Lactose transport in *Escherichia coli*: effect of transmembrane potential difference on apparent substrate affinity

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The lac carrier of *Escherichia coli* enables lactose to pass across the cytoplasmic membrane and to accumulate within the cell. The energy cost of concentrative sugar uptake is borne by the transmembrane proton free-energy gradient (Δ\(\mu^+_p\)) as envisaged by Mitchell (1977) in the form of pH and electrical potential gradients (ΔpH and Δ\(\psi\)). Obligate in nmol of NPG/mg of protein) at pH 5.5, lactose binding, carrier-mediated facilitated diffusion, and substrate translocation in the presence of an electrochemical proton gradient, Δ\(\mu^+_p\).

Lactose binding

The measurement of galactoside binding to the carrier has been facilitated by the use of plasmid-bearing strains, whose carrier content rises to 10–20% of the protein of the cytoplasmic membrane (Teather et al., 1978). No lactose binding to cytoplasmic membranes can be measured up to a lactose concentration of 1 mM, although the half-saturation constant for active transport, \(K_T\), is 0.1 mM (Table 1, columns C). When the ability of lactose to inhibit the binding of another substrate, thiodigalactoside (\(K_T = 50\mu M\)), is examined, an inhibition constant of 18 mM is found for lactose (Fig. 1). Lactose also competitively inhibits the binding and active transport of other substrates. The equilibrium constants (\(K_T\)) for the inhibition of substrate binding by lactose lie between 9 and 18 mM, whereas the half-saturation constants for the inhibition of D-lactate-induced substrate transport are about 0.1 mM (Wright et al., 1979), similar to the value of \(K_T\) for the active transport of lactose. There is thus a discrepancy of a factor of 160 between the half-saturation constant \(K_T\) for active transport and the lactose dissociation constant. Other adventitious substrates exhibit only small or no shifts from \(K_T\) to \(K_T\) for O-nitrophenyl β-D-galactoside and thiodigalactoside, \(K_T/K_T\) = 3 and 1 respectively (Wright et al., 1979).

This was quantified by measuring the inhibition of thiodigalactoside binding (cf. Wright et al., 1979). Thiodigalactoside binding (n) amounts to 3 nmol/mg of protein at saturation. Lactose competitively inhibits thiodigalactoside binding, as evidenced by the change in the slope of the double-reciprocal plot of the degree of binding-site saturation (n, nmol of thiodigalactoside/mg of protein) against thiodigalactoside concentration. Inset: plot of apparent thiodigalactoside dissociation constant (\(K_T\)) against lactose concentration (mM). The intercept on the ordinate is the true thiodigalactoside dissociation constant (50 μM). The lactose dissociation constant (\(K_{T_{\text{app}}}/K_T\)) evaluated from the slope (ratio of thiodigalactoside to lactose dissociation constant) is 18 mM.

Table 1. Lactose binding and translocation

<table>
<thead>
<tr>
<th>pH</th>
<th>(K_T) (Lactose)</th>
<th>(K_T) (NPG)</th>
<th>(n) (NPG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5</td>
<td>11</td>
<td>0.022</td>
<td>1.9</td>
</tr>
<tr>
<td>6.6</td>
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<td>0.23</td>
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</tr>
<tr>
<td>7.6</td>
<td>16</td>
<td>0.019</td>
<td>1.8</td>
</tr>
</tbody>
</table>

(A) Lactose inhibition (\(K_T\), in mM) of β-nitrophenyl α-galactoside (αNPG) binding (\(K_{T_{\text{app}}}/K_T\), in mM) with amount at saturation (\(n\), in nmol of NPG/mg of protein) at pH 5.5, 6.6 and 7.6 in isolated inner-membrane vesicles from strain T185. (B) Lactose influx (\(K_T\), in mM, \(V_{\text{max}}\), in nmol of lactose/min/mg of protein) in the presence of gramicidin (1 μM) at pH 5.5, 6.6 and 7.6 in vesicles from strain ML 308-225. (C) β-Lactate-stimulated active transport of lactose (\(K_T\) and \(V_{\text{max}}\), as above) at pH 5.5, 6.6 and 7.6.
or 'reorientation' effect. If the reorientation of the loaded carrier leads to the cause of some confusion. Previous studies suggested that two classes of galactoside-binding sites exist (Fox & Kennedy, 1965; Carter et al., 1968; Kennedy et al., 1974), based on the ability of substrates, presumably present at saturating concentrations, to protect an essential thiol group. Because $K_r$ is a gross overestimate of the affinity of lactose for the carrier, at least in the absence of $\Delta \mu_H^\pm$, the concentration of lactose present during these experiments (5 mM) did not lead to saturation of the carrier. We have shown that lactose protects this thiol group and that there is no reason to postulate the existence of two classes of galactoside-binding sites (Wright et al., 1979). The problem of the relation of $K_D$ ($\Delta \mu_H^\pm = 0$) and $K_T$ ($\Delta \mu_H^\pm < 0$) remains, however.

Three features of the transport scheme (eqns. 1-4) could account for the shift from the $K_D$ value to the $K_T$ value. Two explanations may be grouped under the heading of 'affinity effects'. If the affinity of the carrier for lactose increases by a factor $R$, then the half-saturation constant for active transport ($K_T$) is related to the reference equilibrium dissociation constant at $\Delta \mu_H^\pm = 0$ as follows

$$K_T = \frac{K_D}{R}$$

The change in affinity may be ascribable to a direct effect of $\Delta \mu_H^\pm$ on the carrier conformation or to the appearance of a heterotropic allosteric interaction between the co-substrates, proton and galactoside, in the presence of $A^{\pm}$. No requirement for heterotropic allosteric interaction between the co-substrates, lactose and proton, in the presence of $A^{\pm}$. No requirement for galactoside binding for simultaneous proton binding has been demonstrated in the absence of $\Delta \mu_H^\pm$. The second explanation for the difference between $K_D$ and $K_T$ is a 'mobility' or 'reorientation' effect. If the reorientation of the loaded carrier (eqn. 2) is faster than that of the unloaded carrier (eqn. 4) by a factor $\rho$, then $K_T$ and $K_D$ are related as

$$K_T = \frac{2}{\rho - 1} K_D$$


**pH-dependence of $K_D$**

Lactose and proton are co-substrates of the carrier. The binding of the two substrates may not be independent. Lactose binding can be measured indirectly by inhibition of $p$-nitrophenyl $\alpha$-galactoside binding. Lactose inhibition manifests a negligible pH-dependence between pH 5.5 and 7.6 (Table 1, columns A). Moreover, neither the affinity nor the extent of binding of $p$-nitrophenyl $\alpha$-galactoside vary significantly (Table 1, columns A). In the absence of $\Delta \mu_H^\pm$, there is no group on the carrier that is titrated between a bulk pH of 5.5 and 7.6 whose ionization state affects the affinity of lactose binding.

**Facilitated diffusion of lactose**

In the absence of an energy source, the carrier catalyses the non-concentrative movement of galactosides across the membrane. Because of obligate proton symport, such a process is necessarily electrogenic when net galactoside flux occurs. The electrogenicity of translocation is not a trivial problem. Viewing the vesicle as a spherical condenser, the translocation of 10-100 protons across the membrane produces a potential difference of nearly 1 mV. For comparison, an intravesicular lactose concentration of 10 mM represents approx. 2 x 10^12 molecules per vesicle. Vesicles become highly permeable to protons and $K^+$ ions in the presence of gramicidin. All facilitated-diffusion measurements were performed in the presence of this uncoupler to prevent the creation of a transmembrane potential difference during lactose translocation.

Four types of transport processes are measured: influx, efflux and equilibrium exchange with inward and outward tracer fluxes. The half-saturation constants for influx are similar to the equilibrium dissociation constants for lactose (Table 1, columns B and A, respectively). Variation of the pH between 5.5 and 7.6 does not cause a decrease in the $K_V$ values of influx towards the values of $K_D$ for active transport (Table 1, columns B and C, respectively). The maximal velocity of influx is equal to or slightly greater than that for active transport (Table 1, columns B and C). The parameters characterizing efflux and equilibrium exchange do not differ significantly from those for influx (results not shown). Were a mobility or reorientation effect operative (i.e. reaction 2 much faster than reaction 4); then the $K_T$ values for influx or efflux would be similar to that for active transport (approx. 0.1 mM), and the maximal velocity of equilibrium exchange would be $p/2$-fold (approx. 100, cf. eqn. 6) faster than influx or active transport (approx. 40 nmol of lactose/min per mg of protein).

Since the $K_T$ values for lactose efflux and influx are similar to $K_D$ and since the rate of lactose exchange at equilibrium is not significantly faster than influx or efflux, the reorientation rates of the loaded and unloaded carrier (eqns. 2 and 4) are nearly identical and the second model (eqn. 6) is not applicable in the absence of $\Delta \mu_H^\pm$.

**Active transport**

In the absence of $\Delta \mu_H^\pm$, neither the binding of proton to the carrier (affinity effect, eqns. 1 and 3) nor the relative reorientation rates of loaded and unloaded carrier (reorientation effect, eqns. 2 and 4) induces the dramatic change with respect to $K_T$ (10-20 mM) in the concentration-dependence of lactose binding and translocation, respectively, observed in active transport ($K_T = 0.1$ mM). Thus one or both of the components of $\Delta \mu_H^\pm$ must be responsible for the change. The magnitude of the components of $\Delta \mu_H^\pm$ can be altered by the choice of respiratory-chain substrate, external pH and ionophore (Ramos & Kaback, 1977a,b,c). At pH 7.6 $\Delta$ lactate oxidation results only in a transmembrane potential difference ($\Delta\psi = -60$ mV). Under these conditions $K_T = 0.17$ mM (Table 1, column C), demonstrating that $\Delta\psi$ alone can induce the change from $K_D = K_T = 20$ mM (Table 1, column B) to the smaller value in $K_T$ (Table 1). An adventitious $-60$ mV in the form of $\Delta\psi$ at pH 5.5 ($\Delta\psi \approx \Delta\psi \approx -60$ mV) fails to elicit a further decrease in $K_T$ (Table 1, column C).

Valinomycin in the presence of $K^+$ can collapse $\Delta\psi$, leaving $\Delta\psi$ relatively unaffected. The half-saturation constant for active transport of lactose at an extravesicular pH of 5.5 increases (0.2 to 18 mM) in the presence of increasing amounts of valinomycin (0-3 $\mu$M, Fig. 2a). Simultaneous measurement of $\Delta\psi$ and $\Delta\psi$ confirms that $\Delta\psi$ is extremely sensitive to valinomycin, decreasing to approx. 0.03 and 0.3 $\mu$M valinomycin while $\Delta\psi$ remains relatively constant (Fig. 2b). In contrast with the observations from the measurements of facilitated diffusion, the maximal velocity of transport decreases slightly as $K_T$ approaches the $K_T$ value. The value of $K_T$ at a valinomycin concentration of 3 $\mu$M is 18 mM, even though the magnitude of $\Delta\psi$ is $-64$ mV (alkaline interior). The transmembrane potential gradient, $\Delta$w, alone shifts the half-saturation constant for active transport, $K_T$, away from the lactose equilibrium dissociation constant, $K_D$.

**Discussion**

The intrinsic affinity of lactose for its carrier in the absence of $\Delta \mu_H^\pm$ is 10-20 mM. This value is a bench mark, to which kinetic constants may be meaningfully referred. The half-saturation constants for facilitated diffusion in the presence of gramicidin (see Table 1 for influx data) are about 20 mM and may be equated to the equilibrium dissociation constant ($K_D$) for lactose. A detailed analysis of lactose translocation in the absence of $\Delta \mu_H^\pm$ is consistent with a simple, nearly symmetrical carrier model in which galactoside binding occurs in a rapid pre-equilibrium. In this model, the affinity of the carrier for
whereas the transmembrane potential difference, $A_{\text{yl}}$, transport conditions the magnitude of $A_{\text{pH}}$ and $A_{\text{yl}}$ were determined (cf. Ramos et al., 1976). The significance of this effect for active transport is that the maximal velocity of lactose uptake (kinetic saturation) is attained at lactose concentrations far below those leading to binding site saturation when the dissociation constant measured in the absence of $A_{\text{yl}}$ is considered. At the present stage, we cannot identify the shift in the half-saturation constant for lactose translocation evoked by $A_{\text{yl}}$ as a reorientation or affinity effect.

Fig. 2. Dependence of half-saturation constant ($K_T$) for lactose active transport on the transmembrane potential difference
(a) The half-saturation constant for lactose uptake, $K_T$ (in mM, ○) increases with increasing concentrations of valinomycin. Active transport was supported by D-lactate (20 mM) oxidation. The protein concentration was 2.5 mg/ml. The value of $K_T$ approaches the value of the equilibrium dissociation constant ($K_D$) for lactose, approx. 20 mM. The maximal velocity of transport ($V_{\text{max}}$) decreases slightly (○). (b) Under identical conditions the magnitude of $\Delta pH$ and $\Delta \psi$ were determined (cf. Ramos et al., 1976) in the absence of lactose. The transmembrane pH difference, $\Delta pH$ (□), decreases only slightly, whereas the transmembrane potential difference, $\Delta \psi$ (○), is completely collapsed between 0.03 and 0.3 μM-valinomycin. The total free energy (in mV) available for lactose accumulation is represented by $\Delta \mu^* = \Delta pH + \Delta \psi$ (○).