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KINETICS OF LACTOSE TRANSPORT INTO *ESCHERICHIA COLI* IN THE PRESENCE AND ABSENCE OF A PROTONMOTIVE FORCE

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1. Introduction

The kinetics of galactoside movements into and out of whole cells of *Escherichia coli* under metabolizing and metabolically-inhibited conditions were described by Winkler and Wilson [1]. They found that the inhibition of metabolism caused the very high app. K_m for efflux to fall to the lower values characteristic of the app. K_m for influx, whereas the app. K_m for influx was not detectably changed. These results were substantially confirmed in [2], but in a neglected paper [3], the presence of uncoupler was reported to cause the app. K_m for influx of thiomethylgalactoside to increase to a much higher value.

In the presence of a protonmotive force membrane vesicles accumulate lactose with an app. K_m similar to that for influx into whole cells [5,6]. In the absence of added respirable substrate there is essentially no protonmotive force and galactosides are not accumulated, though they may still bind to the carrier [6]. Under the latter conditions, the binding constant for lactose was 200-fold greater than the app. K_m for active uptake [6]. Similar results have been reported in [7].

This paper describes experiments designed to examine the kinetics of the lactose carrier in the absence of a protonmotive force but under conditions of net transport. The results rule out the possibility that the difference between the app. K_m and K_d observed in [6] could be due to measurements being made under different conditions (i.e., that the app. K_m was determined under conditions of net turnover while K_d was determined in the absence of turnover). Our results confirm those of in [3] and [8], that the protonmotive force affects the apparent affinity of the carrier for lactose under conditions of net turnover. These experiments also rule out any possibility of the presence of uncouplers directly causing the observed rise in app. K_m for influx.

Abbreviations: app. V, maximum velocity of transport at pH of the experiment; app. K_m, concentration of substrate giving half-maximal transport at the pH of the experiment; K_d, concentration of substrate giving half-maximal binding; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone

2. Materials and methods

Escherichia coli ML308-225 was grown in minimal medium M9 [9] containing 0.5% glycerol to late log phase. The cells were harvested by centrifugation and resuspended in the appropriate medium. After 1 h of shaking at 37°C the suspension was again centrifuged to harvest the bacteria. After 2 washes in 150 mM KCl, 50 mM Tris–HCl (pH 7.0), the bacteria were suspended at a cell density of 10–20 mg dry wt/ml in 100 mM KCl, 50 mM KSCN, 3 mM Tris–HCl (pH 7.0) and brought to pH 7.0 with 20 mM KOH. The suspension was introduced into a large syringe where it was allowed to become anaerobic, and where it was stored for 40 min for equilibration. During that time the suspension was stirred and it was maintained at pH 7.0 by additions of small amounts of 20 mM KOH.

For experiments, 3 ml anaerobic stock suspension were carefully transferred to a closed, nitrogen-flushed chamber containing a pH electrode (Russell Instr.) and a magnetic follower. When the pH reached a steady value (drift, 0.005 pH units/50 s) an addition (1–100 μl) of de-gassed, nitrogen-flushed, 0.5 M lactose solution (in 100 mM KCl, 50 mM KSCN, 3 mM Tris–HCl (pH 7.0) was made as rapidly as possible.

Complete mixing occurred within 2 s, 90% within the first 0.5 s.

The residual protonmotive force across the cell membrane was assessed by making an addition of an ethanolic solution of 2 mM CCCP, to give 10 μM final conc. and monitoring the change in pH of the medium. The ethanol solution was flushed with ethanol-saturated nitrogen for 1.5–2 h before use in order to reduce the amount of dissolved oxygen. A 100 μl addition of thoroughly de-gassed, nitrogen-flushed, 20 mM 2,4-dinitrophenol (dissolved in 150 mM KCl and adjusted to pH 7.0 with 1 M HCl) gave results quantitatively similar to the CCCP additions.

Rates of lactose-induced proton uptake were calculated by drawing tangents to the pH trace and plotting, against time, the logarithms of the tangents. The initial rate was obtained by extrapolation of the logarithmic plot back to zero time (fig.1).

3. Results

Merely starving the bacteria for 1 h by resuspending them in M9 medium without carbon source gave a final suspension of cells which retained a considerable protonmotive force across their cell membranes (fig.2a) and which were able to effect H^+-lactose symport (fig.3a). When the phosphate in the M9 medium was replaced by arsenate the size of the pH excursion after adding CCCP was considerably decreased (fig.2b; note the different scale from fig.2a) and the ability to effect H^+-lactose symport was also reduced (not shown). The addition of 1 mM potassium iodoacetate to the final suspension medium also reduced both the CCCP-induced pH excursion (fig.2c) and H^+-lactose symport (fig.3b). In all these experiments the initial rate of proton uptake produced when lactose was added to the medium showed similar Michaelis-Menten saturation kinetics with similar values for app. K_m and V (fig.4, table 1). Furthermore, the ability of the cells to respire was not inhibited, for the acid pH excursion produced when a small amount of air-saturated KCl solution was

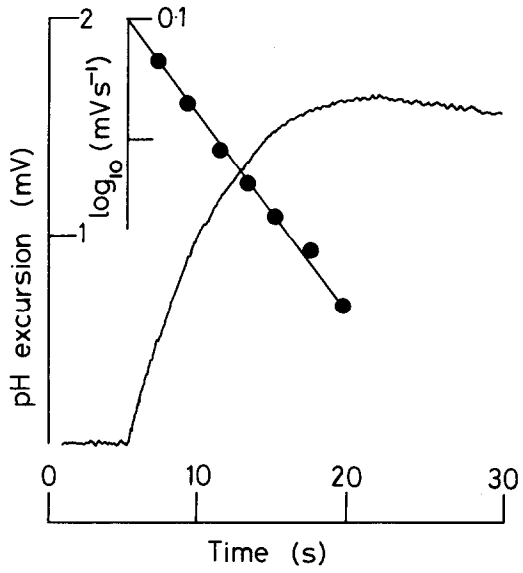

Fig.1. Lactose-induced pH excursion. The trace is a record of the alkalification produced when lactose (to 1.6 mM) is added to an anaerobic suspension of bacteria that had been starved for 1 h in minimal medium. The semi-logarithmic plot of tangents to the trace is also shown, extrapolated to zero time to give the initial rate.

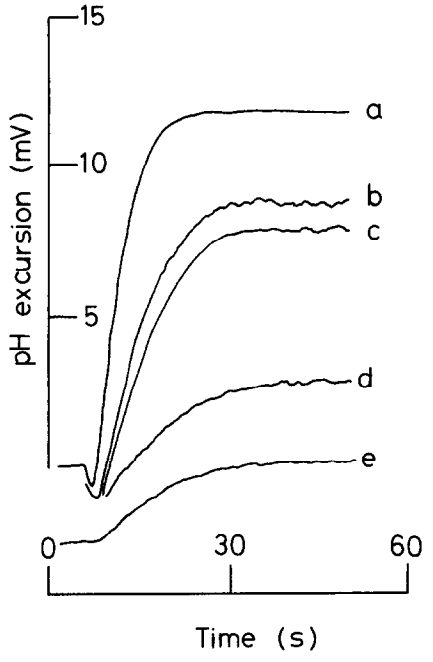

Fig.2. The pH excursion induced by adding uncoupler to anaerobic suspensions of *E. coli*. CCCP (to 10 μM) was added to anaerobic suspensions of bacteria that had been pretreated as follows: (a) incubated in M9 without glycerol for 1 h. This trace was recorded on one half the scale used for the following; (b) incubated in M9 with the phosphate replaced by arsenate, without glycerol for 1 h; (c) as in (b) but with 1 mM potassium iodoacetate added to the final suspension medium; (d) as in (a) but with 30 mM sodium azide present in the M9; (e) as in (c) but with sodium azide present in the incubation medium.

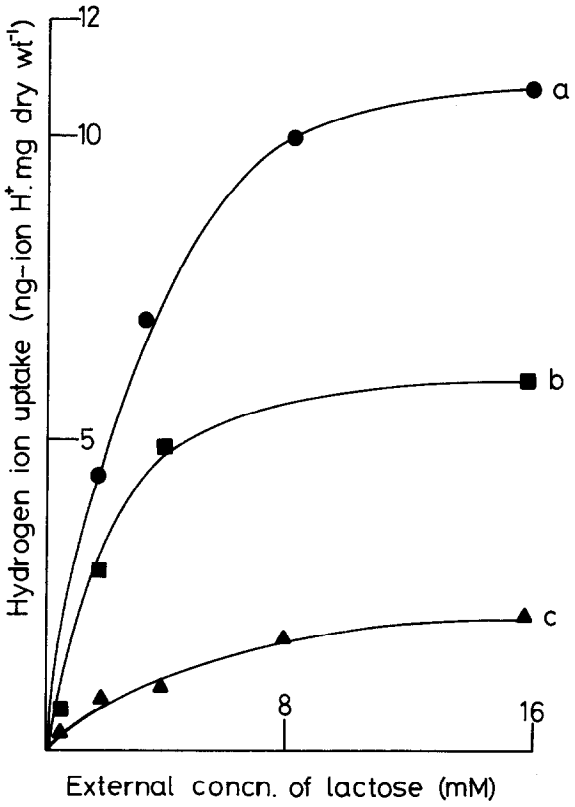

Fig.3. Lactose-induced H^+ uptake. Lactose, to the final concentrations shown, was added to anaerobic suspensions of bacteria pretreated as follows: (a) incubated in M9 without glycerol for 1 h; (b) as in (a) but with the phosphate in the medium replaced by arsenate and with 1 mM potassium iodoacetate present in the final suspension; (c) as in (a) but with 30 mM sodium azide present in the incubation medium.

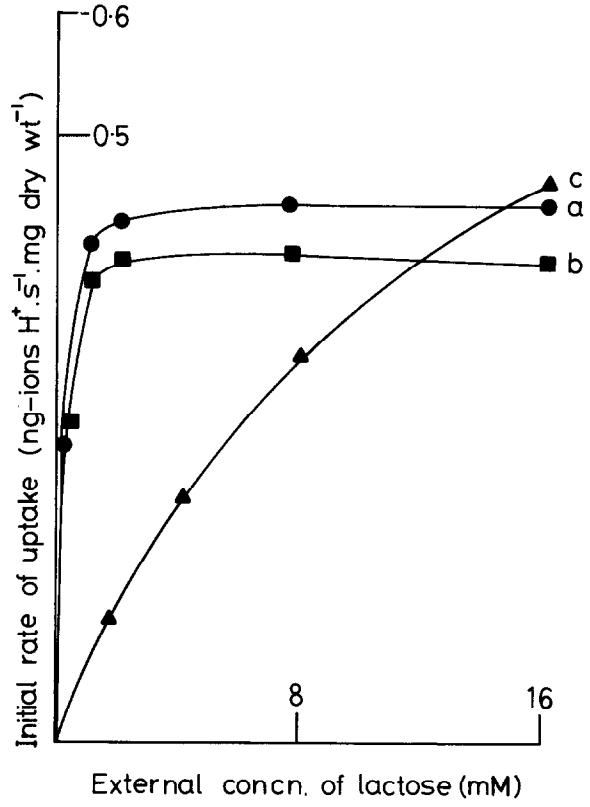

Fig.4. Initial rates of H^+-uptake induced by lactose (see legend to fig.3).

Table 1
Saturation kinetics of lactose-induced proton movements

Treatment	Apparent K_m (mean, (range)) (mM)	Apparent V (mean, (range)) (ng-ion $H^+ . s^{-1}$. mg dry wt^{-1})	No. Expt.
Starved only	0.4, (0.05–0.2)	0.06, (0.2–0.8)	5
Starved in arsenate	0.3, (0.1–0.5)	0.5, (0.2–0.8)	3
Starved plus iodoacetate	0.4, (0.1–0.5)	0.4, (0.1–0.7)	5
Starved plus azide	8, (3–> 16[a])	0.9, (0.5–1.2)	10

[a] This was the highest concentration used and in some experiments the initial rate shows little sign of reaching a plateau value

Fig.5. The pH excursion induced by the addition of air-saturated KCl solution to anaerobic bacterial suspensions. A 100 μl addition of air-saturated 150 mM KCl was made to anaerobic suspension of bacteria. Suspensions (a–d) were pretreated as in the legend to fig.2.

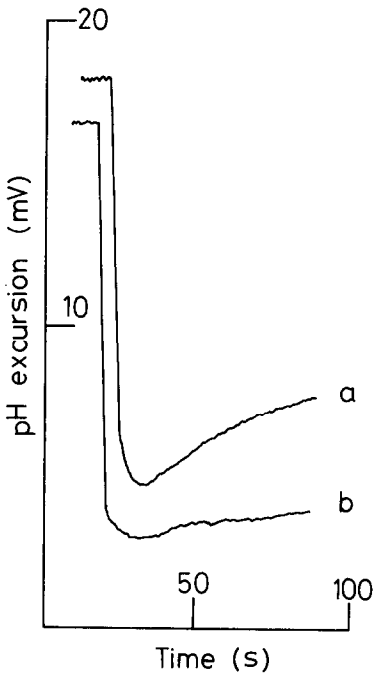

Fig.6. The pH excursion induced by the addition of de-gassed, dilute HCl to anaerobic bacterial suspensions. A 10 μl portion of de-gassed, nitrogen-flushed dilute HCl (100 ng-ions H^+ equiv.) was added to bacterial suspensions pretreated: (a) incubation in M9 1 h; (b) as in (a) but with 30 mM sodium azide present.

injected into the medium followed a similar course with cells prepared by all three methods (fig.5).

Addition of 30 mM NaN_3 to the M9 medium and washes had a profound effect on the pH changes being examined. The CCCP-induced proton movements were reduced to a very low level (fig.2d) and, although the amount of lactose-induced proton-flux was considerably reduced, it indicated that lactose would still enter the cell to approximately the same concentration as in the medium (fig.3c). Addition of iodoacetate and replacement of phosphate by arsenate (fig.2e) gave no further reduction in the uncoupler-induced pH excursion. The azide-treatment alone, while barely affecting the rate or extent of the oxygen-induced acidification, nevertheless caused an appreciable decrease in the apparent proton permeability of the membranes (fig.6). This effect, the opposite of uncoupling, is not understood and has not been further investigated here, though it may be relevant that azide inhibits the H^+-translocating ATPase [10].

The effect of azide-treatment on the saturation kinetics of the lactose-induced pH excursion was very marked. The substrate concentration leading to half-saturation (app. K_m) was 40–100-times higher than observed with cells following any of the other treatments, while the maximum velocity was not markedly affected (table 1).

4. Discussion

In [1] the half-saturation constant (app. K_m) for lactose influx into intact cells was found not to be influenced by the energy state of the cells. They obtained values for the app. K_m of ~0.1 mM both in untreated cells and in cells treated with 30 mM azide and 1 mM iodoacetate. However, in [3] the app. K_m for thiomethylgalactose was 11 mM in azide-treated cells. We have

examined the kinetics of the proton movement associated with lactose influx which has been shown to be stoichiometrically equal to the lactose influx [11–13]. In starving, but apparently still metabolizing cells, the lactose-induced proton movements are large with an app. K_m of ~0.1 mM. However, when the cells are severely depleted of energy, the size of the lactose-induced proton movement is diminished and the app. K_m rises to a much higher value (⩾15 mM). Our results thus confirm those in [3]. In our experiments the period of exposure to azide was longer than that used in [1], which might account for the discrepancy between their results and ours.

The binding of lactose to membrane vesicles in the absence of a metabolic substrate was reported to occur with K_d = 9–20 mM, whilst active transport in the presence of a metabolic substrate has an app. K_m of ~0.1 mM. These values are very similar to our results on whole cells and are in contrast with those in [14] who found K_d = 0.1 mM for membrane vesicles in the presence of sodium azide.

The different apparent affinities of the carrier system for lactose in metabolizing and non-metabolizing states might be due to one or both of two effects [6].

(i) An effect of the protonmotive force on the external binding step, such that the carrier has a greater affinity for its exogenous substrate in the presence of a protonmotive force.

(ii) That arising if the loaded carrier translocates faster than the unloaded carrier. This faster translocation of the loaded carrier might be independent of the protonmotive force or might occur only in the presence of a protonmotive force. In either case the app. K_m would depart from K_d but only in the latter case would it be dependent on the protonmotive force, as observed in our experiments.

We cannot yet determine the relative contribution of the two effects.

The important observations in [6], confirmed here for whole cells, reconcile many problems regarding the lactose carrier which have appeared in the literature during the past 10 years.

Acknowledgements

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