J. Membrane Biol. 80, 235–242 (1984)

The Journal of
Membrane Biology

Transport of an Anionic Substrate by the H^+/Monosaccharide Symport in *Rhodotorula gracilis:* Only the Protonated Form of the Carrier is Catalytically Active

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Summary. The yeast *Rhodotorula gracilis* accumulated glucuronate by an H^+/symport. The transport was electroneutral, driven by the chemical gradient of protons Δ pH. The observed stoichiometry amounted to 1 proton per molecule glucuronate. At pH 4, the half-saturation constant K_T was at its lowest value (K_t = 8 mM), whereas the maximal velocity V_T reached a maximum(V_t = 15 nmol/min × mg dry wt). Monosaccharides competitively inhibited the uptake of glucuronate and vice versa. Hence, the two substrates share the same transport system. The steady-state accumulation of glucuronate reflected the course of the pH gradient. It is concluded that glucuronate is transported as an anionic substrate by the protonated carrier, the driving force being the chemical gradient of the H^+ (ΔpH). The ternary carrier/H^+/glc-COO -complex is electroneutral and independent of the membrane potential. Simultaneous uptake of organic acids (acetic or propionic acid) which is also energized by the pH gradient led to a noncompetitive inhibition of glucuronate transport. Thus, manipulation of the driving force, ΔpH, reduced V_t without affecting K_t. Kinetic and energetic arguments are presented which stronly suggest that only the protonated carrier is catalytically active in *R. gracilis*.

Key Words H^+-cotransport · glucuronate symport · monosaccharide carrier · anionic substrate · carrier protonation · catalytic activity · *Rhodotorula gracilis*

Introduction

The principle of secondary active transport involves the coupling of the uphill movement of one solute to the energetically downhill movement of another substance, usually an ion. This type of energization applies to a wide variety of transport systems (for a survey *see* e.g. the articles by Harold, 1977 or by West, 1980). Especially proton cotransport systems are of universal occurrence (Eddy, 1978, 1982). The driving force of the cotransport is the electrochemical gradient of H^+ consisting of a membrane potential $\Delta\psi$, which is generated e.g. by the electron transport, as in bacteria, or by an electrogenic H^+-translocating ATPase, as in eukaryotes, and of a chemical H^+-gradient ΔpH. Both components have been shown to be equivalent in energizing the secondary active transport.

The coupling of nonelectrolyte transport to the electrochemical proton gradient, the so-called proton symport, results from the joint binding of substrate and proton to the same carrier. The resulting CH^+S complex is susceptible to the membrane potential, and downhill movement of protons drives the uptake of substrate uphill.

In addition to these energetic features, there are some kinetic implications of the symport model. It has been often claimed that the protonation of the carrier should increase its affinity for the substrate (Komor & Tanner, 1974; Höfer & Misra, 1978). This means that the carrier alternates between a protonated high affinity form and a deprotonated state with low affinity for substrate. The model proposes further that both the low- and the high-affinity form a capable of substrate transport catalysis. The activity of the low-affinity form should be manifested under de-energized conditions or at high pH, where the population of the deprotonated carrier is high in comparison to that of the protonated form (Komor & Tanner, 1975; Höfer & Misra, 1978).

The monosaccharide transport in *Rhodotorula gracilis* operates in many respects according to the described model. Proton symport depolarized the membrane potential (Hauer & Höfer,1978) and reduced the pH gradient (Misra & Höfer, 1975; Höfer & Misra, 1978). By measuring the pH-dependent uptake of D-xylose, the half-saturation constant for the protonation of the carrier was estimated to be pK = 6.75 (Höfer & Misra, 1978). This value has been confirmed by measurements in de-energized cells (*unpublished results*). Furthermore, an affinity change of the carrier caused by its protonation has been concluded for D-xylose transport. However, there have been some peculiar features of the transport of monosaccharides which are not compatible with the discussed model. One example is the lack

of passive efflux from preloaded cells on the addition of uncoupling agents which deenergize the cells (Höfer & Kotyk, 1968; Niemietz, Hauer & Höfer, 1981). Thus, the concept of the catalytically active deprotonated carrier appears to be worth questioning.

In the light of these findings, we checked the implication of the above symport model, provided that the substrate itself bears an electrical charge. In the case of glucosamine, a cationic substrate, this question has already been dealt with in a previous publication (Niemietz et al., 1981). The transport of glucosamine turned out to be driven by the membrane potential without a significant contribution of the chemical gradient of H^+ across the plasma membrane. The pH dependence of glucosamine uptake above pH 6.5 reflected the decreasing concentration of the protonated carrier despite a high membrane potential at these pH values. Hence, the conclusion has been drawn that glucosamine, in spite of its own positive charge, is translocated by the protonated carrier.

These results prompted us to extend the investigations for a negatively charged substrate that again shares the same membrane transport system with monosaccharides. This paper deals with the transport of glucuronate (pK = 2.9), an anionic substrate of the monosaccharide carrier in *Rhodotorula gracilis*.

The special significance of these results, in contrast to similar studies with amino acids (*see* e.g. Niven & Hamilton, 1974; Eddy, 1982) consists in the fact that the transport of the three groups of substrates, electroneutral monosaccharides, positively charged glucosamine and negatively charged glucuronate, is mediated by one and the same transport system of the *R. gracilis* plasma membrane. The results are interpreted as indicating that indeed the protonated carrier represents the only catalytic species of H^+-symport in *Rhodotorula gracilis*.

Materials and Methods

Cell Material

The obligatory aerobic yeast *Rhodotorula gracilis*, ATCC 26197 and CBS 6681, (*Rhodosporidium toruloides* mating type a) was grown according to Misra & Höfer (1975). The mutant strain *Rhodotorula gracilis* M67 was cultivated as described previously (Höfer et al., 1982). The cells were grown for 24 and 38 hr, respectively, on a complex medium, which in the case of *Rhodotorula gracilis* M67 additionally contained 150 μg/ml nystatin.

The yeast was harvested by centrifugation (5 min at 5000 × *g*), washed twice in distilled water, and aerated as 5% suspension for 6 hr before use.

Transport Experiments

Transport was measured as described previously (Niemietz et al., 1981). All experiments except those concerning the proton/substrate stoichiometry were carried out in Tris-citrate buffer. 0.3 M Tris-base was adjusted to the appropriate pH with citric acid. All substrates or inhibitors that were present from the beginning, were dissolved double-concentrated in this buffer, further referred to as "incubation medium." The experiment was started by adding this incubation medium to the same volume of aerated yeast.

Experiments in which the H^+/glucuronate stoichiometry was measured were carried out in unbuffered suspensions. The aerated yeast was sedimented again and washed twice in distilled water to reduce the buffer capacity of the yeast cells themselves. It was then resuspended 2.5% (fresh wt/vol) in distilled water and 0.1 mM $CaCl_2$ was added to stabilize the electrode response. The addition of $CaCl_2$ did not affect the transport rates. The pK value for glucuronic acid is 2.9, and so in the pH region used in this work (4.0 to 6.0), more than 90% of the dissolved substance occurred in the form of glucuronate.

H^+ uptake was measured with a glass electrode (GK 2401 C) connected to a pH-meter (PHM-62, both Radiometer, Copenhagen, Denmark) and recorded continuously (Servogor S, Metrawatt, Nürnberg, FRG). The buffer capacity of the cell suspension was calibrated at the end of the experiment by adding known amounts of HCl, as described earlier (Höfer & Misra, 1978).

Sampling Procedure

Samples were taken either by centrifugation for analysis of the external medium: 350 μl samples were centrifuged for 15 sec at 15.000 × *g* in an ECCO Quick centrifuge (Collatz, Berlin, FRG) and the radioactivity of 200 μl supernatant was counted in Packard 3380 liquid-scintillation counter, or by separation of the cells from the medium by means of membrane filtration: 1 ml of cell suspension was filtered, the cells washed twice with ice-cold water and the radioactivity on the filter determined as reported by Heller and Höfer (1975) and Niemietz et al. (1981).

Measurement of the Membrane Potential

The membrane potential was measured as described by Hauer and Höfer (1978). The distribution of the lipophilic cation ^{3}H-TPP^+ was taken as an indicator of the membrane potential. Because TPP^+ adheres to membrane filters, the radioactivity in the supernatant was determined, and the intracellular concentration was calculated by using the factor of 2 μl cell water per mg dry wt of cells (Höfer & Misra, 1978). In experiments with tritiated TPP^+, the cpm values were corrected for unspecific quenching by use of an external standard and the channel ratio method (Brewer, Pesce & Ashworth, 1974).

Chemicals

^{14}C-glucuronic acid was a product of Amersham-Buchler, Braunschweig, FRG. ^{3}H-TPP^+ was a customer synthesis by Hoechst, Frankfurt, FRG. Nystatin (which was dissolved in pro-

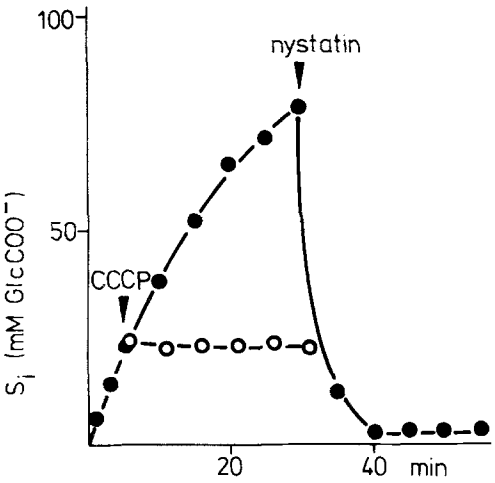

Fig. 1. Effect of the uncoupling agent CCCP (20 μM), ○, and of the polyene antibiotic nystatin (20 μM) on the uptake of glucuronate (5 mM) in Tris/citrate buffer pH 4.5

pane-1,3-diol) was from Serva AG, Heidelberg, FRG. Unlabeled glucuronic acid and carbonylcyanide-*m*-chlorophenylhydrazone (CCCP) were obtained from Sigma, Munich, FRG. The emulsifier scintillator (type 299) was from Packard, Frankfurt, FRG.

All other chemicals were of reagent grade and obtained from Merck, Darmstadt, FRG. All solutions were prepared in glass-distilled water.

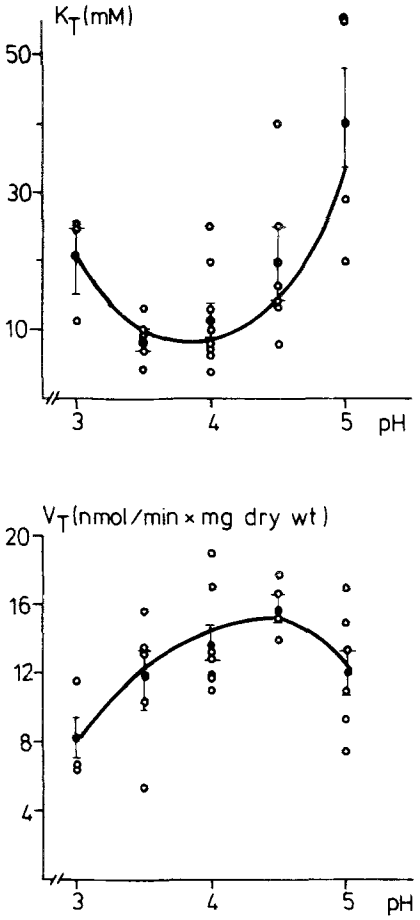

Fig. 2. pH-dependency of transport parameters for glucuronate. Samples were taken at 2-min intervals over a period of 10 min. Uptake was linear up to 15 min. Glucuronate: 3, 10 and 20 mM. The K_T and V_T values at each pH were taken from Lineweaver-Burk (1934) plots and are plotted here both individually (○) and as arithmetic means (●) ± SEM (bars)

Results

Basic Properties of the Uptake System for Glucuronate

Effect of CCCP and Nystatin

Figure 1 shows the energy-dependent uptake of glucuronate. The substrate was accumulated inside the cells. Addition of 20 μM CCCP caused an immediate stop of further accumulation. Any net flux was abolished. To prove that the accumulated glucuronate was osmotically free, cells were treated with 20 μM nystatin. This polyene antibiotic, which renders the membrane permeable to small molecules, brought about a complete and rapid outflow of the substrate, thus demonstrating a true accumulation of glucuronate intracellularly.

pH-Dependency of Transport Parameters

The transport parameters of glucuronate displayed a distinct pH dependency (Fig. 2). At pH 4, the half-saturation constant K_T was at its lowest value, whereas the maximal velocity V_T reached a maximum. The corresponding values were K_T = 8 mM and V_T = 15 nmol/min × mg dry wt. At pH values higher than 5, the transport rates were very low. A graphical analysis of those data was not feasible.

Competition with Monosaccharides

An important point of the following interpretation of the experimental data was the fact that the uptake of glucuronate was really mediated by the monosaccharide carrier. Two substrates sharing the same carrier should compete for transport. Figure 3*A* shows the competitive inhibition of glucuronate uptake by D-galactose. The inhibition constant for

D-galactose K_I was 1.2 M, a value that corresponds to the half-saturation constant for D-galactose uptake (Hauer & Höfer, 1978). Similar competitive inhibition of glucuronate uptake was achieved also by other monosaccharides, e.g. D-xylose (*not shown*). Figure 3*B* demonstrates the complementary competitive inhibition of D-xylose uptake by glucuronate with an inhibition constant of 63 mM (K_T of glucuronate uptake at pH 5 was 20 to 50 mM, Fig. 2). Moreover, monosaccharides brought about an outflow of glucuronate from preloaded cells (*not shown*). Finally, glucuronate and glucosamine, a cationic substrate of the monosaccharide carrier, exhibited a reciprocal competitive inhibition as well (Niemietz, 1982). Hence, glucuronate is transported via the monosaccharide carrier.

Kinetic and Energetic Features of Glucuronate Transport

In the case of an anionic substrate, several hypotheses can be made concerning the energization of transport by symport with protons: One of them, most likely at those pH values used, is that the negatively charged substrate binds to the protonated carrier whereby an electroneutral CH^+S^--complex is formed, which is insensitive to the membrane potential $\Delta\psi$. Consequently, of the two components of the electrochemical proton gradient, $\Delta\mu_{H^+}$, only the pH gradient ΔpH can be used for energization. To test this prediction the following experiments were carried out.

pH-Dependent Accumulation of Glucuronate

Figure 4 shows the pH-dependent accumulation of glucuronate. The pH gradient and the membrane potential at the respective pH values are also indicated. As expected the maximal accumulation reflected the course of the pH gradient. The increase of the membrane potential with rising pH was obviously irrelevant for glucuronate transport.

Stoichiometry H^+/Glucuronate

The proton stoichiometry for glucuronate uptake is recorded in Fig. 5. The cells were suspended in distilled water without added buffer and the substrate (previously adjusted to the appropriate pH with Tris-base) was added. The uptake of glucuronate caused a transient alkalinization of the medium. Both the uptake of protons and of glucuronate was measured simultaneously. The calculated stoichiometry was 1 H^+ take up per molecule of glucuronate.

Influence of Organic Acids on Glucuronate Transport

The results presented so far have demonstrated the importance of the pH gradient as driving force of glucuronate transport. Provided that there is a close correlation between the driving force and the driven process, a decrease of the pH gradient by decreas-

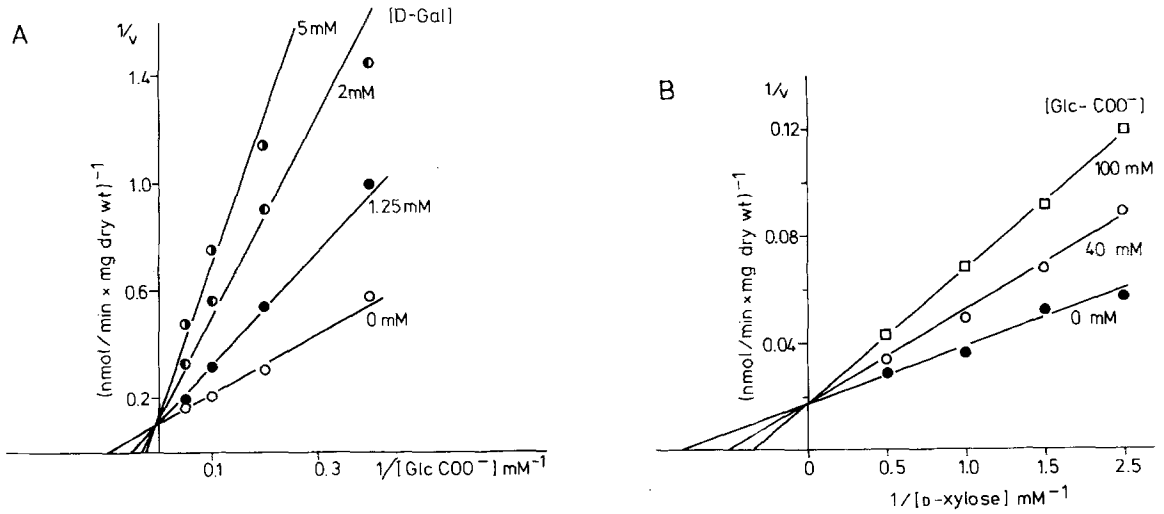

Fig. 3. *A:* Inhibition of glucuronate uptake by D-galactose. The experiment was carried out as described in the legend to Fig. 2. Lineweaver-Burk plot of kinetic data obtained for glucuronate at pH 4.0 in Tris/citrate buffer; galactose concentrations as indicated at the straight lines. K_I for glucuronate 10 mM; K_I for D-galactose 1.2 mM. *B:* Complementary inhibition of D-xylose uptake by glucuronate. Tris/citrate buffer pH 5.0; glucuronate concentrations as indicated at the straight lines. K_T for D-xylose 1.2 mM; K_t for glucuronate 63 mM

ing the intracellular pH_i should be reflected in the uptake parameters for glucuronate.

Organic acids of low molecular weight are assumed to penetrate freely through biological membranes in their protonated, i.e. uncharged form, and to disperse between cell interior and incubation medium according to the existing pH gradient. For our purposes, in order to manipulate the intracellular pH_i, high concentrations (mM) of acetic or propionic acid were used. Due to the completely different chemical structure of the two organic acids compared to the usual substrates of the monosaccharide carrier, it was quite unlikely that the acids would interfere with glucuronate transport at the level of the carrier. In fact, pyruvate and malate were shown to be translocated in *R. gracilis* by a transport system different from the monosaccharide carrier (Höfer & Becker, 1972). On the other hand, the uptake of both glucuronate and the organic acids is energized by the pH gradient. On this level an interference was expected.

Figure 6 shows the influence of propionic acid on the transport parameters for glucuronate. The two substrates sharing the same energy source, i.e. ΔpH, displayed a clearly noncompetitive type of inhibition. The effective affinity of the carrier for glucuronate remained constant whereas the maximal velocity of translocation decreased with rising concentrations of the organic acid. Experiments with acetic acid led to identical results (*data not shown*).

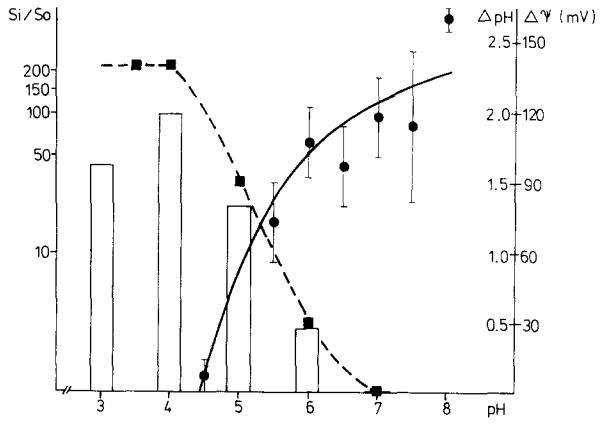

Fig. 4. Accumulation of glucuronate at different pH values. The cells were incubated with 1 mM glucuronate for 3 hr. Samples of cells and of incubation medium were analyzed for glucuronate content. Addition of nystatin (20 μM) in control experiments proved that the substrate was osmotically free inside the cells. The accumulation ratio S_i/S_o, columns, is plotted on logarithmic scale to allow a direct comparison with the pH gradient ■ (according to Höfer & Misra, 1978), and with the membrane potential ● (from Hauer & Höfer, 1978)

Electroneutrality of Glucuronate Transport

It is difficult to prove the electroneutrality of glucuronate transport in *R. gracilis* since at pH values where glucuronate is transported with sufficient affinity and capacity (*cf.* Fig. 4), no or only very low membrane potential can be detected and vice versa. Fortunately, in a nystatin-resistant mutant M67 of

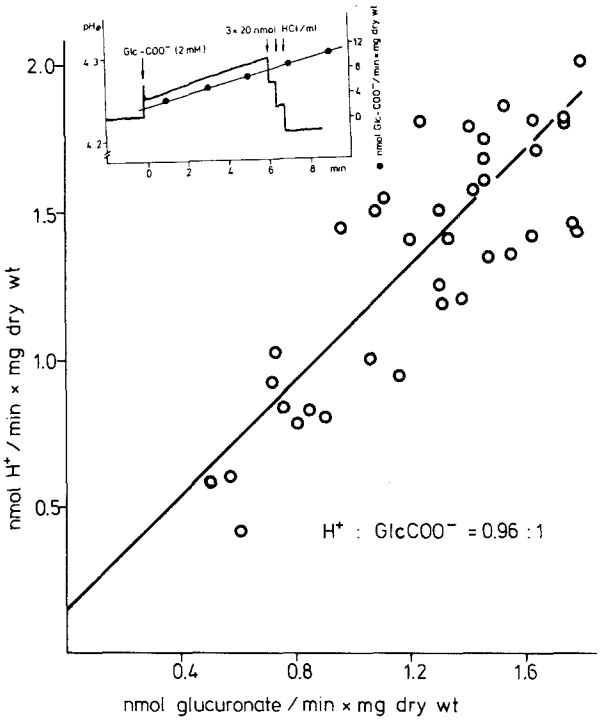

Fig. 5. Stoichiometry H^+/glucuronate. The initial velocity of proton uptake (measured with glass electrode and corrected for buffering capacity of the cell suspension according to Höfer & Misra, 1978) was related to the initial rate of uptake of glucuronate (measured by membrane filtration at 2-min intervals over 10 min). For experimental conditions *see* Materials and Methods. *Insert:* A representative run with 2 mM glucuronate and 5 mg yeast dry wt per ml; pH_e, extracellular pH

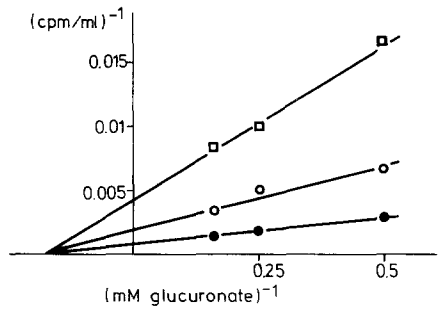

Fig. 6. Effect of propionic acid on glucuronate transport. Experimental conditions were as described in the legend to Figs. 2 and 3*A*, except that propionic acid (0 – ●, 1 – ○, and 2 mM – □) was added instead of D-galactose

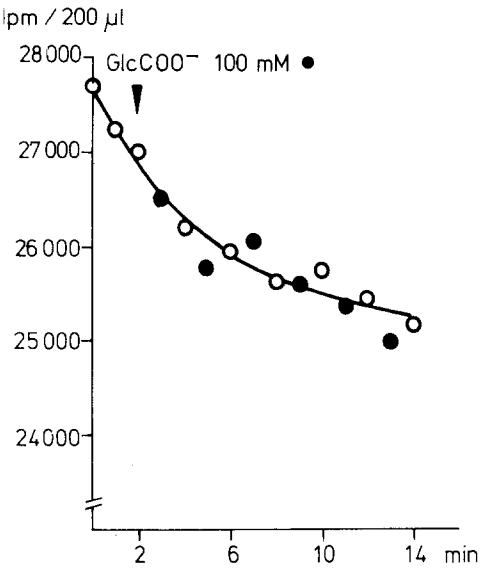

Fig. 7. Effect of glucuronate uptake on the membrane potential in the mutant M67 of *R. gracilis*. Tris/citrate buffer pH 4.5; ^{3}H-TPP$^+$ chloride, 10 µM. For experimental conditions *see* Materials and Methods

the wild strain of *R. gracilis*, membrane potentials could be measured down to pH 4.5 (Höfer, Huh & Künemund, 1983), at which glucuronate transport was optimal. Other transport catalytic properties of the plasma membrane of the mutant seemed to be unaltered from the wild type cells (Höfer et al., 1982). With the mutant, the failure of glucuronate transport to depolarize the membrane potential was clearly demonstrated (Fig. 7). The general ability of mutant cells to transport glucuronate was proved in control experiments (*not shown*).

Discussion

Glucuronate is translocated by the Monosaccharide Carrier with the use of Metabolic Energy

The observed reciprocal competition between glucuronate and monosaccharides on one side, and between glucuronate and glucosamine on the other, as well as the exchange transport of glucuronate and monosaccharides, demonstrated that glucuronate shares the carrier with the monosaccharides. The transport of glucuronate exhibited the same feature known for monosaccharides (Höfer & Kotyk, 1968), i.e. it is completely inhibited by uncoupling agents so that even a downhill movement of substrate is abolished in uncoupled cells. This phenomenon is difficult to reconcile with the concept of a catalytically active deprotonated carrier. If this form was really active, one would expect an efflux of the accumulated substrate from preloaded cells or an influx of substrate up to diffusion equilibrium into empty cells under deenergized conditions. This aspect will be discussed later below.

Glucuronate transport is Electroneutral

In accordance with being transported by the H$^+$-symport for monosaccharides (Fig. 3*A* and *B*) a stoichiometric proton cotransport was observed with the onset of glucuronate uptake (Fig. 5). Furthermore, its accumulation followed the pH gradient (Fig. 4) and was independent of the membrane potential (Fig. 7). Organic acids of low molecular weight, which are taken up as electroneutral molecules, interfered with glucuronate transport. These results indicated that an uncharged $(CH^+S^-)^0$ complex was translocated.

Glucuronate is Transported as an Anionic Substrate by the Protonated Carrier

Transport of glucuronate is optimal at pH 4. At those low pH values where glucuronate transport occurs, the carrier is quantitatively protonated (pK = 6.75; Höfer & Misra, 1978). On the other hand, the substrate is still completely ionized (pK = 2.9). Consequently, the anionic substrate is transported by the protonated form of the carrier.

Deprotonated Carrier is Catalytically Inactive

In the course of this investigation, some arguments were presented which indicated that the deprotonated carrier is catalytically inactive. The uptake of glucuronate as well as of the organic acids (propionic and acetic acid) was energized by the pH gradient. When added together they compete for the common energy source ΔpH.

A comparable inhibition of hexose uptake by butyric acid was reported for *Chlorella vulgaris* (Komor, Schwab & Tanner, 1979). The authors pointed out that the intracellular pH *per se* and not the pH gradient is responsible for the inhibition. They concluded this from the fact that at pH 5, a pH gradient of one unit was less effective in sustaining transport than the same gradient at pH 6. The very likely effect of the increased membrane potential by this pH change was, however, neglected. The experiments of this paper have been carried out at pH 4, where no membrane potential could be measured

in wild type cells, which could additionally influence the kinetic parameters (*cf.* Hauer & Höfer, 1978, and Höfer et al., 1983). Thus, by adding propionic (or acetic) acid, the energy source for glucuronate transport was manipulated without simultaneously changing the membrane potential as was the case in the experiments in *Chlorella* or in those on the pH dependency of transport parameters (*cf.* Fig. 2). The manipulation of the pH gradient at constant external pH had no effect on K_T but changed V_T. The theoretical impact of varying ion gradients and membrane potential on the transport parameters has been thoroughly treated by Heinz and Geck (1978). They constructed different transport models and simulated the influence of artificial ion gradients and of the changing membrane potential upon the kinetic constants of their models.

The results depicted above, indicating that the driving force influenced V_T without changing K_T, coincide with the "velocity type" model of the above-mentioned authors. It is the premise of this model that the driving force (ΔpH) only influences the turnover rate of the carrier without changing its affinity for the substrate. This means that both the protonated and the deprotonated form of the carrier bind the substrate with equal affinity. If this is true, influx by the protonated carrier could always be counteracted by an equally effective substrate efflux using the deprotonated carrier, unless it is catalytically inactive. The observed high accumulation would otherwise result in a high energy dissipation by futile recycling of the substrate. Thus, transport model which does not imply a change in affinity by protonation of the carrier implicitly postulates the binary CS-complex being immobile in the membrane. On the basis of a similar reasoning, the CH^+ form of the carrier has been postulated to be immobile, as well. The latter postulate has been generally accepted, even if never experimentally proved; otherwise the H^+-symport would act as a potent uncoupler.

Kaczorowski and Kabak (1979) observed that the passive efflux of substrate from *E. coli* membrane vesicles occurred via the protonated carrier. This efflux was stimulated by increased external pH and generated a proton motive force, which in turn could be used to drive proline uptake uphill. These results were interpreted as evidence that the protonated carrier served as a catalyst for the passive efflux. The conclusion that the carrier cannot be recycled unless both co-substrates are bound has been drawn for Na^+ and neutral amino acid cotransport in rabbit reticulocytes and pigeon erythrocytes by Thomas and Christensen (1971).

Efflux of β-galactosides from *E. coli* membrane vesicles (Kaczorowski, Robertson & Kaback, 1979) displayed a pattern similar to glucuronate uptake in *R. gracilis*; changes of the proton motive force effected V_T without altering K_T. Conversely, the K_T for β-galactoside influx was reported to be dependent upon energization (Wright, Teather & Overath, 1979; Wright & Overath, 1980). However, these results were questioned by Page and West (1981), who demonstrated by a kinetic analysis that the observed effects of the proton motive force on the apparent saturation constant for β-galactoside can be explained by conventional two-substrate kinetics; the H^+ being a substrate of the transport reaction, provided that the proton availability becomes rate limiting.

Our results, however, were obtained under energized conditions at pH values where the carrier outside was more than 99% saturated with H^+. Due to the missing membrane potential in *R. gracilis* at pH 4 (Hauer & Höfer, 1978), its effect on the transport parameters can be excluded. This does not imply that a membrane potential, in addition to its energetic impact, is generally without influence on the half-saturation constant of transport. Schwab and Komor (1978) reported an enhancement of pK values of the carrier with increasing membrane potential. Under conditions where a membrane potential exists, e.g. in the experiments on the pH-dependency of transport, such effects might also influence transport parameters in *R. gracilis*.

The conclusion that the protonated carrier loses its catalytic activity by deprotonation is supported by several experimental observations: All three groups of substrates, neutral monosaccharides (Hauer & Höfer, 1978; Höfer & Misra, 1978), cationic amino-sugar (Niemietz et al., 1981) and anionic glucuronate (this paper) were accumulated by the protonated carrier. According to the net charge of the ternary carrier-proton-substrate complex, either the membrane potential (amino-sugars), the pH gradient (glucuronate) or both (monosaccharides) were the driving force of uptake. There has been no net translocation of any of the mentioned substrates down their chemical gradient in unenergized cells (Höfer & Kotyk, 1968; Niemietz et al., 1981; Fig. 1 of this paper, respectively).

There is actually only one experimental observation that apparently contradicts the above arguments, viz. the low-affinity electroneutral component of D-xylose transport which became evident at pH values above 6 and at high substrate concentrations (Höfer & Misra, 1978; Hauer & Höfer, 1982). However, this carrier system so far failed to accept any substrate other than D-xylose (*unpublished results*), thus representing obviously a different molecular entity of *R. gracilis* plasma membrane as concluded by Alcorn and Griffin (1978).

This work has been supported by the Deutsche Forschungsgemeinschaft (grant no. Ho 555).

References

Alcorn, M.E., Griffin, C.C. 1978. A kinetic analysis of D-xylose transport in *Rhodotorula glutinis*. *Biochim. Biophys. Acta* **510**:361–371

Brewer, J.M., Pesce, A.J., Ashworth, R.B. 1974. Experimental Techniques in Biochemistry. p. 299. Prentice Hall, Englewood Cliffs, N.J.

Eddy, A.A. 1978. Proton-dependent solute transport in microorganisms. *Curr. Top. Membr. Transp.* **10**:279–360

Eddy, A.A. 1982. Mechanisms of solute transport in selected eukaryotic microorganisms. *Adv. Microb. Physiol.* **23**:1–78

Harold, F.M. 1977. Membranes and energy transduction in bacteria. *Curr. Top. Bioenerg.* **6**:83–149

Hauer, R., Höfer, M. 1978. Evidence for interactions between the energy-dependent transport of sugars and the membrane potential in the yeast *Rhodotorula gracilis* (*Rhodosporidium toruloides*). *J. Membrane Biol.* **43**:335–349

Hauer, R., Höfer, M. 1982. Variable H^+/substrate stoichiometries in *Rhodotorula gracilis* are caused by a pH-dependent protonation of the carrier(s). *Biochem. J.* **208**:459–464

Heinz, E., Geck, P. 1978. The electrical potential difference as a driving force in Na^+-linked cotransport of organic solutes. *In:* Membrane Transport Processes. J.F. Hoffman, editor, Vol. 1, pp. 13–30. Raven, New York

Heller, K.B., Höfer, M. 1975. Temperature dependence of the energy-linked monosaccharide transport across the cell membrane of *Rhodotorula gracilis*. *J. Membrane Biol.* **21**:261–271

Höfer, M., Becker, J.-U. 1972. Regulationsmöglichkeiten des Stoffwechsels von Monosacchariden und Carbonsäuren bei *Rhodotorula gracilis*. *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1: Orig. Reihe A* **220**:374–379

Höfer, M., Huh, H., Künemund, A. 1983. Membrane potential and cation permeability. A study with a nystatin-resistant mutant of *Rhodotorula gracilis* (*Rhodosporidium toruloides*). *Biochim. Biophys. Acta* **735**:211–214

Höfer, M., Kotyk, A. 1968. Tight coupling of monosaccharide transport and metabolism in *Rhodotorula gracilis*. *Folia Microbiol. (Prague)* **13**:197–204

Höfer, M., Misra, P.C. 1978. Evidence for a H^+-sugar symport in the yeast *Rhodotorula gracilis* (*glutinis*). *Biochem. J.* **172**:15–22

Höfer, M., Thiele, O.W., Huh, H., Hunneman, D.H., Mracek, M. 1982. A nystatin-resistant mutant of *Rhodotorula gracilis*. Transport properties and sterol content. *Arch. Microbiol.* **132**:313–316

Kaczorowski, G.J., Kaback, H.R. 1979. Mechanism of lactose translocation in membrane vesicles from *Escherichia coli*. 1. Effect of pH on efflux, exchange, and counterflow. *Biochemistry* **18**:3691–3697

Kaczorowski, G.J., Robertson, D.E., Kaback, R. 1979. Mechanism of lactose translocation in membrane vesicles from *Escherichia coli*. 2. Effect of imposed $\Delta\psi$, ΔpH, and $\Delta\mu_{H^+}$. *Biochemistry* **18**:3697–3704

Komor, E., Schwab, W.G.M., Tanner, W. 1979. The effect of intracellular pH on the rate of hexose uptake in *Chlorella*. *Biochim. Biophys. Acta* **555**:524–530

Komor, E., Tanner, W. 1974. The hexose-proton cotransport system of *Chlorella*. pH-dependent change in K_m values and translocation constants of the uptake system. *J. Gen. Physiol.* **64**:568–581

Komor, E., Tanner, W. 1975. Simulation of a high- and low-affinity sugar-uptake system in *Chlorella* by a pH-dependent change in the K_m of the uptake system. *Planta* **123**:195–198

Lineweaver, H., Burk, D. 1934. The determination of enzyme dissociation constants. *J. Am. Chem. Soc.* **56**:658–666

Misra, P.C., Höfer, M. 1975. An energy-linked proton extrusion across the cell membrane of *Rhodotorula gracilis*. *FEBS Lett.* **52**:95–99

Niemietz, C. 1982. Aktiver Transport geladener Substrate über ein Protonen-Monosaccharid-Symport-System: Transport von Aminozuckern und Uronsäuren bei der Hefe *Rhodotorula gracilis* (*Rhodosporidium toruloides*). Ph.D. Thesis. University of Bonn, West Germany

Niemietz, C., Hauer, R., Höfer, M. 1981. Active transport of charged substrates by a proton/sugar co-transport system. Amino-sugar uptake in the yeast *Rhodotorula gracilis*. *Biochem. J.* **194**:433–441

Niven, D.F., Hamilton, W.A. 1974. Mechanism of energy coupling to the transport of amino acids by *Staphylococcus aureus*. *Eur. J. Biochem.* **44**:517–522

Page, M.G.P., West, I.C. 1981. The kinetics of the β-galactoside-proton symport of *Escherichia coli*. *Biochem. J.* **196**:721–731

Schwab, W.G.M., Komor, E. 1978. A possible mechanistic role of the membrane potential in proton-sugar cotransport of *Chlorella*. *FEBS Lett.* **87**:157–160

Thomas, E.L., Christensen, H.N. 1971. Nature of the cosubstrate action of Na^+ and neutral amino acids in a transport system. *J. Biol. Chem.* **246**:1682–1688

West, I.C. 1980. Energy coupling in secondary active transport. *Biochim. Biophys. Acta* **604**:91–126

Wright, J.K., Overath, P. 1980. Lactose transport in *Escherichia coli*: Effects of transmembrane potential difference on apparent substrate affinity. *Biochem. Soc. Trans.* **8**:279–281

Wright, J.K., Teather, R.M., Overath, P. 1979. Lactose carrier proteins of the two binding sites. *In:* Function and Molecular Aspects of Biomembrane Transport. E. Quagliariello, F. Palmieri, S. Papa and M. Klingenberg, editors. pp. 239–248. Elsevier/North Holland, Amsterdam

Received 12 October 1983; revised 2 February 1984