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LACTOSE TRANSPORT IN *ESCHERICHIA COLI* CELLS

DEPENDENCE OF KINETIC PARAMETERS ON THE TRANSMEMBRANE ELECTRICAL POTENTIAL DIFFERENCE

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We determine the kinetic parameters V and K_T of lactose transport in *Escherichia coli* cells as a function of the electrical potential difference ($\Delta\psi$) at pH 7.3 and $\Delta pH = 0$. We report that transport occurs simultaneously via two components: a component which exhibits a high K_T (larger than 10 mM) and whose contribution is independent of $\Delta\psi$, a component which exhibits a low K_T independent of $\Delta\psi$ (0.5 mM) but whose V increases drastically with increasing $\Delta\psi$. We associate these components of lactose transport with facilitated diffusion and active transport, respectively. We analyze the dependence upon $\Delta\psi$ of K_T and V of the active transport component in terms of a mathematical kinetic model developed by Geck and Heinz (Geck, P. and Heinz, E. (1976) Biochim. Biophys. Acta 443, 49–63). We show that within the framework of this model, the analysis of our data indicates that active transport of lactose takes place with a H^+/lactose stoichiometry greater than 1, and that the *lac* carrier in the absence of bound solutes (lactose and proton(s)) is electrically neutral. On the other hand, our data relative to facilitated diffusion tend to indicate that lactose transport via this mechanism is accompanied by a H^+/lactose stoichiometry smaller than that of active transport. We discuss various implications which result from the existence of H^+/lactose stoichiometry different for active transport and facilitated diffusion.

Introduction

It is now well established that the *lac* permease-catalyzed transport of lactose in *Escherichia coli* is driven by the electrochemical potential difference of protons through a proton symport mechanism as first postulated by Mitchell [1–3] (for a recent review, see also Ref. 4).

The generally accepted scheme consists of the binding of lactose and proton(s) to the *lac* carrier on the external surface, the translocation of the solutes across the membrane, their release inside the cell and the reappearance of binding sites at the external surface. However, at the molecular level, there are numerous uncertainties which include the proton/lactose stoichiometry during transport and the net charge of the *lac* carrier.

Comparing $\Delta\tilde{\mu}_{lactose}$ to $\Delta\tilde{\mu}_{H^+}$ in cytoplasmic membrane vesicles isolated from *E. coli*, Ramos and Kaback [5] observed that a H^+/lactose stoichiometry of 1 was sufficient to account for the observed accumulation of lactose at low pH, but that at high pH values a stoichiometry of 2 was required. They concluded that the H^+/lactose stoichiometry

Abbreviations: Ph_3MeP^+, triphenylmethyl phosphonium ion (bromide salt); CCCP, carbonyl cyanide *m*-chlorophenyl hydrazone; PCMBS, *p*-chloromercuriphenylsulfonic acid (monosodium salt); Mes, 2-(*N*-morpholino)-ethanesulfonic acid.

changed as a function of the external pH. In contrast, Zilberstein et al. [6] and Booth et al. [7] showed that in whole cells of *E. coli,* the observed accumulation ratio of lactose could be accounted for by a H^+/lactose stoichiometry of 1 whatever the external pH. This conclusion was confirmed by direct determination of H^+/lactose stoichiometry [6,7]. With respect to the charge of the carriers Rottenberg [8], in a general theoretical paper, postulated that in the absence of bound solutes they bear a net negative charge. Although challenged by Seaston et al. [9] in the case of the glycine carrier in yeast, the Rottenberg assumption has been favored by Kaback and co-workers [5,10–12] for the *lac* carrier in *E. coli* membrane vesicles.

Further information at the molecular level can be gained by determining how the kinetics of accumulation are affected by the components of the electrochemical potential difference of protons, the chemical potential (ΔpH) and the electrical potential ($\Delta\psi$). Such an approach has been attempted recently by Wright et al. [13] and Robertson et al. [14,15]. The latter authors have carried out a thorough analysis of the behaviour of the kinetic parameters V and K_T of lactose transport in *E. coli* cytoplasmic membrane vesicles as a function of ΔpH and $\Delta\psi$. They show that lactose uptake occurs simultaneously via two components which exhibit high and low K_T values which they associate with facilitated diffusion and active transport, respectively.

Geck and Heinz [16] have proposed a theoretical model of secondary active transport which incorporates the effects of $\Delta\psi$ and of the difference of the chemical potential of the driving ion via several assumptions as to how these parameters control the various steps of transport. They have derived equations between the kinetic parameters V and K_T and the two components of the driving force. They show that the dependence of V upon $\Delta\psi$ is a function of the driving ion/solute stoichiometry and that the dependence of K_T upon $\Delta\psi$ may allow one, theoretically, to distinguish whether the carrier is neutral or negatively charged.

In the present work we first determine, in whole *E. coli* cells, the kinetic parameters of lactose transport as a function of $\Delta\psi$ at $\Delta pH = 0$ for an external pH of 7.3. We show that lactose transport occurs via two components which exhibit a low and a high K_T, which we also associate with facilitated diffusion and active transport, respectively. We test the model of Geck and Heinz [16] against our experimental data. We show that the model accounts for our data provided one assumes a H^+/lactose stoichiometry of active transport of 2 and a neutral empty carrier. On the other hand, our data suggest that the H^+/lactose stoichiometry associated with facilitated diffusion is comparatively smaller than that of active transport.

In view of our results, we discuss some observations reported earlier in the literature, and which taken together hint at the existence of different H^+/lactose stoichiometries for facilitated diffusion and active transport. Finally, our conclusions lead us to reanalyze the various H^+/lactose stoichiometries to be found in the literature.

Material and Methods

Growth condition and cell treatment

Cells of *E. coli* ML 308225 ($i^-z^-y^+a^+$) were grown in minimal medium A containing 1% succinate as the sole carbon source and harvested at an absorbance of 0.8 at 650 nm. The cells were treated with EDTA according to Booth et al. [7] in order to render them sensitive to antibiotics and Ph_3MeP^+. They were resuspended in 5 mM Tris/5 mM Mes/HCl buffer containing 150 mM choline chloride and 1 mM KCl at pH 7.3.

Determination and modification of $\Delta\psi$ and ΔpH

$\Delta\psi$ and ΔpH were determined at 25°C by measuring accumulation of Ph_3MeP^+ and acetate, respectively. 100 μl of EDTA treated cells (1 mg dry weight per ml) were incubated 5 min in the presence of either 70 μM [^{14}C]Ph_3MeP^+ (49.6 Ci/mol) or 100 μM [^{14}C]acetate (47.9 Ci/mol). The cells were then filtered through 0.45 μm EH (Phe_3MeP^+) or HA (acetate) millipores. The filters were counted for radioactivity in a liquid scintillation counter. Blanks were obtained by pretreating the cells with 50 μM of the uncoupler, CCCP. These were filtered immediately after addition of [^{14}C]-Phe_3MeP^+ or [^{14}C]acetate. $\Delta\psi$ and ΔpH were calculated according to Rottenberg [17] assuming an internal cell volume of 1 μl per mg dry weight [7]. Corrections were made for the non-specific binding of Phe_3MeP^+ to the membrane [33].

The experiments were performed at an external pH of 7.3 and a ΔpH of zero. The ΔpH is abolished by the addition of nigericin to the EDTA-treated cells. In the absence of nigericin ΔpH is 1 unit (at pH 7.3). We found that even EDTA treated cells are more impermeable to nigericin than isolated membrane vesicles. Thus, high concentrations of the ionophore had to be used. However, too high a concentration leads to a decrease of $\Delta\psi$ (data not shown). This effect has also been reported for mitochondrial membranes and black lipid films and has been shown to increase with increasing external potassium concentration [18]. Experimentally, we have found that at a K^+ concentration of 1 mM, a nigericin concentration of 10 μM completely collapses ΔpH with only a small effect on $\Delta\psi$.

Variation of $\Delta\psi$ may be obtained by changing the external potassium concentration at a fixed large valinomycin concentration, or vice versa. However, both methods require high K^+ concentrations, leading to a possible uncoupling effect of nigericin. Moreover, in the latter method (varying valinomycin concentration) the change in $\Delta\psi$ occurs over a narrow concentration range (1 to 2 μM) making precise control of $\Delta\psi$ difficult. We have chosen to vary $\Delta\psi$ by allowing high concentrations of the lipophilic cation Ph_3MeP^+ to equilibrate passively across the membrane. The variation of $\Delta\psi$ as a function of the concentration of the lipophilic cation is progressive, reproducible and allows one to change $\Delta\psi$ from 115 mV (no Ph_3MeP^+) to 10 mV (15 mM Ph_3MeP^+).

Lactose transport

100 μl EDTA treated cells (containing 1 mg dry weight per ml) in the presence of 10 μM nigericin were incubated 5 min in the presence of Phe_3MeP^+ (final concentration ranging from 0 mM to 15 mM). [^{14}C]Lactose (1.7 to 5.6 Ci/mol) was then added (final concentration ranging from 0.2 mM to 20 mM) and the cells filtered 20 s later on 0.45 μm HA millipore (25 mm diameter) and washed twice with the suspension buffer. Blanks were obtained by pretreating the cells with 50 μM of the uncoupler, CCCP. These were filtered immediately after addition of lactose. We have verified that at each Ph_3MeP^+ concentration (i.e., for each $\Delta\psi$) the uptake of lactose is linear with time within the 20 s of incubation. The initial rates of lactose uptake were the mean of five independent experiments.

Transport associated with facilitated diffusion was determined in the following way. De-energized cells ($\Delta\psi$ close to zero) are obtained by incubating EDTA-treated cells for 5 min with 50 μM CCCP. To 100 μl of these cells (containing 4 mg dry weight/ml), [^{14}C]lactose (1.7 Ci/mol, concentration 100 mM) was then added (final concentration ranging from 1 mM to 20 mM) and the cells filtered 15 s later on 0.45 μm HA millipores (45 mm diameter) and washed twice with the suspension buffer containing in addition 300 μM PCMBS to prevent efflux during filtration. Blanks were obtained by treating the cells with both CCCP and PCMBS before addition of lactose. These were filtered immediately after addition of [^{14}C]lactose.

Material

[^{14}C]Phe_3MeP^+ and [^{14}C]acetate were obtained from CEA, Saclay (France); [^{14}C]lactose was obtained from Amersham. All other material were of reagent grade and obtained from commercial sources.

Results

Lactose transport as a function of $\Delta\psi$

The kinetic parameters, V and K_T of lactose transport are expected to be a function of $\Delta\psi$, ΔpH and the external pH. Thus, it is essential, while changing $\Delta\psi$ to keep ΔpH and the external pH constant.

The data presented below were obtained at pH 7.3 and for a ΔpH of zero. For each value of $\Delta\psi$ we analyze the transport data in terms of the Eadie-Hofstee representation (v as a function of v/S). Some of these representations at given $\Delta\psi$ values (0 mV, 60 mV, 95 mV, 115 mV) are displayed in Fig. 1.

We focus first on the results obtained for a relatively low value of $\Delta\psi$, 60 mV. The Eadie-Hofstee representation is biphasic indicating the existence of two components of lactose transport with different K_T values. The kinetic parameters associated with the low K_T component can be estimated: $K_T = 0.5$ mM; $V = 13$ nmol/mg dry weight per min. A quantitative determination of the kinetic parameters associated with the high K_T component is beyond our experimental possibilities. It would

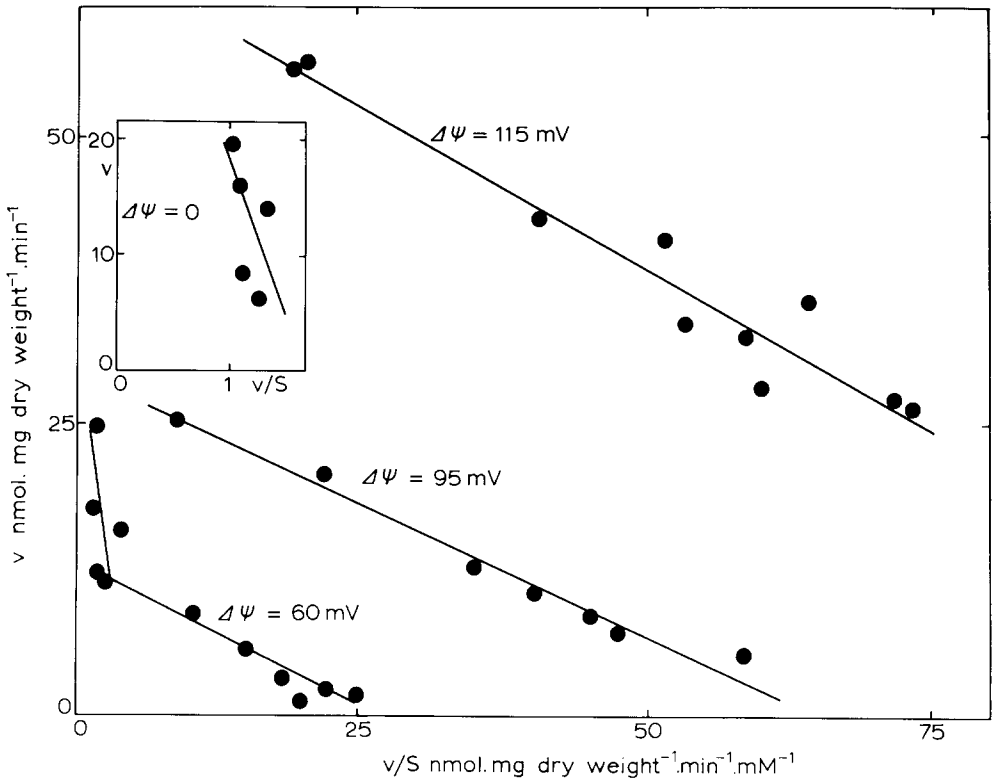

Fig. 1. Initial rate of lactose transport as a function of lactose concentration. The data are displayed using the Eadie-Hofstee representation. They are shown for two extreme values of $\Delta\psi$ (115 mV, the highest value investigated, and 0 mV (inset)), and two intermediate values of $\Delta\psi$ (60 mV and 95 mV).

require measuring transport at lactose concentrations exceeding 20 mM, which we did not attempt. The K_T associated with this component of transport can be estimated to be greater than 10 mM, the corresponding value of V being of course dependent upon the assumed value of K_T.

The kinetic parameters of the low K_T component can be determined for each $\Delta\psi$ value by measuring transport in a range of lactose concentration between 0.2 mM and 2 mM. Except for de-energized cells (see below), the contribution to transport of the high K_T component is negligible in this concentration range. The Eadie-Hofstee representations display a serie of parallel lines, indicating a constancy of K_T, and decreasing values of V as $\Delta\psi$ decreases. In the extreme case, CCCP treated cells ($\Delta\psi$ close to zero), the low K_T component is no longer experimentally detectable. The dependence of V and K_T of the low K_T component of lactose transport as a function of $\Delta\psi$ are shown in Figs. 2 and 3, respectively.

The existence of a component of transport which exhibits a high K_T can, in principle, be assessed by measuring transport in a range of high lactose concentration (3 mM to 20 mM), i.e., under conditions where the low K_T component is nearly saturated. In practice, a quantitative determination of the kinetic parameters of the high K_T component is beyond our experimental possibilities. However, we have assessed its presence by comparing transport at 3 mM and 20 mM lactose concentration. The increased rate of transport at 20 mM as compared to 3 mM is larger than that expected from the contribution of the low K_T component. The data imply the existence of a high K_T component of transport. We show in Table I, for various values of $\Delta\psi$, the

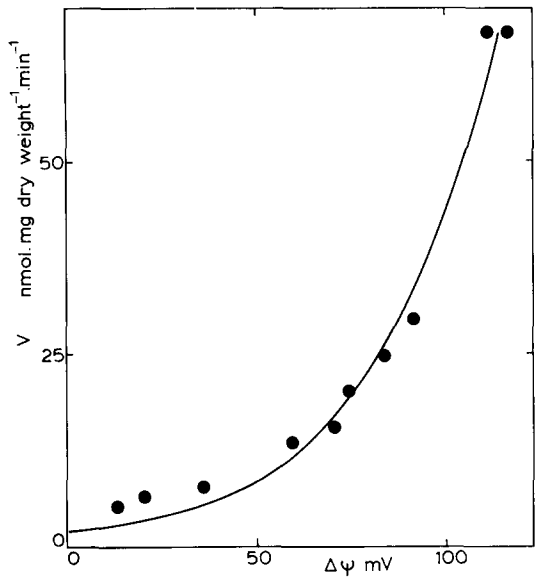

Fig. 2. Dependence upon $\Delta\psi$ of V of the active transport component. The dots correspond to the experimental data, the full line to a curve calculated according to Eqn. 1 (or 1') with $Z = 2$. The values of the parameters A (or A/B) and B (or $1/B$) of Eqn. 1 (or 1') that fit best the experimental data are obtained using a linear representation of these equations: $1/v$ as a function of $\exp((1/2)Z(F\Delta\psi/RT))$.

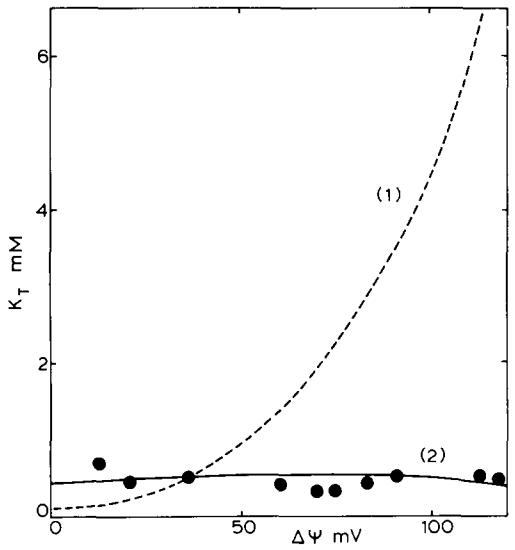

Fig. 3. Dependence upon $\Delta\psi$ of K_T of the active transport component. Curve (1) corresponds to Eqn. 2 with $Z = 2$ and with the value of B that fit Eqn. 1; curve (2) corresponds to Eqn. 2' with $Z = 2$ and with the value of B that fits Eqn. 1'. The dots correspond to the experimental data. The value of C of Eqn. 2 (or 2') has been chosen arbitrarely in both cases so as to give a value of K_T of 0.5 mM for $\Delta\psi = 30$ mV.

TABLE I

DEPENDENCE UPON $\Delta\psi$ OF THE RATE OF LACTOSE TRANSPORT VIA THE ACTIVE TRANSPORT COMPONENT (LOW K_T) AND VIA THE FACILITATED DIFFUSION COMPONENT (HIGH K_T) (LACTOSE CONCENTRATION: 20 mM). THE RATES ARE EXPRESSED IN NMOL/MG DRY WEIGHT PER MIN.

	$\Delta\psi$ (mV)				
	0	40	60	95	115
v (low K_T)	<3	8	13	30	70
v (high K_T)	20	20	15	15	20

contribution to transport at 20 mM lactose concentration of this component and we compare it to the contribution to transport at the same lactose concentration of the low K_T component. The contribution of the former is relatively independent of $\Delta\psi$, whereas that of the latter, as mentioned, increases drastically with increasing $\Delta\psi$.

Following Robertson et al. [14,15], we associate the high K_T component with facilitated diffusion, as it is the only one we observe in de-energized cells, and we associate the low K_T component with active transport. Nevertheless, and although the possibility will not be considered here, it should be mentioned that a system of lactose transport which involves a negative cooperative mechanism will result in apparently biphasic Eadie-Hofstee representations.

Kinetic analysis of lactose active transport as a function of $\Delta\psi$

The different models put forward to account for the symport mechanism of lactose transport all imply that the flux of lactose is energetically linked to a flux of H^+ via the formation of a complex between the *lac* carrier, lactose and proton(s). Within the framework generally accepted for secondary active transport, the kinetic of solute transport will follow a Michaelis-Menten type equation. Even if one assumes that solute transport may occur via two different forms of the carrier (protonated and non-protonated forms in dynamic equilibrium) with different kinetic parameters, the kinetic will follow a single Michaelis-Menten type equation [19,25]. The superposition of two Michaelis type equations,

which is what we report here for lactose transport, will only be obtained if one assumes that there exists two classes of non-interconvertible binding sites for the solute. Therefore, we interpret our data in terms of two classes of lactose binding sites catalyzing facilitated diffusion and active transport, respectively. The sites being independent, it is possible to analyze the kinetic of each component separately. In the following, we analyze in terms of a kinetic model the dependence upon $\Delta\psi$ of V and K_T of active transport.

Geck and Heinz [16] derived the only quantitative relations between the kinetic parameters of transport and $\Delta\psi$ for a secondary active transport for the case of a 1 to 1 stoichiometry between the transported solute and the driving ion. We have chosen to interpret our data in terms of their equations.

Geck and Heinz [16] introduce $\Delta\psi$ in the kinetic equations of transport on the basis of experimental and theoretical results obtained using artificial membranes to study the movement of charged compounds or of ionophore-catalyzed cations. Basically, it is assumed that $\Delta\psi$ will not affect the binding or the release of the transport solutes but that it will affect the mobility (or the probability of translocation) of a species of charge Z across the membrane by a Boltzmann factor $\exp(\pm(1/2)Z(F\Delta\psi/RT))$. As far as the nature of the charged species is concerned, two cases have been considered. Either the empty carrier bears a net negative charge and senses $\Delta\psi$, or the empty carrier is neutral and it is the complex which bears a net positive charge which senses $\Delta\psi$. In both cases, $\Delta\psi$ will drive accumulation of the solute but the dependence of K_T of transport upon $\Delta\psi$ will differ, thus theoretically allowing one to distinguish between the two possibilities.

We have adapted the 'reduced' model of Geck and Heinz [16] to the particular case of active transport of lactose in *E. coli* by making the following additional assumptions: only the empty carrier and the ternary carrier-lactose-H^+ complex can cross the membrane (no inner leakage); the inner concentration of the driving ion is not zero since we are dealing with protons. Furthermore, we do not distinguish between affinity and velocity type models. Finally, we assume, for the time being, as Geck and Heinz do for their model, a 1 to 1 stoichiometry between the transport solute (lactose) and the driving ion (H^+).

The various constants involved in the model are displayed in Fig. 4.

The basic equations to obtain the general rate equations are:

(a) the conservation equation,

$$[C'] + [CL'] + [CH'] + [CHL'] + [C''] + [CL''] + [CH''] + [CHL''] = [C_T]$$

(b) the steady-state equation,

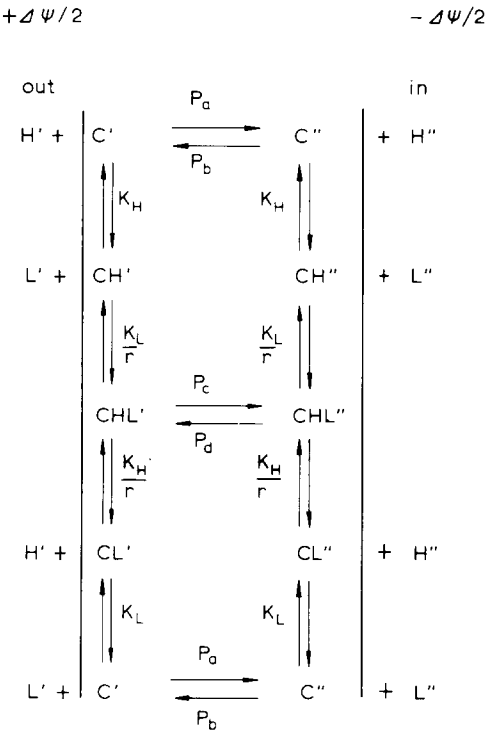

Fig. 4. Model of active transport of lactose. C, H and L represent the carrier, proton and lactose, respectively. K_H and K_L are dissociation constants of the binary complexes. r, represents the 'affinity factor' accounting for a change in affinity between binding site and ligand in the ternary complex as compared to the two binary complexes. P_a, P_b, P_c and P_d are mobility constants which are function of $\Delta\psi$. In the case of a negatively charged carrier: $P_a = P_0 \exp(-(1/2)Z(F\Delta\psi/RT))$, $P_b = P_0 \exp(+(1/2)Z(F\Delta\psi/RT))$, $P_c = P_d = P_1$. In the cae of a neutral carrier: $P_a = P_b = P_0$, $P_c = P_1 \exp(+(1/2)Z(F\Delta\psi/RT))$, $P_d = P_1 \exp(-1/2)Z(F\Delta\psi/RT))$.

in the case of a negatively charged carrier

$$P_0 \exp(-(1/2)Z(F\Delta\psi/RT))[C'] + P_1[CHL'] = P_0 \exp(+(1/2)Z(F\Delta\psi/RT))[C''] + P_1[CHL'']$$

in the case of a neutral carrier

$$P_0[C'] + P_1 \exp(+(1/2)Z(F\Delta\psi/RT))\,[CHL'] = P_0[C''] + P_1 \exp(-(1/2)Z(F\Delta\psi/RT))\,[CHL'']$$

(c) the six equilibrium equations between carrier species and solutes at the interfaces (see Fig. 4),

(d) the transport equation,

in the case of a negatively charged carrier

$$J_L = P_1[CHL'] - P_1[CHL'']$$

in the case of neutral carrier

$$J_L = P_1 \exp(+(1/2)Z(F\Delta\psi/RT))\,[CHL'] - P_1 \exp(-(1/2)Z(F\Delta\psi/RT))\,[CHL'']$$

The eight equations allow one to express J_L (last equation) as a function of the solute concentrations.

Under conditions of initial rate ($L'' = 0$), the relation between J_L and external lactose concentration is expressed by a Michaelis-Menten equation. The relations between V and $\Delta\psi$ and K_T and $\Delta\psi$ at fixed external pH and for a $\Delta pH = 0$ are, with $Z = 1$:

in the case of a negatively charged carrier,

$$V = \frac{A}{1 + B \cdot \exp(-(1/2)\,Z(F\,\Delta\psi/RT))} \qquad (1)$$

$$K_T = C(\exp(-(1/2)\,Z(F\,\Delta\psi/RT)) + \exp(+(1/2)\,Z(F\,\Delta\psi/RT))) \cdot [B + \exp(+(1/2)\,Z(F\,\Delta\psi/RT))]^{-1} \qquad (2)$$

in the case of a neutral carrier,

$$V = \frac{A/B}{1 + (1/B) \cdot \exp(-(1/2)\,Z(F\,\Delta\psi/RT))} \qquad (1')$$

$$K_T = \frac{2C \cdot \cosh(+(1/2)\,Z(F\,\Delta\psi/RT))}{B + \exp(+(1/2)\,Z(F\,\Delta\psi/RT))} \qquad (2')$$

The parameters A, B and C are function of the various constants displayed in Fig. 4.

It should be mentioned that the electrical potential difference relevant in all the above equations is the transmembrane potential difference whereas the experimentally determined electrical potential difference is the one between bulk phases [20]. We have made no attempts to distinguish between the two possibilities.

Our experimental data, V plotted as a function of$\Delta\psi$ (Fig. 2), can in no way be fitted by Eqn. 1 (or 1′). An apparent fit may be obtained but only for meaningless negative values of A and B. This lack of fit is due to the large increase of V taking place over a narrow range of $\Delta\psi$. Indeed, the rate of increase of V with $\Delta\psi$ is controlled by the exponent of the exponential in Eqn. 1 (or 1′) and its absolute value is too small to account for the observed rate of increase. As we will show below, the experimental data could be fitted by an equation similar to Eqn. 1 (or 1′) provided one increases the absolute value of this exponent.

The factor 1/2 in the exponent of exponential in Eqn. 1 (or 1′) is the consequence of the shape of the osmotic barrier across the membrane which is assumed to be triangular and symmetrical. It is the largest possible ratio, all other barrier types leading to smaller ones [21]. The only other way to increase the exponent (at constant $\Delta\psi$ and temperature) is to assume that the charge Z of the membrane species responding to the electrical field (empty carrier or complex) is greater than one. This could be achieved if the empty carrier bears two negative charges or alternatively, if the complexed carrier bears two positive charges ($Z = 2$). Assuming that the H^+-lactose stoichiometry of transport is one, both the empty carrier and the complexed carrier would bear a positive charge (C^+ and CHL^{2+}) or, alternatively, a negative charge (C^{2-} and CHL^-). Although these possibilities cannot be ruled out, they appear unlikely and have not been taken into consideration in the literature, as one step in the translocation process would be retarded by the trans-membrane potential.

The other possibilities are to assume a H^+/lactose stoichiometry of 2.

A H^+/lactose stoichiometry other than one complicates, of course, the model derived by Geck and Heinz [16] and requires its reformulation taking

into consideration the fact that more than one membrane species may respond to the electrical potential difference. For example, in the case of a neutral empty carrier both the ternary complex (carrier-lactose-H^+) and the quaternary complex (carrier-lactose-H^+-H^+) will respond to the electrical field. We may, however, make the simplifying assumption that only the empty carrier and the quaternary complex can cross the membrane. In this case, the relations between V and $\Delta\psi$ and K_T and $\Delta\psi$ remain essentially the same as those derived in the case of a H^+/lactose stoichiometry of 1, except that a factor of 2 in introduced in the exponent of the exponential, i.e., $Z = 2$ in Eqns. 1, 1′, 2 and 2′.

The experimentally observed dependence of V upon $\Delta\psi$ can now be fitted by Eqn. 1 (or 1′) with meaningfull positive values for A and B. The curve calculated with $A = 170$ and $B = 140$ (Eqn. 1) or $A/B = 170$ and $1/B = 140$ (Eqn. 1′) is shown in Fig. 2.

It should be noted that according to these equations, a transport via the active transport component still takes place for $\Delta\psi = 0$. The V of this transport is close to 1 nmol/dry weight/min.

The same parameter B appears in the equation relating K_T and $\Delta\psi$ (Eqn. 2 or 2′). Substituting into these equations that value of B which provides a good agreement between the theoretical and experimental curves for the dependence of V upon $\Delta\psi$, we observe that the shapes of the curves are quite different depending upon whether one assumes a neutral or a negatively charged carrier. The calculated curves are shown in Fig. 3 together with the experimental data. Clearly, our data are more in agreement with a neutral carrier which becomes positively charged upon binding protons.

Discussion

The existence of two components of transport which exhibit a low and a high K_T, respectively, has been reported recently by Robertson et al. [14, 15] for lactose transport in cytoplasmic membrane vesicles isolated from *E. coli*. We confirm here their findings in the case of whole *E. coli* cells and extend them in several respects.

Robertson et al. [14,15] have associated the component which exhibits the high K_T (20 mM) with facilitated diffusion as it is the only one they observe in non-energized vesicles. Similarly, we report here that in de-energized cells transport takes place via a high K_T component (K_T larger than 10 mM). Robertson et al. [14,15] have shown that the K_T of the component which exhibits the low K_T is nearly independent of $\Delta\psi$ whereas the corresponding V increases with increasing $\Delta\psi$. They have associated this component with active transport. We report here the same observation in whole cells. On the basis of these data, these authors propose that there exists a dynamic equilibrium between two forms of the carrier catalyzing facilitated diffusion and active transport; they suggest that an increase in $\Delta\psi$ shifts the equilibrium towards the active form. Thus, the observed increase of V with $\Delta\psi$ would partly result from an increase in concentration of the form catalyzing active transport at the expense of the form catalyzing facilitated diffusion.

It is important to stress the similarity of the experimental data in whole *E. coli* cells and in cytoplasmic membrane vesicles. However, we propose an alternative interpretation of our data.

Indeed, we have made the additional observation that the high K_T component is present whatever the value of $\Delta\psi$ and that its contribution to transport (as determined for a lactose concentration of 20 mM) is nearly independent of $\Delta\psi$. This is not in favor of an interconversion of a facilitated diffusion form into an active transport form as $\Delta\psi$ increases. Moreover, in the models generally accepted for transport, the existence of a dynamic equilibrium between the two forms of the carrier should lead, as we have already mentioned, to a Michaelis type equation of transport displaying a single K_T, and not, as observed, to a superposition of two Michaelis type equations with two different K_T values. Two different K_T values will be obtained if two distinct and non-interconvertible classes of binding sites for lactose are present. The binding on one class of sites would result in facilitated diffusion, the binding on the other class would result in active transport. The different sites may be on the same carrier or on different carriers. The latter possibility leads to two distinct carrier populations, one catalyzing active transport, the other catalyzing facilitated diffusion. Consequently, we interpret our data in terms of the existence of two non-interconvertible classes of

binding sites for lactose which we associate with facilitated diffusion and active transport, respectively. The dependence upon $\Delta\psi$ of V of active transport is the result of a change of the microscopic kinetic constants associated with this mechanism of transport, and not of a change in the concentration of that form. Therefore, it is justified to analyze the macroscopic constants V and K_T of active transport in terms of a kinetic model.

From the analysis of our data, near constancy of K_T and large increase of V with increasing $\Delta\psi$, we conclude that the H^+/lactose stoichiometry of active transport is 2 and that the empty *lac* carrier associated with this transport is neutral. These conclusions are, of course, dependent upon the validity of the assumptions implicit in the model.

Regarding the charge of the empty carrier, Kaczorowski et al. [12] strongly suggest that the *lac* carrier in *E. coli* membrane vesicles is negatively charged. Indeed, these authors have found that the efflux of lactose is not influenced by $\Delta\psi$ under exchange conditions where the *lac* carrier remains protonated, indicating that this complex is neutral and thus, that the empty carrier is negatively charged. On the other hand, Seaston et al. [9] have interpreted the constancy of K_T of glycine transport in yeast, under conditions where $\Delta\psi$ should vary, in terms similar to ours, i.e., a neutral empty carrier. This conclusion was reached on the basis of a model similar to that of Geck and Heinz [16] with the notable exception that the effect of $\Delta\psi$ was not quantitatively formulated.

Regarding the H^+/lactose stoichiometry, the large change of V upon $\Delta\psi$ cannot be accounted for by the model assuming a stoichiometry of 1. This led us to introduce additional assumptions. The H^+/lactose stoichiometry of 2 that we report, rests upon their validity. Nevertheless, the changes of V upon $\Delta\psi$ are bound to reflect the H^+/lactose stoichiometry, the larger the changes, the greater the stoichiometry. This allows a qualitative comparison between the stoichiometries of active transport and facilitated diffusion.

We have mentioned, that in view of experimental limitations, we could not determine the kinetic parameters of transport associated with facilitated diffusion. We show, however, that its contribution to transport as measured at 20 mM lactose concentration is nearly independent of $\Delta\psi$. This constancy does not imply constant values for V. However, a dependency of V of facilitated diffusion upon $\Delta\psi$ similar to that of active transport (an increase by a factor of at least 20 as $\Delta\psi$ increases from 0 mV to 115 mV) would require, in the same $\Delta\psi$ range, an unrealistic increase of K_T of facilitated diffusion. Thus, we conclude that the V of facilitated diffusion responds less, if at all, to $\Delta\psi$ than the V of active transport.

In view of these results, we suggest that the H^+/lactose stoichiometry associated with facilitated diffusion is smaller (if not zero) than that associated with active transport.

This conclusion is conforted by various reports indicating that in other organisms the H^+/sugar stoichiometry accompanying transport of different sugars is smaller for facilitated diffusion than for active transport. Thus, 6-deoxyglucose transport in *Chlorella vulgaris* and xylose transport in *Rhodotorula gracilis* have been shown to occur by active transport via the protonated form of the carrier and by facilitated diffusion via the non-protonated form of the carrier [22,23]. In *Saccharomyces fragilis*, sorbose transport has been shown to take place with a H^+/sorbose stoichiometry of 0.5 which is explained by a simultaneous transport of the sugar via active transport with a stoichiometry of 1 and via facilitated diffusion with a stoichiometry of zero [24]. Interestingly, an additional kinetic analysis suggests that sorbose active transport and sorbose facilitated diffusion take place through distinct carrier populations [25].

In the case of lactose transport in *E. coli*, our conclusion that the H^+/lactose stoichiometry is smaller in the case of facilitated diffusion than in the case of active transport is reenforced by a certain number of observations which have been reported in the literature, and which taken together, strongly hint that lactose efflux down its concentration gradient takes place via the carrier with a H^+/lactose stoichiometry that is smaller than that associated with active lactose influx.

Thus, it has been shown that in *E. coli* cytoplasmic membrane vesicles the uptake of lactose is accompanied by a decrease of both components of $\Delta\tilde{\mu}_{H^+}$ [26,27] indicating a net influx of protons. Moreover, under certain conditions of limiting

supply of reducing substrates, *E. coli* cells display a respiratory control (low rate of oxygen consumption) [28]. Uptake of lactose results in a release of this respiratory control which is also indicative of a net influx of protons [29]. Both these phenomena are still observed under conditions of steady-state levels of lactose accumulation, that is, under conditions where the lactose efflux is equal to the lactose influx. Thus, the efflux of lactose is accompanied by a smaller number of protons than the corresponding influx. Passive efflux across the hydrophobic core of the membrane which takes place without protons cannot be responsible for the net influx of protons observed as it has been shown to be negligible: inhibition of the *lac* carrier by PCMBS completely blocks exit of lactose from preloaded cells [30]. Thus, efflux of lactose takes place via the *lac* carrier with a H^+/lactose stoichiometry smaller than that associated with lactose influx. Lactose influx takes place via the active transport component, as indeed the cells are energized and the external lactose concentration is low. Lactose efflux is expected to take place significantly via the facilitated diffusion component as the internal lactose concentration is relatively high. Thus, the H^+/lactose stoichiometry associated with active transport is larger than the H^+/lactose stoichiometry associated with facilitated diffusion.

The fact that lactose uptake may take place by two mechanisms displaying different stoichiometries, and that the relative contribution to transport of the two mechanisms depends upon the experimental conditions (lactose concentration, state of cell energisation), makes it necessary to reevaluate the H^+/lactose stoichiometries reported in the literature.

Two apparoches have generally been used to determine H^+/lactose stoichiometries: comparison under steady-state conditions of $\Delta\tilde{\mu}_{H^+}$ and $\Delta\tilde{\mu}_{lactose}$ and comparison under initial rate conditions of H^+ influx and lactose influx.

Determination of the stoichiometries by comparison of $\Delta\tilde{\mu}_{H^+}$ and $\Delta\tilde{\mu}_{lactose}$ rests upon the thermodynamic consideration that at steady-state levels of lactose accumulation: $|\Delta\tilde{\mu}_{lactose}| \leqslant n.|\Delta\tilde{\mu}_{H^+}|$, n being the H^+/lactose stoichiometry. This comparison will only yield a lower limit for the stoichiometry as thermodynamic equilibrium does not necessarily prevail. Contradictory results have been reported in the literature, namely a stoichiometry of 2 at alkaline pH values in the case of cytoplasmic membrane vesicles [5] and a stoichiometry of 1 at the same pH values in the case of whole *E. coli* cells [6,7]. These contradictory data can be reconciled in terms of a H^+/lactose stoichiometry of 2, which is the one we report, provided one assumes that the efficiency of coupling is different in both cases. The efficiency of coupling, q, defined as $|\Delta\tilde{\mu}_{H^+}/\Delta\tilde{\mu}_{lactose}| = q \cdot n$ [31] may depend on a variety of parameters which could be different in whole cells and in isolated membrane vesicles.

Determination of H^+/lactose stoichiometry by comparison of rates of proton and lactose influx have been carried out by various authors who systematically report a stoichiometry close to 1 [6,7,32]. This stoichiometry is determined on de-energized cells for a lactose concentration between 1 and 3 mM. We have mentioned that transport via the active transport component still take place for $\Delta\psi = 0$ (de-energized cells). From our data, it can be estimated that, in de-energized cells and for a lactose concentration of 1 mM, transport of lactose will occur with similar rates via the facilitated diffusion and the active transport components. Thus, we propose that the reported H^+/lactose stoichiometry of 1 is the result of a H^+/lactose stoichiometry larger than 1 associated with active transport (possibly 2) and of a H^+/lactose stoichiometry smaller than one (possibly 0) associated with facilitated diffusion.

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