soluble in octyl-β-D-glucopyranoside and SDS but which was only slightly soluble in Zwittergent 312 (cf Figs. 2a, 3c and 4c). The large horizontal brackets indicate a region containing octyl-β-D-glucopyranoside-solubilized polypeptides not observed on the Zwittergent gel.

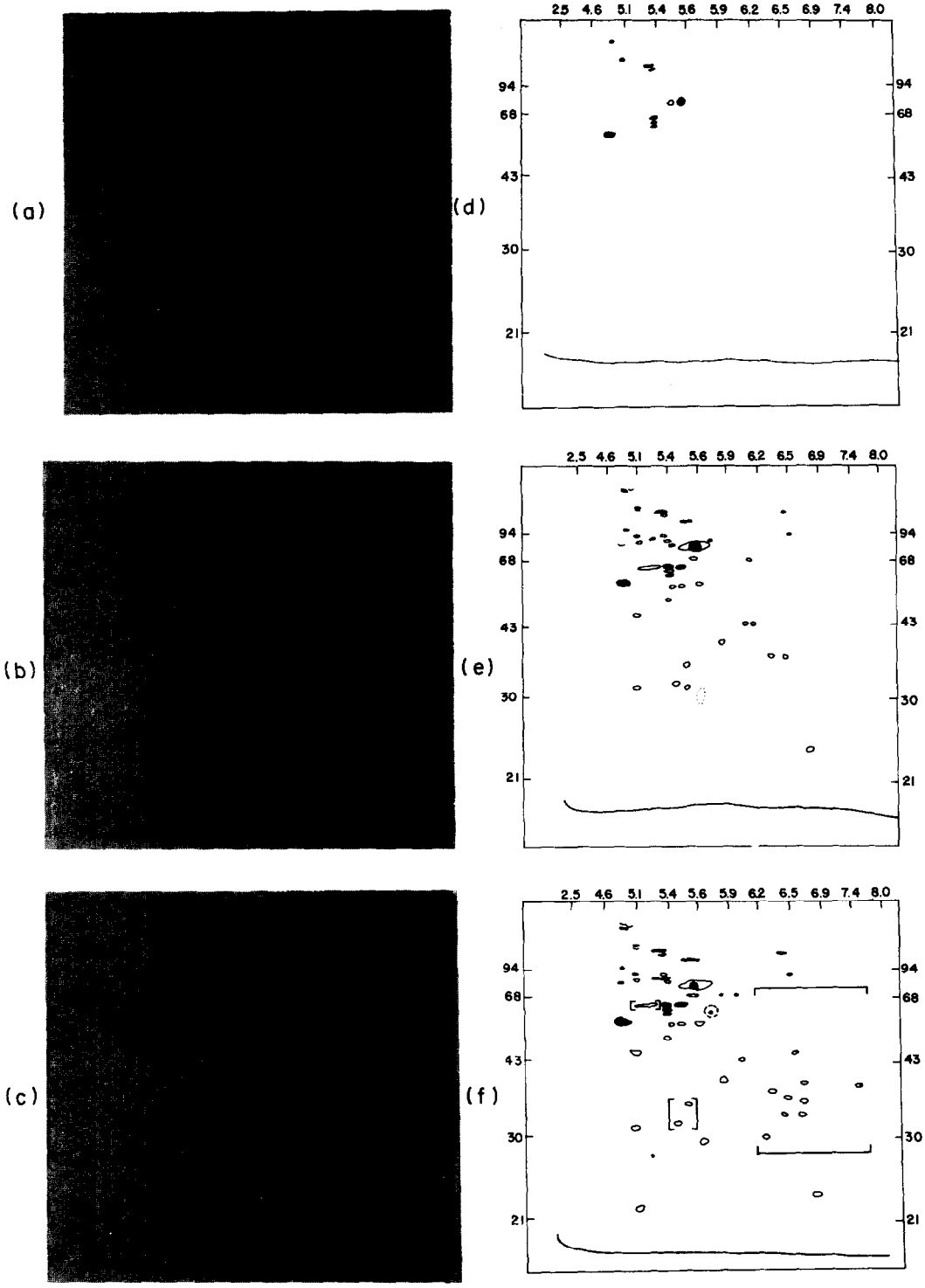

Fig. 4. Whole root plasma membrane polypeptides solubilized by three concentrations of Zwittergent 312. (a) 0.05% gel, below the CMC; (b) 0.5% gel, just above the CMC; (c) 1.0% gel, far above the CMC; (d) tracing of the 0.05% gel, only the easily solubilized polypeptides are shown; (e) tracing of the 0.5% gel; (f) tracing of the 1.0% gel. See legend to Fig. 3 for further details.

[15, 16, 20]. However, technical difficulties have precluded similar studies with higher plant plasma membrane proteins until the recent development of a solubilization medium and two-dimensional gel electrophoresis system for soybean root plasma membrane proteins [11]. That study was expanded to consider the potential for selective solubilization by other detergents, and to determine whether plasma membrane proteins could be effectively solubilized by non-denaturing detergents.

Table 1. Number of polypeptides solubilized from enriched soybean root plasma membrane vesicles

Detergent	Concentration (%) 0.05	0.5	1.0
Octyl-β-D-glucopyranoside	50	61	71
Zwittergent 312	42	43	50

Solubilized polypeptides were subjected to isoelectric focusing in the first dimension followed by SDS-polyacrylamide gel electrophoresis in the second dimension.

Some selective solubilization was demonstrated; however, most examples of selectivity consisted of relatively minor differences between the observed gel patterns. Two factors likely contributed to this result. The high ionic strength of the solubilization buffer may have hindered the selective effect of a particular detergent concentration or type of detergent. In order to obtain adequate resolution, the buffer contained 0.375 M Tris–Cl. Preliminary work with a 0.015 M Tris–Cl solubilization buffer suggested that a higher degree of selective solubilization may be possible, but the results were not conclusive. Secondly, the two-dimensional gel electrophoresis system is very sensitive to low levels of protein. Thus, polypeptides with low solubility in a particular solubilization buffer may be released in high enough concentration to stain on the second dimension slab gel, thus obscuring the selective effect.

Although some selective solubilization was evident, the similarity of the polypeptide patterns obtained with all detergents suggests that plasma membrane polypeptides can be effectively solubilized with detergents other than SDS. Thus, alternative detergents may prove useful in plant plasma membrane studies which require non-denaturing conditions.

EXPERIMENTAL

Plasma membrane vesicles were isolated from whole roots of 4-day-old, dark-grown soybean seedlings (*Glycine max* (L.) Merr. cult. Wells or Wells II) by differential and discontinuous sucrose density gradient centrifugation, according to the procedures outlined in ref. [11] except that Tris–MES was used in place of Tris–HEPES, and EGTA–Tris (pH 6.8) was used in place of Na_2EDTA. The concns of each in the grinding medium were as in ref. [11], but the EGTA–Tris was lowered to 10 μM in all other solns including the solubilization buffers.

Plasma membrane polypeptides were solubilized with SDS or other detergents and electrophoresed in two dimensions as described in ref. [11], a procedure based on refs. [8] and [9]. Vesicles were washed by centrifugation at 100 000 *g* (Spinco T-65 rotor) with an equal vol. of 0.4 M NaCl buffer [11], so that the final concns of components in the wash were 0.2 M NaCl, 10 mM Tris–MES (pH 7.2), 1 mM dithiothreitol, 10 μM EGTA and 100 μg/ml butylated hydroxytoluene. The proper amount of 1% or 10% detergent was added to equal aliquots of pelleted plasma membrane vesicles. Solubilization buffer with no detergent (0.375 M Tris–Cl, (pH 8.9), 1 mM dithiothreitol, 10 μM EGTA and 100 μg/ml butylated hydroxytoluene) was added to 200 μl and the vesicles were suspended by stirring with a glass rod. The prepns were held at room temp. for 1 hr before centrifugation at 100 000 *g* for 30 min to remove the membrane residue. The entire amount of solubilized protein was diluted with 1 vol. of dilution buffer [11] and applied to the isoelectric focusing gels. For SDS-solubilized proteins focusing was carried out for 16 hr at 300 V plus 1 hr at 400 V. For polypeptides solubilized with other detergents the voltage was held at 300 V for 2 hr and then increased to 500 V for 22 hr. The increase in V·hr for these solubilization buffers was necessary to complete the isoelectric focusing. Otherwise the proteins did not migrate to the bottom (acidic) end of the tube gel.

Determination of the pH gradients in the isoelectric focusing gels, equilibration of the focusing gels, SDS slab gel electrophoresis, staining, destaining and drying were as in ref. [11]. Protein was determined according to the procedures of ref. [27].

The detergents used in this study were: SDS, electrophoresis quality from Bio-Rad.; Nonidet P-40 from Bethesda Research Labs.; Zwittergent 312, Zwittergent 314 and octyl-β-D-glucopyranoside from Calbiochem; and Zonyl FSK, a perfluoroalkyl-substituted betaine, a generous gift from duPont Chemicals, Dyes and Pigments Department, Wilmington, Delaware. All electrophoresis chemicals were electrophoresis quality from Bio-Rad. Tris, MES and HEPES buffers were Ultrol grade from Calbiochem. All other chemicals were reagent grade.

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