Plant Physiol. (1982) 70, 1429–1435
0032-0889/82/70/1429/07/$00.50/0

# Plasma Membranes from Oats Prepared by Partition in an Aqueous Polymer Two-Phase System[1]

## ON THE USE OF LIGHT-INDUCED CYTOCHROME *b* REDUCTION AS A MARKER FOR THE PLASMA MEMBRANE

Received for publication March 8, 1982 and in revised form July 21, 1982

SUSANNE WIDELL, TOMAS LUNDBORG, AND CHRISTER LARSSON
*Department of Plant Physiology, University of Lund, S-220 07 Lund, Sweden (S. W., T. L.), and Department of Biochemistry, University of Lund, S-220 07 Lund, Sweden (C. L.)*



### ABSTRACT

**Presumptive plasma membrane fractions have been prepared from oat (*Avena sativa* L. cv. Brighton) roots and shoots, respectively, by partition of microsomal fractions in a dextran-polyethylene glycol two-phase system. The plasma membranes had a high affinity for the polyethylene glycol-rich upper phase, whereas membranes from mitochondria and other organelles partitioned in the dextran-rich lower phase or at the interface. Thus, relatively pure plasma membranes were obtained by only two partition steps, and within 3 hours from homogenization of the material.**

**The plasma membranes from both organs were enriched in $K^+$-stimulated $Mg^{2+}$-dependent ATPase and glucan synthetase II, two tentative markers for the plant plasma membrane. Silicotungstic acid, an indicative stain for the plasma membrane, stained the vesicles recovered from the upper phase, but failed to stain the membranes partitioning in the lower phase or at the interface.**

**The plasma membranes were also enriched in a light-reducible *b*-cytochrome. This *b*-cytochrome can be measured by its light-induced absorbance change and may serve as a marker for the plant plasma membrane.**

There is an increasing interest in the regulating and synthesizing activities of the plasma membrane. Most of the biochemical studies on this membrane are performed with postmitochondrial fractions either used directly or after further fractionation on density gradients (*e.g.* 9, 14, 15, 19, 24, 31). However, the enrichment of plasma membranes in these fractions has been uncertain due to the lack of a reliable plasma membrane marker, and the maximal reported purities are 75 to 80% presumptive plasma membranes (for review, see Ref. 23).

Different markers have been proposed for the plant plasma membrane: $K^+$-stimulated $Mg^{2+}$-dependent ATPase assayed at pH 6.0 (15) or 6.5 (19), glucan synthetase II (24, 31), phosphotungstic or silicotungstic acid staining (26, 27), and naphthylphthalamic acid binding (13). A blue light-induced absorbance change indicating reduction of a membrane bound *b*-Cyt (and with a possible connection to blue light physiology) has been proposed to be located in plasma membranes (6). It has also been shown that red light and methylene blue can be used as substitutes for blue light and endogenous photoreceptor, respectively (7).

We have recently obtained a presumptive plasma membrane fraction with very low contamination of mitochondria and ER (20, 30). These plasma membranes were purified from microsomal fractions by partition in a dextran-PEG two-phase system. By this method, membrane particles are separated according to their surface properties (1, 2), and the procedure has been used for the purification of several plant organelles and membranes (for reviews, see Refs. 16 and 17). Phase partition has also been used to further purify barley root plasma membranes obtained by sucrose gradient centrifugation (8) in which case one partition step reduced the contamination of mitochondria by 65%.

With all species and tissues investigated (corn coleoptiles, corn shoots, barley shoots, cauliflower inflorescences, and wheat roots), we found that a certain part of the methylene blue-mediated LIAC[2] activity (20–50% of total) co-purified with the presumptive plasma membranes. In the plasma membrane preparation from corn coleoptiles, the *b*-Cyt related to LIAC constituted as much as 90% of total dithionite-reducible Cyt. This can be compared with a figure of about 40% for the corresponding preparation obtained by sucrose gradient centrifugation (29). The plasma membranes and the LIAC associated with it had a high affinity for the upper phase (*i.e.* had a high partition ratio) in the two-phase systems used, whereas the rest of LIAC together with mitochondria and ER were distributed to the interface plus lower phase. It was therefore suggested that LIAC in combination with a high partition ratio of the material could serve as a marker for the plasma membrane (30).

The present investigation was undertaken to characterize further our plasma membrane preparation and to determine whether LIAC could be a useful marker for the plasma membrane. We have therefore compared the distribution of LIAC and other membrane markers in different fractions obtained by phase partition. In a survey of plants, oats were found to have relatively high activities of both $K^+$-ATPase and GS II (T. Lundborg and S. Widell, unpublished). Oats were therefore chosen for this investigation. To get a comparison between different organs, both shoots and roots were used for membrane preparations.

## MATERIALS AND METHODS

**Preparation of Plant Material.** Oats (*Avena sativa* L. cv Brighton) were grown in darkness as described elsewhere for wheat (20). After a week, the seedlings were harvested and divided into shoots and roots. Sixty g of each material was homogenized with

[1] This work was supported by the Swedish Natural Science Research Council.

[2] Abbreviations: LIAC: light-induced absorbance change $\Delta$ ($A_{428} - A_{410}$ induced by 10-s red light in the presence of methylene blue; $K^+$-ATPase: $K^+$-stimulated $Mg^{2+}$-dependent ATPase; $U_3$, $L_3$, etc., see Figure 1; STA, silicotungstic acid; GS I, glucan synthetase I; GS II, glucan synthetase II.

a Sorvall Omni-Mixer in 300 ml 0.25 M sucrose, 10 mM Tris-HCl, 1 mM EDTA, pH 7.5. The homogenates were filtered through four layers of cheesecloth and were centrifuged for 15 min at 10,000*g*. The resulting supernatants were spun at 30,000*g* for 1 h, and the pellets were resuspended in 0.5 or 1.2 ml 0.25 M sucrose, 5 mM K-phosphate, pH 7.8. All preparation steps were done in green light at 5°C.

**Countercurrent Distribution.** The phase composition earlier found to be optimum for purification of wheat root plasma membranes (20) was used: 6.3% (w/w) dextran T 500, 6.3% (w/w) PEG 4000, 0.25 M sucrose, 5 mM K-phosphate, pH 7.8 (5°C). A phase system of 200 g was prepared in a separatory funnel, by weighing from stock solutions. After temperature equilibration at 5°C, the phase system was thoroughly mixed by several inversions, and was left to settle overnight in the cold room at 5°C. The lower and upper phases were collected and stored separately.

From a suspension of microsomal membranes, 0.5 ml was added to a 3.5-g phase mixture to give a 4.0-g phase system with the same final composition as above, except for the biological material. This small phase system was mixed by several inversions of the tube, and its contents were divided equally between the first three chambers of an automatic thin layer countercurrent distribution apparatus (1, 2). The other chambers received equal volumes of lower and upper phase obtained from the 200-g phase system. The phase volumes were such that the lower phase and the interface were kept stationary, and about 80% of the upper phase was transferred. (For details in handling of the apparatus, see Refs. 2 and 3.) After 57 transfers, the fractions were collected and diluted with 1 volume of preparation medium. Absorbance at 280 nm was measured on every second fraction. Then, the 60 fractions were pooled to give four main fractions (Fig. 2). These four fractions were further diluted, and the material was collected by centrifugation at 75,000*g* for 1 h. Resuspended pellets were stored frozen at −23°C until used for enzyme assays.

**Batch Procedure.** The batch procedure is outlined in Figure 1. One ml of a microsomal suspension was added to a 7.0-g phase mixture to give an 8.0-g phase system with the same final composition as above. The phase system was mixed by 20 inversions of the tube, and was spun in a swinging bucket rotor at 1000*g* for 4 min to facilitate phase settling. About 90% of the upper phase ($U_1$) was carefully removed with a Pasteur pipette without removing any material collected at the interface, and was added to a tube containing 4 ml new lower phase (obtained from the 200-g phase system described above). To the remaining lower phase, interface and 10% upper phase ($L_1$), 4 ml new upper phase was added. After mixing and phase settling, the procedure was repeated with $U_2$ and $L_2$ to produce $U_3$ and $L_3$, respectively. The "wash phases" *i.e.* $U_2L$, $UL_2$, $U_3L$, and $UL_3$, were all saved for calculation of total recovered activities. Thus, all phases were diluted with 8 volumes of preparation medium, and the material was pelleted at 75,000*g* for 1 h. Each pellet was suspended in 1 ml preparation medium, and these suspensions were stored at −23°C until used for protein determination and marker assays.

**Electron Microscopy.** Thin sections (600–700 Å) of glutaraldehyde and osmium-tetroxide-fixed membrane fractions embedded in Agar 100 epoxy resin (Agar Aids, Stansted, Essex, England) were collected on Ni-grids. The sections were stained for plasma membrane with 1% (w/v) periodic acid (15 min, 20°C) and 1% (w/v) silicotungstic acid (10 min, 37°C) at pH 3.0 according to Roland (26). Nonspecific staining of membranes was obtained with uranyl acetate and lead citrate.

**Marker Assays.** LIAC was measured as $\Delta$ ($A_{428} - A_{410}$) induced by 10-s irradiation with red light (667 nm) under saturating conditions. The assay medium contained 3 mM EDTA, 10 to 20 μM methylene blue and the sample was diluted until signal size was proportional to dilution. The actinic light was generated by a 150 w xenon lamp connected to a Bausch and Lomb high intensity monochromator (1350 grooves/mm) and equipped with 6-mm slits. A red glass filter (RG 645, 2 mm; Schott and Jena) was used to filter the light and the resulting irradiance was 10 to 15 w $m^{-2}$.

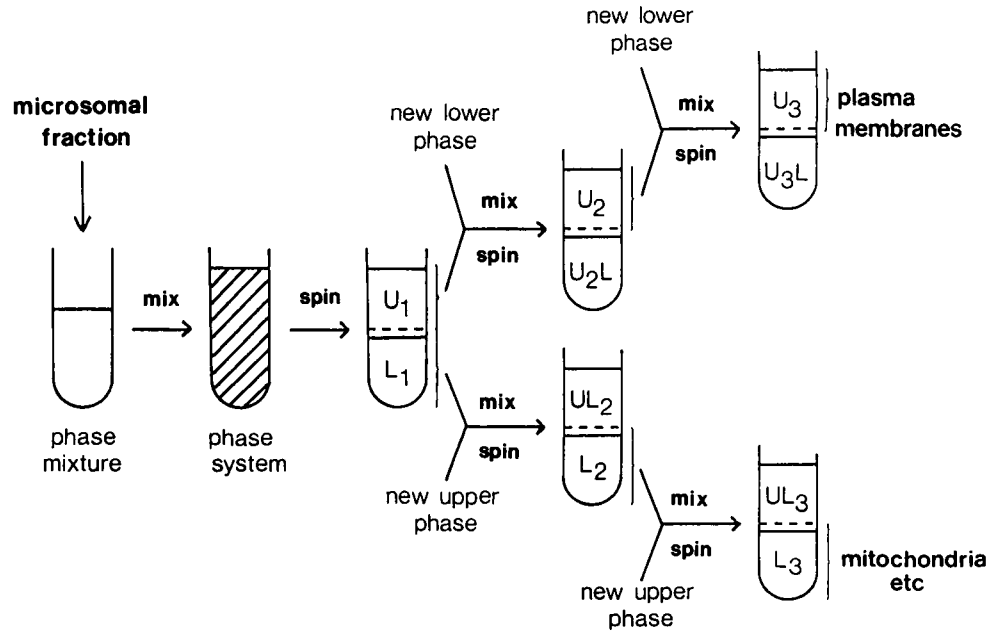


FIG. 1. Batch preparation of plasma membranes by partition in a dextran-PEG two-phase system. One ml of a suspension of a microsomal fraction from oat roots (or shoots) in 0.25 M sucrose, 5 mM K-phosphate, pH 7.8, was added to a 7.0-g phase mixture to produce an 8.0-g phase system with a final composition of 6.3% (w/w) dextran T 500, 6.3% (w/w) PEG 4000, 0.25 M sucrose, 5 mM K-phosphate, pH 7.8. The phase system was mixed by 20 inversions of the tube, and was centrifuged at 1000*g* for 4 min to facilitate phase settling. Ninety per cent (indicated by the dashed line) of the upper phase, $U_1$, was removed with a Pasteur pipette and added to a tube containing 4 ml new lower phase. The remaining lower phase, interface and 10% upper phase, $L_1$, received 4 ml new upper phase. The two new phase systems so obtained were treated as above to finally produce a twice-washed upper phase, $U_3$, enriched in plasma membranes, and a twice-washed lower phase, $L_3$, containing other membranes.

The light hit the cuvette at right angles to the measuring beam. The photomultiplier was protected from the actinic light by a combination of blue glass filter (BG 12.2 mm, Schott and Jena) and two layers of blue-green cellulose acetate. An opaque quartz plate was also inserted between the cuvette and the photomultiplier.

Cyt *c* oxidase was measured as described by Appelmans *et al.* (5) with 0.05% (w/w) Triton X-100 present.

$K^+$-ATPase was determined according to the method of Hodges and Leonard (14), with some modifications. The reaction volume was 1 ml, and ATP ($H^+$ form) was added to a concentration of 1.0 mM in 40 mM histidine HCl, pH 6.7. Concentrations of $MgSO_4$ and KCl were 0.1 and 25 mM, respectively, and the reaction proceeded for 30 min at 30°C. This modification of the assay medium gives lower background values of $Mg^{2+}$-ATPase, whereas the $K^+$-ATPase activity in the plasma membrane fraction remains relatively unaffected (T. Lundborg, unpublished). Assays without KCl were used as blanks.

GS I and GS II were assayed essentially according to Ray (25). GS I was determined in the presence of 0.5 μM UDP-glucose and 10 mM $Mg^{2+}$ and GS II was determined in the presence of 0.1 mM UDP-glucose and 0.3 M cellobiose. Assays with boiled membranes were used as blanks for both activities.

Protein was determined according to Potty (22) after solubilization of the material with 0.02% (w/v) deoxycholate and precipitation with 6% (w/v) TCA.

**Chemicals.** Dextran T 500 (mol wt ~ 500,000) was obtained from Pharmacia Fine Chemicals, and polyethylene glycol 4000 (mol wt ~ 3100–3700) was purchased from Union Carbide as Carbowax PEG 4000 (now available as Carbowax PEG 3350).

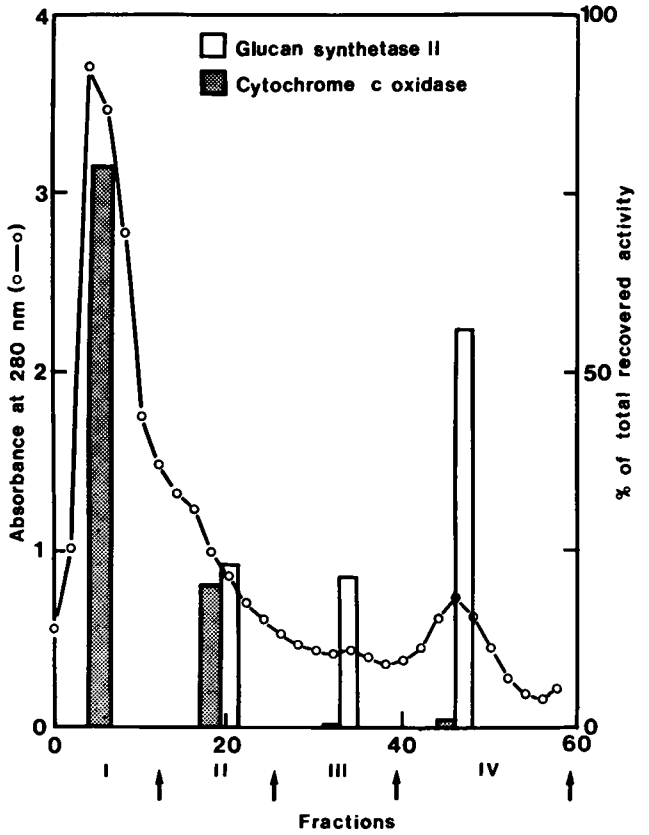


FIG. 2. Countercurrent distribution in a dextran-PEG two-phase system of a microsomal fraction from oat roots. The lower phase plus interface were kept stationary, and 57 transfers of the upper phase were made. After measuring the *A* 280 nm on every second fraction, the 60 fractions were pooled into four main fractions (I–IV) as indicated. Cyt *c* oxidase and GS II were assayed in the pooled fractions.

## RESULTS

**Countercurrent Distribution.** When a microsomal preparation from oat roots was separated by countercurrent distribution (Fig. 2), most of the material ($A_{280}$) was recovered in the first fractions. A second small peak of material was found at fraction 46. Marker analysis on pooled fractions (I–IV, Fig. 2) showed that the bulk of mitochondrial membranes was associated with the first peak, whereas most of the plasma membrane marker GS II was found under the small peak to the far right. This small peak was presumed to be mainly plasma membranes. Similar results have been obtained with corn coleoptiles (30) and wheat roots (20). In these cases, LIAC was used as marker for the plasma membrane.

The countercurrent distribution experiment was performed with the lower phase plus interface as stationary phase and the upper phase as mobile phase. This means that material which partitioned mainly to the lower phase plus interface (*i.e.* had a low partition ratio) was recovered in the first fractions, whereas material with a high affinity for the upper phase (*i.e.* with a high partition ratio) was found in the fractions to the far right in the diagram. Since the presumptive plasma membranes had a high partition ratio and were well separated from the bulk of the material, they could be purified in a few steps only, using the batch procedure of Figure 1.

**Batch Procedure.** Microsomal fractions from roots and shoots were subjected to the batch procedure (Fig. 1). All subfractions obtained were then analyzed with respect to protein and some membrane markers (Table I). For comparison with the results obtained by countercurrent distribution, it should be noted that $L_3$ and $U_3$ correspond largely to fractions I and IV (Fig. 2), respectively, with some overlap of adjacent fractions. The main parts of fractions II and III will be recovered in the 'wash phases.'

Only a few per cent of total protein was recovered in $U_3$ with both roots and shoots, whereas $L_3$ in both cases contained about half of total protein. In contrast, relatively large parts (30–52%) of the total activities for the three tentative plasma membrane markers, LIAC, $K^+$-ATPase, and GS II, were recovered in $U_3$ from roots. For shoots, these figures were lower (17–24%), and larger parts (32–47%) of all three markers were in fact found in $L_3$. GS I, a suggested marker for the Golgi apparatus, was found mainly in $L_3$ with both materials. This was the case also with Cyt *c* oxidase (a marker for the mitochondrial inner membrane), which was extremely distributed to $L_3$.

It should be observed that although all markers were either mainly recovered in $U_3$ or $L_3$, they all showed a dual distribution. For instance, the 17% $K^+$-ATPase in $L_3$ of roots had a low partition ratio ($UL_3$ divided by $L_3 \approx 0.2$), whereas the 52% in $U_3$ had a high partition ratio ($U_3/U_3L \approx 10$), indicating that the $K^+$-ATPase activities in $L_3$ and $U_3$ were associated with membrane vesicles having different surface properties.

The specific activities (Table II) of the three plasma membrane markers were much higher in $U_3$ than in $L_3$. This was the case with both materials, although with shoots the total activities were higher in $L_3$. In comparison with the calculated initial activities of the microsomal fractions, the plasma membrane markers were enriched 6 to 11 times in $U_3$, whereas $L_3$ always showed a depletion of these markers. GS I, the Golgi marker, was also enriched in $U_3$ and depleted in $L_3$. However, compared to the activities of GS II, the activities of GS I were very low, which may call into question its value as a marker in these experiments. Cyt *c* oxidase, finally, was enriched in $L_3$ and depleted in $U_3$.

STA-staining was used to selectively stain plasma membranes in thin sections of $U_3$ and $L_3$ material from roots (Fig. 3, a–d) and shoots (Fig. 4, a and c). These micrographs show a majority of well stained vesicles in $U_3$, whereas $L_3$ from both organs contain

Table I. *Distribution of Protein and Membrane Markers between Fractions Obtained by the Batch Procedure of Figure 1*

Figures are the mean of two experiments, except for GS I, which was assayed in one of the preparations only.

| Fraction | Protein | | LIAC | | $K^+$-ATPase | | GS II | | GS I | | Cyt *c* Oxidase | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *mg* | % | $\Delta\Delta A \cdot 10^3$ | % | *nmol·min*$^{-1}$ | % | *nmol·min*$^{-1}$ | % | *nmol·min*$^{-1}$ | % | *nmol·min*$^{-1}$ | % |
| Roots | | | | | | | | | | | | |
| $U_3$ | 0.28 | 5 ± 0 | 26 | 30 ± 3 | 114 | 52 ± 5 | 14 | 42 ± 0 | 0.007 | 13 | 38 | 1.3 ± 0.5 |
| $U_3L$ | 0.09 | | 3 | | 11 | | 2 | | 0.003 | | 4 | |
| $UL_3$ | 0.74 | | 9 | | 6 | | 2 | | 0.006 | | 236 | |
| $L_3$ | 2.92 | 50 ± 4 | 22 | 35 ± 3 | 36 | 17 ± 2 | 5 | 14 ± 2 | 0.018 | 34 | 1970 | 72 ± 5 |
| Shoots | | | | | | | | | | | | |
| $U_3$ | 0.22 | 3 ± 1 | 19 | 17 ± 2 | 16 | 21 ± 4 | 53 | 24 ± 1 | 0.017 | 9 | 6 | 0.3 ± 0.0 |
| $U_3L$ | 0.08 | | 2 | | 0 | | 10 | | 0.006 | | 1 | |
| $UL_3$ | 0.84 | | 15 | | 5 | | 15 | | 0.013 | | 143 | |
| $L_3$ | 3.75 | 56 ± 1 | 51 | 42 ± 6 | 37 | 47 ± 7 | 75 | 32 ± 4 | 0.076 | 42 | 1650 | 79 ± 2 |

Table II. *Specific Activities and Relative Enrichment of Membrane Markers in Fractions Obtained by the Batch Procedure of Figure 1*

All activities are per mg protein and are the mean of two experiments, except for GS I, which was assayed in one of the preparations only. The initial specific activities of the microsomal fractions were calculated from the total recoveries of protein and markers.

| | LIAC | $K^+$-ATPase | GS II | GS I | Cyt *c* Oxidase |
|---|---|---|---|---|---|
| | $\Delta\Delta A \cdot 10^3$ | *nmol·min*$^{-1}$ | *nmol·min*$^{-1}$ | *nmol·min*$^{-1}$ | *nmol·min*$^{-1}$ |
| Roots | | | | | |
| Initial | 15 ± 2 | 38 ± 3 | 6 ± 2 | 0.010 | 470 ± 30 |
| $U_3$ | 90 ± 7 | 400 ± 20 | 49 ± 18 | 0.025 | 130 ± 60 |
| Enrichment | 6 ± 0 | 11 ± 1 | 9 ± 0 | 3 | 0.3 ± 0.1 |
| $L_3$ | 8 ± 2 | 12 ± 1 | 2 ± 1 | 0.006 | 680 ± 50 |
| Enrichment | 0.5 ± 0.1 | 0.3 ± 0.0 | 0.3 ± 0.1 | 0.6 | 1.4 ± 0.0 |
| Shoots | | | | | |
| Initial | 18 ± 4 | 12 ± 1 | 35 ± 15 | 0.025 | 310 ± 10 |
| $U_3$ | 92 ± 20 | 78 ± 17 | 260 ± 60 | 0.100 | 30 ± 10 |
| Enrichment | 6 ± 2 | 6 ± 1 | 8 ± 2 | 4 | 0.1 ± 0.0 |
| $L_3$ | 14 ± 5 | 10 ± 1 | 21 ± 11 | 0.018 | 440 ± 10 |
| Enrichment | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.5 ± 0.0 | 0.7 | 1.4 ± 0.1 |

only very few well stained vesicles. For comparison, sections stained with uranyl acetate and lead citrate are shown in Figure 4, b and d. The specificity of STA-staining for the plasma membrane is evident in sections from whole tissue (Fig. 3e and 4e), although some parts of the plasma membrane are only poorly stained. However, what is more important, under the conditions used here no membrane other than the plasma membrane showed a pronounced staining. Thus, the presence of positively stained vesicles should indicate the presence of plasma membranes. Interestingly, many of the vesicles in the $U_3$ fraction seem to be dumbbell-shaped rather than spherical (Fig. 3b), indicating exocytotic (or pinocytotic) activity of the membranes, which should be a property of the plasma membrane.

## DISCUSSION

Results obtained earlier with phase partition of different plant materials have shown that the plasma membrane has a high partition ratio compared to other membranes. Thus, intact protoplasts from *Digitaria sanguinalis* partition in the upper phase, while chloroplasts and cell debris collect at the interface (12); and plasma membranes have been prepared from several species and tissues utilizing this high partition ratio for the plasma membrane (20, 30). Similarly, in the present study on oats, plasma membrane fractions with high partition ratios could be isolated from both roots and shoots. These membrane fractions ($U_3$; Tables I and II) could be identified as plasma membranes not only by their high partition ratio but also by their enrichment in the tentative plasma membrane markers LIAC, $K^+$-ATPase, and GS II (Table II). The main part of the vesicles in the $U_3$ fractions was also positively stained by STA (Figs. 3 and 4), the specific plasma membrane stain (26), quite unlike the material in the $L_3$ fractions, where only very few positively stained vesicles were found.

Judging from the STA-staining, the lower phase material, $L_3$, was practically devoid of plasma membranes. Still, quite a lot of the total activities of LIAC, $K^+$-ATPase and GS II was recovered in this fraction (Table I). Calculated on a protein basis, however, a content of less than 15% plasma membranes in $L_3$ could account for these activities (Table II).

We have suggested earlier (30) that the dual distribution of LIAC could be due to the presence of right-side-out and inside-out plasma membrane vesicles in the microsomal fraction. Since phase partition separates vesicles according to their surface properties, the possibility exists that right-side-out and inside-out vesicles could be separated due to a transverse asymmetry of the plasma membrane, as have been found with erythrocyte membranes (28) and chloroplast thylakoid (4) membranes. In both these cases, right-side-out vesicles were found to have a high partition ratio compared to inside-out vesicles. An alternative explanation is that LIAC is not an absolute marker of the plasma membrane as has earlier been suggested for $K^+$-ATPase and GS II (for review, see Ref. 23). The $L_3$ fractions also contained, besides mitochondria, ER (20) and plastids, as measured by Pchlide fluorescence (S. Widell, unpublished). This fraction therefore contained several *b*-type Cyt which theoretically could give rise to LIAC. Low temperature (77 K) spectra on $U_3$ and $L_3$ fractions from cauliflower showed that only one Cyt with an $\alpha$-band at 556 nm was reduced by light in $U_3$ (S. Widell, R. Caubergs, and C. Larsson in preparation). Dithionite reduced an additional Cyt with $\alpha$-band at 566 nm. The latter Cyt corresponded to about 10% of the 556-nm component. In $L_3$ on the other hand, two peaks (at

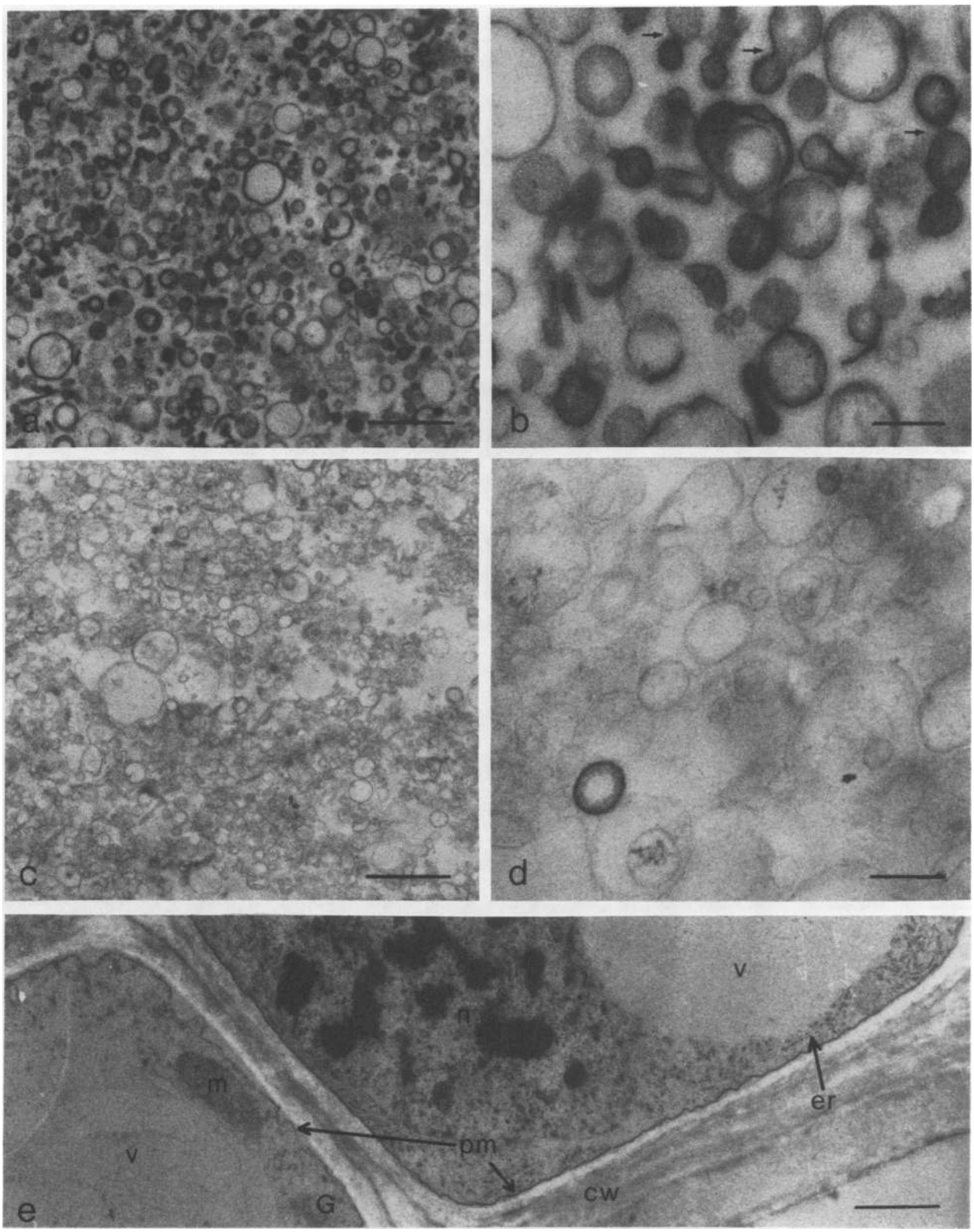


FIG. 3. Electron micrographs of subfractions obtained from a microsomal fraction of oat roots and of root tissue. Subfractionation was achieved by subjecting the microsomal fraction to phase partition in a dextran-PEG two-phase system according to Figure 1. All sections were stained with STA, which is a specific stain for the plasma membrane (26). a and b, Subfraction $U_3$; c and d, subfraction $L_3$; e, whole tissue. Bar represents 1 μm in a, c, and e, and 0.2 μm in b and d. Observe the presence of dumbbell-shaped vesicles in the plasma membrane fraction (b, arrows) indicating exocytotic (or pinocytotic) activity. cw, cell wall; er, endoplasmic reticulum; G, Golgi; m, mitochondrion; n, nucleus; pm, plasma membrane; v, vacuole.

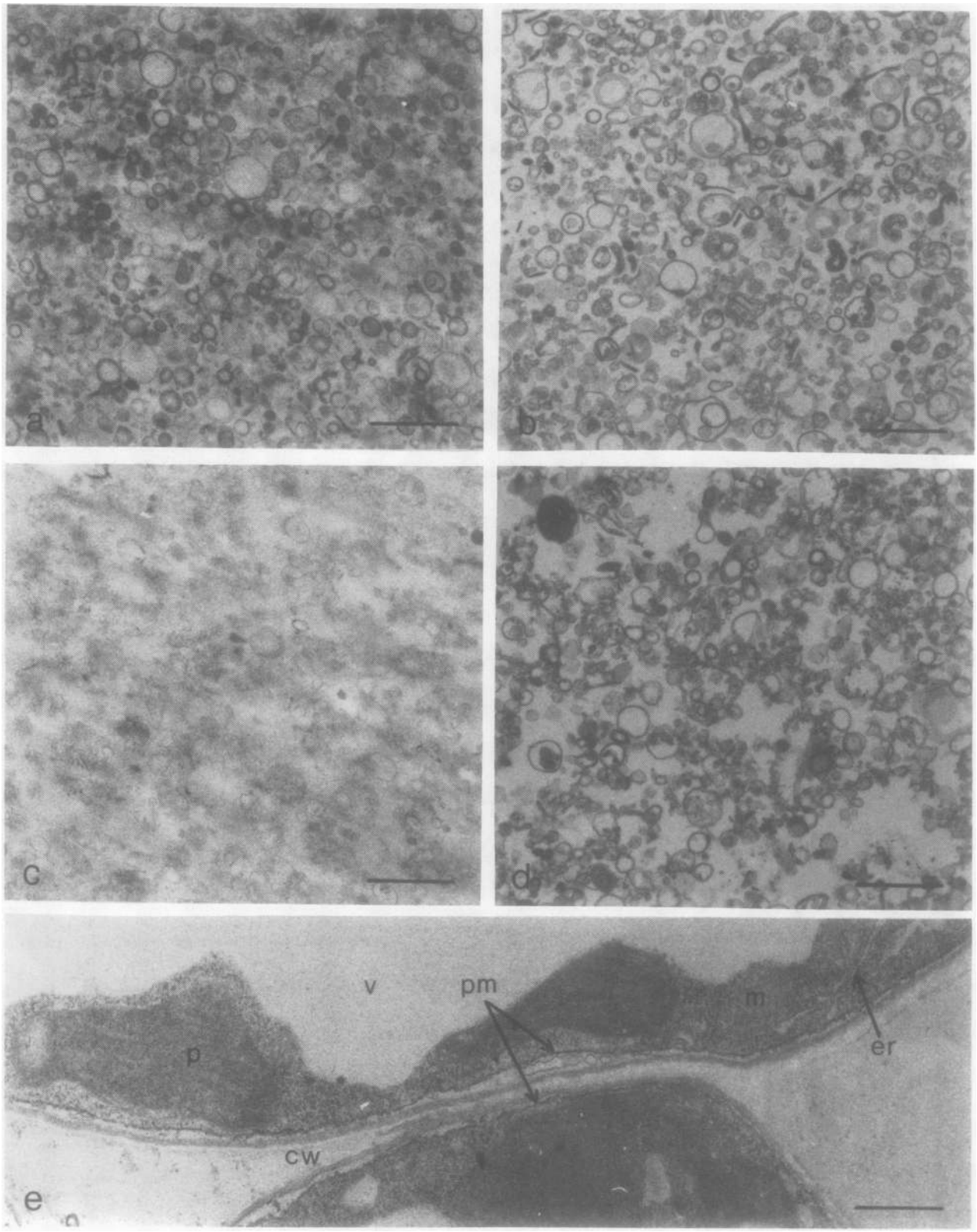


FIG. 4. Electron micrographs of subfractions obtained from a microsomal fraction of oat shoots and of shoot tissue. Subfractionation was according to Figure 1. The sections in a, c, and e were stained with STA, a specific stain for the plasma membrane (26). The sections in b and d were stained with uranyl acetate and lead citrate. a and b, Subfraction $U_3$; c and d, subfraction $L_3$; e, whole tissue. All bars represent 1 $\mu$m. p, plastid; other abbreviations as in Figure 3.

551 and 557 nm) of about equal size were obtained upon photoreduction. Thus, LIAC activity in $U_3$ and $L_3$ are at most partly identical in this species. A recent investigation on the correlation between NADPH-dependent, antimycin A-resistant Cyt *c* reductase and LIAC (S. Widell and C. Larsson, in preparation) showed no correlation between these activities in $U_3$ fractions, but a relatively good correlation in $L_3$ fractions. Therefore, the LIAC activity in $L_3$ may be associated with the ER.

A $K^+$-ATPase with a pH optimum around 9 is associated with mitochondrial membranes (14). The $K^+$-ATPase activities in $L_3$ may therefore be explained by an overlap in the assays of these two $K^+$-ATPases. Interestingly, the specific activities in the $L_3$ fractions were similar for roots and shoots, whereas the root plasma membranes showed a 5-fold higher activity compared to the plasma membranes from shoots. This is in agreement with the suggested function of $K^+$-ATPase in ion uptake into plant roots (10).

GS II, on the other hand, gave much higher activities with shoot material than with roots. This may be correlated to a higher rate of cell division and cell wall synthesis in the shoot at this stage of development.

Only very low activities of GS I, a suggested Golgi marker (24, 31), were found. GS II is assayed at high (0.1 mM) UDP-glucose concentration and no added $Mg^{2+}$, whereas glucan synthetase I is assayed at low (0.5 μM) UDP-glucose and high (10 mM) $Mg^{2+}$ concentration, assay conditions which most probably lead to a certain overlap of the activities. This is a problem discussed by several investigators (*e.g.* Refs. 11, 21, 23, and 25) and the conclusion is that neither of the glucan synthetases can serve as an absolute marker. For instance, the enrichment of GS I in the $U_3$ fractions (Table II) is completely accounted for if the GS II is assayed with 0.1% efficiency in the assay for GS I. Therefore, in our opinion, the enrichment of GS I in the plasma membrane fractions is no evidence for the enrichment of Golgi membranes in these fractions. Rather, when the total activities (Table I) are regarded, it is evident that the large part of GS I is recovered in $L_3$, indicating a low partition ratio for Golgi membranes, while GS II is mainly recovered in $U_3$, at least with roots.

Also, Cyt *c* oxidase, as well established marker for the mitochondrial inner membrane, had a dual distribution (Table I), even if this was much less pronounced than for the other markers. The most probable explanation for this is that, during homogenization of the material, mitochondrial membranes are enclosed within larger vesicles of plasma membranes and thereby achieve a high partition ratio. Enclosure of whole organelles, such as chloroplasts and mitochondria, within plasma membrane sacs has earlier been observed in spinach chloroplast preparations (18); and Ray (25) has reported plasma membrane vesicles containing RER. This may also be the explanation for the small amounts of antimycin A-resistant NADH-Cyt *c* reductase which we found earlier to be associated with the corresponding plasma membrane fraction from wheat roots (20).

In summary, the results suggest that neither LIAC, $K^+$-stimulated $Mg^{2+}$-ATPase or GS II can be used with safety as an absolute marker for the plasma membrane. However, in combination with a high partition ratio in a suitable two-phase system, all three seem to be useful plasma membrane markers, although their usefulness may differ among species and organs. On the other hand, silicotungstic acid almost stained only membranes with a high partition ratio with both root and shoot material, and it therefore seems to be a more specific marker for the plasma membrane.

It is also evident from the results above that a relatively pure plasma membrane fraction is obtained by this method. Furthermore, the method does not seem to be very species- or organ-dependent, and has the advantages that no sophisticated equipment is needed, that it is rapid (plasma membranes within 3 h from homogenization), and that it can easily be scaled up.

*Acknowledgments*—We wish to thank Mrs. Ann-Christine Holmström, Mrs. Inger Rohdin, Mrs. Adine Karlsson, and Mrs. Ulla Wulf for skillful technical assistance. We are also indebted to Mrs. Lena Eriksson for typing the manuscript.

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