LACTOSE TRANSPORT IN *ESCHERICHIA COLI* CELLS

Evidence in favor of a permease-catalyzed efflux of lactose without protons

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1. Introduction

The mechanism of β-galactoside transport in *E. coli* is a proton symport: the membrane-located carrier (lactose permease) cotransports a proton(s) and a molecule of lactose [1,2]. The entry of protons under an electrochemical potential difference across the cytoplasmic membrane is the exergonic process which drives the endergonic process, the accumulation of lactose. The original hypothesis was that the lactose permease catalyzed the reaction:

$$H^+_{out} + \text{lactose}_{out} \rightleftarrows H^+_{in} + \text{lactose}_{in}$$

so that at steady-state, thermodynamic equilibrium is reached: $\Delta\tilde{\mu}_{H^+} = \Delta\mu_{\text{lactose}}$ [3]. Later, it was shown that under most experimental conditions, lactose accumulation does not achieve thermodynamic equilibrium with the driving force, i.e., $\Delta\tilde{\mu}_{H^+} > \Delta\mu_{\text{lactose}}$ [4].

The absence of equilibrium suggests that a leakage exists of lactose out of the cells without proton(s). Since the membrane is impermeable to lactose by passive diffusion [5], the efflux without proton(s) is via the permease.

As a consequence, at steady-state levels of lactose accumulation when lactose influx and efflux are balanced there should be a deficit in proton efflux, i.e., a net influx of protons.

We report here the results of experiments demonstrating the existence of such net influx of protons under conditions of steady-state levels of lactose accumulation. In addition, we show that there exists a clear correlation between the magnitude of net proton influx and the internal lactose concentration. We propose that the efflux of lactose without proton(s) takes place via a component having a half-saturation constant (K_t) <5 mM.

Abbreviations: Ph_4P^+, tetraphenyl phosphonium ion; Ph_3MeP^+, triphenylmethyl phosphonium ion; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; pCMBS, *p*-chloromercuribenzene sulfonate; Mes, 2-morpholinoethanesulfonic acid

2. Material and methods

2.1. *Growth of bacteria and EDTA treatment*

Escherichia coli ML 308225 cells were grown as in [6]. EDTA-treated cells were prepared according to [4] and resuspended in 5 mM Tris–5 mM Mes–1 mM KCl–150 mM choline adjusted at pH 7.6. The same buffer was used throughout this study.

2.2. *Lactose accumulation*

EDTA-treated cells (1 mg dry wt/ml) were suspended in the suspension buffer (pH 7.6) at 25°C in the presence of D-lactate (20 mM final conc.). At time zero, [^{3}H]lactose (10 mCi/mmol, final conc. 5 μM–5 mM) was added. Aliquots of 100 μl were pipetted at given intervals, immediately diluted with 4 ml washing buffer (suspension buffer containing in addition 300 μM pCMBS) and filtered on HA millipore filters (0.45 μM diam.). The filters were washed twice with 4 ml washing buffer and counted for radioactivity. Each accumulation has been corrected by subtracting blanks obtained in identical conditions except that the cells were pretreated with 50 μM CCCP and 200 μM pCMBS. Whatever the lactose concentration (in range investigated) the steady-state levels of lactose accumulation were reached within 10 min. The inter-

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nal lactose concentrations were calculated assuming an internal cytoplasmic volume of 2 μl/mg dry wt for EDTA and energized cells [6].

2.3. *Determination of* $\Delta\psi$

$\Delta\psi$ was determined by the accumulation of [^{14}C]-Ph_4P^+. EDTA-treated cells (1 mg dry wt/ml) were incubated in the suspension buffer as above. When needed, non-radioactive lactose (final conc. 0–5 mM) or non-radioactive Ph_3MeP^+ (final conc. 0–2 mM) was added at time zero. At time 10 min, [^{14}C]Ph_4P^+ (175 mCi/mmol, final conc. 2.8 μM) was added and at given intervals 100 μl aliquots were pipetted, filtered, washed, counted and corrected for blanks as above. Accumulation of Ph_4P^+ reached a plateau within 5 min. $\Delta\psi$ was calculated from that plateau value using the Nernst equation.

2.4. *Initial rate of proline uptake*

EDTA-treated cells (100 μl containing 1 mg dry wt/ml) were incubated in the suspension buffer in the presence of chloramphenicol (80 μg/ml). When needed, non-radioactive lactose or non-radioactive Ph_3MeP^+ was added (see above). After 10 min [^{14}C]proline (175 mCi/mmol, final conc. 5.1 μM) was added and 30 s later the sample was diluted, filtered, washed, counted for radioactivity and corrected for blanks as above.

2.5. *Material*

[^{14}C]Phe_4P^+, [^{14}C]proline and [^{3}H]lactose were obtained from CEA, Saclay. All other materials were of reagent grade and obtained from commercial sources.

3. Results

3.1. *Depolarization at steady-state levels of lactose accumulation*

The steady-state level of lactose accumulation is a saturable function of the external lactose concentration [7]. Fig.1 displays the saturation curve for external lactose concentrations up to 5 mM. Under our experimental conditions (EDTA-treated cells, pH 7.6) the external concentration for half-saturation is 0.1–0.2 mM while the internal lactose saturates at some 15 mM.

Fig.1 also displays the values of $\Delta\psi$ as determined by the accumulation of [^{14}C]Ph_4P^+ as a function of the external lactose concentration. $\Delta\psi$ is determined once the steady-state levels of lactose accumulation have been reached (see section 2). It is seen that as the external lactose concentration is increased from 0–0.5 mM, $\Delta\psi$ decreases from 155–135 mV. A further increase of the external lactose concentration (up to 5 mM, the highest concentration investigated in this study) does not result in a further decrease of $\Delta\psi$. As shown in fig.1, the variations of $\Delta\psi$ and of the internal lactose concentration take place over the same external lactose concentration range, i.e., between 0–0.5 mM. In [8], $\Delta\tilde{\mu}_{H^+}$ was determined at the steady-state level of lactose accumulation at pH 6. $\Delta\tilde{\mu}_{H^+}$ was independent of external lactose to 1 mM [8]. Although we will not elaborate on the difference between the data in [8] and ours, it may be due to the different pH-values used in the two studies. At pH 6, both $\Delta\psi$ and ΔpH contribute significantly to $\Delta\tilde{\mu}_{H^+}$ while at pH 7.6 (our experimental conditions) $\Delta\psi$ is the sole contributor to $\Delta\tilde{\mu}_{H^+}$ [4,9].

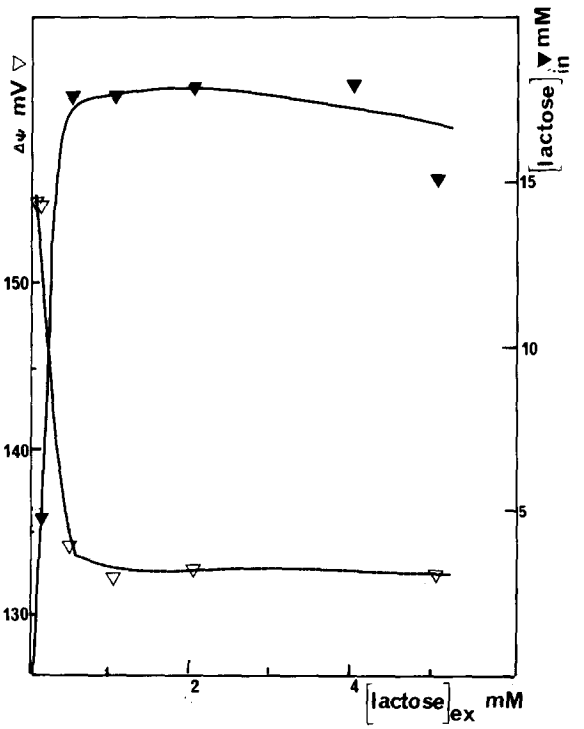

Fig.1. Steady-state internal lactose concentration (right ordinate, ▼) and $\Delta\psi$ (left ordinate, ▽) as a function of the external lactose concentration. Total volume/assay was 100 μl containing 0.1 mg dry wt.

3.2. *Inhibition of the initial rate of proline uptake at steady-state levels of lactose accumulation*

The initial rate of proline uptake, a solute whose transport is coupled to $\Delta\tilde{\mu}_{H^+}$ is dependent upon the value of $\Delta\Psi$ [10].

We first determined the dependence of the initial rate of proline uptake upon $\Delta\Psi$ in the range of interest here (~150 mV). Thus, we incubated cells with varying concentrations of Ph_3MeP^+, whose accumulation in response to $\Delta\Psi$ partially depolarizes the membrane [6], and determined on aliquots $\Delta\Psi$ and initial rate of proline uptake. The results are shown in table 1. It can be seen that as external Ph_3MeP^+ is raised from 0–2 mM, $\Delta\Psi$ decreases from 157–93 mV while the initial rate of proline uptake decreases from 100 (arbitrary units) to 40.

We have taken advantage of this phenomena to substantiate the existence of a decrease of $\Delta\Psi$ as the result of lactose accumulation. The data in fig.2 display the initial rate of proline uptake as a function of the external lactose concentration. The uptake is determined once the steady-state levels of lactose accumulation have been reached. As external lactose is increased from 0–0.5 mM, the initial rate of proline uptake decreases from 100 (arbitrary units) to 50 and it remains stable at that value as external lactose is further increased from 0.5–5 mM. For comparative purposes we display in fig.2 the variations of the internal lactose concentration as a function of the external lactose concentration previously displayed in fig.1. It can be seen that both phenomena, increase in internal lactose concentration and decrease in the initial rate of proline uptake, take place over the same external lactose concentration range.

Table 1
Dependence of $\Delta\Psi$ and initial rate of proline uptake in EDTA-treated cells upon the external concentration of added Ph_3MeP^+

Ph_3MeP^+ (mM)	0	0.5	1.0	2.0
$\Delta\psi$ (mV)	157	142	113	93
V_i (proline)	100	71	52	40

Ph_3MeP^+ was allowed to accumulate 15 min in the presence of the cells prior to the determination of $\Delta\psi$ and of the initial rate of proline uptake. The initial rate of proline uptake was set arbitrarily at 100 in the absence of Ph_3MeP^+ (V_i (proline)).

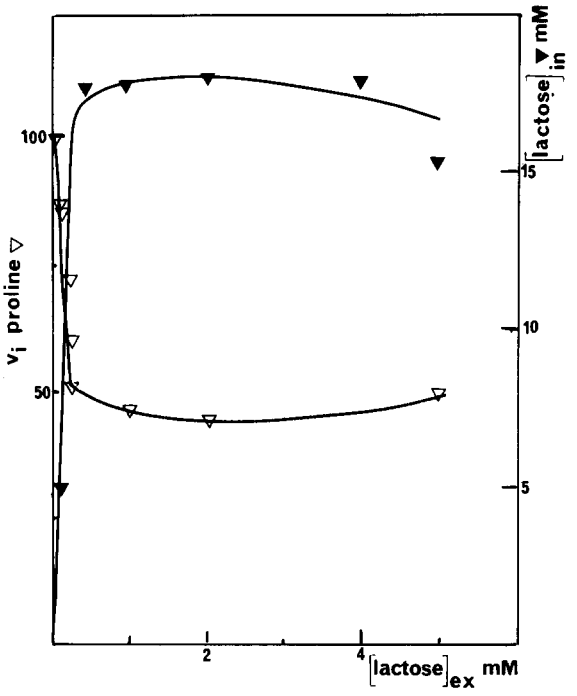

Fig.2. Steady-state internal lactose concentration (right ordinate, ▼) and initial rate of proline uptake (V_i proline) (left ordinate, ▽) as a function of the external lactose concentration. Total volume/assay was 100 μl containing 0.1 mg dry wt. The initial rate of proline uptake was set arbitrarily at 100 in the absence of external lactose.

4. Discussion and conclusions

The H^+ symport mechanism of lactose uptake implies that during the process of lactose accumulation, as long as lactose influx predominates over lactose efflux, there is a net influx of protons. Direct evidence for the existence of such net influx of protons has been presented [1,2]. Moreover, it has been shown that in cytoplasmic membrane vesicles isolated from whole cells there is a depolarization of the membrane as a consequence of the net influx of protons [11,12]. However, if one assumes that the system reaches thermodynamic equilibrium ($\Delta\tilde{\mu}_{H^+} = \Delta\mu_{lactose}$), then the net influx of protons should stop once influx and efflux of lactose are balanced, that is, once the steady-state level of lactose accumulation has been reached. However, comparison of $\Delta\tilde{\mu}_{H^+}$ and $\Delta\mu_{lactose}$ have clearly indicated that a thermodynamic equilibrium is not reached [4] suggesting that a leakage exists of lactose without protons. As a conse-

quence, even at steady-state level of lactose accumulation there is a net deficit in proton efflux or conversely a net influx of protons.

These data demonstrate the existence of such a net deficit in proton extrusion. Indeed, we show that at steady-state levels of lactose accumulation there exists a depolarization of the membrane which we can detect either directly by measurements of $\Delta\Psi$ or indirectly by the inhibition of the initial rate of proline uptake.

In addition to demonstrating the existence of a net deficit in proton extrusion, our data indicate a correlation between the extent of depolarization and the internal lactose concentration. Thus, we do not observe an appreciable depolarization for internal lactose at <5 mM. At >5 mM internal lactose, the extent of depolarization increases and stabilizes in parallel with the internal lactose concentration. Therefore, we conclude that the exit of lactose without protons which is responsible for the net deficit in proton extrusion and leads to the inefficiency of coupling between $\Delta\tilde{\mu}_{H^+}$ and $\Delta\mu_{lactose}$ takes place via a route displaying a K_t of efflux >5 mM.

Based on different experimental evidence, we reached a similar conclusion in [13]. Indeed, influx of lactose has been shown to occur by two different routes in cytoplasmic membrane vesicles [14] as well as in whole cells [13]. Two components of transport, one displaying a low K_t (0.2–0.5 mM) and the other a high K_t (>10 mM) have been observed. These have been associated with active transport and facilitated diffusion, respectively. In addition, our study of the kinetics of lactose uptake in whole cells [13] led us to the conclusion that the H^+/lactose stoichiometry of facilitated diffusion is smaller than that of active transport (we suggested a stoichiometry of 0 *vs* 2). The facilitated diffusion component is expected to function in efflux as well as in influx. Thus we suggested that at steady-state levels of lactose accumulation where there is a large internal lactose concentration, efflux by this component should predominate over influx leading to a net deficit in proton extrusion.

We propose that this efflux via the facilitated diffusion component of lactose transport is the one responsible for the depolarization reported here.

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