Journal of Bioenergetics and Biomembranes, Vol. 21, No. 3, 1989

Analysis of the H^+/Sugar Symport in Yeast under Conditions of Depolarized Plasma Membrane

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Received May 31, 1988; revised August 23, 1988

Abstract

H^+/sugar symport in the obligatory aerobic yeast *Rhodotorula glutinis* was analyzed under conditions where the plasma membrane was selectively depolarized by the lipophilic cation tetraphenylphosphonium (TPP^+). Control experiments showed that this treatment did not impair the transmembrane ΔpH, the cell energy charge, and the function of plasma membrane H^+-ATPase. The kinetic data were fitted to elementary functions derived from a model constructed on the basis of some simplifying premises for ordered (either $C + H^+ + S$ or $C + S + H^+$) and random reaction mechanisms. In addition, the comparison of the kinetic parameters in fully energized and depolarized cells provided information about the free carrier charge. It was concluded that the binding sequence of formation of the ternary carrier/H^+/substrate complex follows a random mechanism and that the carrier bears a negative charge.

Key Words: H^+-symport; membrane potential; depolarized cells; model construction; reaction mechanism; carrier charge; *Rhodotorula glutinis*.

Introduction

It has been shown (Höfer and Misra, 1978; Hauer and Höfer, 1978; Höfer, 1989) that the secondary active transport of monosaccharides in the obligatory aerobic yeast *Rhodotorula glutinis* is driven by the electrochemical potential difference of protons across the plasmalemma

$$\Delta\tilde{\mu}_{H^+} = F\Delta\psi + RT \ln([H^+]_i/[H^+]_0)\ [J\,mol^{-1}]$$

where $\Delta\psi$ is the membrane potential and F, R, T have the usual meaning. The electrical component of $\Delta\tilde{\mu}_{H^+}$ was estimated by means of the lipophilic cation

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0145-479X/89/0600-0321$06.00/0

tetraphenylphosphonium (TPP^+), which has been shown to be a suitable indicator of $\Delta\psi$ in *R. glutinis*, when used in micromolar concentrations (Höfer and Künemund, 1984). Recently, Höfer and coworkers (1985) measured the intracellular pH_i by ^{31}P-NMR (nuclear magnetic resonance) spectrometry and found that, when the extracellular pH_0 was changed from 4 to 8, the magnitude of $\Delta\tilde{\mu}_{H^+}$ ($\Delta G^{0\prime}$) decreased only moderately. At low pH_0 values, $\Delta\tilde{\mu}_{H^+}$ consists solely of ΔpH ($\Delta\psi \approx 0$ at pH_0 4.5), increasing pH_0 leads to a decline of ΔpH; however, this is being compensated for, to a great extent, by a concomitant increase of $\Delta\psi$ ($\Delta pH \approx 0$ at pH_0 7.7). This work was aimed at ascertaining the significance of the individual components of $\Delta\tilde{\mu}_{H^+}$ for the kinetics of secondary active uptake of nutrients by an independent manipulation of either $\Delta\psi$ or ΔpH. Direct measurement of $\Delta\psi$ with glass microelectrodes in yeasts, apart from a few exceptions (Vacata *et al.*, 1981; Höfer and Novacky, 1986; Bakker *et al.*, 1986; Lichtenberg *et al.*, 1988), has so far failed because of the small size of yeast cells and their mobility in a suspension. Moreover, attempts to manipulate membrane potential by using valinomycin in the presence of K^+ were unsuccessful since the ionophore exhibited no effect on *R. glutinis* cells (Künemund and Höfer, 1983). Hence, in order to obtain information about the effect of $\Delta\psi$ on the kinetics of substrate uptake, we exposed the cells to millimolar extracellular concentrations of TPP^+. Under the influence of the existing membrane potential, a massive inflow of TPP^+ is induced and consequently the plasma membrane becomes depolarized. Control experiments showed that, when proper concentrations of TPP^+ were used, $\Delta\psi$ was selectively dissipated without other physiological functions of the cells being affected.

This work deals with the application of this procedure to study the kinetics of the secondary active uptake of 2-deoxy-D-glucose in *R. glutinis*. The results are interpreted within the framework of the classical ion/substrate symport models (Heinz *et al.*, 1972; Geck and Heinz, 1976; Geck, 1978; Turner, 1981, 1985).

Materials and Methods

Growth Conditions

Rhodotorula glutinis (ATCC 26194, CBS 6681) was grown, harvested, and aerated as described previously (Kotyk and Höfer, 1965; Heller and Höfer, 1975).

Continuous Measurement of pH

The velocity of H^+ extrusion or uptake was measured in unbuffered 10% cell suspensions (wet wt/vol). In order to stabilize the signal of pH

electrodes (GK 2401, Radiometer Copenhagen, connected to pH-meter PHM-62, Radiometer Copenhagen), $CaCl_2$ at a final concentration of 0.1 mM was added to the suspensions and the initial pH was adjusted to ~ 6.6 by the addition of 0.1 M $Ca(OH)_2$.

Measurement of Cell Respiration and of Energy Charge (EC)

Oxygen uptake by the cells was measured by the manometric technique (Umbreit, 1949). For determination of intracellular adenosine nucleotide concentrations, yeast cells were extracted as described by Hauer *et al.* (1981). ATP, ADP, and AMP concentrations were measured fluorometrically in a coupled enzymatic assay as described preivously (Trautschold *et al.*, 1985; Jaworek and Welsch, 1985). The energy charge of yeast cells was calculated as follows:

$$EC = \frac{[ATP] + 0.5\,[ADP]}{[ATP] + [ADP] + [AMP]}$$

Transport Experiments

Uptake of D-xylose was measured by the orcinol method as described before (Heller and Höfer, 1975; Misra and Höfer, 1975; Hauer and Höfer, 1978). 2-Deoxy-D-glucose (2-DOG) uptake experiments were carried out with 1% yeast suspensions (wet wt/vol) buffered with 150 mM Tris–citrate adjusted to the desired pH value. Yeast suspensions were incubated in a water-bath shaker at 28°C to provide sufficient aeration and constant temperature. Each experiment was started by adding ^{3}H-labeled 2-DOG. Samples of 1-ml volume were withdrawn after 10, 30, 50 and 70 sec, filtered through Sartorius membrane filters (cellulose nitrate; pore size, 0.8 μm) under suction, and washed twice with 0.5 ml ice-cold distilled water to inhibit further uptake of 2-DOG and to remove the radioactivity possibly bound to cell surface. The pellets on filters were transferred into scintillation vials containing 10 ml scintillation cocktail (299 M, Packard). The samples were counted for radioactivity in a packard TRICARB 460C automatic liquid scintillation counter. Recorded cpm values were corrected for unspecific quenching by the channel ratio method (Neame and Homewood, 1974). In some cases, the external 2-DOG concentration was so low that it decreased during the uptake experiment, thus leading to a curved plot of inner substrate concentration $[S]_i$ over time. Initial velocity of uptake was then estimated by fitting a tangent at the point $t = 10$ sec.

The uptake rates are expressed in nmol min^{-1} (μl cell water)$^{-1}$; 1 mg cell dry weight corresponds to 2 μl cell water (Höfer and Misra, 1978).

Chemicals

^{3}H-labeled 2-deoxy-D-glucose (2-DOG) was purchased from Amersham-Buchler, unlabeled 2-DOG was obtained from Calbiochem-Behring, TPP^+

was delivered by FLUKA, and carbonylcyanide *m*-chlorophenylhydrazone (CCCP) was obtained from Serva. Enzymes and substrates used in enzymatic assays were products of Boehringer. All other chemical compounds were of analytical grade and were obtained from Merck.

Theoretical Section

Several models have been constructed to describe the symport of organic substrates with ions (either Na^+ or H^+) (Heinz *et al.*, 1972; Geck and Heinz, 1976; Geck, 1978; Turner, 1981, 1985). All of these models rely on the following fundamental assumptions: (1) The symporter protein (= carrier) exists in two functional states; in one state, it binds its ligands only from the outside and, in the other state only from the inside, of the separating membrane. (2) The translocation of the carrier or of its complex with bound ligands is the rate-limiting step of the transport process; the ligand-binding reactions are in equilibrium. (3) The total amount of all carrier forms is constant. (4) The transport system is in a steady state. (5) The electrical field of the membrane potential does not affect the structure of the membrane; however, it influences the probability of permeation of the charged carrier or the charged carrier/ligand complex according to the Eyring's mechanism with a symmetric energy barrier. Consequently, the constants of the charge translocating step have to be corrected in relation to those for tranport at $\Delta\psi = 0$:

$$\vec{k} = \vec{k}_0 e^{-z\mu} \quad \text{(inward)}$$
$$\overleftarrow{k} = \overleftarrow{k}_0 e^{z\mu} \quad \text{(outward)}$$

where z is the net charge of the complex and μ the standardized membrane potential [$\mu = F\Delta\psi(RT)^{-1}$; $\Delta\psi = \psi_i - \psi_0$ by definition; F, R, T have the usual meaning].

On the basis of our experimental data (see *Results*), we have assumed, moreover, that both the free charged carrier and the binary carrier/substrate complex (without bound ion) are effectively immobile. Hence, the substrate uptake can be adequately described by flux equations characterizing its dependence on the concentration of ligands on the two sides of the membrane and on the membrane potential.

In order to explain further the present model, some special features of the particular experimental object used in this study should be taken into account: The intracellular pH_i of *R. glutinis* was fairly constant over a wide range of extracellular pH_0 (Höfer *et al.*, 1985), so that pH_i cannot be adjusted to a desired value by changing pH_0. Similarly, due to peculiarities of 2-DOG metabolism in *R. glutinis* (Woost and Griffin, 1983), a defined intracellular

$$V_{T,S}([H^+]) = \frac{a[H^+]}{b + [H^+]}; \quad \lim_{[H^+]\to 0} V_{T,S}([H^+]) = 0, \quad \lim_{[H^+]\to \infty} V_{T,S}([H^+]) = a$$

$$K_{T,S}([H^+]) = \frac{c}{d + [H^+]}; \quad \lim_{[H^+]\to 0} K_{T,S}([H^+]) = \frac{c}{d}, \quad \lim_{[H^+]\to \infty} K_{T,S}([H^+]) = 0$$

$$V_{T,S}([H^+]) = \text{const}$$

$$K_{T,S}([H^+]) = f + \frac{g}{[H^+]}; \quad \lim_{[H^+]\to 0} K_{T,S}([H^+]) = \infty, \quad \lim_{[H^+]\to \infty} K_{T,S}([H^+]) = f$$

$$V_{T,S}([H^+]) = \frac{h[H^+]}{l + [H^+]}; \quad \lim_{[H^+]\to 0} V_{T,S}([H^+]) = 0, \quad \lim_{[H^+]\to \infty} V_{T,S}([H^+]) = h$$

$$K_{T,S}([H^+]) = \frac{k + l[H^+]}{m + n[H^+]}; \quad \lim_{[H^+]\to 0} K_{T,S}([H^+]) = \frac{k}{m}, \quad \lim_{[H^+]\to \infty} K_{T,S}([H^+]) = \frac{l}{n}$$

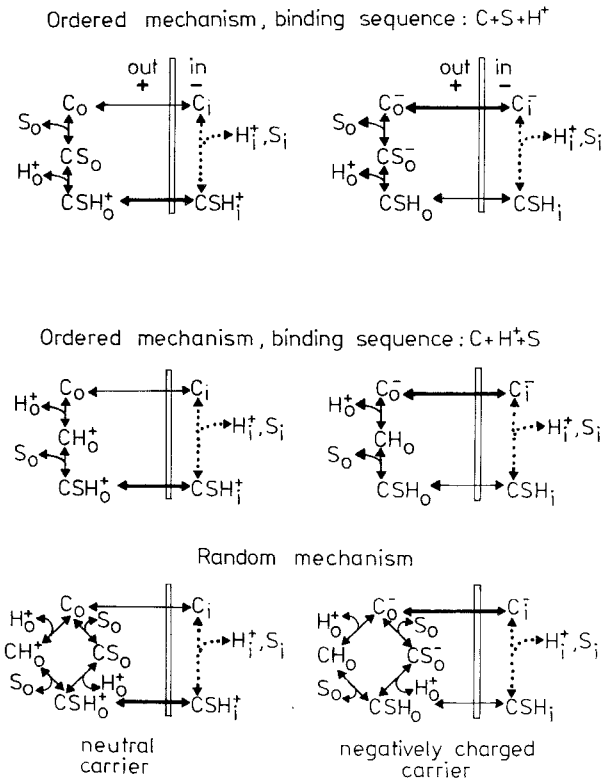

Fig. 1. Schematic representation of the model for two possible net charges of the symporter and three different binding sequences. Boldface arrows denote the individual $\Delta\psi$-driven transport steps. The kinetics of substrate uptake follow the Michaelis-Menten function: $d[S]_i/dt = V_{T,S}[S]_0/(K_{T,S} + [S]_0)$ in all three alternatives. The apparent kinetic parameters $V_{T,S}$ and $K_{T,S}$ for substrate uptake show specific patterns of their dependence on extracellular H^+ concentration for each of the alternative sequences; corresponding elementary functions $V_{T,S}([H^+])$ and $K_{T,S}([H^+])$ for each sequence scheme are given on the left. The numbers $a, b \ldots n$ used in the equations represent products of the euilibrium and rate constants of the individual steps involved in the respective transport cycle.

concentration of 2-DOG also cannot be settled. Consequently, only initial rates of uptake into cells under conditions of varying pH_0 or extracellular substrate concentrations, or the magnitude of $\Delta\psi$ (set up by TPP^+), were measured. These conditions guaranteed identical initial pH_i (= constant) and $[S]_i (= 0)$.

The model (scheme in Fig. 1) makes it possible to draw conclusions from our experimental data as to the sequence of ligand binding on the outside of the plasma membrane and as to the charge of the free carrier. The sequence of dissociation reactions of the ternary complex on the inside of the plasma membrane could not be determined due to the impossibility to adjust pH_i and $[S]_i$ to defined values. However, this appears to be irrelevant for the problems addressed in the present work, as long as pH_i is constant and $[S]_i = 0$.

In regard to its net charge, the carrier is usually assumed to exist in two states, either neutral or negatively charged. When dealing with the ligand binding sequence, three cases are considered (Fig. 1). Fitting of experimental data to the elementary functions given in Fig. 1 enabled us to ascertain the binding sequence for the H^+/sugar symporter in *R. glutinis*. The question as to the net charge of the free carrier, on the other hnd, could be answered by means of flux equations comparing the kinetic parameters in fully energized cells with those in cells depolarized by TPP^+. Following the general mathematical treatment by Geck and Heinz (1976) in the case of an uncharged symporter the membrane potential affects the half-saturation constant of transport of a neutral substrate without having any significant effect on the maximal transport velocity. In the case of a negatively charged symporter, the effects are just opposite (substantial changes of V_T, insignificant changes of K_T). The differences are usually distinct enough to justify the assignment of the experimental data to one of the alternatives. However, one should bear in mind that the increase of V_T in the latter case depends strongly on the ratio of the rate constants for translocation of free carrier (C^-) and of the ternary complex (CHS) at $\Delta\psi = 0$. If the rate constant for C^- is smaller than that for CHS (the translocation of C^- becomes rate limiting), the effect of $\Delta\psi$ on V_T is correspondingly more pronounced.

Results

TPP^+ Depolarizes Yeast Cells

It was shown in earlier experiments (Hauer and Höfer, 1978) that TPP^+, when applied in millimolar concentrations, depolarized *R. gutinis* cells. It was imperative to find a depolarizing TPP^+ concentration that would not yet affect the transmembrane ΔpH, the cell energy charge, and the membrane ATPase function.

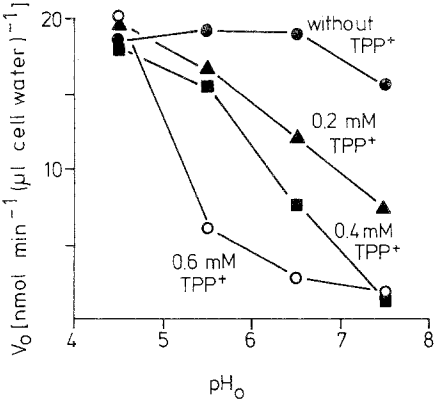

Fig. 2. Dependence of the pH profile of D-xylose uptake rate on the concentration of TPP^+. Experimental conditions: Cell suspension (4 mg cell dry wt ml^{-1}) in 50 mM Tris–citrate buffer, 10 mM D-xylose. Numbers at curves denote TPP^+ concentration in mM.

Choice of the Appropriate TPP^+ Concentration

Determination of the uptake rate for D-xylose (10 mM) and 2-DOG (0.5 mM) in the presence of various concentrations of TPP^+ revealed 0.5 mM to be the optimal concentration of TPP^+ for our experiments. Whereas no effect on the initial rate of uptake was detectable at pH 4.5, 0.5 mM TPP^+ inhibited strongly the uptake of either 2-DOG or D-xylose at pH_0 7.5 (Fig. 2 and Table I). Higher TPP^+ concentrations inhibited the uptake also

Table I. Comparison of the Effects of the Lipophilic Cation TPP and the Uncoupler CCCP on the Cell Energy Charge, Respiration, and Sugar Uptake at Two Different pH_0 Values[a]

	Control	0.5 mM TPP^+	50 µM CCCP
pH 4.5			
EC	0.83	100%	28%
Q_{O_2} (µl O_2 min^{-1} mg dry wt^{-1})	0.28	161%	235%
Uptake (nmol min^{-1} $µl^{-1}$)			
2-DOG (0.5 mM)	12.7	108%	0%
Xylose (10 mM)	18.5	112%	0%
pH 7.5			
EC	0.7	96%	32%
Q_{O_2} (µl O_2 min^{-1} mg dry wt^{-1})	0.24	233%	300%
Uptake (nmol min^{-1} $µl^{-1}$)			
2-DOG (0.5 mM)	11.5	25%	10%
Xylose (10 mM)	15.7	20%	8%

[a] For experimental conditions see *Materials and Methods*. TPP^+ and CCCP were added 2 min before the start of experiments. The results of control experiments (without added agents) were taken as 100%. They are representative of 3–4 experiments.

at pH_0 4.5 although no membrane potential could be measured at this pH_0 value (Höfer and Künemund, 1984; Höfer *et al.*, 1985). Neither the density of yeast suspensions (1–2.5% wet wt/vol) nor the concentration of the buffer (50–150 mM Tris–citrate) influenced the described effects. Virtually the same results were obtained with 2-DOG (cf. Table I.).

Effect of TPP^+ on the pH Gradient (ΔpH)

In order to check whether 0.5 mM TPP^+ affects the ΔpH across the plasma membrane, unbuffered yeast suspensions were treated either with TPP^+ or with the protonophore CCCP (50 μM) (Fig. 3). Following the adjustment of pH_0 to 6.6 by $Ca(OH)_2$ [Ca^{2+} at this concentration does not depolarize *R. glutinis cells* (Hauer and Höfer, 1978)], cells acidify the extracellular medium by energy-dependent H^+ extrusion. The process of acidification was not affected by 0.5 mM TPP^+ whereas uncoupling of yeast mitochondria by 50 μM CCCP (and concomitant increase of plasma membrane permeability for H^+) led to an immediate dissipation of ΔpH. Evidently, 0.5 mM TPP^+ exhibits no inhibitory effect on the plasma membrane ATPase in *R. glutinis*.

Effect of TPP^+ on the Energy Charge (EC) and on Cell Respiration

The energy charge was determined at two defined physiological states of *R. glutinis* cells: at pH 4.5 ($\Delta\psi \approx 0$; ΔpH high) and at pH 7.5 ($\Delta pH \approx 0$; $\Delta\psi$ high). The effect of both 0.5 mM TPP^+ and 50 μM CCCP thereon was also measured (Table I). TPP^+ was without effect on EC at either pH_0 value. In contrast, CCCP induced a distinct drop in the energy charge. The results, taken together with those of Fig. 3 showing the maintenance of ΔpH across the plasma membrane in the presence of TPP^+, provide evidence that the energy metabolism of *R. glutinis* cells, and especially the supply of plasma membrane ATPase with energy, was not impaired in the presence of 0.5 mM

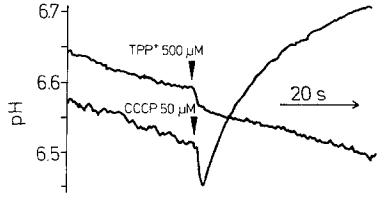

Fig. 3. Effects of TPP^+ and the uncoupler CCCP on the H^+ extrusion (and, hence, ΔpH) by the plasma membrane ATPase. Experimental conditions: Unbuffered cell suspension (8 mg cell dry wt ml^{-1}); the initial pH_0 was adjusted to about 6.6 by $Ca(OH)_2$; additions of agents are indicated by arrows. The traces represent of five experiments.

TPP^+. The maintenance of EC regardless of TPP^+ present is likely to reflect an increased rate of mitochondrial oxidative phosphorylation, as indicated by the enhanced cell respiration with TPP^+ (Table I).

Kinetics of 2-DOG Uptake and $\Delta\psi$ Effect Thereon

2-DOG Uptake in R. glutinis The suitability of 2-deoxy-D-glucose as a nonmetabolizable glucose analog for studies of H^+/glucose symporters, especially for short kinetic experiments, was discussed by Woost and Griffin (1984) [cf. also Höfer and Nassar (1987)]. The kinetics of 2-DOG uptake was measured in a pH_0 range of 5–8. The data were treated by means of the BMDP-software package (Dixon, 1983) according to the following three functions:

a) Monophasic saturation kinetics

$$V = \frac{V_T[S]}{K_T + [S]}$$

b) Biphasic saturation kinetics

$$V = \frac{V_{T1}[S]}{K_{T1} + [S]} + \frac{V_{T2}[S]}{K_{T2} + [S]}$$

c) Monophasic sturation kinetics with superimposed diffusion term

$$V = \frac{V_T[S]}{K_T + [S]} + P[S]$$

where K_T is the half-saturation constant of transport, V_T its maximal velocity, $[S]$ the concentration of the transported substrate, and P the permeability coefficient. Contrary to the results reported by Taghikhani *et al.* (1984), our experimental data did not show a conclusive indication of biphasic kinetics. Consequently, all measurements given below were interpreted in terms of monophasic saturation kinetics.

Stoichiometry of H^+/2-DOG Symport The stoichiometry was measured over a pH_0 range of 4.5–6.5 at 0.5 mM 2-DOG. It should be recalled that, by increasing the pH_0 of cell suspension, the proportion of the two components of $\Delta\tilde{\mu}_{H^+}$ changes as mentioned in the *Introduction* (see also Höfer *et al.*, 1985). Throughout the pH_0 range tested, the ratio of 2-DOG to cotransported H^+ taken up was 1 : 1. This stoichiometry corresponds to that for H^+/glucose symport in the 2-DOG-tolerant mutant of *R. glutinis* (Mahlberg *et al.*, 1985).

These experiments demonstrated, moreover, that, at $pH_0 > 8$ ($\Delta pH = 0$, $\Delta\tilde{\mu}_{H^+} = \Delta\psi$) in the presence of 0.5 mM TPP^+ ($\Delta pH = 0$, $\Delta\psi = 0$), no

uptake of 2-DOG was detectable. From this fact two important conclusions can be drawn: (a) The translocation of each 2-DOG molecule is strictly coupled to the cotransport of H^+ or, in other words, the unprotonated carrier/substrate complex cannot penetrate the plasma membrane (cf. Niemietz and Höfer, 1984). (b) The operation of the H^+/substrate symport is strictly dependent on the existence of an electrochemical potential difference of H^+ across the plasma membrane.

pH Dependence of the Kinetic Parameters of 2-DOG Uptake and Effect of $\Delta\psi$ Thereon

The dependence of the kinetic parameters $K_{T,S}$ and $V_{T,S}$ of 2-DOG uptake on the pH_0 of suspensions of fully energized cells and cells depolarized by TPP^+ is shown in Fig. 4A and B. The maximal velocity of 2-DOG uptake was fairly independent of external pH_0, decreasing significantly only at $pH_0 > 8$. This correlates with the pH_0 profile of the overall $\Delta\tilde{\mu}_{H^+}$ (Höfer *et al.*, 1985). However, in the presence of 0.5 mM TPP^+, the drop in the maximal velocity with increasing pH_0 was distinctly stronger; above pH_0 7.7, no 2-DOG uptake could be measured. The $K_{T,S}$ values of 2-DOG uptake in fully energized cells increased with increasing pH_0 whereas, in cells depolarized by TPP^+, the $K_{T,S}$ values were maintained relatively constant at a somewhat lower level, albeit with a slightly rising tendency.

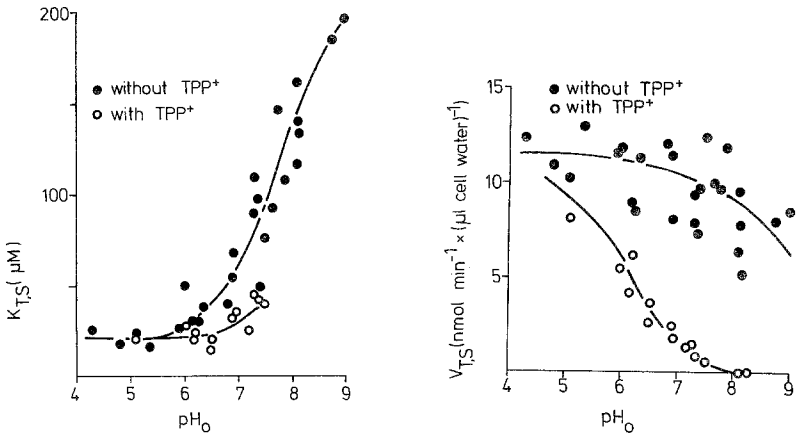

Fig. 4. pH dependence of (A) the half-saturation constant $K_{T,S}$ and (B) the maximal velocity $V_{T,S}$ of 2-DOG uptake in fully energized and TPP^+-depolarized cells. Experimental conditions: Cell suspension (1.5 mg cell dry wt ml^{-1}) in 150 mM Tris–citrate buffer with (depolarized cells, ○) and without 0.5 mM TPP^+ (fully energized cells, ●). Each point was calculated from uptake rates measured with at least five concentrations of 2-DOG.

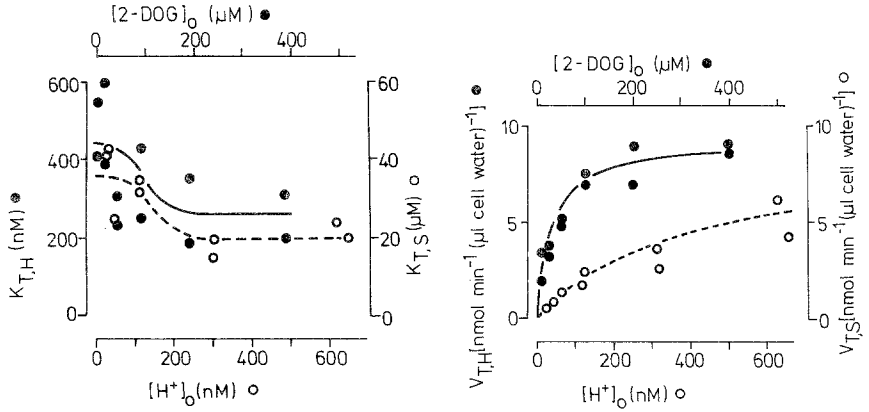

Fig. 5. Dependence of (A) the half-saturation constant and (B) the maximal velocity for 2-DOG uptake on extracellular H^+ concentration ($K_{T,S}$; $V_{T,S}$) and for H^+ cotransport on 2-DOG concentration ($K_{T,H}$; $V_{T,H}$) in cells depolarized by 0.5 mM TPP^+. Experimental conditions as in Fig. 4. The rate of H^+ influx at a fixed 2-DOG concentration at different extracellular H^+ concentrations was estimated from the rates of 2-DOG uptake, assuming a strict one-to-one stoichiometry (see p. 329).

The dependence of both the half-saturation constants and of the maximal velocities of either cosubstrate in TPP^+-depolarized cells on a changing concentration of the respective other cosubstrate matched (Fig. 5A and B). As discussed in detail below, the results presented in Fig. 4 indicate that the free carrier bears a negative charge. The other point derived from the kinetic data of Fig. 5 is that the binding sequence for the two cosubstrates, H^+ and 2-DOG, follows a random mechanism.

Discussion

TPP^+ Depolarizes Specifically the Plasma Membrane

Earlier experiments showed that about millimolar concentrations of TPP^+ depolarize *R. glutinis* cells (Hauer and Höfer, 1978). The present results demonstrated that the lipophilic cation did not affect the chemical component (ΔpH) of the $\Delta\tilde{\mu}_{H^+}$ nor was the cellular energy charge dissipated in its presence. Hence, the pumping efficiency of the plasma membrane ATPase was not impaired, as was also demonstrated by the equal rates of acidification of cell suspensions before and after TPP^+ application. On the other hand, the fact that TPP^+ completely inhibited substrate transport at and above pH_0 7.7 ($\Delta pH \approx 0$; $\Delta\tilde{\mu}_{H^+} = \Delta\psi$) proved the total dissipation of $\Delta\psi$ by the TPP^+ concentration used. Moreover, the pH dependence of the inhibitory effect of TPP^+ corresponded to that of $\Delta\psi$ as measured by Höfer

et al. (1985); in particular, there was no inhibition of symport at pH_0 4.5. Taken together, these findings proved unambiguously that TPP^+ depolarized specifically the plasma membrane of *R. glutinis* cells and, consequently, transport in such cells proceeded under depolarized conditions.

Monophasic or Biphasic Kinetics of 2-DOG Uptake

Taghikhani *et al.* (1984) postulated at least two distinct transport systems for 2-DOG uptake in *R. glutinis*. The statistical evaluation of our experimental data failed to confirm their postulate. Indeed, it was possible in individual cases to fit our data to biphasic kinetics (based on the greater number of degrees of freedom), but the resulting transport parameters of the individual subsystems were not reproducible. In contrast, when the same results were fitted to monophasic kinetics, the transport parameters of the resulting single transport system were well reproducible. The difference between our data and those of Taghikhani *et al.* (1984) could be due to different experimental conditions such as cell growth, aeration period, choice of buffers, storage of cells on ice, or the technique of determination of the initial transport rates.

Interpretation of Experimental Data by Means of the Model

The interpretation of the measured kinetic data was based, as in earlier cases (see references cited in the *Theoretical Section*), on the assumption that the translocation step was rate limiting in the overall transport process. In the light of the observed transport behavior, different reaction mechanisms can be identified. In experimental work, one is always limited by the rather narrow substrate concentration range suitable for measurements, which is due to the physiological properties of the tested organism. Moreover, the shapes of the above elementary functions depend considerably upon the size of the individual functional parameters. Hence, it was in some cases possible to fit several elementary functions to a given series of experimental data. However, the application of a combined treatment using four different elementary functions, each characterizing a specific reaction mechanism, enabled an unambiguous determination of the binding sequence (see the *Theoretical Section*).

Conclusions as to the reaction mechanism of formation of the carrier/H^+/substrate complex in *R. glutinis* were drawn from the experimental data measured in depolarized cells; the binding sequence is seen to be random. Since $\Delta\psi$ considerably affects the transport kinetics, being itself strongly pH_0 dependent, the determination of the reaction mechanism from the kinetic data obtained with fully energized cells, as in the case described by van den Broek and van Steveninck (1980), is not feasible.

A comparison of the data obtained under depolarizing conditions with those from measurements in fully energized cells demonstrated that the membrane potential exerted a "velocity effect" [according to Geck and Heinz (1976)] on the transport kinetics, which implies a negatively charged carrier molecule. It should be recalled (see the *Theoretical Section*) that the effect of the membrane potential will be the stronger the greater the difference is between the rate constants of the free carrier (C^-) and of the ternary carrier/H^+/substrate complex (CHS) in depolarized cells.

The asusmption of translocation being the rate-limiting step of transport and, hence, of the binding reactions of the ligands to the symporter being in a pseudo-equilibrium was criticized by Sanders *et al.* (1984) and Sanders (1986). The authors have not found any compelling experimental evidence for such an assumption. Indeed, the hitherto published model constructions relied rather on the common-sense premise that the process of translocation, i.e., "movement" across a distance by a conformational change, lasts longer than the chemical process of binding. Another advantageous consequence of this assumption is the considerably simpler mathematical treatment of the models. When the complex general model of symport mechanism developed by Sanders and coworkers, who avoided any simplifying assumption, was applied to our results, the data fitted each of the ordered mechanisms and any of the possible charges of free carrier. Despite the critical comments, the simplified sets of conditions were again used as basic tenets for model construction in the recent literature (Turner, 1985; Jauch and Läuger, 1986). In the present work, this approach led to a satisfactory explanation of the observed kinetic phenomena. The meaning of model construction in general consists, in our opinion, in interpreting the measured membrane-transport properties on the basis of simple physicochemical rules.

Regardless of the above discussion as to the proper premises for the construction of models, the present results demonstrated that yeast cells can be selectively depolarized by TPP^+. The present method enabled a successful analysis of the effects of either ΔpH or $\Delta\psi$ on the kinetics of H^+/substrate symport in *R. glutinis*.

Acknowledgments

Introductory discussions with Dr. R. Strasser as well as critical comments by Dr. P. Geck during the running work are greatly appreciated. Our thanks are also due to Drs. D. Richter and D. Söndgerath for providing the statistical software as well as to Dr. K. Sigler for careful reviewing of the English manuscript. This work was supported by the Deutsche Forschungsgemeinschaft (grant Ho 555/12).

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