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Functional reconstitution of the malate carrier of barley mesophyll vacuoles in liposomes

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The malate carrier of barley (*Hordeum vulgare* L.) mesophyll vacuoles was highly purified by chromatography on hydroxyapatite followed by affinity-chromatography using 5-amino-1,2,3-benzenetricarboxylic acid as ligand. The carrier, reconstituted in asolectin liposomes, had properties similar to those described previously for the carrier in intact vacuoles (Martinoia, E., Flügge, U.I., Kaiser, G., Lieber, U. and Heldt, H.W. (1985) Biochim. Biophys. Acta 806, 311–319). The apparent K_m for malate uptake was 2–3 mM, and the uptake was inhibited by other carboxylic acids (preferentially tricarboxylic). The sulfhydryl reagent, *p*-chloromercuribenzenesulfonate, as well as the anion transport inhibitor 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid, also inhibited malate uptake. The transport was dependent on the membrane potential with an optimum at about 35 mV.

Introduction

Fluxes of ions across the vacuolar membrane of plant cells are involved in the regulation of cell turgor and cytoplasmic homeostasis [1–3]. One of the predominant organic anions is malate which can accumulate to concentrations of more than 200 mM in plants exhibiting Crassulacean acid metabolism (CAM) [4]. In CAM plants, malic acid accumulates during the night, and this process is reversed during the daytime. In contrast, in leaves of C_3 plants, levels of malate are high at the end of the day and low in the morning [5]. For C_3 plants it has been shown that the fluctuation in the malate concentration results from the dynamics of the vacuolar malate pool, whereas the cytosolic malate concentration is kept constant [5]. Similar conclusions have been drawn indirectly from experiments with CAM plants [4].

Recently, malate uptake into vacuoles of C_3 and CAM plants has been investigated using either isolated vacuoles or tonoplast vesicles. An apparent K_m of 1.5 to 5 mM has been determined for mesophyll vacuoles from C_3 plants, and of 1 to 10 mM for vacuoles from CAM plants, respectively [6–10]. Malate uptake was not specific for the natural L-enantiomer of the acid. D-Malic acid and other di- and tricarboxylic acids act as competitive inhibitors. Uptake of malate is ATP-dependent, suggesting that the tonoplast-bound ATPase [11,12] is involved in the energization of the transport process.

No experimental data are available on the mechanism controlling the flux of malate across the tonoplast. Such regulatory mechanisms must, however, be postulated on the basis of the observed dynamics of vacuolar malate concentrations.

In the present study we have purified and reconstituted the malate carrier with the objective to obtain further information on the mechanism of malate compartmentation and its regulation.

2. Materials and Methods

Plant material. Barley (*Hordeum vulgare* L. cv Gerbel) was grown in a growth cabinet with 12 h fluorescent light (45 μmol m^{-2} s^{-1}) at 22°C and 12 h dark at 18°C; relative humidity was 75%.

Chemicals. Percoll and Ficoll were supplied by Pharmacia (Uppsala, Sweden), benzenetricarboxylic acid by Aldrich Chemie (Steinheim, F.R.G.), asolectin by

Abbreviations: CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate; pCMBS, *p*-chloromercuribenzenesulfonate; DIDS, 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; Mes, 4-morpholinoethanesulphonic acid.

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Fluka AG (Buchs, Switzerland), Triton X-100 by Boehringer (Mannheim, F.R.G.), hydroxyapatite by Bio-Rad (Richmond, CA). Dowex 1X8-100 was from Sigma (St. Louis, MO) and was used in the chloride form. L-[U^{14}C]Malate (2TBq mol^{-1}) was from Amersham International, U.K.

Isolation of vacuolar membranes. Primary leaves of 8-day-old barley plants were harvested at the beginning of the light period. Mesophyll protoplasts were prepared as described [13]. Mesophyll vacuoles were isolated and purified by a slight modification of the method described by Martinoia et al. [14]. Briefly, protoplasts were suspended in 10 ml of a solution containing 500 mM sorbitol, 30% (v/v) Percoll, 1 mM $CaCl_2$, and 10 mM 4-morpholinoethanesulphonic acid (Mes) (pH 6.0). This suspension was overlayered with 8 ml medium A (400 mM sucrose, 3% (w/v) Ficoll, 2 mM EDTA, 1 mM Mg-gluconate and 30 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Hepes-KOH, pH 7.6)) and 4 ml medium B (400 mM sorbitol, 30 mM K-gluconate, 2 mM EDTA, 1 mM Mg-gluconate and 20 mM Hepes-KOH (pH 7.6). After centrifugation for 3 min at $200 \times g$ and 4 min at $1200 \times g$, the protoplasts were recovered from the upper interphase and forced through a needle (100 mm × 0.8 mm) to liberate the vacuoles.The vacuoles were purified by flotation. For this, the lysate was overlayered with 10 ml of medium B and 2 ml of medium C (as medium B but 400 mM glycinebetaine instead of 400 mM sorbitol). The vacuoles were collected from the upper interphase. Batches recovered from several density gradients were mixed with Percoll (final concentration 10% v/v) containing 500 mM sorbitol and 15 mM Hepes-KOH (pH 7.6) and a gradient as described for the purification of the vacuoles was formed. The purified and concentrated vacuoles were again recovered from the upper interphase and mixed with an equal volume of medium C. After a further centrifugation step (20 s at $10000 \times g$) the vacuoles were recovered as a compact sediment. They were either used directly or stored in liquid nitrogen. Contamination of the vacuolar preparation with other cell constituents was less than 1% as assessed by the determination of the activity of marker enzymes [15].

Vacuolar membranes (tonoplasts) were prepared by mixing one part of the sedimented vacuoles with three parts of 20 mM Tricine-imidazole (pH 7.0) containing 4 mM $MgCl_2$. After a 5 min incubation on ice, the tonoplasts were sedimented by centrifugation for 30 min at $100000 \times g$ at 4°C.

Solubilisation and reconstitution of the malate carrier. Vacuolar membranes corresponding to approx. 400 μg of protein were mixed with 0.5 ml of medium D (380 mM sorbitol, 10 mM K-gluconate and 10 mM Tricine-imidazole (pH 7.0)). The membranes were solubilised by the addition of 0.5 ml of medium D containing 4% (v/v) Triton X-100 and 4 mg ml^{-1} asolectin (soybean lectin). The suspension was kept on ice for 20 min. For the incorporation of the carrier into liposomes the Triton X-100 concentration was reduced to 0.5% by the addition of medium D, and the K-gluconate concentration was adjusted to 20 mM.

Reconstitution of the proteins into liposomes was achieved by a slight modification of the method described by Kaplan and Pedersen [16]. Briefly, 115 mg ml^{-1} asolectin was added to medium D containing 20 mM K-gluconate and 360 mM sorbitol. The tube, after flushing with nitrogen, was sealed and vortexed vigorously for 10 s. The lipid was dispersed in a bath sonicator for about 45 min and subsequently sonicated with a probe sonicator for a total of 14 s (2×7 s pulses, 7 s intervall, 50% duty). Equal volumes either of the solubilised membranes or of the purified fractions (see below) were mixed with the dispersed lipid and rapidly frozen in liquid nitrogen. Prior to the assay the samples were allowed to thaw on ice and were then sonicated for a total of 14 s (2×7 s pulses, 3 s intervall, 50% duty).

Malate uptake. To energize the accumulation of malate in the proteoliposomes an inside positive membrane potential was generated by an asymmetric potassium distribution in the presence of 10^{-7} M valinomycin. For this, one part of medium E (200 mM K-gluconate, 10 mM Tricine-imidazole (pH 7.0), unless stated otherwise) was added to two parts of proteoliposomes. [^{14}C]Malate uptake (3.7 kBq/100 μl assay medium, 74 MBq mmol^{-1}) was calculated from the difference of label taken up in the presence or absence of 50 μM 4,4'-diisothiocyano-2,2'-stilbenedisulfonic acid (DIDS). The samples were preincubated for four minutes at 20°C in the presence or absence of DIDS.

To separate the proteoliposomes from the incubation medium, 100 μl (three or four replicates) of the assay medium were loaded on a Dowex 1X8-100 column (400–500 μl) and eluted with 500 μl of 450 mM sorbitol and 10 mM Tricine-imidazole (pH 7.2). Where indicated, the elution buffer was adjusted to the K-gluconate concentration of the incubation medium. A 500-μl aliquot of the eluate was used to determine the radioactivity taken up by the liposomes.

Hydroxyapatite chromatography. Dry hydroxyapatite (500 mg) in a small plastic column was cooled to 4°C. The column was loaded with 500 μl of the solubilised membranes with the final Triton X-100 concentration adjusted to 1% by the addition of medium D. The malate carrier was eluted with medium D containing 1% Triton X-100 and 3 mg ml^{-1} asolectin, and the first 600 μl of the eluate were collected. For incorporation into proteoliposomes the concentration of Triton X-100 and K-gluconate were adjusted to 0.5% and 20 mM, respectively.

Synthesis of the ligand, 5-amino-1,2,3-benzenetricarboxylic acid. Nitration of 1,2,3-benzenetricarboxylic acid was achieved by a slight modification of the method

described by Prelog and Schneider [17]. 1,2,3-Benzenetricarboxylic acid (8 g) was added to 45 ml of concentrated H_2SO_4 monohydrate. The mixture was heated to 60–70°C and 15.6 g of KNO_3 were added in small portions over a period of about 2 h. The temperature was raised to 110–120°C and the mixture was refluxed for 12 h. After cooling, ice cubes were added and the mixture was extracted with diethyl ether. The diethyl ether extract was reduced to dryness, the product again dissolved in diethyl ether, and petroleum ether (40–60°C) was added. The product crystallized overnight with a final yield of 5.8 g. The ^{1}H-NMR spectrum of the product in ^{2}H-dimethylformamide confirmed its structure as 5-nitro-1,2,3-benzenetricarboxylic acid. Two g of the product were dissolved in 50 ml of methanol, mixed with 0.2 g 10% palladium/charcoal in a round-bottom flask, and flushed with hydrogen gas under atmospheric pressure and vigorous mixing. When H_2 consumption had terminated, the reaction mixture was diluted with 150 ml of ethanol and filtered. The filtrate was reduced to dryness by rotary evaporation and the residue was dissolved in a minimum of boiling water. After crystallization at 4°C overnight the slightly yellow crystalls were collected by filtration and washed with ice-cold water. The yield of the product, identified as 5-amino-1,2,3-benzenetricarboxylic acid by ^{1}H-NMR, was 700 mg.

Coupling of the ligand to the resin. Fifty mg of 5-amino-1,2,3-benzenetricarboxylic acid, dissolved in 3 ml dimethylformamide, were added to 3 ml of *N*-hydroxysuccinimide activated resin (Affi-Gel 10, Bio-Rad, Richmond, CA) which had been washed with dimethylformamide. After 2 h of incubation (1 h at 4°C, 1 h at 30°C) the resin was washed with 100 mM Tris-HCl (pH 7.5). The binding of the ligand was confirmed by comparison of the absorption spectra of the resin before and after the coupling.

Affinity chromatography. An aliquot (1–3 ml) of the eluate from the hydroxyapatite column was loaded onto the benzenetricarboxylate-Sepharose column, washed three times with 1.2 ml of washing buffer (medium D containing 1% Triton X-100 and 3 mg ml^{-1} asolectin as described above) and then eluted with washing buffer containing 40 (fractions 5–9), respectively, 60 (fractions 10–12) mM benzenetricarboxylic acid. From 1.2-ml fractions, 200 μl were used for the assay of malate transport activity. They were loaded onto a Dowex 1X8-100 (300 μl) column to remove benzenetricarboxylic acid and then eluted with 300 μl of medium D. The remaining portions were frozen in liquid nitrogen and subsequently used for uptake experiments or for gel electrophoresis.

Gel electrophoresis. SDS-PAGE was performed according to Laemmli [18] using a 12.5% or 13.5% polyacrylamide separation gel and piperazine diacrylamide as crosslinker. Silver staining was performed according to Morrisey [19]. In the absence of proteins, two silver-stained bands were visible which originated from the buffer system used. Various attempts to avoid this unspecific staining failed however. Eluates from the hydroxyapatite and from the affinity column were first freeze-dried in silanized tubes and then dissolved in 200–400 μl water. Proteins were concentrated by phenol extraction followed by ammonium acetate-methanol precipitation [20]. Samples were suspended directly in SDS sample buffer.

Protein determination. Protein was determined according to the method of Spector [21] or estimated from Coomassie-stained gels. Since the amount of protein was too low to be accurately determined in the highly purified fractions the data of some of the uptake experiments were based on the amount of vacuoles rather than protein to allow a comparison with experiments in which intact vacuoles were used (10^8 vacuoles correspond to 1 mg of chlorophyll or to approx. 100 μg of vacuolar protein).

Results and Discussion

Solubilisation and purification of the malate carrier

Best solubilisation of vacuolar membranes was achieved using 2% Triton X-100. Other detergents tested failed to solubilise the membranes to the same extent (octyl glucoside) or turned out to be unsuitable in further purification steps (CHAPS, deoxycholate). The Triton X-100 concentration was reduced to 0.5% before mixing the solubilised membranes with the dispersed lipid. This procedure reduced the nonspecific uptake of [^{14}C]malate to a minimum, and a higher accuracy of the experiments was achieved.

A chromatographic step using dry hydroxyapatite has been shown effective in the purification of several mitochondrial carriers [16,22–24]. The vacuolar malate carrier could also be enriched by this procedure. About 80% of the malate-transport activity was recovered in the eluate, whereas more than 90% of the total protein was retained on the column. An exact determination of the protein content in the hydroxyapatite eluate was not possible due to the very low amount of polypeptides and to the presence of Triton X-100 in this fraction. A comparison of the protein pattern and of stain intensity shows, however, how powerful this first purification step is (Fig. 1). Lane a (vacuolar membranes) of this figure corresponds to approx. 2 μg of protein. In lane b, the same amount of vacuolar polypeptides had been loaded on the hydroxyapatite column, and the entire eluate was then loaded on the gel. Lane c shows the polypeptide pattern from the whole eluate from the hydroxyapatite column to which 25 μg of vacuolar membrane proteins had been loaded.

Like the mitochondrial tricarboxylate carrier, the vacuolar malate carrier is inhibited competitively by

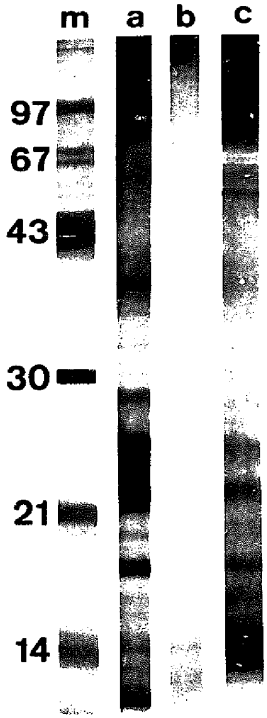

Fig. 1. Polypeptide patterns of purified tonoplasts and hydroxyapatite eluates containing the malate carrier activity. (a) Tonoplast membranes corresponding to $2 \cdot 10^5$ vacuoles (2 μg protein); (b) hydroxyapatite eluate loaded directly on the gel, the same number of vacuoles as in (a) was used as starting material; (c) concentrated hydroxyapatite eluate from $2.5 \cdot 10^6$ vacuoles (25 μg protein). Phosphorylase *b* (97 kDa), bovine albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (29 kDa) and trypsin inhibitor (21 kDa) were used as molecular markers (m). Polyacrylamide concentration was 13.5%. For experimental details see Materials and Methods.

1,2,3-benzenetricarboxylic acid [26]. We took advantage of this property and synthesized an affinity column with 5-amino-1,2,3-benzenetricarboxylic acid as ligand (see Materials and Methods).

One to three ml of hydroxyapatite eluate (starting from 400–700 μg membrane protein) were loaded onto the affinity column and fractions of 1.2 ml were collected. As shown in Fig. 2, the malate transport activity eluted in two peaks. The activity in the first peak eluted in the absence of 1,2,3-benzenetricarboxylic acid, which probably indicates that one fraction of the carrier is incorporated into the Triton X-100 micelles with the cytoplasmic face outside and the other with the vacuolar face outside. Since the malate carrier is a uniporter, the binding properties of both faces may be different. We have some indication that the affinity of the malate carrier to malate inside the vacuole is low, since malate efflux from isolated vacuoles is very low [25]. The first peak containing malate uptake activity eluting from the affinity column may therefore represent Triton X-100 micelles in which the vacuolar face of the carrier is exposed to the ligand. It follows that the second activity peak which is specifically eluted by 1,2,3-benzenetricarboxylic acid may represent the Triton X-100 micelles with the cytosolic face of the carrier exposed to the outside. As is evident from Fig. 3, lane a, most of the proteins in the hydroxyapatite eluate are not bound to the affinity column. Thus, the polypeptide pattern of lane a corresponds largely to that observed in the hydroxyapatite eluate (Fig. 1, lane c). In contrast, only a few polypetides which are scarcely visible (Fig. 3, lane b) appear in the two peaks containing malate transport activity. However, we still do not know which of the polypeptides is (are) involved in malate transport across the vacuolar membrane. Attempts using *N*-ethyl[^{14}C] maleimide or pyridoxal phosphate followed by [^{3}H]BH_4 reduction to covalently label a polypeptide involved in malate transport have failed so far. Both these agents are known to inactivate malate transport (Ref. 26, and Martinoia, unpublished data).

Only a rough estimate can be made of the purification factor. The hydroxyapatite column gave at least a 20–30-fold purification. Purification by the affinity column can only be estimated by comparing the intensity of the staining of the unbound fraction with that of the fraction eluted with benzenetricarboxylic acid and containing 40 to 60% of the malate transport activity (see Fig. 3). Even taking into acount that silver stainining is not proportional for different polypeptides, a purifica-

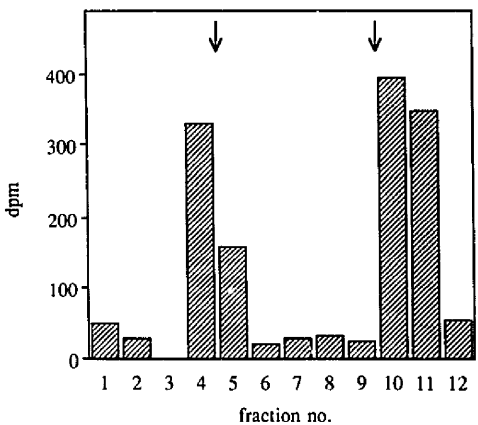

Fig. 2. Uptake of malate (after reconstitution) in the fractions obtained from the affinity chromatography. 3 ml of hydroxyaptite eluate were loaded on the benzenetricarboxylate-Sepharose column and eluted as described in Materials and Methods. The arrow after fraction 4 indicates addition of 40 mM benzenetricarboxylate to the buffer. From fraction 10 (arrow) to 12 the benzenetricarboxylate concentration was raised to 60 mM.

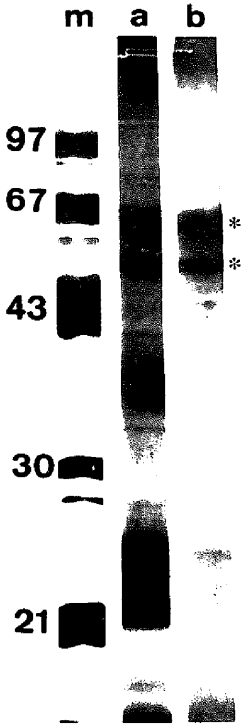

Fig. 3. Polypeptide pattern of eluates from the affinity chromatography column. (a) material not bound by the column (fraction 1 from Fig. 2); (b) fraction containing the malate carrier activity (fraction 10 and 11 from Fig. 2). The two stained regions marked with an asterisk are due to the buffer system used and do not represent vacuolar polypetides (for this, and for experimental details see Materials and Methods). Molecular markers (m) as in Fig. 1.

tion factor of at least 20 can be estimated. Furthermore, as the vacuolar membrane of mature plants contains less than 1% of the total cellular protein [15], an overall purification factor, relative to the total protein of a mesophyll cell, for the affinity purified malate carrier of about 30000 to 50000 appears realistic.

Characterization of the purified and reconstituted malate carrier

The purified functional malate carrier was reconstituted in liposomes (see Materials and Methods).

As the vacuolar malate carrier is not an antiport system, uptake of labelled malate cannot be driven by loading liposomes with unlabelled counter-metabolites [23,24]. We had therefore to generate a driving force. A positive $\Delta\psi$ can be generated by an asymmetrical potassium gradient in the presence of valinomycin [27]. To assess the size of the membrane potential generated by this procedure we have determined the amount of the permeable anion [^{14}C]SCN$^-$ (Fig. 4) taken up at equilibrum. The linear relationship observed for SCN$^-$ present in the liposomes as a function of the $K^+_{outside}/K^+_{inside}$ ratio (K_0^+/K_i^+) means that the membrane potential generated by the asymmetrical potassium gradient behaves ideally in our system. Uptake of malate has an optimum at a K_0^+/K_i^+ ratio of about 4 (Fig. 5). The same behaviour can be observed when sorbitol or sodium gluconate was used to maintain the osmotic pressure of the external medium. Therefore an influence of ionic strength can be excluded. The dependence of the malate uptake rate on the $K^+_{outside}/K^+_{inside}$ ratio indicates that malate uptake into the liposomes is voltage-dependent and is therefore mediated by a transfer system. The optimal membrane potential for malate uptake was found to be approx. +35 mV. Interestingly, this is the membrane potential found in isolated vacuoles [28]. Since potassium concentrations are known to vary in plant vacuoles, a dependence of the malate transport on the membrane potential is difficult to demonstrate with isolated, intact vacuoles. No data are available on the membrane potential between the cytosol and the vacuole in higher plant cells in situ. The hypothesis that changes in the membrane potential between the cytoplasm and the vacuole may be involved in the regulation of malate exchange across the tonoplast requires the confirmation that the membrane potential across the vacuolar membrane fluctuates in vivo.

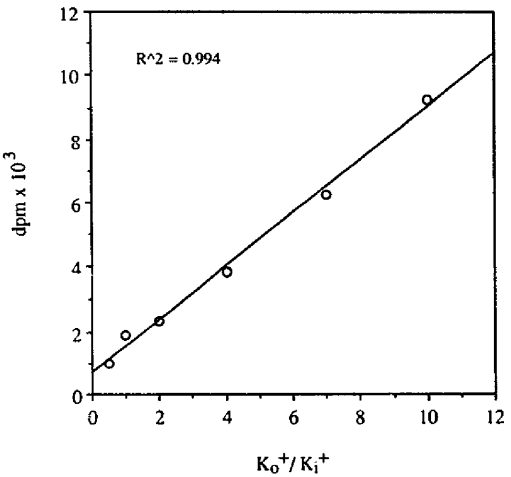

Fig. 4. Uptake of [^{14}C]SCN$^-$ as a function of different potassium outside [K_0^+] to potassium inside [K_i^+] ratios. Liposomes or proteoliposomes were incubated until equilibrum was reached (three minutes) in the presence of 30 μM [^{14}C]SCN$^-$ (2.1 GBq/mmol). To remove [^{14}C]SCN$^-$ not taken up, the liposomes or proteoliposomes were loaded on Dowex 1X8-100 columns (400–500 μl) Equilibration of the columns and elution were carried out with media adjusted with respect to potassium to the concentrations of the respective incubation medium.

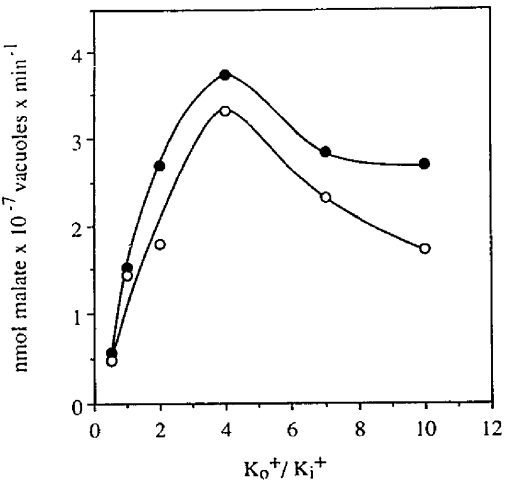

Fig. 5. Rate of [^{14}C]malate uptake into proteoliposomes as a function of membrane potential. The potassium concentration inside the liposomes was 10 mM. The osmotic potential of the external medium was kept constant by varying the sorbitol concentration (○) or by maintaining a constant salt concentration and varying the sodium gluconate concentration (●). Proteoliposomes generated from the eluate of the affinity chromatography column were incubated for 1 min in the presence of 0.5 mM [^{14}C]malate (74 MBq/mmol) at various membrane potentials. Similar results were obtained using the hydroxyapatite eluate to generate proteoliposomes. 10^7 vacuoles correspond to 100 μg total tonoplast protein. For experimental details and calculation of the uptake rates see Materials and Methods.

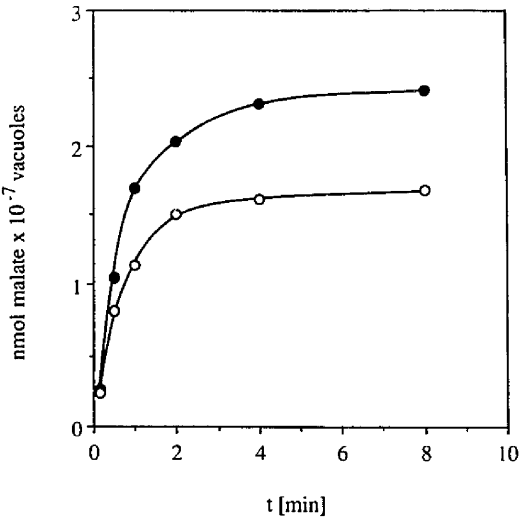

Fig. 6. Time-dependent malate uptake into proteoliposomes. Proteoliposomes were incubated in the presence of 0.5 mM [^{14}C]malate (74 MBq/mmol) for the times indicated. The results of two experiments are shown. Uptake was calculated by subtracting the values obtained in the presence from those obtained in the absence of 50 μM DIDS (●) or from the difference between proteoliposomes and pure liposomes (○).

Uptake of malate into proteoliposomes is time-dependent and is completed within three minutes (Fig. 6). Similar results were obtained when the unspecific label taken up by liposomes was subtracted from that taken up by the proteoliposomes containing the malate carrier. Nonspecific uptake of radioactive label accounted for 20 to 35 percent of the total label taken up by liposomes containing the functional malate carrier. The rates of uptake observed differed from one preparation to the other and were about 10–30% of those observed with intact vacuoles [7]. Protein fractions incorporated into liposomes after they had been heated for five minutes in a boiling water bath showed no malate uptake activity.

TABLE I

Inhibition of [^{14}C]malate uptake into proteoliposomes by di- and tricarboxylates

Proteoliposomes prepared from the eluate of the affinity column were incubated for 1 min in the presence of 0.5 mM labelled malate and of the respective inhbitor at 5 mM concentrations. The control rate was 3.4 nmol malate/10^7 vacuoles per min.

Treatment	% Uptake
Control	100
Malonate	50
Phenylmalonate	78
Phenylsuccinate	28
Benzenetricarboxylic acid	8
Citrate	6

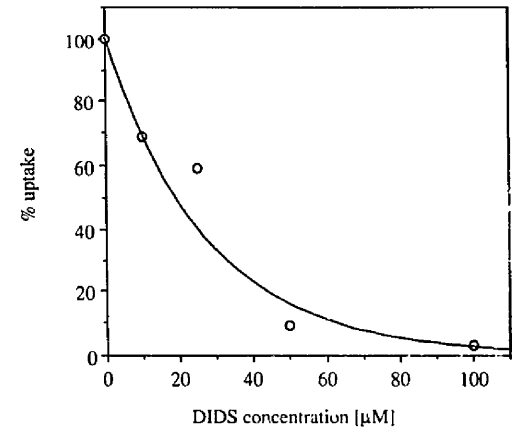

Fig. 7. Concentration-dependent inhibition of malate uptake by DIDS. Proteoliposomes generated from the polypeptide in the eluate of the affinity chromatography were preincubated for 4 min at the DIDS concentrations indicated. Malate uptake was determined using 0.5 mM [^{14}C]malate (74 MBq/mmol). 100% correspond to 2.1 nmol malate/10^7 vacuoles per min.

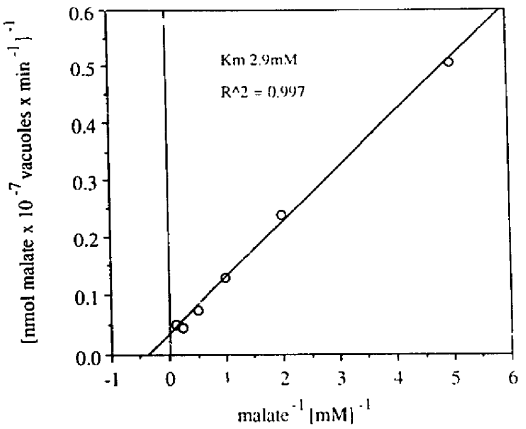

Fig. 8. Lineweaver-Burk plot of malate uptake. Proteoliposomes generated from the eluate of the affinity chromatography were incubated for 1 min in the presence of [^{14}C]malate (37 kBq/ml) at the concentrations indicated and uptake was determined as described in Materials and Methods.

Malate uptake was inhibited by the anion transport inhibitor DIDS [29] (Fig. 7) as was previously observed with intact vacuoles [26]. Inhibition was usually almost complete at a concentration of 50 μM. As with intact vacuoles, 1 mM pCMBS completely inhibited malate uptake (not shown).

Malate uptake exhibited saturation kinetics (Fig. 8) with an apparent K_m of 2.0–3.2 mM, which is similar, though slightly higher, to that observed with intact vacuoles [7]. The slightly higher K_m value observed in the reconstituted system may be due to the difficulty to exactly determine the initial rates of uptake.

Various inhibitors also had effects comparable to those seen with isolated, intact vacuoles (Table I). Tricarboxylates, such as citrate or 1,2,3-benzenetricarboxylic acid, are strong inhibitors of malate uptake indicating that the vacuolar malate carrier has some similarity to the mitochondrial tricarboxylate carrier [30,31]. Phenylsuccinate also acts as a strong inhibitor, whereas phenylmalonate and malonate, which are both good inhibitors of the malate/malate exchange of the mitochondrial dicarboxylate carrier [24] showed only slight inhibition.

Chloride, also at higher concentrations (40 mM), had only a slight inhibitory effect (not shown). As malate and chloride cross the tonoplast by different carriers [26] this effect is probably due to a partial dissipation of the imposed membrane potential

Conclusions

A highly purified malate carrier from barley mesophyll vacuoles has been reconstituted into liposomes and been shown to exhibit characteristics similar to those of the carrier described for intact vacuoles. The membrane potential-dependence of the transport suggests that this parameter may be involved in the regulation of malate transport into the vacuole.

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