Biochimica et Biophysica Acta, 978 (1989) 56–64
Elsevier

BBA 74257

The sucrose carrier of the plant plasma membrane. I. Differential affinity labeling

Olivier Gallet [1], Rémi Lemoine [1], Christer Larsson [2] and Serge Delrot [1]

[1] *UA CNRS 574, Laboratoire de Biologie et Physiologie Végétales, Université de Poitiers, Poitiers (France) and* [2] *Department of Plant Physiology, University of Lund, Lund (Sweden)*

(Received 30 June 1988)
(Revised manuscript received 29 September 1988)

Key words: Sucrose carrier; Sucrose transport; Plant plasmalemma; Plant plasma membrane; Protein, 42 kDa; Differential affinity labeling; (Sugar beet)

Plasma membranes of sugar beet leaves were purified by partition in an aqueous polymer two-phase system. The fractions obtained were devoid of chlorophyll and cytochrome *c* oxidase (EC 1.9.3.1) and contained most of the initial vanadate-sensitive ATPase (EC 3.6.1.3) and glucan synthase II activities (EC 2.4.1.34). The yield of plasma membranes (30–36 mg protein, starting from 250 g leaf tissue) was much higher than that reported with the other species investigated so far using the same technique. The sucrose carrier of the plant plasma membrane contains a SH-group protected by sucrose in the vicinity of its active site (M'Batchi, B., Pichelin, D. and Delrot, S. (1985) Plant Physiol. 79, 537–542). In order to characterize the polypeptides specifically protected by sucrose, the plasma membranes were submitted to a double labeling procedure by *N*-[1,2-^{14}C]ethylmaleimide and *N*-[2-^{3}H]ethylmaleimide ([^{14}C]NEM and [^{3}H]NEM) in the presence of sucrose, or in the presence of a non-transported sucrose analogue, palatinose. After differential labeling, the membranes were washed in 10 mM ethyleneglycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetra-acetic acid (EGTA) and in 1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate (CHAPS) to produce a fraction of intrinsic proteins. The different fractions obtained were analyzed by denaturating polyacrylamide gel electrophoresis, and the differential labeling pattern was studied by monitoring the $^{3}H/^{14}C$ ratio. A 42 kDa polypeptide, particularly apparent in the fraction solubilized by CHAPS, was differentially labeled in the presence of sucrose, but not of palatinose. Together with previous results, the data suggest that this polypeptide is a component of the sucrose carrier of the plant plasma membrane.

Introduction

Sugar transport from the assimilating cells to the receiving organs (fruits, tubers, roots) harvested for animal or human consumption is an important process for plant productivity [1]. Long distance transport of carbohydrates in plants is thought to occur by a mass-flow mechanism, and to be driven by the active uptake of sucrose in the conducting complex of the leaf (loading) and exit of sucrose out of the sieve tube in the receiving organ (unloading). Therefore, long distance transport involves several carrier-mediated steps through the plasma membrane of the assimilating cells, of the conducting elements of the phloem, and of the storage cells [1–4]. In many plants, sucrose is the major form of carbohydrate. In leaves exporting assimilates, sucrose synthesized in the mesophyll cells leaks into the apoplast before being loaded actively through the plasma membrane of the conducting complex [1–4]. Sucrose is not hydrolysed during transport from the mesophyll cell to the conducting bundle. It is also transported without hydrolysis in the phloem. In the receiving organs, sucrose moves from the sieve tube to the apoplast, and may be accumulated by the storage cells without hydrolysis, as in sugar beet root [5] or after hydrolysis by a cell wall invertase, as in sugar cane stem [6].

Membrane transport of sucrose therefore plays a key role in long distance transport of carbohydrates, but also of other phloem mobile compounds such as amino acids, since it drives mass-flow translocation. There is

Abbreviations: CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate; DTT, dithiothreitol; EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; NEM, *N*-ethylmaleimide; PAGE, polyacrylamide gel electrophoresis; PCMBS, *p*-chloromercuribenzenesulfonic acid; PEG, polyethylene glycol; PVPP, polyvinylpolypyrrolidone; SDS, sodium dodecyl sulfate.

Correspondence: S. Delrot, Laboratoire de Biologie et Physiologie Végétales (CNRS UA 574), 25 rue du Faubourg St-Cyprien, 86000 Poitiers, France.

evidence that sucrose is taken up with proton symport through the plasma membrane of various plant cells [7–11]. However, in spite of various attempts [12,13], the plasma membrane protein(s) mediating transport of organic solutes in plants have not yet been characterized with certainty, partly due to difficulties in preparing plant plasma membranes with satisfactory yield and purity until recently, and partly due to the lack of probes useful for tracing the sucrose carriers. The work presented here takes advantage of recent progress concerning these two points, and attempts to characterize by differential affinity labeling polypeptides involved in sucrose transport. In the accompanying paper [14], the involvement of a 42 kDa polypeptide in sucrose transport is demonstrated by an immunological approach.

Phase partition of microsomal fractions in Dextran-PEG two-phase systems, introduced a few years ago [15–19] allows the preparation of plant plasma membranes with high yield and purity (approx. 95% plasma membranes). The use of the slowly-permeant, reversible, thiol reagent PCMBS has shown, that the sucrose carrier of broad bean leaf contains a thiol protectable by sucrose in (or close to) its active site [20–22]. Studies on the binding of [^{203}Hg]PCMBS by leaf tissues of this species have shown that the differential labeling obtained in the presence of sucrose (or of other sugars and analogues recognized by the sucrose carrier, i.e. maltose, raffinose, α-phenylglucoside) is not released by EGTA but solubilized by CHAPS, suggesting that the differential label is associated with intrinsic proteins [21–23]. However, the reversible binding and the low specific activity of [^{203}Hg]PCMBS make it impossible to characterize more precisely these proteins. Work with a microsomal fraction prepared from broad bean leaf has suggested that two membrane polypeptides (33 and 42 kDa) are differentially labeled in the presence of sucrose by the irreversible thiol reagent NEM [24].

The work presented here and in the accompanying paper [14] was aimed at identifying the sucrose carrier of the plant plasma membrane by indirect (differential labeling) and functional (immunological) evidence. Sugar beet (*Beta vulgaris* L.), a species expected to possess the enzymatic equipment needed to support high rates of sucrose transport was used to prepare membrane fractions highly enriched in plasma membrane.

Materials and Methods

Isolation of plasma membranes. Sugar beet (*Beta vulgaris* L.) plants were kindly supplied by Hilleshög AB, Sweden, or grown in Poitiers. In the latter case, the plants (*Beta vulgaris* L., variety Aramis, type multigerm) were grown either in the field or in vermiculite under controlled conditions (16 h of light at 25°C; dark period at 20°C). Plants were watered daily with a nutrient solution [25] and mature exporting leaves were excised after 4 to 5 weeks of culture. Plasma membranes were isolated essentially as described in Ref. 19. Briefly, leaves (250 g) were homogenized four times for 15 s with a Braun homogenizer in 275 ml of a medium consisting of 0.33 M sorbitol, 1 mM phenylmethylsulfonyl fluoride, 0.6% PVPP, 1 mM DTT and 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) adjusted to pH 7.8 with 1 M KOH; DTT, phenylmethylsulfonyl fluoride and PVPP were added as solids just before use. The resulting slurry was filtered through a 240 μm nylon cloth; intact chloroplasts and mitochondria and whole thylakoids were pelleted at $10000 \times g$ for 10 min. A microsomal fraction was obtained from the supernatant by centrifugation at $50000 \times g$ for 30 min. With 250 g of leaves, we obtained approx. 200 mg protein in the microsomal fraction, which was divided equally on two phase systems (each 36 g final weight) with a final composition of 6.5% (w/w) Dextran T 500, 6.5% (w/w) PEG 3350, 0.33 M sorbitol, 5 mM KCl, 1 mM DTT, 5 mM potassium phosphate (pH 7.8). These two phase systems were processed in parallel using the three-step batch procedure described previously [19]. In this procedure the plasma membranes are enriched in the upper phase, while intracellular membranes remain at the interface or in the lower phase. The final plasma membrane and intracellular membrane fractions were stored in liquid N_2. To preserve enzyme activities all media contained 1 mM DTT, freshly added. Dextran T500 was obtained from Pharmacia Fine Chemicals (Sweden), and PEG 3350 (earlier designated 4000) from Union Carbide (New York).

Membrane markers. Vanadate-inhibited, K^+-stimulated, Mg^{2+}-dependent ATPase (EC 3.6.1.3) was assayed at pH 6.0 essentially according to Gallagher and Leonard [26], using 1 mM azide and 0.1 mM molybdate to suppress mitochondrial ATPase and acid phosphatases, respectively. Triton X-100 [0.025% (w/v)] was used to obtain total activities, since a large part of the plasma membrane ATPase activity is latent due to the right-side-out orientation of the plasma membrane vesicles and to the fact that the active site of the ATPase is on the cytoplasmic side of the membrane [27]. Vanadate inhibition was achieved with 0.1 mM orthovanadate. Glucan synthase II (1,3-β-glucan synthase, EC 2.4.1.34) was assayed according to Kauss and Jeblick [28] using 16 mM cellobiose, 0.8 mM spermine and 80 μM Ca^{2+} to activate the enzyme. Since the active site of glucan synthase II is also on the cytoplasmic side of the plasma membrane [27], 0.015% (w/v) digitonin was included to solubilize the vesicles. Cytochrome *c* oxidase (EC 1.9.3.1) was measured according to Hodges and Leonard [29], in the presence of 0.01% (w/v) Triton X-100. Protein was determined according to Bearden [30] using 0.01% (w/v) Triton

X-100 to solubilize membrane proteins, and bovine serum albumin as standard. Chlorophyll was determined according to Arnon [31].

Differential labeling of plasma membranes. The method of labeling, slightly modified from [24] was basically the same as that used for the study of carriers in bacteria or in animal cells (see, for example, Ref. 32). 10 mg of plasma membranes were suspended in 20 ml of a resuspension medium containing 0.33 M sorbitol, 0.5 mM $CaCl_2$, 0.25 mM $MgCl_2$, 20 mM disodium citrate adjusted to pH 5.0 with 10 mM disodium phosphate. The resulting suspension was divided equally into two fractions which were centrifuged at $150000 \times g$ for 30 min. One of the two pellets obtained was resuspended in 2 ml of the same medium (unprotected = control) while the other one (protected) was suspended in 2 ml of a medium containing 0.25 M sucrose + 0.08 M sorbitol (protecting medium), instead of the usual 0.33 M sorbitol. The media used for resuspension were preequilibrated at room temperature, and the vesicles were allowed to stay 2 min at room temperature so that some sucrose could diffuse into the protected vesicles. 20 μl of a freshly prepared 10 mM NEM solution (unlabeled) were then added to both batches, and they were placed for 5 min at 4°C before dilution with 10 ml of resuspension medium and centrifugation at $150000 \times g$ for 30 min. In the control batch, this prelabeling step blocks most of the thiols available with unlabeled NEM, while in the other batch sucrose will protect some thiol groups, particularly those of the active site of the sucrose carrier, and prevent at least partially NEM binding. The control pellet was then incubated for 60 min with 0.1 mM [^{14}C]NEM (total radioactivity: 0.22 MBq; final specific activity: 1.1 GBq · mmol^{-1}) in 2 ml of sucrose medium and the protected batch was incubated with 0.1 mM [^{3}H]NEM (total radioactivity: 3.1 MBq; final specific activity: 15.5 GBq · mmol^{-1}) in 2 ml of resuspension medium. The labeling step was done at room temperature. During this step, [^{14}C]NEM labels the few thiol groups of the control batch which did not react during prelabeling, except the sucrose-protected proteins, since sucrose is present during labeling of the control batch. Therefore, [^{14}C]NEM traces the 'background' proteins (i.e., proteins not protected by sucrose). In the protected batch, [^{3}H]NEM labels the thiol groups which had been protected by sucrose during prelabeling. At the end of incubation, both membrane suspensions were diluted to 13 ml with resuspension medium also containing 50 μM unlabeled NEM (to desorb and to dilute any remaining adsorbed labeled NEM) and pelleted at $150000 \times g$ for 30 min. The unprotected pellet and the protected pellet were then resuspended and pooled into a single centrifugation tube, filled (12 ml) with resuspension medium. The double-labeled pellet was recovered by another centrifugation and resuspended in 2 ml of 0.33 M sorbitol, 5 mM Tris-maleate (pH 7.8). 1 ml of the suspension was frozen in liquid nitrogen and kept at −70°C. 1 ml was treated as described below.

Treatment with EGTA and CHAPS. About 4.5 mg protein of double-labeled plasma membranes were resuspended in 1 ml of solubilization medium (0.33 M sorbitol, 0.5 mM $CaCl_2$, 0.25 mM $MgCl_2$, buffered at pH 6.0 with 20 mM sodium phosphate/disodium phosphate). A stock solution of 15 mM Na EGTA was prepared in the same medium. 2 ml of this solution were added dropwise, at 4°C and under constant stirring, to the membranes. After 20 min on ice, the insoluble pellet was recovered by centrifugation and resuspended in 2.7 ml of resuspension buffer. The EGTA supernatant was frozen in liquid nitrogen and stored at −70°C. 300 μl of a 10% stock solution of CHAPS prepared in the solubilization medium were added dropwise to the resuspended pellet, under constant stirring. After 20 min on ice, the unsolubilized membrane proteins were pelleted and the supernatant was either directly frozen into liquid nitrogen or first concentrated to 0.3 ml by dialysis under reduced pressure (Microprodicon equipped with Prodimem membranes, cut-off size = 10 000; Beaverton, OR, U.S.A.) against the solubilization medium containing 1% CHAPS and 0.05 mM NEM.

Polyacrylamide gel electrophoresis. The membranes were solubilized and separated by SDS-PAGE according to [33] with a stacking gel of 4.75% acrylamide and a running gel containing a linear gradient of 10 to 22% acrylamide. The gels were stained with Coomassie blue [33] or with silver nitrate [34]. Molecular weights were determined using a marker kit (Dalton VII, Sigma). For radioactivity measurements, about 100 μg of plasma membrane protein was deposited in each well. After electrophoresis, each lane was cut into 2 mm-thick slices. To increase sensitivity of detection, each membrane sample was run in triplicate (3×100 μg protein) on the gel. The corresponding slices of the three lanes were pooled, and the ^{14}C and ^{3}H were fully separated and recovered by combustion in an oxidizer (Intertechnique IN 4101). Samples were counted for 10 min and radioactivity measurements were corrected for background and counting efficiency using the external standard method. For each slice, a differential labeling index was calculated according to the formula: [(^{3}H on the band)/(^{14}C of the band)]/[(total ^{3}H of the lane)/(total ^{14}C of the lane)]. *N*-[1,2-^{14}C]Ethylmaleimide (1.9 GBq · mmol^{-1}) was purchased from CEA (Saclay, France) and *N*-[2-^{3}H]ethylmaleimide (2.0 TBq · mmol^{-1}) was from NEN France (Paris).

Results and Discussion

Yield and characterization of purified plasma membranes

A relatively large proportion (15–20%) of the total protein in the microsomal fraction was recovered in the

TABLE I

Distribution of protein, chlorophyll, and total activities ($\mu mol \cdot min^{-1}$) of some marker enzymes between the plasma membrane (PM), intracellular membrane (ICM), and microsomal fraction (MF) from sugar beet leaves

Percentage recovery within brackets (MF: 100%). Data from from one representative experiment.

Fraction	Protein (mg)	Chl (mg)	Glucan synthase	ΔVO_4^--ATPase	Cyt *c* oxidase
PM	33	0.0	56	15	0.17
	(17)	(0.0)	(230)	(83)	(0.4)
ICM	130	8.7	0.9	2.6	34
	(65)	(77)	(2)	(14)	(94)
MF	200	11.3	24	18	36

plasma membrane fraction, together with most of the two plasma membrane markers, vanadate-inhibited K^+, Mg^{2+}-ATPase and glucan synthase II (Table I). Markers for the two main intracellular membrane systems in the microsomal fraction, chlorophyll for chloroplast thylakoids and cytochrome *c* oxidase for mitochondrial inner membranes, were almost exclusively distributed to the intracellular membrane fraction, and only negligible amounts of cytochrome *c* oxidase were recovered with the plasma membranes (Table I).

A problem with sugar beet leaves is the content of phenolic compounds. Inclusion of PVPP in the homogenization medium and the presence of PEG in the phase system were not sufficient to remove all phenolics, and it was necessary to include 1 mM DTT throughout the purification procedure to help preserve enzyme activities. Still, the relatively low activities of glucan synthase II in the intracellular membrane and microsomal fractions, and hence the apparent high recovery (200%) in the plasma membrane fraction, were probably due to a more rapid inactivation of the enzyme by phenolics in the two former, crude fractions.

Taken together, the distribution of the membrane markers used (Table I) demonstrate that the plasma membrane preparation from sugar beet leaves is of high purity, in agreement with earlier results with other materials (for reviews, see Refs. 18 and 19). The high yield of plasma membranes obtained with sugar beet leaves makes this a very favourable starting material, and the last seven preparations during autumn 1987 (when all parameters had been optimized) all gave 30–36 mg of protein in the plasma membrane fraction, starting from 250 g of leaves.

Differential labeling of fractions enriched in plasma membrane

Differential labeling by NEM has been used successfully in the study of several proton/sugar symporters in bacteria [35,36], but its application to the sucrose carrier of the plant plasma membrane had to overcome two main difficulties. The first one is the high concentration of sucrose needed to protect the carrier from NEM binding, due to the high apparent K_m of the carrier for sucrose (about 25 mM [2,7]). The concentrations of substrates used to protect enzymes from NEM are usually 10–20-fold higher than the K_m [35,37]. Using such a high concentration of sucrose as 250 mM in the protecting medium may change the sensitivity towards NEM also of proteins which are not related to sucrose transport, and thus result in 'unspecific protection' of thiol groups. Therefore, a parallel set of experiments was run using palatinose instead of sucrose as the 'protecting' sugar. Palatinose (6-*O*-α-D-glucopyranosyl-D-fructofuranose), which differs from sucrose by the location of the bond between glucose and fructose, is neither recognized [22] nor transported [38,39] by the sucrose carrier of plant tissues. The rationale was that the polypeptide band(s) corresponding to the sucrose carrier should be differentially labeled in the presence of sucrose, but not of palatinose, and that peaks of differential label appearing with palatinose cannot be ascribed to the sucrose carrier.

The sensitivity of the method was the second problem. While ^3H counts measured in each slice were high enough to be reliable, the low specific activity of [^{14}C]NEM commercially available resulted in low counts in the fractions obtained after solubilization of the membranes by CHAPS, even when these fractions were concentrated by dialysis under reduced pressure. This yielded peaks which were sometimes erratic. Therefore, all labeling experiments were made at least three times independently, and the gels of all experiments were compared. A peak was taken into consideration only if it was found in at least two out of the three experiments. Some examples representative of individual gels will be given below before presenting diagrams summarizing whole sets of experiments.

Fig. 1 shows an example of the differential labeling patterns observed with native plasma membrane in the presence of sucrose (Fig. 1A) or in the presence of palatinose (Fig. 1B). In the example shown in Fig. 1A, several bands exhibit a higher differential labeling index than the background, with a large region of low molecular mass polypeptides (0–10 kDa), and more discrete bands at 34, 36, 42, 62, 72 and 89 kDa. Thus, these peaks may correspond to polypeptides protected by sucrose. Conversely, several polypeptides (15–19, 23, 52 kDa) exhibit a low differential labeling index in the presence of sucrose, which may result from an increased sensitivity to NEM due to the conformational changes induced directly or indirectly by this sugar. Repeated analysis of the same membrane sample in different gels gave consistent patterns with S.E. not exceeding 10% of the means of differential labeling index measured for a given slice (data not shown). Yet, this pattern may vary somewhat from one batch of labeled membranes to

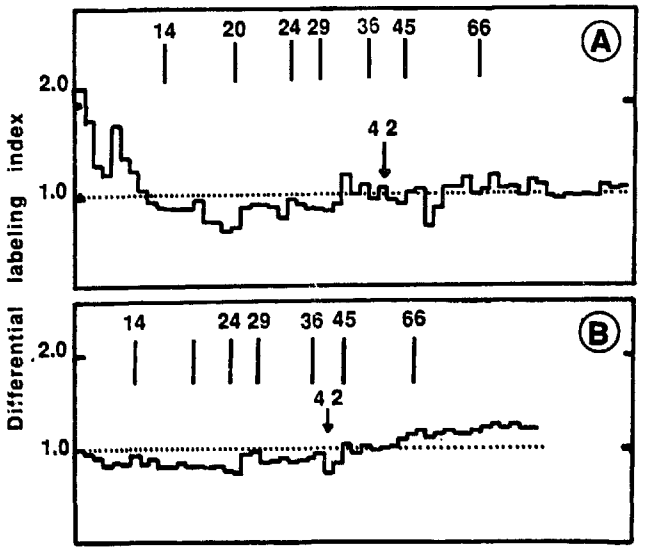

Fig. 1. Examples of different labeling patterns of native plasma membrane. Sugar beet plasma membranes were differentially labeled by [^{3}H]NEM/[^{14}C]NEM in the presence of sucrose (A) or of the non-transported analogue palatinose (B). The membrane polypeptides were separated by SDS-PAGE, the gels were sliced into 2 mm-thick bands, and the ^{3}H/^{14}C ratio of each band was monitored. The data ar expressed by a differential labeling index (^{3}H/^{14}C of the band divided by the ^{3}H/^{14}C ratio of the whole track). Protection of a polypeptide by the sugar tested results in a high differential labeling index. The dotted line indicates the ^{3}H/^{14}C ratio of the whole track and the numbers at the top of the figure refer to the molecular weight markers.

another one. The differential labeling index pattern found in the presence of palatinose (Fig. 1B) is flatter than that obtained with sucrose and shows some unspecific rise in differential labeling index for high molecular mass polypeptides (62–150 kDa).

Since previous data [23] suggest that the proteins differentially labeled by the impermeant thiol reagent PCMBS may be partially purified by a sequential treatment with EGTA and CHAPS, the same treatment was applied to plasma membranes. Table II gives an account of the distribution of the proteins and of the ^{3}H and ^{14}C contents of the various fractions recovered from membranes differentially labeled in the presence of sucrose or palatinose. The EGTA treatment solubilized about 44% of the plasma membrane proteins, and contained a much higher amount of ^{3}H and ^{14}C than the CHAPS supernatant. The CHAPS treatment solubilized more ^{14}C than ^{3}H. This suggests that the protecting sugars are less efficient for intrinsic proteins than for extrinsic proteins, since the amounts of ^{3}H and ^{14}C recovered in the EGTA fraction were equal. The distribution of the label between the different fractions was the same for membranes differentially labeled in the presence of sucrose and of palatinose. This, together with the low amount of ^{3}H in the CHAPS fraction supposed to contain the intrinsic proteins differentially labeled suggests that these proteins only represent a small percentage of this fraction, and that the labeling procedure used was not optimal. Indeed, would every step of the differential labeling procedure have fulfilled its role completely, the native plasma membrane, and even more the CHAPS fraction should contain one, or at most a few polypeptides highly labeled by ^{3}H, and almost no ^{14}C. Obviously, this was not the case, suggesting that at least one of these steps was not optimal, possibly the prelabeling step (neutralization of 'background' thiols by unlabeled NEM). However, a longer prelabeling step, or the use of higher NEM concentrations would have resulted in a smaller amount of radioactivity bound in the labeling step, thus decreasing the sensitivity of counting. Therefore, inasmuch as the labeling procedure yielded consistent results, it was considered as a good compromise and used throughout.

The peaks of differential labeling index found for the polypeptides solubilized by CHAPS are higher than those found for the native plasma membranes, and the main peaks were observed at 11, 13, 36, 42, 45, 49, 74, 89 and 95 kDa for the proteins solubilized from plasma membrane differentially labeled in the presence of sucrose (Fig. 2A). For the polypeptides solubilized from

TABLE II

Distribution of protein, ^{3}H and ^{14}C in the different fractions solubilized from plasma membranes differentially labeled by [^{3}H]NEM/[^{14}C]NEM in the presence of sucrose (S) or palatinose (P)

Data are means ± S.E. ($n = 3$). Numbers given for 'total' are means of the 'total' values of separated experiments and therefore differ from the 'total' of each column.

Fraction	% Recovery					
	protein		^{3}H		^{14}C	
	S	P	S	P	S	P
Native plasma membrane	100	100	100	100	100	100
EGTA supernatant	43.8 ± 5.5	50.9 ± 4.6	51.9 ± 3.0	56.1 ± 14.1	54.2 ± 0.8	58.3 ± 13.8
CHAPS supernatant	4.7 ± 0.5	8.7 ± 3.2	6.0 ± 0.1	7.8 ± 0.7	10.3 ± 3.1	12.0 ± 0.51
Residual pellet	30.4 ± 3.3	23.7 ± 6.2	35.7 ± 2.1	32.9 ± 13.2	37.7 ± 11.5	29.9 ± 0.81
Total	84.5 ± 12.0	83.3 ± 3.5	93.6 ± 1.0	102.1 ± 26.6	98.4 ± 1.8	100.2 ± 18.1

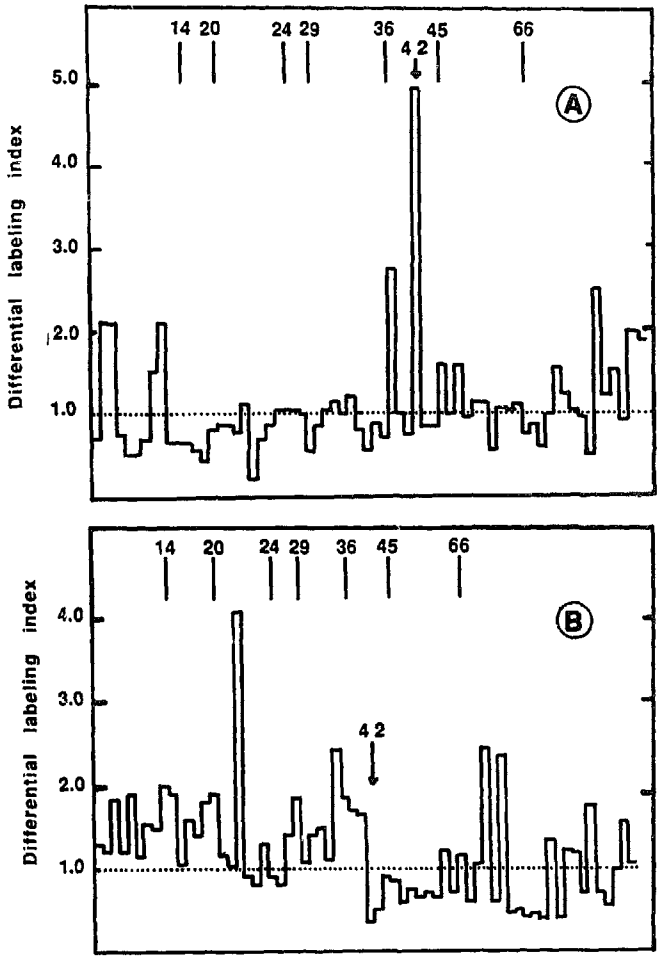

Fig. 2. Examples of differential labeling patterns of the CHAPS-soluble fraction of plasma membrane. Sugar beet plasma membranes differentially labeled as in Fig. 1 were solubilized first by 15 mM EGTA and then by 1% CHAPS. The proteins recovered in CHAPS were analyzed as described in Fig. 1. The plasma membranes were differentially labeled in the presence of sucrose (A) or palatinose (B).

plasma membrane differentially labeled in the presence of palatinose, peaks are observed at 11, 12, 14, 18, 20, 27, 30, 33, 38, 71 and 77 kDa (Fig. 2B).

Because of the numerous peaks observed, the peaks which are consistently and specifically protected by sucrose, but not by palatinose cannot be distinguished by a simple assessment of Figs. 1 and 2. The experiments were therefore repeated, the positions of the differentially labeled peaks and of the bands visualized on each gel were noted, compared, and the data were summarized as presented in Figs. 3–6. The S.E. found for a given peak of differential labeling among three labeling experiments did not exceed 25% of the mean.

In native plasma membrane differentially labeled in the presence of sucrose (Fig. 3A), the 55, 60 and 72 kDa peaks were found in two out of three experiments while the other peaks were found in all experiments. The 33 and 42 kDa peaks have already been noted after differential labeling of a crude microsomal fraction of broadbean leaves [24]. In native plasma membrane differentially labeled in the presence of palatinose, only three peaks (each found in two out of three experiments) were detected at 49, 67 and 72 kDa (Fig. 3B). Consequently, the 49 and 72 kDa peaks are not specific for sucrose, and are not likely candidates for the sucrose carrier.

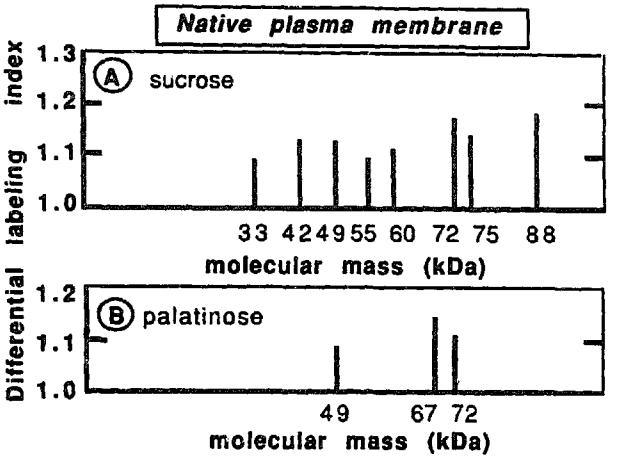

Fig. 3. Cumulative analysis of the differential labeling pattern of native plasma membrane. The labeling was done in the presence of sucrose (A) or palatinose (B). The figure combines the data of three experiments with each sugar. Only the peaks appearing at least in each of the three experiments are shown.

Solubilization by EGTA of plasma membranes differentially labeled in the presence of sucrose yielded six peaks (Fig. 4A), including a 33 kDa peak. Since the sucrose carrier is not expected to be solubilized by EGTA, this polypeptide may also be discarded when considering potential candidates. The 30 and 33 kDa peaks present in this fraction (Fig. 4A) were also found in the EGTA supernatant of palatinose-treated membranes (Fig. 4B), although they were less apparent.

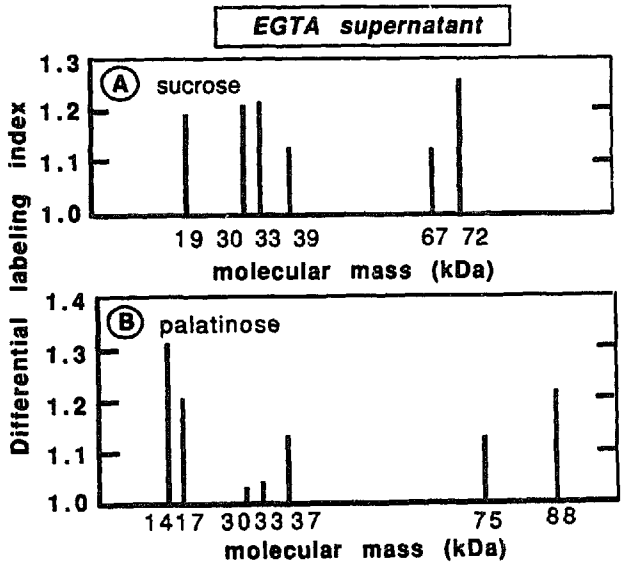

Fig. 4. Cumulative analysis of the differential labeling pattern of the EGTA-soluble fraction of plasma membrane. Other details as in the legend of Fig. 3.

Unlike the EGTA fraction of sucrose-treated membranes (Fig. 4A), the EGTA supernatant of palatinose-treated membranes contained a 75 and an 88 kDa peak. Both these peaks, which had already been noted in native plasma membrane labeled in the presence of sucrose (Fig. 3A) are therefore not specific for sucrose. At this step of the analysis, the data of Fig. 3 and Fig. 4 suggest that the 42 and the 60 kDa peaks are possible candidates for the sucrose carrier.

Among the numerous peaks found in the CHAPS supernatant of plasma membranes differentially labeled in the presence of sucrose (Fig. 5A), only the 19, 42, 49 and 95 kDa peaks seem specific for sucrose (compare with Fig. 5B). The 60 kDa peak, which had been retained as a possible candidate after the analysis of Fig. 3 and 4 is absent in the CHAPS supernatant. Since previous data suggest that the sucrose carrier is solubilized by CHAPS [23], the 60 kDa peak cannot be ascribed to differential labeling of the sucrose carrier.

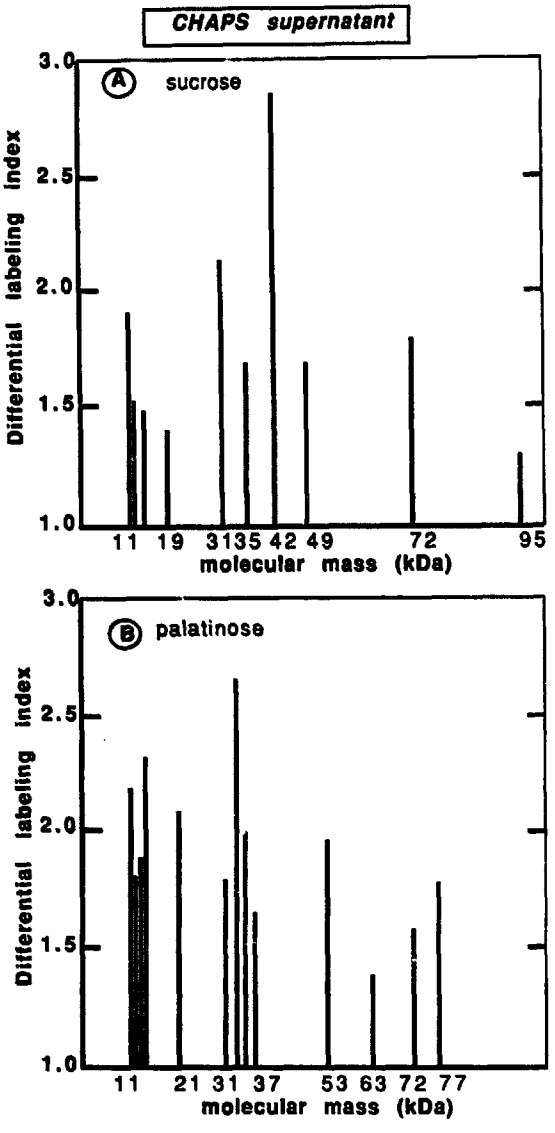

Fig. 5. Cumulative analysis of the differential labeling pattern of the CHAPS-soluble fraction of plasma membrane. Other details as in the legend of Fig. 3.

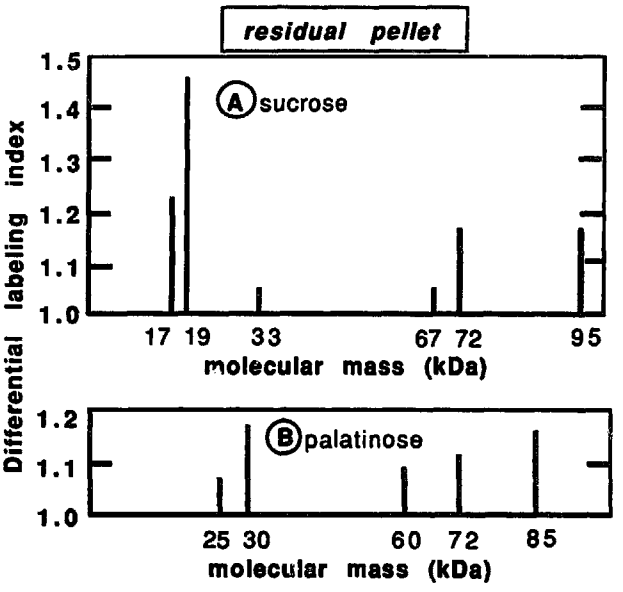

Fig. 6. Cumulative analysis of the differential labeling pattern of the fraction insoluble in EGTA and in CHAPS. Other details as in the legend of Fig. 3.

Analysis of the residual pellets remaining after solubilization by EGTA and by CHAPS (Fig. 6) shows that the 19 and the 95 kDa peaks previously noted in the CHAPS supernatant of sucrose treated membranes (Fig. 5A) are also present in the corresponding residual pellet (Fig. 6A). Because the sucrose carrier is expected to be completely solubilized by CHAPS [21], the 19 and 95 kDa polypeptides are not likely components of this carrier.

In spite of the difficulty of analysis, altogether, the data presented in Figs. 3–6 suggest that the only polypeptide differentially labeled in the presence of sucrose, not solubilized by EGTA, solubilized by CHAPS, and absent in the residual pellet is a 42 kDa polypeptide. Figs. 3–6 summarize the results of three experiments. Additional analysis were made on the CHAPS fraction obtained from two additional labeling experiments conducted with sucrose. When all data concerning the CHAPS supernatants are pooled, the values found for the differential labeling index of the 42 kDa peak increase up to 3.58 ± 0.85 ($n = 5$) for sucrose-protected membranes, and to 1.65 ± 0.40 ($n = 6$) for palatinose-protected membranes.

Photographs of SDS-PAGE gels obtained with the different fractions show that indeed the 42 kDa band, visible in the native plasma membrane (Fig. 7A) is absent in the EGTA supernatant (Fig. 7B), in the residual pellet (Fig. 7D), but more apparent in the CHAPS fraction (Fig. 7C). These data thus confirm that the sucrose-induced differential labeling, which is absent of the EGTA supernatant and much more apparent in the fraction solubilized by CHAPS is associated with a visible polypeptide.

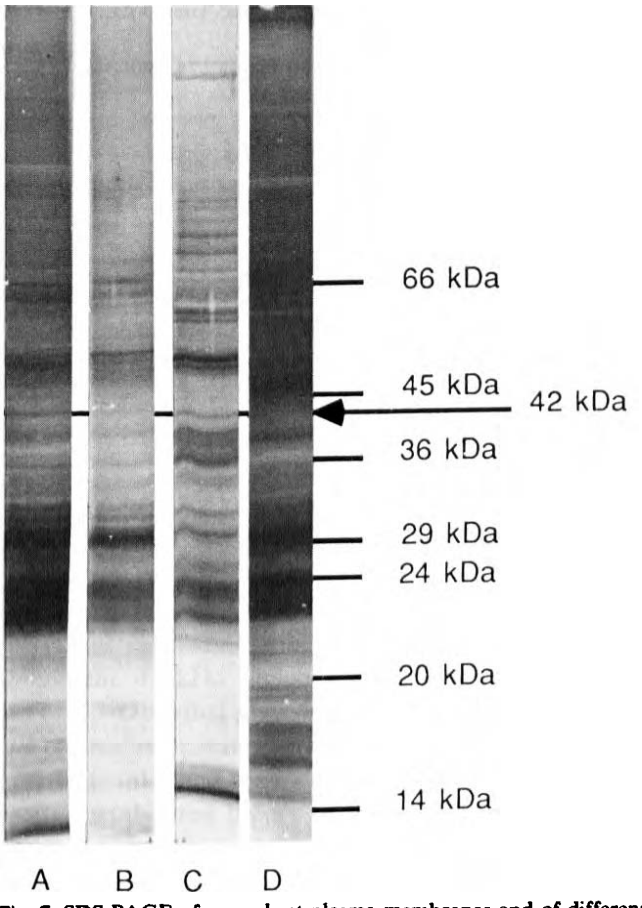

Fig. 7. SDS-PAGE of sugar beet plasma membranes and of different plasma membrane fractions obtained after solubilization. Lane A: native plasma membrane; lane B: EGTA-soluble fraction; lane C: CHAPS supernatant; lane D: residual pellet remaining after sequential treatment of plasma membrane by EGTA and CHAPS. The position of the 42 kDa polypeptide is indicated by the arrows. Fifteen μg protein was deposited in each lane; the gels were silver stained.

In conclusion, the data presented here show the existence, in purified plasma membranes of sugar beet, of a 42 kDa polypeptide differentially labeled in the presence of sucrose, but not in the presence of the non-transported sucrose analogue palatinose. The assumption that this polypeptide is a component of the sucrose transport system of the plasma membrane is strengthened by its distribution after a sequential treatment by EGTA and CHAPS, which is that expected from indirect evidence gathered on the effect of these treatments on the sucrose carrier of broad bean leaves [21]. In the accompanying paper [14], we have used an immunological approach to test by functional means the identity of this polypeptide.

Acknowledgements

We are grateful to Mrs. Janine Bonmort, Aline Karlsson and Ann-Christine Holström for preparing large amounts of plasma membrane. This work was supported by grants from the Organization for Economic Cooperation and Development (R.L.) from the Swedish Natural Science Research Council (C.L.) and the Carl Tesdorpf Foundation (C.L.).

References

1 Giaquinta, R.T. (1980) in The Biochemistry of Plants (Preiss, J., ed.), Vol. 3, pp. 271–320, Academic Press, New York.
2 Geiger, D.R. (1975) in Transport in Plants (Zimmermann, M.H. and Milburn, J.A., eds.), Vol. 1, pp. 395–431, Springer Verlag, Berlin.
3 Delrot, S. and Bonnemain, J.L. (1984) in Membranes and Compartmentation in the Regulation of Plant Function (Boudet, A.M., Alibert, G. and Lea, P.J., eds.), pp. 69–87, Clarendon Press, Oxford.
4 Delrot, S. and Bonnemain, J.L. (1984) Physiol. Vég. 23, 199–200.
5 Lemoine, R., Daie, J. and Wyse, R. (1988) Plant Physiol. 87, 575–580.
6 Bowen, J.E. and Hunter, J.E. (1972) Plant Physiol. 49, 789–793.
7 Giaquinta, R.T. (1977) Plant Physiol. 59, 750–755.
8 Komor, E. (1977) Planta 137, 119–131.
9 Delrot, S. (1981) Plant Physiol. 68, 706–711.
10 Delrot, S. and Bonnemain, J.L. (1981) Plant Physiol. 67, 560–654.
11 Lin, W. (1985) Plant Physiol. 78, 41–45.
12 Sauer, N. (1986) Planta 168, 139–144.
13 Payne, J.W. and Walker-Smith, D.J. (1987) Planta 170, 263–271.
14 Lemoine, R., Delrot, S., Gallet, O. and Larsson, C. (1989) Biochim. Biophys. Acta 978, 65–71.
15 Widell, S. and Larsson, C. (1981) Physiol. Plant. 51, 368–374.
16 Yoshida, S., Uemura, M., Niki, T., Sakai, A. and Gusta, L.V. (1983) Plant Physiol. 72, 105–114.
17 Kjellbom, P. and Larsson, C. (1984) Physiol. Plant 62, 501–509.
18 Larsson, C. (1985) in Cell Components, Modern Methods in Plant Analysis, New Series (Linskens, H.F. and Jackson, J.F., eds.), Vol. 1, pp. 85–104, Springer Verlag, Berlin.
19 Larsson, C., Widell, S. and Kjellbom, P. (1987) Methods Enzymol. 148, 558–568.
20 Delrot, S., Despeghel, J.P. and Bonnemain, J.L. (1980) Planta 49, 144–148.
21 M'Batchi, B. and Delrot, S. (1984) Plant Physiol. 75, 154–160.
22 M'Batchi, B., Pichelin, D. and Delrot, S. (1985) Plant Physiol. 79, 537–542.
23 M'Batchi, B., Pichelin, D. and Delrot, S. (1987) Plant Physiol. 83, 541–545.
24 Pichelin-Poitevin, D., Delrot, S., M'Batchi, B. and Everat-Bourbouloux, A. (1987) Plant Physiol. Biochem. 75, 597–608.
25 Snyder, F.W. and Carlson, G.E. (1978) Crop Sci. 18, 657–669.
26 Gallagher, S.R. and Leonard, R.T. (1982) Plant Physiol. 70, 1335–1340.
27 Larsson, C., Kjellbom, P., Widell, S. and Lundborg, T. (1984) FEBS Lett. 171, 271–276.
28 Kauss, H. and Jeblick, W. (1986) Plant Sci. 43, 103–107.
29 Hodges, T.K. and Leonard, R.T. (1974) Methods Enzymol. 32, 392–406.
30 Bearden, J.C., Jr. (1978) Biochim. Biophys. Acta 533, 525–529.
31 Arnon, D.I. (1949) Plant Physiol. 24, 1–15.
32 Poiree, J.C., Mengual, R. and Sudaka, P. (1978) Biochem. Biophys. Res. Commun. 90, 1387–1392.
33 Laemmli, U.K. (1970) Nature 227, 680–685.
34 Guevara, J., Jr., Johnston, D.A., Ramagali, L.S., Martin, B.A., Capetillo, S. and Rodriguez, L.V. (1982) Electrophoresis 3, 197–205.
35 Fox, C.F. and Kennedy, E.P. (1965) Proc. Natl. Acad. Sci. USA 54, 891–899.

36 Henderson, P.J.F., Bradley, S., MacPherson, A.J.S., Horne, P., Davis, E.O., Daruwalla, K.R. and Jones-Mortimer, M. (1984) Biochem. Soc. Trans. 12, 146–148.
37 Bowman, E.J., Mandala, S., Taiz, L. and Bowman, B.J. (1986) Proc. Natl. Acad. Sci. USA 83, 48–52.
38 Schmitt, M.R., Hitz, W.D., Lin, W. and Giaquinta, R.T. (1984) Plant Physiol. 75, 941–946.
39 M'Batchi, B. and Delrot, S. (1988) Planta 174, 340–348.