Aerobic and Anaerobic Uptake of Sugars in *Schizosaccharomyces pombe*

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Aerobic cells of the yeast *Schizosaccharomyces pombe* accumulated 2-deoxy-D-glucose (2-DOG) and glucosamine, but were unable to accumulate 3-O-methyl-D-glucose, 6-deoxy-D-glucose, D-xylose and D-arabinose. Uptake of all sugars tested displayed saturation kinetics; however, only 2-DOG, glucosamine and D-glucose exhibited mutual competitive inhibition of uptake and the phenomenon of exchange transport. Thus, they share a common 'glucose transport system'. Uncouplers inhibited sugar accumulation or induced sugar outflow from preloaded cells. The uptake of sugars of the glucose transport system was pH dependent and was accompanied by a stoichiometric cotransport of H⁺. Since millimolar concentrations of the lipophilic cation tetraphenylphosphonium (which depolarizes the membrane potential) prevented sugar accumulation, the transport was electrogenic. Thus, the glucose carrier is a H⁺-symporter. Accumulation of sugars was inhibited by the plasma membrane ATPase inhibitors Dio-9 and *N,N'*-dicyclohexylcarbodiimide. Anaerobic cells of *S. pombe* were virtually unable to transport 2-DOG and glucosamine. However, when energized by glucose, the accumulation of both sugars was completely restored. This anaerobic transport was also catalysed by the glucose carrier.

INTRODUCTION

The transport of sugars across yeast plasma membranes has been studied only in a few yeast species (for a review see Eddy, 1982). In most of the transport systems studied, the driving force for sugar uptake is the electrochemical proton gradient, $\Delta\mu_{\text{H}^+}$, generated by a plasma-membrane-bound H⁺-ATPase (Goffeau & Boutry, 1986). The sugar molecules are translocated in symport with H⁺.

Among the plasma-membrane-bound H⁺-ATPases in fungi (for a review see Goffeau & Slayman, 1981) two have been investigated in membrane vesicle studies. Scarborough (1976) used plasma membrane vesicles from *Neurospora crassa* to prove that the ATPase functions as an electrogenic H⁺-pump. Villalobo and co-workers used reconstituted proteoliposomes to demonstrate that the plasma membrane ATPase of the fission yeast *Schizosaccharomyces pombe* very likely catalyses the electrogenic H⁺-efflux and the compensating K⁺-influx (Villalobo *et al.*, 1981; Villalobo, 1982). However, neither of these systems were used for studies of the energetic coupling between the H⁺-ATPase and the H⁺/sugar symport.

In *S. pombe*, sugar transport has not been studied in detail. Goffeau and his group (Foury & Goffeau, 1975; Goffeau *et al.*, 1975) reported that uptake of sucrose and of 2-deoxyglucose by the respiratory mutant *S. pombe* COB5 displays saturation kinetics, and is dependent on intracellular ATP as well as on a pH gradient across the plasma membrane. No attempt was made to elucidate the transport mechanism. Since plasma membranes of *S. pombe* can readily be

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Abbreviations: 2-DOG, 2-deoxy-D-glucose; 3-OMG, 3-O-methyl-D-glucose; 6-DOG, 6-deoxy-D-glucose; DNP, 2,4-dinitrophenol; CCCP, carbonylcyanide *m*-chlorophenylhydrazine; TPP⁺, tetraphenylphosphonium; DCCD, *N,N'*-dicyclohexylcarbodiimide; $\Delta\psi$, membrane potential (difference of electrical potential across the plasma membrane); $\Delta\mu_{\text{H}^+}$, electrochemical gradient of H⁺.

fractionated from cell-free homogenates (Delhez *et al.*, 1977), their reconstitution as plasma membrane vesicles is of potential importance for the following aim: provided that sugars are translocated in symport with protons, the energetic link between the primary H^+ -pumping ATPase (Villalobo *et al.*, 1981) and the secondary H^+ /sugar symport can be established.

In this paper we analyse the kinetics and characterize the energetics of sugar uptake in *S. pombe*. Since *S. pombe* is a facultative anaerobic yeast, transport properties were compared under both aerobic and anaerobic conditions. This comparison is also important with regard to the so-called Kluyver effect and the problem of oxygen requirement for active sugar transport in yeasts (cf. Sims & Barnett, 1978; Barnett & Sims, 1982).

METHODS

Organism and growth. *Schizosaccharomyces pombe* (972 h⁻) was grown in a medium containing 6% (w/v) glucose and 2% (w/v) yeast extract. The pH was adjusted to 4.5 with HCl before autoclaving. A preculture (10 ml) was inoculated with one loop of cells and cultivated for 15–17 h on a gyratory shaker at 30 °C; the preculture was used to inoculate the main culture (100 ml). After a further 24 h, the cells were harvested by centrifugation, washed three times with distilled water, and aerated for 1–4 h as a 5% (fresh wt/vol.) suspension.

Transport experiments. The uptake and accumulation of ¹⁴C-labelled 2-deoxy-D-glucose (2-DOG), glucosamine, 3-O-methyl-D-glucose (3-OMG) and 6-deoxy-D-glucose (6-DOG) were measured as described by Niemietz *et al.* (1981); the uptake of D-xylose and D-arabinose was measured by photometrical analysis with orcinol as described by Heller & Höfer (1975); D-glucose uptake was measured by enzymic analysis of supernatants of reaction suspensions as described by Höfer & Dahle (1972). The reaction suspension, kept at 30 °C, contained (unless otherwise stated in the legends to Figures or Tables) 5–8 mg dry wt yeast cells ml⁻¹ and 120 mM-potassium phosphate buffer, pH 4.5 (or, in experiments with glucosamine, 120 mM-Tris/citric acid buffer, pH 6.0). Transport was started by addition of the particular sugar at the chosen concentration. H^+ -cotransport was monitored in unbuffered cell suspensions with a pH electrode connected to a PHM 62 pH meter (both from Radiometer, Copenhagen, Denmark); the H^+ /sugar stoichiometry was calculated as described in detail by Höfer & Misra (1978) and Hauer & Höfer (1982).

ATP determination. The intracellular ATP content was determined enzymically (test combination, Boehringer) in HClO₄-extracts (0.5 M) of yeast cells after neutralization with 1 M-triethanolamine/10 M-KOH/1 M-acetic acid (1.5:0.4:0.1, by vol.). The increase in absorbance due to NADPH was measured at 366 nm in an Eppendorf photometer.

Anaerobic transport. Experiments under anaerobic conditions were done in a closed acrylic-glass cuvette with 30 ml reaction suspension (10–15 mg dry wt yeast cells ml⁻¹) kept at constant temperature (30 °C) under a flow of purified nitrogen (99.96% pure; Messer Griesheim, Düsseldorf, FRG) for at least 15 min before addition of transport substrates. All additions were made either as solids or in solutions deaerated with nitrogen. Samples withdrawn for analysis were quickly treated in the same way as samples from aerobic experiments.

Chemicals. All enzymes and coenzymes were from Boehringer; ¹⁴C-labelled carbohydrates were purchased from Amersham. [³H]6-DOG was kindly supplied by Dr W. Tanner, University of Regensburg, FRG. Oligomycin was from Serva, carbonylcyanide *m*-chlorophenylhydrazone (CCCP) and 2,4-dinitrophenol (DNP) were from Sigma, Dio-9 was from Gist-Brocades (Delft, The Netherlands). All other chemicals were of reagent grade (Merck). Solutions were made up using deionized glass-distilled water.

RESULTS

Transport under aerobic conditions

Kinetic characterization of the glucose transport system. The uptake of all sugars tested exhibited saturation kinetics (Table 1). Values of K_T for D-glucose, 2-DOG and glucosamine were considerably lower than those for other sugars. Moreover, only 2-DOG and glucosamine were accumulated intracellularly, reaching maximum intracellular concentrations of 50 and 44 mM at corresponding external concentrations of 2 and 3 mM, respectively. D-Glucose competitively inhibited the uptake of 2-DOG and glucosamine and vice versa. Contrary to this, D-glucose had no significant effect on the transport of the other sugars, which in turn, were without effect on D-glucose uptake. These results demonstrate that in *S. pombe* there is an accumulating transport system for glucose, 2-DOG and glucosamine, and a facilitated-diffusion system of rather low effective affinity for the other monosaccharides. The phenomenon of exchange transport observed when D-glucose was added to cells preloaded with either 2-DOG or glucosamine (Fig. 1a, b) further supports the above conclusion. In addition, only D-glucose and 2-DOG but not 3-OMG nor 6-DOG induced exchange transport in cells preloaded with glucosamine (Fig. 1b).

Table 1. Accumulation and kinetic parameters of various sugars in *S. pombe*, and mutual inhibition of sugar uptake by glucose or of glucose uptake by other sugars

All experiments were done under aerobic conditions at 30 °C in 120 mM-potassium phosphate buffer, pH 4.5 (except for glucosamine: Tris/citric acid buffer, pH 6) with a 5% (fresh wt/vol.) yeast suspension (about 8–10 mg dry wt ml⁻¹). The cells were de-energized by adding 50 µM-CCCP or 0.5 mM-DNP.

Transport substrate	K_T (mM)	V_T [nmol min ⁻¹ (mg dry wt) ⁻¹]	Intracellular accumulation	Outflow after addition of uncoupler	Inhibition of uptake	
					Sugars	Glucose
D-Glucose	3	50	—*	—*		
2-Deoxy-D-glucose	2	36	25-fold	+	+	+
Glucosamine	17	5	15-fold	+	+	+
3-O-Methyl-D-glucose	178	135	—	—	—	—
6-Deoxy-D-glucose	45	29	—	—	—	—
D-Xylose	206	130	—	—	—	—
D-Arabinose	283	167	—	—	—	—

* Since glucose is rapidly catabolized there was virtually no free intracellular glucose detectable. Hence, neither accumulation nor outflow from preloaded cells could be demonstrated.

These results demonstrated that glucose, 2-DOG and glucosamine are translocated by a single common carrier, which was designated as the 'glucose transport system'. To characterize the energetics of the glucose transport system 2-DOG, and occasionally glucosamine, were chosen as the transport substrate. The carrier possessed the highest effective affinity for 2-DOG, which is not metabolized intracellularly (Foury *et al.*, 1977) and only slowly phosphorylated to a very small extent (unpublished results). Glucosamine, on the other hand, was potentially a useful substrate since it bears a net positive charge due to association with H⁺ at the pH values (between 5.0 and 6.5) used ($pK_a = 7.75$). Experiments with [¹⁴C]glucosamine proved that it also was not catabolized by *S. pombe* cells since no radioactivity was released as CO₂ (results not shown), and the radioactivity was completely recovered in cell suspensions when an uncoupler was added to preloaded cells (Fig. 2).

Energetics of the glucose transport system. The time course of 2-DOG accumulation is shown in Fig. 2. Similar results were also obtained for glucosamine (not shown). The accumulation of 2-DOG and glucosamine was prevented by uncouplers, when added before the substrate. Uncouplers induced an outflow of accumulated 2-DOG and glucosamine down to the diffusion equilibrium when added to preloaded cells. Fig. 2 also demonstrates that the accumulation of 2-DOG in control suspensions was reduced after about 15 min incubation, indicating an inhibition of cell metabolism by accumulated 2-DOG. This was confirmed by measurements of cell respiration which was stimulated up to 8-fold during the first 5 min following 2-DOG addition and then rapidly declined to the level of resting cells after about 30 min (data not shown). However, in the presence of ethanol (the solvent for the uncouplers) the accumulation of 2-DOG continued, eventually reaching higher values than in control suspensions. Correspondingly, cell respiration was also stimulated by added ethanol (about 12-fold). Hence, ethanol was used as an advantageous energy source in subsequent aerobic experiments in *S. pombe* because it penetrates biological membranes by physical diffusion and does not interfere with any carrier mechanism.

By comparison of the transport kinetics in energized (by ethanol) and de-energized (by uncoupler) cells the effect of energy supply on the carrier mechanism can be assessed. Energy supply did not significantly change the effective affinity of the carrier for substrate (2-DOG in this experiment); however, the maximum influx was enhanced 6-fold [from 5.9 to 35.7 nmol min⁻¹ (mg dry wt)⁻¹ (energized compared with de-energized cells)]. Thus, the supply of energy enhanced the turnover rate of the carrier.

Evidence for H⁺/substrate cotransport. When 2-DOG was added to unbuffered cell suspensions energized by either ethanol (120 mM) or glucose (5 mM), the onset of 2-DOG uptake

Fig. 1. (a) Inhibition by D-glucose of 2-DOG uptake and the phenomenon of exchange transport. ●, Uptake of 2-DOG (2 mM); ○, uptake of 2-DOG in the presence of 50 mM-D-glucose; ▲, outflow of 2-DOG from preloaded cells induced by the addition of 50 mM-D-glucose as indicated by the arrow (exchange transport). (b) Exchange transport of glucosamine (5 mM) induced by 50 mM-2-DOG (▲) or 50 mM-D-glucose (○) added as indicated; 50 mM-3-OMG (□) or 50 mM-6-DOG (△) failed to induce glucosamine outflow from preloaded cells. The dotted lines correspond to the diffusion equilibria. The curves given are representative of three (a) or four (b) repeated experiments.

Fig. 2. Effect of uncouplers on 2-DOG accumulation. Uncouplers were dissolved in ethanol; its concentration in cell suspensions was 120 mM. ●, Control uptake of 2-DOG (2 mM) without further additions; ■, uptake of 2-DOG in the presence of 120 mM-ethanol; ▲, outflow of 2-DOG from preloaded cells induced by the addition of either 50 μM-CCCP or 0.5 mM-DNP (arrowed); ○, 2-DOG accumulation inhibited by uncoupler added 5 min before the sugar. Similar results were obtained in cell suspensions incubated with glucosamine instead of 2-DOG. The dotted line corresponds to the diffusion equilibrium. The results given are representative of three repeated experiments.

was accompanied by a distinct alkalinization of the suspensions. This indicated H^+ -cotransport induced by 2-DOG. Fig. 3 shows increasing rates of alkalinization with increasing concentration of added 2-DOG. No H^+ -cotransport was induced by sugars transported by the facilitated-diffusion system, viz. 3-OMG or D-xylose (see Fig. 8). A double reciprocal plot of H^+ -cotransport rates against 2-DOG concentration measured in *S. pombe* cells energized either with glucose (Fig. 3 inset) or with ethanol (not shown) was linear with a half-saturation constant close to that of 2-DOG uptake. This is indicative of coupled transport of H^+ and 2-DOG. Comparison of the fluxes of H^+ and of 2-DOG revealed stoichiometries of $H^+/2\text{-DOG}$ cotransport of 0.3 and 0.4 in cells energized by glucose or ethanol, respectively. The stoichiometry was fairly constant under various physiological conditions, e.g. when the reaction temperature was lowered to 10 °C or when anaerobic conditions or certain metabolic inhibitors were applied (Table 2). These

Fig. 3. Continuous recording of the pH of unbuffered cell suspensions during 2-DOG uptake. Cells were energized with 5 mM-D-glucose. Numbers above individual curves give the 2-DOG concentration added at the arrow. The curves are tracings of original recordings shifted to one common origin at the arrow. At the end of each run the pH recording was calibrated by successive additions of known amount of H^+ (0.5 μ mol HCl into 30 ml cell suspension) in order to calculate the initial influx of H^+ induced by 2-DOG transport. Simultaneously, samples of the cell suspension were withdrawn and analysed for 2-DOG influx. The tracings shown are representative of two experiments with cells energized with D-glucose and three additional experiments with cells energized with ethanol. Inset: double reciprocal plot of the kinetics of both the initial H^+ ($\blacktriangle$) and 2-DOG ($\bullet$) influx. The similarity of the two half-saturation constants [$K_T(2\text{-DOG}) = 2.1$ mM, $K_T(H^+) = 3.7$ mM] indicates $H^+/2\text{-DOG}$ cotransport. The ratio of the maximum initial influx of H^+ [$9.6 \mu\text{mol min}^{-1} (\text{mg dry wt})^{-1}$] and of 2-DOG [$33.3 \mu\text{mol min}^{-1} (\text{mg dry wt})^{-1}$] gives an $H^+/2\text{-DOG}$ stoichiometry of 0.3.

Table 2. *Stoichiometry of $H^+/2\text{-DOG}$ symport under different physiological conditions*

Experimental conditions were as in Table 1. The inhibitors (50 μ g Dio-9 ml^{-1} , 10 μ g oligomycin ml^{-1} , both dissolved in methanol, and 1 mM-DCCD, dissolved in ethanol) were added 45 min before the sugars. Aerobic cells were energized with 120 mM-ethanol, anaerobic cells with 5 mM-D-glucose. r_0 , Initial influx; n , number of experiments.

Conditions	Temperature ($^{\circ}\text{C}$)	r_0 [nmol $\text{min}^{-1} (\text{mg dry wt})^{-1}$]		Stoichiometry $H^+/2\text{-DOG}$	n
		H^+ uptake	2-DOG uptake		
Aerobic					
1 mM-2-DOG	30	3.9	11.5	0.35	3
5 mM-2-DOG	30	8.3	16.5	0.5	4
	10	2.5	6.3	0.4	1
+ Oligomycin	30	2.2	7.6	0.3	3
+ Dio-9	30	2.3	6.5	0.35	3
+ DCCD	30	0	0	0	3
Anaerobic					
5 mM-2-DOG	30	6.8	14.7	0.45	2
	10	2.6	5.8	0.45	1

results suggest that $\Delta\bar{\mu}_{H^+}$ is the driving force for substrate accumulation by the glucose transport system in *S. pombe*.

The electrogenicity of $H^+/2\text{-DOG}$ cotransport was tested by applying millimolar concentrations of the lipophilic cation tetraphenylphosphonium (TPP^+) (Lieberman & Topali, 1969). Due to its hydrophobic nature, TPP^+ readily penetrates biological membranes by physical diffusion, whereby its mass flow depolarizes the membranes (Hauer & Höfer, 1978; Prasad & Höfer, 1986). In cell suspensions at pH 6, TPP^+ (10 or 20 mM) completely prevented 2-DOG

Fig. 4.

Fig. 4. Outflow of 2-DOG (2 mM) from preloaded cells induced by depolarizing concentrations of the lipophilic cation TPP⁺. ●, No TPP⁺; ▲, 10 mM-TPP⁺; ○, 20 mM-TPP⁺. Experimental conditions were as in Fig. 2, pH = 6.0. The dotted line corresponds to the diffusion equilibrium. The results given are representative of two repeated experiments.

Fig. 5.

Fig. 5. Dependence of the initial influx (v_0) of 2-DOG (2 mM) on the pH of the cell suspension (120 mM-potassium phosphate buffer). The data plotted are means from four repeated experiments.

accumulation or, when added to preloaded cells, caused a complete outflow of accumulated sugar (Fig. 4). Virtually the same results were obtained with glucosamine (data not shown). The effect of TPP⁺ on 2-DOG accumulation was considerably less pronounced at pH 4.5 (data not shown), where the proton electrochemical gradient generally consists mainly of a Δ pH (Höfer *et al.*, 1985).

Since the uptake of substrates induced a stoichiometric cotransport of H⁺ and was dependent on the membrane potential it was concluded that the glucose transport system of *S. pombe* is a H⁺-symporter. H⁺-symport mechanisms display a characteristic pH dependence of transport velocity which slows down as the population of protonated carrier molecules decreases at higher pH values. The effect of extracellular pH on 2-DOG influx in energized *S. pombe* cells is shown in Fig. 5. When the initial rates of 2-DOG uptake, at pH-values between 5.5 and 7.5, were plotted against the H⁺ concentration in a double-reciprocal plot, a straight line was obtained. From its intercept with the abscissa the apparent half-saturation constant of the carrier with H⁺ was calculated to be 10⁻⁷ M ($pK_a = 7.0$). The initial influx of D-glucose exhibited a similar pH dependence. A distinctly different pattern of pH dependence was obtained for the initial influx of D-xylose, a substrate of the facilitated-diffusion system. D-Xylose influx decreased steadily from pH 4.5 to 9.5, where it was still about one-third of that at pH 4.5 (data not shown).

Effect of inhibitors of the plasma membrane ATPase. A H⁺/substrate symport depends on a functioning plasma membrane ATPase generating the driving force of substrate translocation, $\Delta\bar{\mu}_{H^+}$. Two of the known inhibitors of the plasma membrane ATPase in *S. pombe*, Dio-9 and *N,N*-dicyclohexylcarbodiimide (DCCD) (Delhez *et al.*, 1977; Foury *et al.*, 1977) as well as oligomycin, an inhibitor of the mitochondrial ATPase, were tested for their effect on 2-DOG transport in cells energized by ethanol (Fig. 6). Cells were preincubated with inhibitor for 45 min before addition of 2-DOG. Both 2-DOG uptake (Fig. 6a) and H⁺-cotransport (Fig. 6b) were measured. After preincubation with inhibitors, three samples were withdrawn for measurement of the intracellular ATP concentration. DCCD was the most powerful inhibitor of

Fig. 6. Effect of inhibitors of the plasma membrane ATPase on $H^+/2\text{-DOG}$ symport. (a) Uptake of 2-DOG (5 mM) in cells energized with 120 mM-ethanol. Dio 9 ($50 \mu\text{g ml}^{-1}$) (●) or oligomycin ($10 \mu\text{g ml}^{-1}$) (■), both dissolved in methanol, or 1 mM DCCD (1 mM) (○), dissolved in ethanol, were added 45 min before the transport substrate; ▲, control without inhibitors. The dotted line corresponds to the diffusion equilibrium. (b) Tracings of the pH recordings corresponding to the uptake curves in (a). The tracings were shifted to one common origin indicated by the arrow. The pH values of the particular cell suspensions (unbuffered) at the time of 2-DOG addition were as follows: control, 5.5; oligomycin, 6.5; Dio-9, 6.5; and DCCD, 6.9. At the end of each run the H^+ recordings were calibrated by successive additions of $0.5 \mu\text{mol HCl}$ (suspension volume 30 ml). The results shown are representative of three repeated experiments (cf. also Table 2).

transport; intracellular ATP concentration was also lowered to 0.3 mM. Dio-9 and oligomycin both inhibited uptake by about 50%. The intracellular ATP concentration was 1.8 mM in the presence of either of these two inhibitors (ATP concentration in energized cells without inhibitor was 9.9 mM). Corresponding to the inhibited uptake of 2-DOG, H^+ -cotransport was also lowered, maintaining the $H^+/2\text{-DOG}$ stoichiometry constant at about 0.4 (Table 2). The fact that the symport stoichiometry was maintained at different extents of inhibition of 2-DOG uptake further supported the conclusion that the glucose transport system is an H^+ -symporter.

Transport under anaerobic conditions

Under anaerobic conditions ethanol cannot serve as a metabolic substrate. *S. pombe* cells without an external energy source were virtually incapable of uptake of both 2-DOG (Fig. 7a) and glucosamine (data not shown). However, anaerobic uptake of D-glucose was reduced by only 30% and was still inhibited by high 2-DOG concentrations (Fig. 7b), indicating that the same glucose transport system was operating as under aerobic conditions. When 10 mM-D-glucose was used as anaerobic energy source the accumulating capacity of the H^+ -symporter was completely restored (Fig. 7a). The occurrence of H^+ -cotransport induced by the onset of 2-DOG transport (Fig. 8) demonstrated that $\Delta\bar{\mu}_{H^+}$ was also the driving force for transport under anaerobic conditions. The anaerobic $H^+/2\text{-DOG}$ symport displayed a stoichiometry of 0.45 which was close to that observed under aerobic conditions (Table 2).

Fig. 7. Anaerobic transport and accumulation of 2-DOG (a) and anaerobic consumption of D-glucose (b). ○, Uptake in anaerobic cells without further additions; ●, transport in anaerobic cells as control; ▲, uptake in anaerobic cells (a) of 2-DOG energized with D-glucose (5 mM), and (b) of D-glucose inhibited by 2-DOG (20 mM); substrate concentration, 5 mM. The dotted line in (a) corresponds to the diffusion equilibrium. Similar results also were obtained with glucosamine instead of 2-DOG. The data given are representative of four (a) or three (b) repeated experiments.

Fig. 8. Continuous recording of the pH of unbuffered anaerobic cell suspensions during 2-DOG uptake. Conditions were as in Figs 3 and 6(b) except that cell suspensions were kept anaerobic throughout the experiment. Note that 5 mM-D-xylose, a sugar not translocated by the glucose transport system, failed to trigger H^+ -cotransport. The curves shown are representative of a total of nine measurements with two different cell batches.

DISCUSSION

The experimental work presented in this paper provided evidence that D-glucose and its analogues 2-DOG and glucosamine are transported in *S. pombe* by an H^+ -symporter. 2-DOG and glucosamine are accumulated in energized cells and, unlike the yeast *Rhodotorula glutinis* (Höfer, 1971), the molecular operation of the symporter is independent of energy supply, catalysing facilitated-diffusion under de-energized conditions. In this respect, it resembles some bacterial transport systems (Winkler & Wilson, 1966). The D-glucose analogues 6-DOG and 3-OMG, as well as the aldopentoses D-xylose and D-arabinose, enter the cells by a facilitated-diffusion system independent of metabolic energy. The apparent half-saturation constant of the H^+ -symporter with protons ($pK_a = 7$) corresponds to those reported for *R. glutinis* ($pK_a = 6.7$; Höfer & Misra, 1978) and for the green alga *Chlorella vulgaris* ($pK_a = 6.9$; Komor & Tanner, 1974).

The $H^+/2\text{-DOG}$ stoichiometry of the *S. pombe* H^+ -symporter was rather low (0.3–0.5; cf. Table 2). This is doubtless due to recycling of protons by the plasma membrane H^+ -ATPase. The mass flow of positive charges into the cells by the H^+ /substrate cotransport must be electrically compensated for during the transport process. Only at the early stage of cotransport can charge compensation be effected, for example by a transient efflux of K^+ as demonstrated in *R. glutinis*

($H^+ : K^+ : D$ -xylose stoichiometry was 1:1:1 for the initial 60–90 s; Hauer & Höfer, 1982). In *S. pombe* no K^+ efflux was detected following the onset of 2-DOG uptake. Obviously, the electrogenic $H^+/2$ -DOG cotransport was electrically compensated for from the beginning mainly by proton recycling, thus reducing the measurable stoichiometry. The physiological significance of the measured $H^+/2$ -DOG stoichiometry is apparent from the evidence for coupled fluxes of H^+ and the transported substrate. Our attempts to measure $\Delta\psi$ in *S. pombe* by using micromolar TPP^+ concentrations (Hauer & Höfer, 1978; Boxman *et al.*, 1982; Höfer & Künemund, 1984; Prasad & Höfer, 1986) failed. Nevertheless, the results obtained with high TPP^+ concentrations strongly suggest the participation of $\Delta\psi$ in energizing the uptake and accumulation of both 2-DOG and glucosamine. The effect of inhibitors (DCCD, Dio-9 and oligomycin) of membrane-bound ATPases on 2-DOG and glucosamine accumulation is also consistent with their translocation by an H^+ -symporter. However, since the inhibitors also lowered the intracellular ATP concentration their impact on transport may be secondary due to a primary effect on mitochondrial ATP production.

The glucose transport system operated under aerobic and anaerobic conditions. However, endogenous anaerobic metabolism could not provide sufficient energy for transport. When energy supply was restored (by adding D-glucose) full transport capacity was regained. In this respect, the anaerobic transport in *S. pombe* closely resembles that in *Debaryomyces polymorphus* (Schulz & Höfer, 1986). In this yeast, it is the inability of anaerobic metabolism to energize disaccharide uptake that prevents disaccharide utilization under anaerobic conditions (Kluyver effect; cf. Sims & Barnett, 1978). Although our work on *S. pombe* did not deal with transport and utilization of disaccharides, it has a bearing on the elucidation of the Kluyver effect, since the results obtained confirmed the strict dependence of anaerobic sugar uptake on a sufficient energy supply, which thus determines the ability to utilize sugars anaerobically.

Anaerobic 2-DOG uptake induced a measurable H^+ -cotransport in cells energized with D-glucose. Superficially, this observation might be surprising since D-glucose was also taken up in symport with protons. However, any measurable H^+ -cotransport is always transient as long as the plasma membrane ATPase does not fully compensate for the symported protons. When the steady state between H^+ -expulsion by the ATPase and H^+ -uptake by the symporter becomes disturbed by an additional load on the symporter (2-DOG addition) another transient non-compensated H^+ -cotransport is likely to be observed.

It was the aim of this work to test whether or not sugar uptake in *S. pombe* is mediated by an H^+ -symporter. The results presented have established the prerequisite for further investigation of the mechanism of energy coupling between the plasma membrane H^+ -ATPase and the H^+ -symporter by using reconstituted plasma membrane vesicles. Preliminary experiments showed that plasma membrane vesicles reconstituted with pure phospholipids generated a $\Delta\mu_H$ (inside acidic and positive) on adding ATP to a vesicle suspension.

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