The Effect of β -Galactosides on the Protonmotive Force and Growth of *Escherichia coli*

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The effect of three β -galactosides on the components membrane potential ($\Delta\psi$) and pH gradient (ΔpH) of protonmotive force and growth of *Escherichia coli* has been examined. A good correlation between the reduction of the protonmotive force and growth inhibition was observed. Thus some galactosides had little effect on either the protonmotive force or growth while lactose diminished the protonmotive force and caused growth inhibition. This effect of lactose was dependent on the ionic composition of the growth media. In Medium A (77 mM- Na^+ , 85 mM- K^+) lactose diminished $\Delta\psi$ but had no effect on ΔpH . Growth inhibition was transient at an external pH 6.0 but complete at pH 7.5. In medium KA (approximately 1 mM- Na^+ , 162 mM- K^+) ΔpH was diminished and $\Delta\psi$ was not affected and consequently growth inhibition was complete at pH 6.0. In medium NA (163 mM- Na^+ , 20 mM- K^+) lactose had little effect on $\Delta\psi$, ΔpH or growth. These data support Skulachev's hypothesis of buffering of the protonmotive force by K^+ and Na^+ gradients.

INTRODUCTION

Bacterial cells use energy during growth both for biosynthesis and for the maintenance of essential gradients. The principle gradients are those of K^+ and amino acids (high inside), Na^+ and H^+ (low inside) and charge (negative inside) (Harold & Altendorf, 1974). High intracellular K^+ concentrations are a consequence of the mechanism of osmoregulation (Epstein & Laimins, 1980). This cation may have ancillary roles in activation of cellular enzymes and the regulation of intracellular pH (Plack & Rosen, 1980). The gradients of H^+ (ΔpH) and charge ($\Delta\psi$) constitute the protonmotive force (Δp) (Mitchell, 1963; Hamilton, 1975; Harold, 1982) which is the energy coupling intermediate between respiratory, phosphorylative and certain transport processes. The Na^+ gradient participates in some transport processes (Stock & Roseman, 1971; Lanyi, 1979). However, a direct role for Na^+ in growth has not been detected, although it may function in regulation of the cytoplasmic pH (Padan *et al.*, 1982; Booth & Kroll, 1983). In *Streptococcus faecalis* it has been demonstrated that these gradients may be dissipated without impairing growth. In rich media at alkaline pH (pH_0 7.8, high external K^+ and low external Na^+) growth occurs rapidly in the absence of the ion and charge gradients (Harold & Van Brunt, 1977). On the other hand Skulachev has proposed that the role of K^+ and Na^+ gradients is to buffer $\Delta\psi$ and ΔpH . During growth energy is utilized to accumulate K^+ and expel Na^+ . Under starvation conditions K^+ and Na^+ flow down their electrochemical gradients, via a uniport and a Na^+/H^+ antiport, respectively, to stabilize $\Delta\psi$ and ΔpH (Skulachev, 1977, 1980). Thus it is envisaged that the major solute gradients and the protonmotive force are interconvertible.

Recently it has been suggested that excessive solute uptake may lead to growth inhibition. A well established example is that of lactose inhibition of *Escherichia coli* strains which are consti-

Abbreviations: p-CMBS, para-chloromercuribenzenesulphonic acid; TDG, thiodigalactoside; TMG, thiomethylgalactoside; TPMP⁺, triphenylmethyl-phosphonium cation; TPP⁺, tetraphenylphosphonium cation; $\Delta\psi$, membrane potential; ΔpH , pH gradient; Δp , protonmotive force; pH_0 , external pH; pH_i , cytoplasmic pH.

tutive for the β -galactoside permease (Hofsten, 1961; Wilson *et al.*, 1980, 1981). Inhibition was greatest at pH₀ 6.0 and was ascribed to the collapse of the pH gradient due to H⁺ entry with lactose (Wilson *et al.*, 1980). However, since inhibition also occurred at pH₀ 7.9 (Wilson *et al.*, 1981) where most of the protonmotive force is in the form of $\Delta\psi$, it is likely that this gradient is also affected. Thus the gradients of pH and charge are partially exchanged for one of lactose, leading to growth inhibition. In addition, Wilson *et al.* (1981) made the interesting observation that the degree of growth inhibition was dependent on the identity of the β -galactoside. However, no explanation of this observation was put forward.

We have previously reported that lactose provoked a reduction of $\Delta\psi$ by 53 mV in endogenously respiring cells incubated at pH₀ 6.0 (Ahmed & Booth, 1981*b*). Simultaneously Δ pH rose by 37 mV and consequently Δp was only reduced by 16 mV. These observations appeared to contradict those of Wilson *et al.* (1981). In view of this apparent contradiction we further investigated the phenomenon of lactose inhibition of growth.

The data presented here demonstrate that the degree of inhibition of growth of *E. coli* by lactose is a function of the ionic composition (Na⁺, K⁺) of the growth medium. The effect of lactose on the components of Δp is similarly affected by the relative balance of Na⁺ and K⁺. The data support the Skulachev hypothesis that gradients of Na⁺ may act as a buffer against sudden changes in intracellular pH. Additionally, a specific prediction of the hypothesis that a large K⁺ gradient assists maintenance of $\Delta\psi$ has been tested and found to be correct.

METHODS

Bacterial strains. *Escherichia coli* ML308 (*lac*⁻*z*⁺*y*⁺*a*⁺) and ML308-225 (*lac*⁻*z*⁻*y*⁺*a*⁺) were obtained from Dr I. C. West, University of Newcastle-upon-Tyne, U.K. The strains were maintained as freeze-dried cultures at -20 °C and on Yudkins slopes at 4 °C.

Growth conditions and media. Cells were grown on three media of differing ionic composition. *Medium A* contained KH₂PO₄·3H₂O, 31 mM; KH₂PO₄, 22 mM; trisodium citrate, 1 mM; MgSO₄·7H₂O, 0.4 mM; (NH₄)₂SO₄, 7.6 mM and di-sodium succinate was present as the carbon source at 37 mM. *Medium KA* contained the components of Medium A with the K⁺ salts of succinate and citrate replacing the sodium salts. *Medium NA* contained Medium A with the sodium phosphates used in place of the K⁺ salts. KCl (20 mM) was added to this medium to supply the essential K⁺ for growth. Overnight growth was always initiated at pH₀ 7.0. For experiments at different pH₀ values the media were adjusted with NaOH, KOH or HCl as appropriate.

Cultures were grown overnight on one of the media described above at 30 °C on a New Brunswick shaking incubator. These cultures were then used as inocula for growth experiments by dilution to an optical density of approximately 0.2 at 650 nm, as measured in 1 ml plastic cuvettes using either a Unicam SP600 or Cecil spectrophotometer. An OD₆₅₀ of 2.4 equals 1 mg dry wt ml⁻¹. The cell population was allowed to double at 30 °C prior to any experimental manipulation.

The cytoplasmic volume of growth cells. The cytoplasmic volume of growing cells was measured by the method of Stock *et al.* (1977) after concentrating cells from cultures at 0.15 mg dry wt ml⁻¹ and 0.33 mg dry wt ml⁻¹ to 4 mg dry wt ml⁻¹ in growth medium. This was necessary as the method is not sensitive enough to detect small differences between the ³H₂O and [¹⁴C]sucrose. The volumes were estimated to be (mean $\pm$ S.E.) 1.30 $\pm$ 0.34 (*n* = 3) μ l (mg dry wt)⁻¹ and 1.35 $\pm$ 0.15 (*n* = 4) μ l (mg dry wt)⁻¹, respectively, by sucrose exclusion (Booth *et al.*, 1979).

The membrane potential ($\Delta\psi$), pH gradient (Δ pH) and solute accumulation gradient. These gradients were measured for growing cells by using centrifugation-based assays. Samples (1.25 ml) were withdrawn from the 100 ml cultures in 250 ml Erlenmeyer flasks in shaking water baths and transferred to a stirred glass vessel at 30 °C. [¹⁴C]TPP⁺ [10 μ M, 8.7 Ci mol⁻¹ (1 Ci = 3.7 $\times$ 10¹⁰ becquerels)] and [¹⁴C]benzoate (6 μ M; 24.4 Ci mol⁻¹) were used to measure $\Delta\psi$ and Δ pH, respectively.

It has been assumed that TPP⁺ does not penetrate the outer membrane of *E. coli* (Zilberstein *et al.*, 1979; Wilson *et al.*, 1981) and therefore EDTA treated cells need to be used to measure $\Delta\psi$ with this probe. However, we have shown that in *E. coli* ML308-225 TPP⁺ can be used as a quantitative probe for $\Delta\psi$ without the need for EDTA treatment (Ahmed & Booth, 1981*a*). Thus we have employed TPP⁺ to measure $\Delta\psi$ in growing cells. ³H₂O was present at a final activity of approximately 1–2 μ Ci ml⁻¹. Catalase was present at 250 μ g ml⁻¹ final concentration. After 10 min incubation at 30 °C 1 ml samples were transferred to Eppendorf centrifuge tubes containing 10 μ l 2 vol. H₂O₂. Catalase and H₂O₂ acted as an oxygen generating system and maintained aerobic conditions during centrifugation. The cell suspensions were then centrifuged for 20 s in an Eppendorf microfuge. The supernatants and pellets were treated as previously described (Ahmed & Booth, 1981*a*). $\Delta\psi$ was calculated using the Nernst equation after a correction for uncoupler insensitive binding of TPP⁺ had been made (Ahmed & Booth, 1981*a*).

Solute concentration gradients were measured in an identical manner except that the appropriate radioactively

labelled β -galactoside was added as a probe: [^{14}C]gluconolactose (4 mM, 0.072 Ci mol $^{-1}$), [^{14}C]thiomethylgalactoside (4 mM, 0.05 Ci mol $^{-1}$) and [^3H]thiodigalactoside (4 mM, 6 mCi mol $^{-1}$).

Rates of β -galactoside uptake in growing cells. Duplicate 1 ml samples of growing cultures at an OD $_{650}$ of 0.4 were transferred to test-tubes incubated at 30 °C, containing the radioactive β -galactoside at a final concentration of 4 mM. The test-tubes were incubated 30 °C for 1 min and then rapidly diluted with 5 ml growth medium (Medium NA, KA or A) containing 200 μM *p*-CMBS. The cells were filtered from the growth medium and washed twice with 5 ml of the *p*-CMBS containing media. Radioactivity on the filters was assessed as previously described (Booth & Hamilton, 1980).

Respiratory rates. The respiratory rate of growing cells was estimated using a Clark oxygen electrode. The electrode was calibrated with air-saturated distilled water at 30 °C. Samples (3 ml) of growing cultures were rapidly transferred to the electrode and the cap inserted. The initial rate of oxygen consumption was recorded over the first minute.

Materials. All materials were as previously specified (Booth *et al.*, 1979; Booth & Hamilton, 1980; Ahmed & Booth, 1981*a*) with the following additions: thiodigalactoside and thiomethylgalactoside were purchased from Sigma; [^{14}C]thiomethylgalactoside was purchased from Amersham; [^3H]thiodigalactoside was the generous gift of Dr P. Overath (Max-Planck Institut für Biologie, Tübingen, F.R.G). TPMP $^{+}$ was purchased from K and K Laboratories, Plainway, N.Y., U.S.A.

RESULTS

Magnitude of $\Delta\psi$ and ΔpH during growth

At constant external pH (pH $_0$ 6.0 and pH $_0$ 7.5) $\Delta\psi$ and ΔpH were essentially constant during growth from OD $_{650}$ 0.4 (0.16 mg dry wt ml $^{-1}$) to OD $_{650}$ 1.0 (0.45 mg dry wt ml $^{-1}$) (data not shown). Thus this range of growth was used in all subsequent experiments.

During growth on succinate the medium pH became progressively more alkaline. In the experiments described below this change was approximately 0.5 pH unit over the course of the experiment. This drift, caused by the consumption of succinate, was used to measure the effect of external pH on $\Delta\psi$, ΔpH , Δp and cytoplasmic pH in growing cells (Fig. 1).

Effect of lactose on growth

Cells of *E. coli* ML308-225 (β -galactosidase negative) growing in Medium A (77 mM-Na $^{+}$, 85 mM-K $^{+}$) at pH $_0$ 6.0 suffered only transient inhibition by lactose (Fig. 2*a*) whereas cells growing at pH $_0$ 7.5 were strongly inhibited (Fig. 2*b*). The degree of inhibition at either pH $_0$ was dependent on the concentration of lactose added. With either 2 or 4 mM lactose there was a lag of 1.0 h and 0.5 h, respectively, before inhibition was evident. At higher concentrations (20 mM) there was no lag prior to the onset of inhibition. Addition of 4 mM-TDG during the lag period completely prevented inhibition (Fig. 2*a, b*). At pH $_0$ 6.0 the duration of growth stasis was a function of the lactose concentration, being shorter at the lower concentrations (Fig. 2*a*). There appeared to be no recovery at pH $_0$ 7.5 during the time period of the experiment (Fig. 2*b*).

In *E. coli* ML308, a strain possessing β -galactosidase, lactose provoked no growth inhibition at either pH $_0$ 6.0 or pH $_0$ 7.5 (data not shown).

Our results contrast with the findings of earlier workers (Hofsten, 1961; Wilson *et al.*, 1980, 1981). We therefore sought discrepancies between the experiments. The major difference was found to be in the ionic composition of the growth media. In earlier studies the media (Medium 63) contained only contaminating Na $^{+}$ (Hofsten, 1961; Wilson *et al.*, 1980, 1981). Thus we repeated our experiments in Medium KA in which all Na $^{+}$ salts were exchanged for their K $^{+}$ equivalent.

In Medium KA (approx. 0.6–1 mM-Na $^{+}$, 162 mM-K $^{+}$) the pattern of growth inhibition was changed relative to Medium A, and was similar to that observed by earlier workers. At pH $_0$ 6.0 both ML308 and ML308-225 were severely inhibited by lactose (Fig. 3). There was no significant lag before inhibition became evident and there was no recovery with strain ML308-225 (Fig. 3) in contrast to strain ML308 (data not shown). Addition of NaCl to the growth medium reversed the inhibition at low concentrations (10 mM-NaCl) and restored the lag at higher concentrations (100 mM-NaCl). Thus the severity of growth inhibition at pH $_0$ 6.0 depended upon the external Na $^{+}$ concentration.

Fig. 1. $\Delta\psi$ (■), ΔpH (●), Δp (○) and pH_i (□) as a function of pH_0 in growing cells of *E. coli* ML308-225. Samples were removed at the appropriate times from cultures growing on Medium A and $\Delta\psi$ and ΔpH were assayed as described in Methods. Δp was calculated by using the relationship $\Delta p = \Delta\psi - Z\Delta\text{pH}$. pH_i was calculated by using the following equation: $\text{pH}_i = \text{pH}_0 + \Delta\text{pH}$. Samples were taken at an OD_{650} of approximately 0.4, 0.6, 0.8 and 1.0. The initial pH_0 was set at four different values (5.5, 6.5, 7.5 and 8.0). pH_0 thus varied from 5.7–8.5 during growth. Each point represents an individual experiment carried out in duplicate. ΔpH values below zero were measured by [^{14}C]benzoate exclusion.

Fig. 2. Effect of different concentrations of lactose on growth at pH_0 6.0 (a) and pH_0 7.5 (b) in *E. coli* ML308-225. Lactose was added as indicated by the first arrow at the following concentrations: 2 mM (○), 4 mM (□), 20 mM (■), 4 mM followed by 4-mM TDG (●). To one culture 4 mM-TDG was added 0.5 h after the addition of lactose (second arrow). Control points are omitted for clarification. The growth rate in the absence of lactose was almost identical to the culture in which both lactose and TDG were added.

Fig. 3. Effect of external Na^+ concentration on growth inhibition by lactose in *E. coli* ML308-225 at pH_0 6.0. No addition (●), 4 mM-lactose added in Medium KA (approx. 0.6–1 mM- Na^+ , 162 mM- K^+) (■), Medium KA plus 10 mM- Na^+ (□) and Medium KA plus 100 mM- Na^+ (○). Cells were grown overnight and for one generation in Medium KA with (10 mM or 100 mM- Na^+) or without Na^+ .

At pH_0 7.5 lactose inhibition of growth in low Na^+ medium was only transient in agreement with previous reports (Hofsten, 1961; Wilson *et al.*, 1980, 1981).

Respiratory rate

The respiratory rate of growing cells was not affected by the composition of the growth medium and was in the range 160 to 210 nmol oxygen consumed $(\text{mg dry wt})^{-1} \text{ min}^{-1}$ at pH_0 6.1. Lactose addition to cultures of ML308-225 caused an immediate lowering of respiratory rate in all media. Severest inhibition was in Medium KA at pH_0 6.1 and was approximately 48%, increasing over 30 min to 64% inhibition. In Medium A initial inhibition was 16% increasing to 25% over 30 min.

Effect of lactose on $\Delta\psi$ and ΔpH

The effects of lactose on $\Delta\psi$ and ΔpH were investigated to ascertain how they correlated with growth inhibition (Fig. 4). It was evident that the major effect of lactose depended upon the Na^+/K^+ balance of the growth medium. Thus in Medium A (74 mM- Na^+ , 85 mM- K^+) the primary effect of lactose was to diminish $\Delta\psi$. The cytoplasmic pH was not significantly affected (Fig. 4a). This accords with greater growth inhibition at pH_0 7.5 than at pH_0 6.0 since $\Delta\psi$ was the major component of Δp at the higher pH value (Fig. 1). In the K^+ -based medium (KA, 0.6–1 mM- Na^+ , 162 mM- K^+) the cytoplasmic pH fell to approximately pH_i 6.4 within seconds of addition of lactose (Fig. 4b). In the presence of added Na^+ (10 mM) pH_i fell slightly less, to a value between 6.8 and 7.0 but more significantly, recovered to normal values over a period of 4 h (data not shown). The growth rate was not restored to that of the control culture.

These effects of Na^+ on lactose transport-mediated changes in ΔpH are as predicted by Skulachev (1977, 1980), namely, that the presence of a Na^+ gradient buffers the pH gradient against collapse. The other aspect of the Skulachev hypothesis was that with a maximal K^+ gradient, the buffering effect of this gradient on $\Delta\psi$ would be increased. We thus manipulated the external K^+ concentration to test this aspect of the hypothesis with lactose inhibition. A minimal concentration of 15–20 mM- K^+ was required to allow growth on succinate. Addition of lactose to cells growing in Medium NA (162 mM- Na^+ , 20 mM- K^+) resulted in a lesser inhibition of growth (data not shown) and a diminished effect on $\Delta\psi$ and ΔpH (Fig. 4c). However,

Fig. 4. Effect of lactose on the protonmotive force in *E. coli* ML308-225. $\Delta\psi$ and ΔpH were measured as described in Methods in cells growing in (a) Medium A (77 mM- Na^+ , 85 mM- K^+), (b) Medium KA (approx. 0.6–1 mM- Na^+ , 162 mM- K^+) and (c) Medium NA (162 mM- Na^+ , 20 mM- K^+). Zero time refers to the point of addition of 4 mM-lactose. The $\Delta\psi$ is expressed as a percentage of the control at the same value of extracellular pH (range of values as in Fig. 1). The pH gradient is expressed as the intracellular pH. Open symbols have lactose added at zero time; filled symbols indicate the control; pH_0 6.0 ($\triangle$, $\blacktriangle$) and pH_0 7.5 ($\bullet$, $\circ$).

inhibition appeared to be delayed rather than prevented. Thus growth slowed after 1.5–2 h and $\Delta\psi$ fell relative to the control cells (Fig. 4c). The data are consistent with buffering of $\Delta\psi$, by the K^+ gradient although the effect here may be transient.

Effect of TMG and TDG on growth and the protonmotive force

The lactose analogues TDG and TMG, were also tested for their ability to inhibit growth. Only TMG (4 mM) had any effect, causing a 15–20% decrease in the growth rate of *E. coli* ML308-225 at both pH_0 6.0 and pH_0 7.5 (data not shown). The effects of TMG and TDG on growth correlated with their effect on the protonmotive force. Thus TDG did not affect the protonmotive force at all while TMG reduced $\Delta\psi$ by approximately 10 mV (data not shown).

Transport and accumulation of β -galactosides

Of the three principle β -galactosides used to investigate the *lac* permease, TMG, TDG and lactose, only the last causes significant growth inhibition. If all three were accumulated in the same manner then each should be equivalent. The transport and accumulation of these three analogues in growing cells was investigated.

The pattern of the initial rates of transport of the analogues (4 mM final concentration) paralleled that of their effect on growth. Thus lactose was transported at a rate of 1.5- to 2-fold faster than that of TMG and 4- to 5-fold faster than that of TDG (Table 1).

The steady-state intracellular concentrations of the three analogues followed the same pattern as their rates of uptake (Table 2). Thus TDG was accumulated poorly, TMG somewhat better and lactose to the greatest extent. At pH_0 7.5 lactose achieved approximately the same intracellular concentration as TMG (Table 2) yet the difference in growth inhibition was considerable. None of the sugars examined was accumulated to a level which equalled the magnitude of the protonmotive force. However, by virtue of the collapse of $\Delta\psi$, lactose accumulation was the closest to equilibrium (Table 2).

Table 1. *Initial rates of β -galactoside uptake in growing cells of *E. coli* ML308-225*

Cells were allowed to grow for a generation in the appropriate media and then 2×1 ml samples of the 100 ml cultures were transferred to test-tubes containing 4 mM radioactive β -galactoside. After incubation for 1 min with the radioactive β -galactoside the cells were separated by filtration and the radioactivity was assayed as detailed in Methods.

[Na ⁺] mM	[K ⁺] mM	Initial pH	Rate of β -galactoside uptake [nmol min ⁻¹ (mg cells) ⁻¹]		
			Lactose	TMG	TDG
77	85	6.0	352 $\pm$ 44	175 $\pm$ 27	69 $\pm$ 36
77	86	7.5	320 $\pm$ 21	230 $\pm$ 29	83 $\pm$ 12
1	162	6.0	264 $\pm$ 48	ND	ND
162	20	6.0	430 $\pm$ 83	ND	ND

ND, Not determined.

Table 2. *Accumulation of β -galactosides in growing cells of *E. coli* ML308-225*

Accumulation of β -galactosides was assayed as described for $\Delta\psi$ and ΔpH (Methods). Accumulation in mV was calculated using the following equation:

$$\Delta\text{p}_{\beta\text{-gal}} \text{ (mV)} = Z \log ([\beta\text{-gal}]_i)/([\beta\text{-gal}]_o)$$

$[\beta\text{-gal}]_i$ and $[\beta\text{-gal}]_o$ are the internal and external concentrations of β -galactoside, respectively. All the data refer to cells growing in Medium A (77 mM-Na⁺, 85 mM-K⁺).

β -Galactoside	pH	[S] _i * (mM)	Accumulation (mV)	Δp (mV)
Lactose	6.0	300	111	138
	7.5	212	102	118
TMG	6.0	100	83	198
	7.5	160	95	173
TDG	6.0	48	64	205
	7.5	32	53	176
Control	6.0	—	—	205
	7.5	—	—	176

* Intracellular concentration of solute.

DISCUSSION

The data presented here support the conclusion of Wilson *et al.* (1980, 1981) that growth inhibition by lactose is a result of diminution of the protonmotive force. However, it is clear that the inhibition phenomenon is complex. Only when a large decrease in intracellular pH occurred (Fig. 4*b*) was growth inhibition instantaneous (Fig. 3). When $\Delta\psi$ was lowered growth inhibition was delayed even though the changes in $\Delta\psi$ were detected within a few minutes of addition of lactose. Interestingly when the lipophilic cation, TPMP⁺ was used to collapse ΔpH to the same extent the pattern of growth inhibition was identical to that caused by lactose (unpublished data). In this case the inhibition might not result directly from the decrease of $\Delta\psi$, but be a consequence of the fall in either the $\Delta\psi$ or Δp as a whole.

The studies of the respiratory rate indicate that a feature of the growth inhibition is a decrease in the capacity to maintain Δp . During growth on succinate Δp is required not only to synthesize most of the ATP but also to ensure a continued supply of the respiratory substrate. In this case a fall in Δp is likely to be compounded by a decrease of the respiratory rate due to a lower rate of uptake of succinate.

The reduction of the respiratory rate in cells growing on succinate may explain the greater sensitivity to lactose of such cells than of their counterparts growing on glycerol (Hofsten, 1961).

Since glycerol uptake is not Δp -dependent a continued supply of respiratory substrate would occur during lactose-mediated decrease of Δp . Additionally, the extra ATP available from substrate-level phosphorylation during metabolism of glycerol may lessen the inhibition.

The rate of H^+ entry consequent upon β -galactoside transport is clearly also a major determinant in inhibition of growth. Given a K_m for lactose of 0.7 mM then 2 mM-lactose is transported at approximately 90% of the rate at 4 mM-lactose, yet growth inhibition sets in later at the lower concentration and is less severe (Fig. 2*a, b*). Indeed 1 mM-lactose provokes virtually no growth inhibition although the rate of uptake is 70% of that at 4 mM-lactose. This rate dependency of inhibition may explain the failure of TMG and TDG to provoke growth inhibition. These analogues were found to be transported at rates which were 50% (TMG) and 20% (TDG) of that of lactose. That TMG does provoke a slight growth inhibition may be due either to the proton leak induced by this analogue (Eddy, 1980) or to the high intracellular concentration of the analogue.

Clearly the major determinant of the severity of the growth inhibition by lactose is the composition of the growth medium. The observations reported here are consistent with the hypothesis of Skulachev (1977, 1980). This is clearest in the case of the Na^+ gradient which he proposed would buffer ΔpH . As reported above the presence of Na^+ in the medium prevented the collapse of the pH gradient (Fig. 4*a, b*) during lactose-mediated proton entry. Two factors make it difficult to place precise limits on the mechanism of this protective effect. Firstly, respiration, and hence the capacity to extrude protons, is not as severely inhibited as in the absence of Na^+ . This paradoxically may result from the maintenance of ΔpH at the expense of $\Delta\psi$ since the former comprises up to 75% of the driving force ($\Delta\psi - 3Z\Delta pH$) for succinate uptake at pH 6.0. Secondly, the maintenance of an intracellular pH favourable to metabolism will increase the viability of the cell during lactose inhibition. Thus, as a consequence of these two factors the cell is eventually capable of striking a new balance between the pumps and sinks for protons and can re-establish growth.

In the Skulachev model Na^+ buffering of the pH gradient takes place via the Na^+/H^+ antiport. Our data suggest that if this is correct then the antiport must possess specific kinetic properties. From the data on lactose influx (Tables 1 and 2) one may calculate that the duration of net sugar uptake is maximally 1 min. In order that Na^+ can exchange for the protons with no significant change in ΔpH then the antiport must possess kinetics similar to those of lactose influx and must be sensitive to either changes in ΔpH or a decrease in the intracellular pH. This must be the case to allow the exchange of the Na^+ gradient for a proton gradient during the period of net lactose influx.

Such properties would appear to contradict the model for pH_i regulation discussed by Padan *et al.* (1982). However, studies of pH regulation are still in their infancy and the essential properties of the regulatory system have not been elucidated (see Booth & Kroll, 1983).

Less convincing data were obtained in support of the K^+ gradient restoring $\Delta\psi$. The effect of increasing the initial gradient was transient possibly due to the progressive dissipation of the K^+ gradient. Consistent with this is the observation that the onset of inhibition was associated with a lowering of $\Delta\psi$. However, the overall result was consistent with the Skulachev hypothesis.

It has been reported that mutants in the *lac* permease can be selected on the basis of the resistance they confer to lactose (Müller-Hill *et al.*, 1968). Subsequently it was observed that the use of succinate as carbon source increased the inhibition and thus the selective advantage of mutants (Smith & Sadler, 1971). Now we can add that careful attention to the Na^+ content of the growth medium, and of the pH, is likely to enhance the selective pressure.

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REFERENCES

- AHMED, S. & BOOTH, I. R. (1981*a*). Quantitative measurements of the protonmotive force and its relationship to steady-state lactose accumulation in *Escherichia coli*. *Biochemical Journal* **200**, 573–581.
- AHMED, S. & BOOTH, I. R. (1981*b*). The effects of partial and selective reduction in the components of the protonmotive force on lactose uptake in *Escherichia coli*. *Biochemical Journal* **200**, 583–589.
- BOOTH, I. R. & HAMILTON, W. A. (1980). Quantitative analysis of proton-linked transport systems: β -galactoside exit in *Escherichia coli*. *Biochemical Journal* **188**, 467–473.
- BOOTH, I. R. & KROLL, R. G. (1983). pH homeostasis in bacteria. *Biochemical Society Transactions* **11**, 70–72.
- BOOTH, I. R., MITCHELL, W. J. & HAMILTON, W. A. (1979). Quantitative analysis of proton-linked transport systems: the lactose permease of *Escherichia coli*. *Biochemical Journal* **182**, 687–696.
- EDDY, A. A. (1980). Slip and leak models of gradient-coupled solute transport. *Biochemical Society Transactions* **8**, 271–273.
- EPSTEIN, W. & LAIMINS, L. (1980). Potassium transport in *Escherichia coli*: diverse systems with common control of osmotic forces. *Trends in Biochemical Sciences* **5**, 21–23.
- HAMILTON, W. A. (1975). Energy coupling in microbial transport. *Advances in Microbial Physiology* **12**, 1–53.
- HAROLD F. M. (1982). Pumps and currents: a biological perspective. *Current Topics in Membranes and Transport* **16**, 485–516.
- HAROLD, F. M. & ALTENDORF, K. (1974). Cation transport in bacteria. Potassium, sodium and protons. *Current Topics in Membranes and Transport* **5**, 1–49.
- HAROLD, F. M. & VAN BRUNT, J. (1977). Circulation of protons and potassium across the plasma membrane is not obligatory for bacterial growth. *Science* **197**, 372–373.
- HOFSTEN, V-B. (1961). The inhibitory effect of galactosides on the growth of *Escherichia coli*. *Biochimica et biophysica acta* **48**, 164–171.
- LANYI, J. K. (1979). The role of sodium in transport processes of bacterial membranes. *Biochimica et biophysica acta* **559**, 656–661.
- MITCHELL, P. (1963). Molecule, group and electron translocation through natural membranes. *Biochemical Society Symposium* **22**, 142–168.
- MÜLLER-HILL, B., CRAPO, L. & GILBERT, W. (1968). Mutants that make more lac repressor. *Proceedings of the National Academy of Sciences of the United States of America* **59**, 1259–1264.
- PADAN, E., ZILBERSTEIN, D. & SCHULDINER, S. (1982). pH homeostasis in bacteria. *Biochimica et biophysica acta* **605**, 151–166.
- PLACK, H. P., JR & ROSEN, B. P. (1980). Cation/proton antiport systems in *Escherichia coli*. *Journal of Biological Chemistry* **255**, 3824–3825.
- SKULACHEV, V. P. (1977). Transmembrane electrochemical proton potential as a convertible energy source for the living cell. *FEBS Letters* **87**, 171–179.
- SKULACHEV, V. P. (1980). Membrane electricity as a convertible energy currency for the cell. *Canadian Journal of Biochemistry* **58**, 161–175.
- SMITH, T. F. & SADLER, J. R. (1971). The nature of lac operator constitutive mutations. *Journal of Molecular Biology* **59**, 273–303.
- STOCK, J. B. & ROSEMAN, S. (1971). A sodium dependent sugar co-transport system in bacteria. *Biochemical and Biophysical Research Communications* **44**, 132–136.
- STOCK, J. B., RAUCH, B. & ROSEMAN, S. (1977). Periplasmic space in *Salmonella typhimurium* and *Escherichia coli*. *Journal of Biological Chemistry* **252**, 7850–7861.
- WILSON, D. M., KUSCH, M. & WILSON, T. H. (1980). Control of lactose transport in *Escherichia coli*. *FEBS Letters (supplement)* **117**, K34–K44.
- WILSON, D. M., PUTZRATH, R. M. & WILSON, T. H. (1981). Inhibition of growth of *Escherichia coli* by lactose and other galactosides. *Biochimica et biophysica acta* **649**, 377–384.
- ZILBERSTEIN, D., SCHULDINER, S. & PADAN, E. (1979). Proton electrochemical gradient in *Escherichia coli* cells and its relation to active transport of lactose. *Biochemistry* **18**, 669–673.