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Maltose/proton co-transport in *Saccharomyces cerevisiae*

Comparative study with cells and plasma membrane vesicles

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Maltose/proton co-transport was studied in intact cells and in plasma membrane vesicles of the yeast *Saccharomyces cerevisiae*. In order to determine uphill transport in vesicles, plasma membranes were fused with proteoliposomes containing cytochrome *c* oxidase as a proton-motive force-generating system. Maltose accumulation, dependent on the electrical and pH gradients, was observed. The initial uptake velocity and accumulation ratio in vesicles proved to be dependent on the external pH. Moreover, kinetic analysis of maltose transport showed that $V_{max.}$ values greatly decreased with increasing pH, whereas the K_m remained virtually constant. These observations were in good agreement with results obtained with intact cells, and suggest that proton binding to the carrier proceeds with an apparent pK of 5.7. The observation with intact cells that maltose is co-transported with protons in a one-to-one stoichiometry was ascertained in the vesicle system by measuring the balance between proton-motive force and the chemical maltose gradient. These results show that maltose transport in vesicles prepared by fusion of plasma membranes with cytochrome *c* oxidase proteoliposomes behaves in a similar way as in intact cells. It is therefore concluded that this vesicle model system offers a wide range of new possibilities for the study of maltose/proton co-transport in more detail.

INTRODUCTION

A large number of yeast species possess sugar carriers capable of uphill transport. The energy for the accumulation of these sugars comes from the proton-motive force (PMF), which is created by the action of the plasma membrane ATPase. In *Saccharomyces cerevisiae*, however, almost all sugars are transported by carrier-mediated diffusion. A notable exception is maltose transport, and the uptake and metabolism of maltose are inducible. When the growth medium contains maltose, and no other more favourable sugars, such as glucose, are present, the maltose permease and α-glucosidase are synthesized (Harris & Millin, 1964). Controversy exists concerning the number of maltose carriers. High-affinity maltose transport is conceived to be a proton symport with a 1:1 stoichiometry and an affinity constant of 1–5 mM (Görts, 1969; Seaston *et al.*, 1973; Postma *et al.*, 1990). The existence of a low-affinity carrier is debatable, but it could be a facilitated transporter with a K_m of 70–80 mM (Busturia & Lagunas, 1985; Cheng & Michels, 1991). The rationale for the presence of a H^+/maltose co-transport mechanism in *S. cerevisiae* is probably the fact that the cytoplasmic maltose-hydrolysing enzyme (α-glucosidase; maltase) has a poor affinity for maltose (K_m = 10–17 mM; Needleman *et al.*, 1978; Postma *et al.*, 1990). Therefore internal accumulation of the substrate is required when the external maltose concentration is low.

Studies on the mechanism of active sugar transport across the plasma membrane of yeasts have so far been limited to intact cells, since a system of plasma membrane vesicles, showing carrier-mediated transport, has only recently become available. Studies in such a model system have the advantage over intact cell studies that sugar transport is not influenced by its subsequent metabolism. Moreover, in a cell-free system, the transport parameters can be varied selectively. So far only the glucose and galactose permease of *S. cerevisiae* have been studied in plasma membrane vesicles in some detail (Franzusoff & Cirillo, 1983; Ongjoco *et al.*, 1987; Ramos *et al.*, 1989). These sugars, however, are transported via carrier-mediated diffusion, or possibly via sugar-kinase-dependent transport.

Studies on energy-coupled solute transport in plasma membrane vesicles have been scarce. PMF-driven uphill leucine transport was observed in vesicles of *S. cerevisiae*, but the accumulation ratios obtained were low (Opekarová *et al.*, 1987; Calahorra *et al.*, 1989). Recently we have developed a procedure to measure energy-coupled uphill galactose transport in isolated plasma membranes of the yeast *Kluyveromyces marxianus* fused with proteoliposomes containing the PMF-generating cytochrome *c* oxidase (Van Leeuwen *et al.*, 1991). This method, based on reconstitution of bacterial transporters (Driessen *et al.*, 1985), yielded vesicles capable of giving high sugar accumulations.

In this paper it is shown that our method of preparing membrane vesicles, containing the co-reconstituted cytochrome *c* oxidase, can also be applied to study the active transport of maltose by the yeast *S. cerevisiae*.

MATERIALS AND METHODS

Strain and growth conditions

S. cerevisiae CBS 8066 was obtained from the Centraal Bureau voor Schimmelcultures (Delft, The Netherlands) and was maintained on malt agar slopes at 4 °C. Chemostat cultivation was performed in a laboratory fermenter (Applikon, The Netherlands) with a working volume of 1 litre at 30 °C and a stirrer speed of 1000 rev./min. The pH was maintained at 5.0 by the

Abbreviations used: PMF, proton-motive force; CL–PMV, hybrid cytochrome *c* oxidase liposome/plasma membrane vesicles; $\Delta\Psi$, transmembrane electrical potential; ΔpH, transmembrane pH gradient; TPP^+, tetraphenylphosphonium; TMPD, *NNN'N'*-tetramethyl-*p*-phenylenediamine; FCCP, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone.

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automatic addition of 1 M-KOH. The fermenter was flushed with air at a flow rate of 1.0 l·min^{-1}. The dissolved oxygen tension was above 50% air saturation. For continuous cultivation the dilution rate was set at 0.1 h^{-1}, and the working volume was maintained by effluent pumping when the culture fluid made contact with a sensor.

The mineral growth medium contained, per litre: 5.0 g of $(NH_4)_2SO_4$, 3.0 g of KH_2PO_4, 0.5 g of $MgSO_4,7H_2O$, 15.0 mg of EDTA, 4.5 mg of $ZnSO_4,7H_2O$, 0.3 mg of $CoCl_2,6H_2O$, 1.0 mg of $MnCl_2,4H_2O$, 0.3 mg of $CuSO_4,5H_2O$, 4.5 mg of $CaCl_2,2H_2O$, 3.0 mg of $FeSO_4,7H_2O$, 0.4 mg of $Na_2MoO_4,2H_2O$, 1.0 mg of H_3BO_3, 0.1 mg of KI and 0.05 ml of silicone antifoam (BDH). After heat sterilization at 120 °C and cooling, a filter-sterilized vitamin solution was added, giving final concentrations per litre of: 0.05 mg of biotin, 1.0 mg of calcium pantothenate, 1.0 mg of nicotinic acid, 25.0 mg of inositol, 1 mg of pyridoxin/HCl, 1.0 mg of thiamin/HCl and 0.2 mg of *p*-aminobenzoic acid. In order to prevent hydrolysis, maltose was heat-sterilized separately (Postma & Van den Broek, 1990) at 110 °C during 10 min and was added to the medium to a final concentration of 10 g·l^{-1}. The above-described conditions resulted in maltose-limited growth at a cell density of 4.7 g dry wt.·l^{-1}.

Vesicle preparation

Isolation of plasma membranes. Membranes were isolated by the procedure described by Van Leeuwen *et al.* (1991), except that the crude membranes were subjected to an additional washing in order to decrease the contamination with soluble cell constituents, especially α-glucosidase. To this end, the pellet of centrifuged (20 min, 6200 *g*) crude plasma membranes was suspended in a large volume (e.g. 100 ml) of buffer A (0.1 M-glycine/0.3 M-KCl at pH 7.0), frozen in liquid nitrogen and thawed at room temperature. After centrifugation (20 min, 6200 *g*), the pellet was resuspended in 15 ml of the same buffer and the procedure continued with the titration.

Isolation of cytochrome *c* oxidase. Cytochrome *c* oxidase was isolated according to Yu *et al.* (1975). The activity was determined by the method of Yonetani (1965).

Purification of lipids. Commercially obtained phospholipids were extracted with acetone/ether (Viitane *et al.*, 1986). The lipids were dissolved in chloroform and stored at −20 °C under nitrogen.

Preparation of liposomes with reconstituted cytochrome *c* oxidase. Cytochrome *c* oxidase-containing proteoliposomes were prepared according to Van Leeuwen *et al.* (1991), using *Escherichia coli* lipids (containing 50% phosphatidylethanolamine).

Fusion of cytochrome *c* oxidase-containing liposomes with yeast plasma membranes. Cytochrome *c* oxidase-containing liposomes (5 mg of lipid) and plasma membranes (0.25 mg of protein) were thawed and mixed in 25 mM-KP_i/1 mM-$MgCl_2$ or 100 mM-KP_i/1 mM-$MgCl_2$ at the indicated pH in a volume of 300 μl in an Eppendorf reaction vessel. The mixture was frozen in liquid N_2, thawed at room temperature, and sonicated for 2 × 10 s in an ultra K42 sonic sonication bath. During this sonication step the white suspension became clear. The obtained hybrid cytochrome oxidase liposome–plasma membrane vesicles (CL–PMV) were used immediately.

Internal volume. The internal volume, determined according to Van Leeuwen *et al.* (1991), was 0.9 μl/mg of phospholipid.

Transport studies

Determination of the transmembrane electrical potential. The membrane potential (ΔΨ) was determined from the distribution of tetraphenylphosphonium (TPP^+), using a TPP^+-selective electrode (Shinbo *et al.*, 1978). Fused vesicles (0.42 mg of phospholipid) were suspended in 1.5 ml of 25 mM-KP_i/1 mM-$MgCl_2$ or of 100 mM-KP_i/1 mM-$MgCl_2$, at the indicated pH and 2 μM-TPP^+. 'Energization' (generation of a PMF) was started with 100 μM-*NNN'N'*-tetramethyl-*p*-phenylenediamine (TMPD), 10 μM-cytochrome *c* and 10 mM-ascorbate (adjusted to the indicated pH with KOH). ΔΨ was calculated according to Lolkema *et al.* (1982) and De Vrij *et al.* (1986), assuming symmetrical binding.

Determination of the pH gradient. The transmembrane pH gradient (ΔpH) was measured according to Clement & Gould (1981), using pyranine (p*K* 7.2) and the same electron donor and buffer system as for the ΔΨ determination.

Assay of sugar uptake in CL–PMV. Uptake of sugar in fused CL–PMV was determined according to Van Leeuwen *et al.* (1991), except that the buffer was 25 mM-KP_i/1 mM-$MgCl_2$ for uptake experiments at tracer (15 μM) concentration of sugar and initial sugar uptake experiments, or 100 mM-KP_i/1 mM-$MgCl_2$ for the 2.5 mM sugar uptake experiments. For kinetic analysis of uptake, the CL-PMV were energized 1 min before sugar addition and the PMF-driven maltose uptake was determined from the amount of label accumulated during the first 60 s at pH 5.5 and the first 90 s at pH 6.7. Diffusion and binding were determined by the same method, using lactose instead of maltose. The reaction was stopped by addition of 2 ml of 100 mM ice-cold LiCl to the reaction mixture, followed by subsequent filtration on cellulose nitrate filters and washing.

Kinetic analysis of sugar uptake in intact cells. Samples of the culture fluid were washed twice in a mineral salts solution ($(NH_4)_2SO_4$ 5.0 g·l^{-1}, KH_2PO_4 3.0 g·l^{-1}, $MgSO_4,7H_2O$ 0.5 g·l^{-1}) adjusted to the indicated pH with 1 M-NaOH or 1 M-phosphoric acid. Maltose uptake and binding of [U-^{14}C]maltose to cells and filter were determined as described by Postma *et al.* (1988), using 200 μl of washed cell suspension (0.9 mg dry weight) per assay.

Kinetic analysis of proton uptake in intact cells. Uptake of protons by intact cells was determined from the H^+ disappearance from the medium, according to Van Urk *et al.* (1989); 5.0 ml of yeast suspension (5 g·l^{-1}) was used. To calculate H^+ uptake rates, alkalinization curves were calibrated with a standard NaOH solution.

Protein determination

Protein was determined by the method of Lowry *et al.* (1951), with BSA as standard.

Materials

The radiolabelled sugars [U-^{14}C]maltose (11.3–20 GBq/mmol), [D-*glucose*-1-^{14}C]lactose (2.1 GBq/mmol), D-[U-^{14}C]-glucose (111 GBq/mmol) and L-[U-^{14}C]glucose (2.04 GBq/mmol) were obtained from Amersham (U.K.).

Miscellaneous chemicals were obtained as follows: L-α-phosphatidylethanolamine (type IX from *E. coli*) (Sigma; P6398), maltose monohydrate (Merck; art 5912), n-octyl β-D-glucopyranoside, TPP^+ bromide, carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP) (Fluka); valinomycin, cytochrome *c*, phenylmethanesulphonyl fluoride and α-glucosidase (Boehringer); TMPD (British Drug House); ascorbic acid (J. T. Baker Inc.); Pyranine (Eastman); nigericin (Sigma); BSA (A-8022, 5.9% water; Sigma); Scintillator 299 (Packard Instrument Co.); Ready Safe Liquid Scintillation Cocktail (Beckman); cellulose nitrate filters, pore size 0.45 μm (Schleicher & Schuell).

RESULTS

In order to determine PMF-driven uphill maltose transport, membrane vesicles were prepared containing a PMF-generating

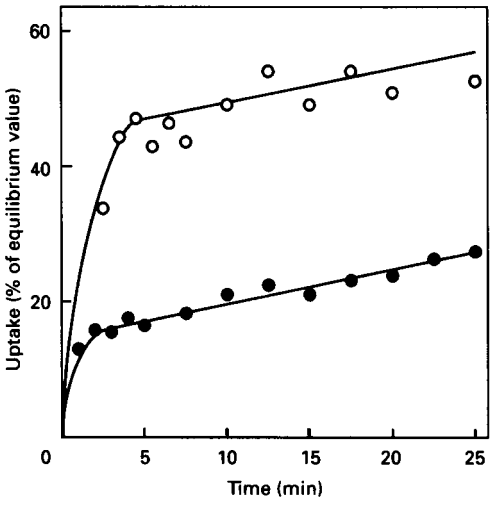

Fig. 1. Maltose (○) and lactose (●) uptake in non-energized CL–PMV

Sugar (2.5 mM) was added at $t = 0$. The fraction of the equilibrium value was calculated from the ratio of the internal sugar concentration per sample and the internal sugar concentration at the diffusion equilibrium.

Table 1. PMF and maltose (2.5 mM) transport activity of CL–PMV upon energization with ascorbate, cytochrome *c* and TMPD

CL–PMV were prepared by fusion of plasma membranes and cytochrome *c* oxidase-containing proteoliposomes at the different pH values indicated. The reconstitution efficiency of the maltose permease did not vary significantly at the different pH values. PMF was measured 2 min after 'energization'. The maltose transport data were corrected for passive diffusion into vesicles not containing a maltose carrier, and are expressed in nmol·min^{-1}·mg of phospholipid^{-1}. *Z* is a factor converting pH units into mV units, and is equal to 60 mV.

pH	ΔΨ (mV)	ZΔpH (mV)	PMF (mV)	Initial rate of maltose uptake	Accumulation ratio
5.5	−74	85	−159	8.7	19
5.9	−98	62	−160	3.7	11
6.2	−106	63	−169	2.7	7.5
6.7	−108	37	−145	1.3	4.7
7.0	−110	31	−141	0.4	2.4

system. Plasma membranes were fused with proteoliposomes containing cytochrome *c* oxidase by freeze/thaw/sonication, as adopted for the preparation of *K. marxianus* plasma membrane vesicles (Van Leeuwen *et al.*, 1991). Using the detection method for membrane fusion described by Hoekstra *et al.* (1984), it was established that fusion of plasma membranes with proteoliposomes had indeed taken place (see also Ongjoco *et al.*, 1987; Van Leeuwen *et al.*, 1991). Contamination of plasma membranes with the maltose-hydrolysing enzyme α-glucosidase (EC 3.2.1.20), which is present in the cell with high specific activity (2.5 μmol·min^{-1}·mg of total protein^{-1}; Postma *et al.*, 1990), was decreased by introducing an extra freeze/thaw and washing step. This resulted in a 100-fold decrease of the enzyme specific activity, to 26 nmol·min^{-1}·mg of membrane protein^{-1}.

A previous study on galactose transport in *K. marxianus* membrane vesicles had shown that not all vesicles contained an active galactose transporter (Van Leeuwen *et al.*, 1991). This was attributed to the fact that maximal galactose transport rates in intact cells were low (35 μmol·min^{-1}·g dry weight^{-1}). However, in the present study, the rate of maltose transport was high (260 μmol·min^{-1}·g dry weight^{-1}). It might therefore be expected that CL–PMV of *S. cerevisiae* contained more active maltose carriers. The percentage of CL–PMV with an active maltose transport carrier was determined by measuring maltose passive transport by the maltose/proton symport carrier in non-energized vesicles. Since *S. cerevisiae* does not contain a permease for lactose, this sugar was used as control for binding and passive diffusion. Fig. 1 shows that maltose initially was taken up faster than lactose. Subsequently there was a slow uptake of maltose occurring at the same rate as passive lactose diffusion. Assuming that the maltose and lactose passive permeability coefficients are identical, the difference between maltose and lactose uptake can be taken as a measure of carrier-mediated transport. According to the method described by Ongjoco *et al.* (1987), it follows from Fig. 1 that approx. 30–35% of the vesicles possessed an active maltose permease. This is significantly higher than the 11% of the *K. marxianus* vesicles containing an active galactose carrier (Van Leeuwen *et al.*, 1991).

Energization of the CL–PMV with TMPD, cytochrome *c* and ascorbate generated a PMF, inside-negative and alkaline. As shown in Table 1, PMF values of about −160 mV could be obtained, identical to the results published for *K. marxianus* CL–PMV (Van Leeuwen *et al.*, 1991). The PMF generated appeared to be maximal, and could not be increased by preparing vesicles at different lipid/protein ratios. This suggests that all vesicles participated in the generation of the PMF. This appears reasonable, since cytochrome *c* oxidase was present in excess, resulting in a vesicle preparation devoid of oxidase-free liposomes, as was shown by Driessen *et al.* (1986) and De Vrij *et al.* (1986). It can therefore be assumed that, in contrast with the maltose permease, apparently all vesicles contained cytochrome *c* oxidase, capable of generating a PMF with externally added cytochrome *c*. To determine PMF-driven transport, maltose (2.5 mM) was added to energized CL–PMV at pH 5.5. The intravesicular maltose concentration was calculated as the difference between maltose uptake and lactose diffusion, divided by the volume of the vesicles containing a maltose-specific transport system (i.e. 30–35% of the internal volume of the total vesicle preparation). Uphill transport resulted in an accumulation ratio of 19 (Fig. 2; Table 1), where glucose (2.5 mM) transport under the same conditions did not exceed the passive diffusion level (results not shown). Addition of the ionophores nigericin and valinomycin, which dissipate the ΔpH and the ΔΨ respectively, lowered the level of accumulation. Moreover, addition of the uncoupler FCCP or of nigericin and valinomycin simultaneously resulted in efflux of accumulated maltose (Fig. 2). This maltose efflux proceeded by apparent biphasic kinetics, as has been also observed for galactose efflux from *K. marxianus* CL–PMV (Van Leeuwen *et al.*, 1991). The rationale for the slow second efflux phase remains as yet unclear. At an external concentration of 15 μM-maltose, accumulation ratios of 30–35 were reached.

To determine the influence of the pH on influx velocity and the accumulation ratio of maltose, CL–PMV were prepared at pH values of 7.0, 6.7, 6.2, 5.9 and 5.5. The total PMF, as measured with TPP^+ distribution (ΔΨ) and the fluorescent probe pyranine (ΔpH), showed only a slight decrease with increasing pH (Table 1). It was found that the electrical component of the PMF (ΔΨ) decreased with decreasing pH, whereas the ΔpH increased with decreasing pH, resulting in a virtually constant internal pH of 7.0–7.5. Although the PMF was high and should account for a high influx velocity and accumulation ratio over the total pH range, the values decreased dramatically with increasing external pH (Table 1). Addition of nigericin inhibited maltose transport at low pH values (see also Fig. 2) whereas, at high pH, transport

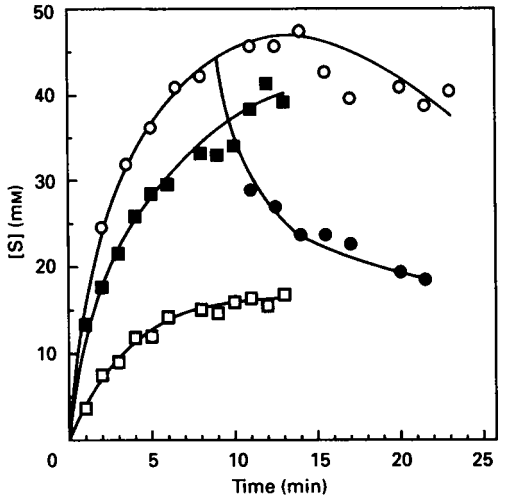

Fig. 2. Effect of ionophores on maltose uptake in energized CL–PMV at pH 5.5

Maltose (2.5 mM) was added at $t = 0$. Nigericin (37 nM; ■) or valinomycin (100 nM; □) was added at $t = -2$ min; ●, simultaneous addition of nigericin (37 nM) and valinomycin at $t = 9$ min; ○, no additions. Energization with ascorbate, TMPD and cytochrome *c* was performed at $t = -1$ min. All measurements were corrected for binding and diffusion. [S] is the internal maltose concentration in those vesicles that contained a maltose carrier.

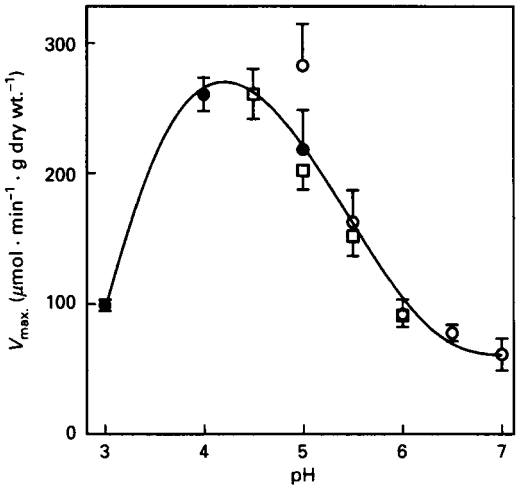

Fig. 3. Effect of pH on the maximal uptake rate of [^{14}C]maltose in *S. cerevisiae*

The different symbols refer to cell suspensions from independent steady states. For the calculation of these parameters, uptake experiments were performed with maltose concentrations ranging from 0.5 to 8.0 mM.

was not inhibited or even slightly stimulated. The stimulation is due to the fact that the high $\Delta\Psi$ value at pH 6.7 and 7.0 increased upon addition of nigericin.

The kinetic constants of maltose transport in both CL–PMV and intact cells was measured as a function of the external pH. In CL–PMV, initial uptake rates of maltose and lactose at sugar concentrations ranging from 0.5 to 20.0 mM at pH 5.5 and 6.7 were determined. Analysis of these data by non-linear regression revealed that the affinity constant did not vary with pH ($K_m = 9.9 \pm 2.4$ mM at pH 5.5 and 10.6 ± 1.2 mM at pH 6.7), whereas the $V_{max.}$ at pH 6.7 was 6 times lower than at pH 5.5 ($V_{max.} = 40.0 \pm 4.3$ nmol · min^{-1} · mg of phospholipid^{-1} at pH 5.5 versus 6.3 ± 0.3 at pH 6.7). In intact cells initial maltose uptake studies were performed at sugar concentrations ranging from 0.5 to 15.0 mM and at pH values ranging from 3.0 to 7.0. Plots of these data also showed a small variation in maltose affinity ($K_m = 2$–4 mM), whereas an optimum in $V_{max.}$ was observed of 260 μmol · min^{-1} · g dry weight^{-1} at pH 4.0–4.5, decreasing to 50 μmol · min^{-1} · g^{-1} at pH 7.0 and to 100 μmol · min^{-1} · g^{-1} at pH 3.0 (Fig. 3).

Table 2. Relationship between PMF and maltose uptake in CL–PMV at pH 5.5

All values were determined at the time steady-state sugar accumulation level was reached. Maltose accumulation concerns only the vesicles containing a maltose carrier.

Addition	$\Delta\Psi$ (mV)	$Z\Delta$pH (mV)	PMF (mV)	$\Delta\mu_{maltose}/F$ (mV)	$n_{app.}$
None	−46	70	−116	91	0.8
Nigericin (37 nM)	−70	-	−70	89	1.3
Valinomycin (100 nM)	-	77	−77	68	0.9

The stoichiometry of the maltose/proton co-transport was determined in two ways. In intact cells the H^+/maltose stoichiometry was determined by measuring proton uptake rates and [^{14}C]maltose uptake rates at concentrations ranging from 0.5–15.0 mM at pH 5.0, and comparing $V_{max.}$ values calculated assuming Michaelis–Menten kinetics. The data obtained resulted in a H^+/maltose stoichiometry of 0.8 ($V_{max.} = 176 \pm 8$ μmol · min^{-1} · g dry weight^{-1} for proton uptake, and 218 ± 30 for maltose influx). In energized vesicles the H^+/maltose stoichiometry was determined from the steady-state maltose accumulation ratio at a tracer external concentration (15 μM) and measurements of PMF at the time at which maximal maltose accumulation was observed, i.e. 15–20 min after sugar addition. Table 2 shows the values of the PMF and the chemical maltose gradient ($\Delta\mu_{maltose}/F$). Since the maltose uptake is both $\Delta\Psi$- and ΔpH-driven, data of experiments in the presence of nigericin or valinomycin are included. The stoichiometry, $n_{app.}$, was calculated by the Mitchell equation (Driessen *et al.*, 1987):

$$n_{app.} = \Delta\mu_{maltose} \cdot (F \times PMF)^{-1}$$

The results are shown in Table 2.

DISCUSSION

S. cerevisiae was grown in chemostat culture under maltose limitation. The use of cells obtained via this cultivation technique offers several advantages for studies on active maltose transport as compared with batch cultures. Due to the low residual maltose concentration in the culture (approx. 0.1 mM), it can be expected that a possible low-affinity (passive) transport is absent, analogous to studies on glucose transport in chemostat-grown *Candida utilis* (Postma *et al.*, 1988). Furthermore, since cells are grown under essentially constant conditions, low batch variation in transport activity can be expected. Indeed, chemostat cultivation allowed for reproducible isolation of batches of plasma membrane vesicles with little variation in transport activity.

The results presented in this paper clearly establish that maltose uptake proceeds through a PMF driven process. In vesicles this was concluded from the fact that PMF generation caused intravesicular accumulation of maltose. Accumulation ratios of 30–35 ($\Delta\mu_{maltose}/F = \pm 91$ mV) were reached at low external concentrations of maltose (15 μM) (Table 2). Since steady-state maltose accumulation ratios obtained are dependent on the

maltose concentration used (Fig. 2 and Table 2), this strongly suggests that passive transmembrane solute leakage becomes more important at high concentrations, as was concluded before (Driessen *et al.*, 1987; Van Leeuwen *et al.*, 1991).

The influence of the extracellular or extravesicular pH on [^{14}C]maltose uptake was very pronounced. Although the pH at which the vesicles were fused hardly affected the total PMF generated, it influenced the $\Delta\Psi$ and the ΔpH in opposite ways (Table 1). A similar relationship between $\Delta\Psi$, ΔpH and external pH was found for *Bacillus subtilis* membrane vesicles (De Vrij *et al.*, 1987). In contrast to the PMF, both the accumulation ratio and the initial influx velocity were negatively affected by increasing pH (Table 1). This effect has also been observed for amino acid/proton symport carriers in membrane vesicles of *Streptococcus cremoris* (Driessen *et al.*, 1987). The apparent influence of the pH on maltose accumulation is probably due to a counterbalancing of maltose uptake by passive maltose efflux. At high pH values, maltose influx is slow compared with outward diffusion, leading to a low accumulation ratio.

Kinetic studies showed that the K_m in vesicles is higher than in intact cells. A similar difference has also been found for galactose transport in *K. marxianus* and for galactose uptake in *S. cerevisiae* (Ramos *et al.*, 1989; Van Leeuwen *et al.*, 1991). This difference might indicate that the lipid environment of the carrier in vesicles is suboptimal compared with intact cells. Kinetic analysis in both vesicles and cells showed that, whereas the affinity of the carrier for maltose is not affected by pH, its maximal velocity decreases with decreasing external proton concentration. Studies on the effect of the external pH on the growth parameters of steady-state maltose-limited continuous culture of *S. cerevisiae* revealed similar conclusions (Olivero *et al.*, 1982). Plots of $V_{max.}$ values of cells (Fig. 3) and the initial influx velocity of vesicles (in the pH range 5.5–7.0) (Table 1) against the external proton concentration revealed apparent pK values of 5.7 and 5.5 respectively. These values are slightly lower than those found for the sorbose/ and galactoside/proton symporters of *K. marxianus*, which were 6.2–6.4 (Van den Broek & Van Steveninck, 1980, 1982). For these carriers, proton binding was suggested to proceed to the imidazole group of histidine, which has a pK of 6.0. Since the pK value for maltose transport is also close to 6, a histidine residue might be involved in H^+ binding in maltose/proton symport of *S. cerevisiae*. It should be noted that a pH-sensitive $V_{max.}$ and a pH-insensitive K_m has also been observed for other sugar transport systems in yeast (Van den Broek & Van Steveninck, 1980, 1982). This suggests that the symport mechanism in these yeasts might be similar, and possibly has a random reaction sequence. Further studies are, however, needed to fully clarify the reaction mechanism.

The H^+/maltose stoichiometry of the maltose symporter was estimated to be about 1, from the ratio of H^+ to maltose influx into intact cells (Eddy *et al.*, 1977; Serrano, 1977). This was confirmed in our cell experiments as well as in CL–PMV studies. It is noteworthy that the latter value follows from the relationship between the chemical gradient of solute ($\Delta\mu_{solute}/F$) and the PMF or its components (Table 2). The data with CL–PMV in particular clearly show that maltose transport is a PMF-driven process with a 1:1 H^+/maltose stoichiometry. Moreover, the fact that maltose accumulation and the PMF reach a thermodynamic equilibrium in vesicles, as well as the fact that maltose uptake kinetics in vesicles resemble those of intact cells, shows that our method of preparing plasma membrane vesicles from *S. cerevisiae* successfully reconstitutes maltose transport. Therefore it can be expected that studies with CL–PMV will greatly advance the knowledge of active transport mechanisms of *S. cerevisiae*.

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