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The sucrose carrier of the plant plasma membrane. II. Immunological characterization

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Polyclonal antibodies were raised against either whole plasma membrane of sugar beet (*Beta vulgaris* L.) leaves or against the 42 kDa region polypeptides of the plasma membrane. The effect of these antibodies on the uptake of sucrose, 3-*O*-methyl glucose and α-aminoisobutyric acid by broad bean (*Vicia faba* L.) leaf protoplasts was tested. The anti-plasma membrane serum did not affect the uptake of these solutes, while the anti-42 kDa serum markedly and selectively inhibited the uptake of sucrose without effect on the absorption of hexoses or of amino acids. Sucrose is very poorly metabolized before or after its penetration into the cells, and the inhibition of uptake cannot be explained in terms of side-effects on invertase or glucan synthase activities. The data provide functional evidence confirming the previous hypothesis (Gallet, O., Lemoine, R., Larsson, C. and Delrot, S. (1989) Biochim. Biophys. Acta 978, 56–64) that the 42 kDa polypeptide is a component of the sucrose uptake system of plant plasma membrane. The immunoglobulins purified from the serum were unable to inhibit sucrose uptake, suggesting that the activity of the antibody subclass responsible for inhibition was lost during purification or that an additional component of the serum is necessary to observe this inhibition.

Introduction

The sucrose carrier of the plant plasma membranes plays a key role in the transport of carbohydrate from the assimilating leaf to storage organs and fruits [1]. The active site of this protein possesses a thiol which is protected from thiol reagents by sucrose and by other recognized substrates [2,3]. Based on this property, attempts have been made to identify polypeptides possibly involved in sucrose transport by differential labeling of membranes with the SH-reagent NEM in the presence of various sugars. Using a microsomal fraction prepared from broad bean leaves, Pichelin-Poitevin et al. [4] showed that a 33 kDa and a 42 kDa polypeptide were differentially labeled in the presence of sucrose. The 42 kDa polypeptide has been found after differential labeling of purified plasma membranes prepared from sugarbeet leaves [5]. This finding demonstrated the plasma membrane origin of this polypeptide and its occurrence in various plant species. Moreover, the 42 kDa polypeptide is not solubilized by chelating agents, but it is solubilized by 1% 3-[(3cholamidopropyl)dimethylammonio]-1-propanesulfonic acid (CHAPS), suggesting that it is an intrinsic polypeptide, as expected for a transport protein [5].

However, no functional evidence is still available to show that this polypeptide is involved in sucrose transport. Other enzymes binding sucrose (e.g., invertase or glucan synthase II) could be differentially labeled by NEM in the presence of sucrose. This is especially true for glucan synthase II, a plasma membrane protein which possesses an allosteric site for sucrose and has been suggested to have a molecular mass of about 42 kDa [6]. In the present paper, we have studied the

Abbreviations: α-AIB, α-aminoisobutyric acid; BSA, bovine serum albumin; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propane sulfonate; DTT, dithiothreitol; EGTA, ethyleneglycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; Ig, immunoglobulin; NEM, *N*-ethylmaleimide; 3-*O*-MeG, 3-*O*-methylglucose; PAGE, polyacrylamide gel electrophoresis. PCMBS, *p*-chloromercuribenzenesulfonic acid; PBS, phosphate-buffered saline; PEG, polyethylene glycol; PVPP, polyvinylpolypyrrolidone; SDS, sodium dodecyl sulfate.

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effects of polyclonal antibodies directed against the 42 kDa polypeptide on the uptake and on the metabolism of sucrose by leaf protoplasts.

Materials and Methods

Plant material. Broad bean (*Vicia faba* L.) plants were grown in a controlled environment as already described [3] and used when they were 3 to 4 weeks old. Sugar beet (*Beta vulgaris* L.) plants were grown and used as detailed in Ref. 5.

Antigen preparation. Rabbit polyclonal antibodies were prepared against either purified plasma membrane or the 42 kDa region of the plasma membrane. Plasma membranes were purified by phase partitioning of a microsomal fraction prepared from sugar beet leaves [5]. The 42 kDa antigen was obtained from preparative SDS-polyacrylamide slab gels (10–22% acrylamide). The gels were prepared and Coomassie-stained according to standard procedures [7]. Each gel (1.5 mm thick) was loaded with 3 mg purified plasma membranes of sugar beet. After staining, a 2 mm band including the 42 kDa polypeptide was cut from the gel, cut into 5 mm long pieces, and electroeluted at 200 V in a Biotrap device (Schleicher and Schull) [8]. Preliminary experiments were run with plasma membrane labeled by [^{3}H]NEM [5] to study the time-course of elution of the 42 kDa polypeptide from the 42 kDa band. The elution medium was composed of 50 mM Tris buffered at pH 8.0 with H_3BO_3. Elution was completed after 8 h, with a yield of 90% of the initial radioactivity content of the polyacrylamide band. Ten preparative gels loaded with 3 mg plasma membranes were electrophoresed and eluted in this way to prepare one dose of 42 kDa antigen. After elution, the antigen was concentrated in the Microprodicon (Bio Molecular Dynamics, Beaverton, OR, U.S.A.) by dialysis against a 150 mM NaCl solution.

Immunization of rabbits. Antisera were prepared by subcutaneously injecting rabbits with 0.4 ml (100–200 μg of protein, either total plasma membrane or 42 kDa antigen) mixed with 0.4 ml complete Freund's adjuvant. The same amounts of proteins (without adjuvant) were injected 1, 2, 8, 9 and 10 weeks after the first injection. Blood was collected from the ear 10 days after the last injection. A clot was allowed to form for 2 h at room temperature and was discarded. The serum was then centrifuged at 300 × *g* for 2 min, and the supernatant was frozen in aliquots at −20 °C until further use.

Immunoglobulins were precipitated with ammonium sulfate (40% final concentration), dialyzed against a buffer containing 17.5 mM sodium/disodium phosphate (pH 7.5) and sodium azide (1 : 10000, w/v), and chromatographed in the same buffer on a DEAE-Sephacryl column (1.6 × 20 cm). The immunoglobulins were eluted in the first fractions and their purity was checked by SDS-PAGE and immunoelectrophoresis.

Immunoblotting procedures. After separating the membrane polypeptides by SDS-PAGE, they were transferred to nitrocellulose paper (0.45 μm) in a Trans-Blot apparatus (Bio-Rad Laboratories, Richmond, CA, U.S.A.) as described by Burnette [9]. The nitrocellulose paper was then incubated for 12 h at 4 °C in PBS (10 mM sodium phosphate/disodium phosphate + 150 mM NaCl, pH 7.2) containing 0.1% (w/v) NaN_3 in order to decrease unspecific reactions [10]. After 5 × 12 min rinses in PBS containing 0.3% (w/v) Tween 20 and 3% (w/v) defatted dry milk [11] as blocking agents, the nitrocellulose paper was incubated with either anti-42 kDa serum or pre-immune serum (both at a 1/300 dilution) or purified IgG (5 μg/ml) during 16 h at 4 °C in the same blocking buffer [10]. The nitrocellulose strips were rinsed for 3 × 10 min in PBS with 0.3% (w/v) Tween 20 and then incubated for 2 h at room temperature with the second antibody, horseradish peroxidase-conjugated goat antibodies to rabbit Igs (Bio-Rad) diluted to 1 : 3000 in the same blocking buffer. The strips were then rinsed 3 × 10 min in PBS containing 3% (w/v) defatted dry milk, and 2 × 3 min in PBS to remove milk in excess.

Both primary and secondary antibody incubations were performed in heat-sealed plastic bags to minimize the volume of the antibody solutions. Rinsing of the nitrocellulose paper and detection of peroxidase activity were conducted according to the manufacturer's instructions (Bio-Rad, HRP Color development reagent). After development of the bands, the nitrocellulose paper was thoroughly rinsed with H_2O. The position of the bands was compared with molecular weight standards stained with amido-black [12].

Protoplast preparation. Several attempts using various cellulolytic and pectinolytic enzymes as well as various conditions to infiltrate the tissues did not result in satisfactory preparations of protoplast from sugar beet leaves. Since broad bean leaf tissues also contain a 42 kDa membrane polypeptide which is differentially labeled in the presence of sucrose [4], we decided to turn to broad bean protoplasts to test the effects of the anti-42 kDa serum on solute uptake. The lower epidermis of mature leaves of broadbean was peeled off with fine forceps to facilitate infiltration of the incubation solutions. The peeled leaves (about 2 g) were cut into 4 to 5 cm^2 pieces with a razor blade and plasmolyzed for 45 min by flotation on 90 ml of a medium containing 500 mM mannitol, 0.5 mM $CaCl_2$, 0.25 mM $MgCl_2$, 10 mM disodium citrate adjusted to pH 5.6 with 20 mM disodium phosphate (P medium). The tissues were then incubated for 60 to 90 min at 30 °C in 90 ml of P medium containing 0.5% (w/v) polyvinylpyrrolidone K25, 0.1% (w/v) bovine serum albumin, 2.5% (w/v) cellulase YC and 0.1% (w/v) pectolyase Y23. This medium was filtered through a 0.4 μm Millipore filter before use. The enzymes were purchased from Seishin

Ltd (Tokyo, Japan). At the end of enzymatic digestion, the samples were passed through a metallic filter (200 μm frame), and the protoplasts were sedimented by centrifugation at 100 × *g* for 3 min. The medium containing the enzymes was recovered, filtered through a 0.4 μm filter and stored at 4° C until further use. The same medium could be used up to 5 times. The sedimented protoplasts were washed in 30 ml of P medium containing 5 mM KCl, centrifuged and resuspended in 15 ml of the same medium. The protoplast density of the suspension obtained was estimated by counting 25 fields in a Malassez cell. The suspension was diluted accordingly to obtain a density of $0.5 \cdot 10^6$ protoplasts · ml^{-1}. The overall procedure yielded about $15 \cdot 10^6$ protoplasts with a medium containing fresh enzymes and $10 \cdot 10^6$ protoplasts after the fifth utilization of the same medium. Cell wall contamination of the protoplasts preparations was checked by staining with the fluorescent brightener Calcofluor White ST (0.1%) in 0.5 M mannitol [13]. Microscopic examination of the stained specimens showed virtually no cell wall contamination.

Uptake assay. Protoplasts (1.8 ml ≈ $0.9 \cdot 10^6$ protoplasts) were incubated for 15 min in P medium in the presence of the studied antiserum (diluted to 1/256, on the basis of preliminary experiments). Cell viability was checked at this moment with the Evans Blue exclusion test. At least 95% of the protoplasts excluded Evans Blue, and none of the antibodies tested affected cell viability. In some experiments, the protoplasts were preincubated with 0.5 mM PCMBS, which decreased the proportion of living cells down to 90%. All uptake data were corrected for cell viability.

Uptake was then initiated by addition of 18 μl (148 KBq) [6,6′(n)-^{3}H]sucrose or 3-*O*-methyl-D-[1-^{3}H]glucose or α-[*methyl*-^{3}H]aminoisobutyric acid. The solution added also contained 100 mM unlabeled subtrate, so that the final concentration of the substrate in the incubation medium was 1 mM. Uptake was conducted with constant stirring, under fluorescent tubes. Aliquotes (0.4 ml) of the protoplast suspension were sampled at selected times (2.5, 5.5, 8.5 and 11.5 min, unless stated otherwise) and layered in an Eppendorf 1.5 ml microtube over 500 μl silicone oil (AR 200, Wacker Chemie, München, F.R.G.). The protoplasts were sedimented for 30 s at 6500 rpm (about 6500 × *g*) with a Beckman M11 Microfuge. The incubation medium and the silicone oil were carefully removed by aspiration with a Pasteur pipette, and the bottom of the tube containing the protoplasts was cut off and put in a scintillation minival (6 ml). After addition of 150 μl 65% $HClO_4$ and 300 μl 33% H_2O_2, the samples were digested overnight at 55° C. They were counted by liquid scintillation spectroscopy after addition of 6 ml scintillation cocktail (PCS II, Amersham France, Les Ulis). The data were corrected for background and for efficiency using the external standard method.

In parallel experiments, the amount of incubation medium trapped into the pellet with the protoplasts was determined by incubating the protoplasts with [1,2-^{3}H]polyethyleneglycol (mol. wt. 800–1000) as the tracer. This amount was calculated from the number of protoplasts pelleted, from the amount of radioactivity trapped, and from the volumic activity (18.5 kBq · ml^{-1}) of the [^{3}]polyethyleneglycol solution.

Chromatographic analysis. The metabolic fate of the adsorbed sucrose was studied by incubating $2.5 \cdot 10^6$ protoplasts with 410 kBq [U-^{14}C]sucrose (1 mM) for 20 min. The protoplasts were centrifuged as described above, and extracted in methanol/chloroform/water (12 : 5 : 3, v/v/v) according to Dickson [14]. The extract was centrifuged at 1200 × *g* for 10 min and the aqueous (lower) phase was concentrated in a Rotary evaporator and taken up in 500 μl 10 mM morpholineethanesulfonic acid buffered at pH 5.0 with 1 M NaOH. One half of this sample was deposited directly on a paper chromatogram while the other half was first hydrolyzed for 2 h at 37° C in the presence of 0.25 μkat invertase (Sigma Grade X). Descending chromatography was run for 36 h in ethyl acetate/acetic acid/water (3 : 3 : 1, v/v) with sucrose, glucose and fructose as standards. The chromatograms were sprayed with a mixture of 2% (v/v) aniline in absolute ethanol/0.2 M citric acid (1 : 1, v/v) and heated for 10 min at 105° C. The radioactive tracks were cut in 1 cm bands and their radioactivity was measured with a gas-flow counter (Manu 16, Numelec, Versailles).

Proteins were measured according to Bearden [15].

Results

Characterization of sucrose uptake by broad bean leaf protoplasts

A time-course study showed that uptake of sucrose into the protoplasts was linear for about 15 min and was rapidly saturated within 45–60 min (Fig. 1). When the label was added in the medium 30 min after the beginning of incubation in sucrose, the initial rate of uptake was similar to that found in Fig. 1 (data not shown). This suggests that the rapid decrease of uptake observed after 15 min is due to equilibration between influx and efflux of the label. From the volume of the protoplasts and from the amount of radioactivity taken up, it can be calculated that after 30 min incubation, the intracellular concentration of sucrose concentration of sucrose was 1.06 mM. However, this does not necessarily mean that the uptake was passive, since this calculation takes into account the whole volume of the protoplast, while sucrose may be accumulated first in the cytoplasm (10% of the cell volume), at least during short incubation times. The radioactivity associated with the protoplasts at 'zero' time was due to contamination by extracellular fluid pelleting with the cells, as shown by the use of the impermeant tracer PEG-800. There-

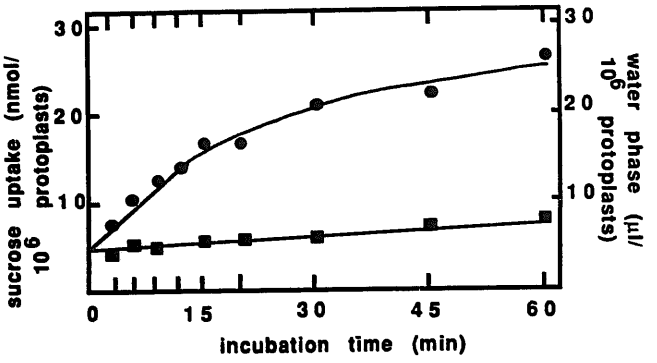

Fig. 1. Uptake of 1 mM [^{3}H]sucrose (●) and [^{3}H]PEG (■) by broadbean mesophyll protoplasts ($0.5 \cdot 10^{-6}$ protoplasts/ml) as a function of time. The volume of the external water phase sedimented with the protoplast was calculated from the volumic activity of [^{3}H]PEG. For a sucrose concentration of 1 mM in the medium, this volume (5 µl/10^6 protoplasts) corresponded to 5 nmol sucrose and was substracted from all measurements in subsequent calculations (see Fig. 3).

fore, in subsequent experiments, the contribution of this extracellular component (5.0 nmol/10^6 protoplasts) was substracted from all uptake measurements. It must be noted however that even PEG 800 seemed to enter slowly into the cells, due to passive diffusion or (and) to possible extracellular hydrolysis into smaller molecules, or to adsorption to the outside of the protoplasts (Fig. 1).

After 20 min incubation in [^{14}C]sucrose, only 3.6% of the total radioactivity of the samples was recovered in the insoluble fraction. If the contribution of the extracellular fluid is taken into account, this means that only 5.2% of the sucrose taken up was converted into insoluble products. Chromatographic analysis of the soluble fraction yielded only sucrose (Fig. 2, A), and upon hydrolysis by invertase, equal amounts of [^{14}C]glucose and [^{14}C]fructose were found (Fig. 2, B). This indicates that sucrose was taken up without prior hydrolysis, and was not hydrolyzed in the intracellular compartment either, at least during short incubation times. The same conclusions were reached when similar experiments were carried out with leaf fragments instead of protoplasts (data not shown). The anti-42 kDa serum used for uptake studies, which was tested for invertase activity, did not hydrolyze sucrose (Fig. 2).

Effects of the anti-42 kDa serum on the uptake of various solutes

The effects of the serum raised against the 42 kDa polypeptide of sugar beet plasma membranes were tested on the uptake of sucrose (Fig. 3A), 3-*O*-MeG (Fig. 3B) and α-AIB (Fig. 3C). These effects were compared with those of the pre-immune serum (obtained from the same rabbit before injection of the antigen). The pre-immune serum exerted a small but significant inhibition of sucrose uptake (Fig. 3A), while it had no effect on the uptake of 3-*O*-MeG (Fig. 3B) nor on that of α-AIB (Fig. 3C). To check if sucrose uptake was not simply sensitive to the presence of proteins in the medium, we tested the effects of BSA (142 µg/ml). This treatment did not inhibit sucrose uptake (data not shown). As will be discussed below in the light of other results, the inhibition exerted by the pre-immune serum must be considered as non-specific. The anti-42 kDa serum inhibited markedly sucrose uptake (Fig. 3A) without affecting the uptake of the two other substrates tested (Figs. 3B and 3C). This inhibition reached 55% of the control rate, and 45% of the uptake rate measured in the presence of pre-immune serum.

Characterization of the specific inhibition of sucrose uptake by the anti-42 kDa serum

To characterize more precisely the effect of the anti-42 kDa serum on sucrose uptake, it was compared with that exerted by a serum directed against all proteins of the plasma membrane, and with that of various compounds known to inhibit sucrose uptake. At the same dilution as the anti-42 kDa polypeptide, the anti-plasma membrane serum had no significant effect on the rate of sucrose uptake (Table I). This lack of inhibition was probably due to a low titer of this serum against the 42 kDa polypeptide, because the 42 kDa protein represents only a small proportion of total plasma membrane proteins.

The supernatant recovered from the serum after precipitation by 40% ammonium sulfate did not inhibit sucrose uptake, suggesting that the inhibition was indeed due to the Igs (Table I). Yet, various concentrations of the purified Igs, alone or in combination with BSA or with the supernatant of the serum were also ineffective (Table I).

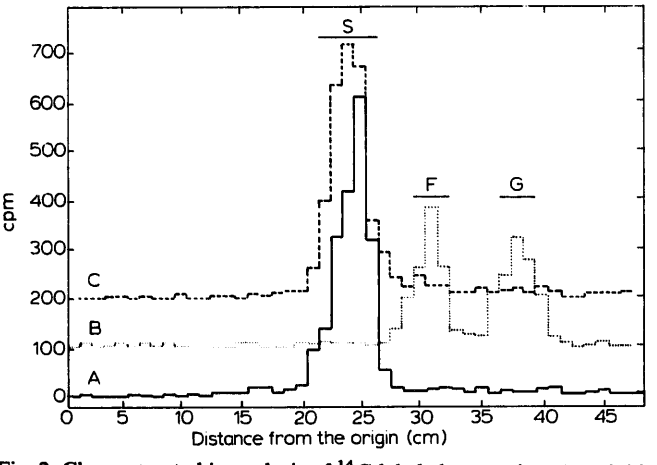

Fig. 2. Chromatographic analysis of ^{14}C-labeled sugars in: (A) soluble extracts from protoplasts after incubation with 1 mM [^{14}C]sucrose; (B) same extracts as (A) subjected to invertase hydrolysis and (C) [^{14}C]sucrose incubated with anti-42 kDa serum. Positions of sucrose (S), glucose (G) and fructose (F) are indicated above the chromatograms. For the purpose of clarity the cpm values at the origin (o) were moved to 100 cpm (B) and 200 cpm (C).

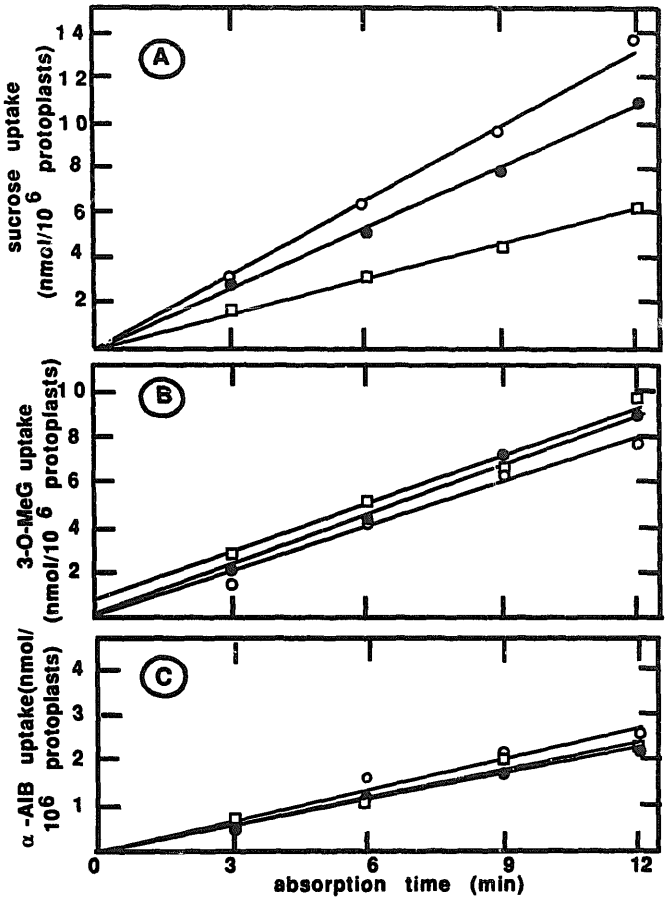

Fig. 3. Effects of the anti-42 kDa serum (□) and the pre-immune serum (●) on sucrose (A), 3-*O*-MeG (B) and α-AIB (C) uptake by broad bean mesophyll protoplasts ($0.5 \cdot 10^{-6}$ protoplasts/ml). All substrates were tritiated and tested at 1 mM, dilution of sera was 1/256. (○): control uptake without sera. Each figure is the mean of 12 (A) or 8 (B, C) different experiments. Free space contribution at zero time was deducted from all measurements. (A) Uptake rate of sucrose in the presence of pre-immune serum (●) was significantly different (0.01%) from the control uptake rate (○) and the uptake rate in the presence of anti-42 kDa serum (□) was significantly different (0.001%) from the uptake rate in the presence of pre-immune serum. (B, C) The uptake rates were not significantly different.

Finally, since PCMBS is a strong inhibitor of sucrose uptake in leaf tissues [16] and is sometimes used as a reference to check the effect of antibodies on sugar uptake in similar experiments [17], we studied the effect of this compound under our experimental conditions. The inhibition caused by 500 μM PCMBS was only 27% of control uptake. This value is much lower than that found in broad bean leaf tissues with the same inhibitor concentration and the same preincubation time (70% [2]).

Specificity of the antiserum and of the antibodies

Electrophoresis of the antigen prepared from the 42 kDa region of preparative SDS-PAGE yielded three apparent bands in the 42 kDa region, together with minor bands in the 50–70 kDa region (Fig. 4A). The appearance of several bands in the 42 kDa region may

TABLE I

Effect of various additions on the rate of sucrose uptake by broad bean leaf protoplasts

The data are expressed as percent of the control rate (no addition). Supernatant is the supernatant recovered from the anti-42 kDa serum after precipitation of the immunoglobulins. Ig refers to the immunoglobulins precipitated from the anti-42 kDa serum, *n* is the number of experiments for each condition. Statistical analysis was made by means of a *t*-test; s, significant; ns, not significant.

Solution added	Rate of sucrose uptake (% of control)	*n*	Significance (0.05 level)
Anti-42 kDa serum (1/256)	46	12	s
Anti-plasma membrane serum (1/256)	84	8	ns
Ig (29 μg/ml)	102	4	ns
Ig (29 μg/ml) + BSA (142 μg/ml)	87	4	ns
Ig (100 μg/ml)	97	4	ns
Ig (100 μg/ml) + BSA (142 μg/ml)	102	4	ns
Ig (60 μg/ml) + supernatant (170 μg/ml)	113	4	ns
Supernatant (170 μg/ml)	84	12	ns
PCMBS (500 μM)	73	12	s

be due to the presence of several polypeptides in the 2 mm-wide strips cut out from the preparative SDS-PAGE and used for electroelution. It is also possible that the 42 kDa polypeptide presents abnormal migration patterns due to hydrophobicity and to aggregation phe-

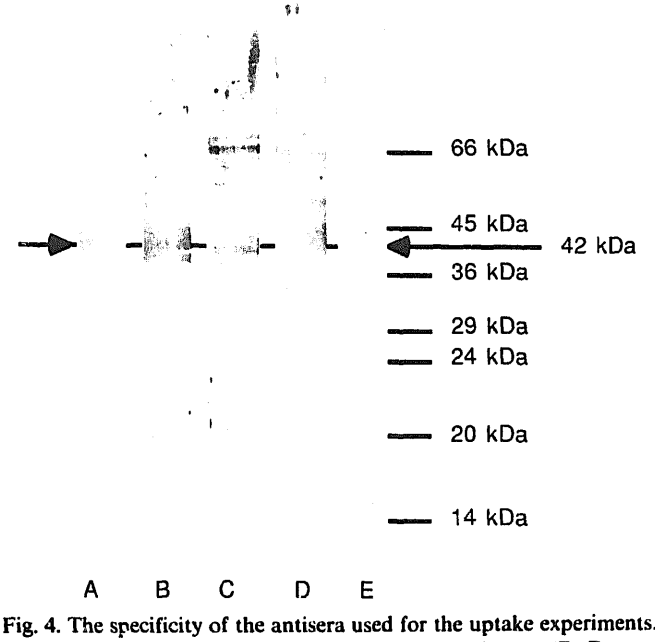

Fig. 4. The specificity of the antisera used for the uptake experiments. The 42 kDa antigen (B, C) or purified plasma membranes (D, E) were separated by SDS-PAGE and then blotted on to a nitrocellulose membrane. The blots were incubated with anti-42 kDa serum (B, C, D) or with pre-immune serum (E) and then with a peroxidase-conjugated goat antirabbit IgG. (A) Silver staining of the 42 kDa antigen.

nomena [17,18], as reported for many transport proteins. Blots of the anti-42 kDa serum directed against the purified 42 kDa polypeptide presented the same reaction pattern, i.e. a major band at 42 kDa (Fig. 4B), sometimes accompanied by several minor bands in the 60–70 kDa region (Fig. 4C), depending on the batch of 42 kDa polypeptide used. The pattern found in the 60–70 kDa region is typical for contamination by keratin [19], and it is therefore possible that some of the 42 kDa antigen samples used to immunize the rabbit contained trace amounts of keratin. Although gloves were used throughout in the preparation of the samples, the presence of reducing agents in the media may have favored this contamination [20]. Compared to lane C, the 60–70 kDa bands were much less apparent in blots using total plasmalemma (lane D), suggesting that keratin contamination occurred mainly during the different steps (electrophoresis, electro elution, dialysis) needed to prepare the antigen starting from the membranes. Blots directed against the plasma membrane showed a major band at 42 kDa, and a fainter reaction in the 50–70 kDa region (Fig. 4D). The pre-immune serum showed no reaction with any of the plasma membrane polypeptides (Fig. 4E). Whatever the origin (either sugar beet or broad bean) of the proteins (42 kDa polypeptide or total plasma membranes) used in the blotting experiments with the anti-42 kDa serum, results similar to those decsribed above were obtained.

Discussion

The main purpose of this paper was to test by functional means the previous hypothesis [5] according to which the 42 kDa polypeptide of the plasma membrane is involved in the absorption of sucrose by the plant cells.

The anti-42 kDa serum was able to inhibit strongly and selectively sucrose uptake (Fig. 3). The extent of the inhibition measured in these conditions (55%) is even greater than that measured with PCMBS (27%, Table I), a powerful inhibitor of sucrose uptake [2,16] in leaf tissues. The relatively weak inhibition exerted by PCMBS in the present experiments with protoplasts, compared to the inhibition found in intact leaf tissue (about 70% [2]) may result from the absence of the vein (phloem) cells in the protoplast preparation, since these cells are a major target in leaf tissues, as shown by autoradiographs [1]. Inhibition of sucrose uptake by the protoplasts in the presence of anti-42 kDa serum cannot be explained by hydrolysis of sucrose, since the serum contained no invertase activity (Fig. 2, C). Neither can it be explained by metabolic effects since the sucrose taken up was not involved significantly in the synthesis of insoluble material (starch, cell wall), and was taken up without hydrolysis (Fig. 2 A and B). Given this poor metabolism, interpretations involving an effect of the serum on glucan synthase or invertase must be discarded.

Since all substrates tested are taken up with proton symport [21,22], it is also possible to exclude inhibition of sucrose uptake via an effect of the serum on the proton motive force, because hexose and amino acid absorption remained unaffected.

The simplest way to explain the data presented here is to conclude that the anti-42 kDa serum blocked a protein essential for sucrose uptake, namely the 42 kDa polypeptide. This conclusion confirms, on a functional basis, the hypothesis emerging from the indirect data of differential labeling [5]. Although the pre-immune serum inhibited slightly but significantly sucrose uptake (Fig. 3A), it did not react with plasma membrane components (Fig. 4E). This inhibition must then be ascribed to a side effect, although specific for sucrose, since it did not appear for hexoses and amino acids (Figs. 3B and 3C).

Lack of inhibition of sucrose uptake by the antibodies purified from the anti-42 kDa serum is more surprising. However, similar data, still unexplained, have been mentioned when monoclonal antibodies to lac permease or to the animal Na^+/glucose symporter are purified and assayed in uptake experiments [23,24]. In some cases, antibodies purified from a strongly inhibiting serum even stimulates the uptake of substrate [24]. Interestingly, this discrepancy between whole serum and purified antibodies only appears when rather specific purification methods are used. For example, Wu and Lever [24] reported that antibodies purified by gel filtration kept their inhibiting activity towards the binding of phlorizin to brush-border membranes while antibodies purified on protein A-Sepharose lost this inhibiting activity. It must be concluded that in our experiments, as in those mentioned above, the serum contains still unidentified components affecting synergistically the binding of the antibodies to the membrane, or that the activity of the antibody subclass responsible for inhibition of uptake is lost during the purification procedure used.

In conclusion, the data presented above and in the accompanying paper [5] concur to provide direct (use of antibodies) and indirect (differential labeling) evidence that a 42 kDa polypeptide may be (part) of the sucrose carrier of the plant plasma membrane. This carrier, which plays an important physiological role, seems to share a number of common properties in broadbean and in sugar beet, since it exhibits the same molecular weight, and since antibodies against the 42 kDa polypeptide of sugar beet are efficient inhibitors of sucrose uptake in broad bean.

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