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Proton-Coupled β-Galactoside Translocation in Non-Metabolizing *Escherichia coli*

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Abstract

Acid-base and electrogenic processes coupled to the flux of β-galactosides into non-metabolizing cells of *Escherichia coli* have been studied.

When β-glactoside was added to non-metabolizing suspensions of *E. coli*, the pH of the suspension medium increased, indicating that the β-galactoside travelled in with acid equivalents. When the cells were made permeable to K^+ ions, this inflow of acid equivalents was accompanied by an equal outflow of K^+ ions, indicating that each acid equivalent carried one positive charge across the membrane, and corresponded to an H^+ ion going in or an OH^- ion coming out. The effective movement of H^+ ions, caused either by a pH difference or by an electrical potential difference across the membrane of the cells, was specifically facilitated by the presence of β-galactoside. These effects of β-galactoside were abolished by N-ethyl maleimide, which is known to inhibit the specific β-galactoside translocation.

The possible involvement of a Na^+-β-galactoside symporter was ruled out by showing that the galactoside-induced inflow of acid was practically independent of Na^+ ion concentration in the range 0.05-50.0 mM, and that Na^+ ions did not flow into the bacteria under the influence of a β-galactoside concentration gradient.

It is concluded that the β-galactoside translocation in *E. coli* is probably mediated by a β-galactoside-H^+ symporter or by a β-galactoside/OH^- antiporter.

Abbreviations: ATPase, adenosine triphosphatase; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; NEM, N-ethyl maleimide; TMG, methyl-β-D-thiogalactoside; ΔH_0^+, quantity of H^+ ions entering unit volume of the outer aqueous phase; pH_0, the pH of the outer aqueous phase. The same conventions are used for potassium (K) and sodium (Na).

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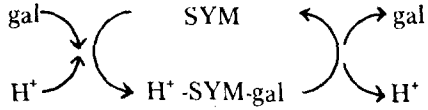
Introduction

Lactose and other β-galactosides may be concentrated up to several hundredfold in the intracellular water of respiring *Escherichia coli*. The translocation of these sugars across the plasma-membrane is dependent on the presence of an inducible (or constitutive) membrane-located protein (the M protein) which combines specifically with β-galactosides [1]. Although, in normal respiring or glycolysing cells, the specific β-galactoside-translocation system maintains the internal concentration of free non-metabolizable β-galactoside much higher than the external concentration, the same translocation system (judged by its genetic, kinetic and inhibitor-sensitivity properties) is responsible for equilibrating the concentration of β-galactosides across the membrane when the cells are treated with agents, such as 2,4-dinitrophenol or azide, which equilibrate the electrochemical activity of H^+ ions across the membrane [1]. This remarkable and specific action of the proton-conducting uncoupling agents [2] on β-galactoside translocation prompted the suggestion some 10 years ago [3] that the carrier responsible for β-galactoside translocation is a β-galactoside-proton symporter (SYM) which can catalyse only the tightly coupled translocation of β-galactoside molecules (gal) and H^+ ions together across the membrane, as follows:



(1)

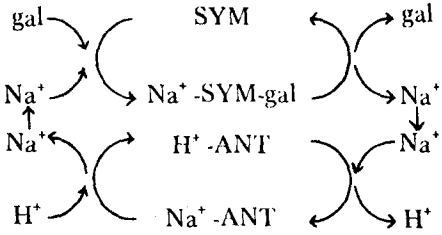
It was assumed that the driving force for the solute uptake through this porter system was the inwardly-directed thermodynamic pressure of H^+ ions resulting from the outward translocation of H^+ ions by the respiratory chain and proton-translocating ATPase systems, as in the case of the proton-coupled anion and cation translocators postulated for osmotic regulation in the membrane systems catalysing oxidative and photosynthetic phosphorylation [2, 4].

The possibility that the catalyst of β-galactoside uptake in *E. coli* might be a β-galactoside-H^+ symporter (or a functionally equivalent β-galactoside/OH^- antiporter) seemed to us to call for further experimental study because: (a) the specific action of the proton-conducting uncouplers cannot be explained by their secondary effect of lowering cellular ATP levels [5]; (b) there is no evidence for involvement of the phosphoenolpyruvate-dependent phosphotransferase system in β-galactoside transport in *E. coli* [6]; (c) β-galactosides do not stimulate ATPase activity in membrane preparations containing the M protein [6].

As the β-galactoside molecules generally used as substrates in the transport studies are electrically neutral and have negligible acid/base

properties near pH 7, the symport of the H^+ ions with the β-galactoside molecules by the postulated symporter should be observable as a transfer of acid or as an equivalent transfer of positive electric charge across the membrane. Some experimental observations on the alkalinization of the external medium accompanying lactose entry into *E. coli*, and on the facilitation of lactose translocation by the permeant thiocyanate ion under non-metabolizing conditions have already been described [7] in support of the type of proton-coupled translocation reaction given by equation (1).

In the present paper we have extended the earlier observations [7]; and we have sought evidence for the following: (1) that the translocation of β-galactoside via the porter under the influence of its own concentration gradient causes both a pH difference and an electric potential difference to develop across the membrane; (2) that β-galactoside is translocated via the porter either in response to a pH gradient or in response to an electric potential gradient across the membrane. We have also tested the possibility that, by analogy with anion translocating systems involving the circulation of phosphate in mitochondria [8], the coupling between β-galactoside and H^+ ion translocation might involve the intermediary circulation of Na^+ ions in a system such as the following:



(2)

where SYM and ANT stand for a Na^+-β-galactoside symporter and a Na^+/H^+ antiporter respectively.

In practice, β-galactoside-H^+ symport and β-galactoside/OH^- antiport are functionally equivalent with respect to acid/base and electrogenic properties; and although they might be distinguished by certain criteria, no attempt was made to do so in the present work. To avoid unnecessary repetition, we have used the phrase "effective proton movements", or equivalent phrases, to describe either the movement of H^+ ions one way or of OH^- ions the other way.

The net translocation process catalysed by a solute/OH^- antiporter is actually the same as that catalysed by a solute-H^+ symporter, when the membrane across which translocation occurs is permeable to H_2O. For this reason, we have conventionally used the phrase "proton-coupled solute translocation" to describe either solute/OH^- antiport or solute-H^+ symport.

Materials and Methods

Reagents

FCCP was a gift from Dr. P. G. Heytler of E. I. du Pont de Nemours and Co., Inc. (Wilmington, Delaware, U.S.A.); choline base type III, choline chloride and carbonic anhydrase were obtained from Sigma London Chemical Co. Ltd. (London, S.W.6); TMG was obtained from Mann Research Laboratories (New York, N.Y. 10006, U.S.A.); valinomycin was obtained from Calbiochem Ltd. (London, W1H 1AS); and the other chemicals were of Analar grade and obtained from Hopkin and Williams (Chadwell Heath, Essex).

Choline thiocyanate was prepared by passing a solution of KSCN over a cation-exchange resin column (Zeokarb 225, H-form) previously converted to the choline form with choline base.

Organism

The bacterial strain used in this work was *E. coli* ML 308-225 (i^-, z^-, y^+) constitutive for β-galactoside accumulation but lacking galactosidase, and was kindly provided by Dr. T. H. Wilson, Harvard Medical School, Boston, Mass., U.S.A.

Culture of Bacteria

Stock cultures were maintained on agar slopes. The liquid culture medium used was that of Mager and Magasanik [9], with succinate as carbon source, and contained (in 1 litre): 7.0 g KH_2PO_4, 3.2 g Na_2HPO_4, 2.0 g $(NH_4)_2SO_4$, 0.41 g $MgSO_4.7H_2O$, 1 mg $CaCl_2$, 2.5 g succinic acid. The medium was brought to pH 7.0 with KOH. A small inoculum from the slope was grown for 24 h at 25° in 4 ml of liquid medium, which was then added to 200 ml of medium in a 1-litre flask. This was incubated at 37° for 17 h under aerated conditions [10]. The stationary phase of growth, corresponding to an extinction at 700 nm of about 1.0 (0.5-0.7 mg cell dry weight/ml), was reached 1 h before harvesting. Cells were harvested by centrifugation at 4° for 5 min at 15,000 x *g*. Under these conditions the harvested cells were depleted of carbohydrate reserves and the rate of endogenous glycolysis was very low.

Preparation of Washed Suspensions of Normal and K^+-Permeable Cells

The harvested cells were washed twice at 4° and suspended at a cell density of 10-15 mg dry weight/ml. For preparation of suspensions of normal cells both the washing and the stock suspension media used were either 150 mM KCl-3.0 mM glycylglycine/KOH at pH 7, or 250 mM sucrose.

For the preparation of suspensions of K^+-permeable cells, a tris-EDTA and valinomycin treatment based on that of Pavlasova and Harold [5] was employed; but care was taken to proceed with the valinomycin treatment immediately after the tris-EDTA preconditioning because it was observed by Leive [11] that the preconditioning effect of the tris-EDTA was only transient. The harvested cells were washed twice with 120 mM tris/HCl buffer at pH 8. They were then suspended in the same tris buffer at 10-15 mg cell dry weight/ml and warmed to 37°. A solution of 100 mM EDTA/KOH at pH 7 was added to give a final EDTA concentration of 0.5 mM, and the suspension was shaken gently for 2 min at 37°. It was then centrifuged at room temperature (18° to 20°) and the cells were washed once with 250 mM sucrose and resuspended at a cell density of 10-15 mg dry weight/ml in 250 mM sucrose. Valinomycin dissolved in ethanol (2 mg/ml) was then added to give 300 μg valinomycin/g cell dry weight, and this stock suspension was then stored at 4°, and, like the stock suspensions of normal cells, was used for experiments for up to 5 h.

Vessel and Systems for Following Solute Translocation by pH and pK Measurement

The experimental vessel, electrodes, electrometers and recording systems were as described previously [12]. The cell suspensions, in the required experimental media, were introduced into the closed temperature-controlled electrode-vessel (final volume 4.0 ml, temperature 25°) from a syringe fitted with a fine plastic "needle" which was passed through the narrow inlet tube of the electrode vessel. Air was then excluded by directing a stream of nitrogen (O_2 free) at the mouth of the inlet tube. The oxygen in the well-stirred suspension was consumed by the bacteria within 1-2 min. Carbonic anhydrase (25 μg/ml of suspension) was routinely added to catalyse the equilibration of the CO_2/carbonic acid/bicarbonate system as described by Scholes and Mitchell [13].

Measurement of Sodium Concentration in Extracellular Media

The sodium concentration in the incubation medium was measured with a flame photometer (Evans Electroselenium Ltd., Halstead, Essex, U.K.) after centrifuging down the cells at room temperature.

Stock Solutions

Stock solutions of NEM (0.2 M), FCCP (1 mM) and valinomycin (2 mg/ml) were made up in ethanol; carbonic anhydrase (20 mg/ml) was

made up in H_2O. For pH titrations in experiments in KCl medium, standard HCl (50.0 mM) and KOH (50.0 mM) were made up in 100 mM KCl, but for experiments in the sucrose medium, 50.0 mM HCl, 50.0 mM choline base, 50.0 mM NaCl and 50.0 mM KCl in water were used as standards. Acid, alkali, Na^+ and K^+ ion standards were freed of oxygen by evacuating, and flushing with nitrogen (O_2 free) in Thunberg tubes. When required, other solutions were freed of oxygen by bubbling with a stream of water-saturated nitrogen for 10 min. Stock solutions and the anaerobic standards were dispensed from Agla micrometer syringes (Burroughs Wellcome and Co., London, N.W.1) fitted with glass needles, as previously described [12].

Results and Discussion

Ion Movements During Galactoside Transport

Figure 1A shows a typical strip-chart recording of the ion-selective electrode responses that indicate the changes of pH and (apparent) pK that occurred in the anaerobic medium of resting suspensions of K^+-permeable cells (prepared as described under Materials and Methods) when a pulse of anaerobic TMG solution was added. The pK traces have not been corrected for the significant, but low, reactivity of the K^+ ion-selective electrode to H^+ ions. This experiment shows that H^+ ions leave (or OH^- ions enter) the medium, presumably as a result of the entry of the TMG into the cells, confirming a similar observation using lactose [7]. It also shows that during the effective entry of H^+ ions there is an exit of K^+ ions from the K^+-permeable cells, the half-time of this process being about 35 sec, corresponding to that of the effective H^+ ion entry. In a control experiment, shown in Fig. 1B, inhibition of the β-galactoside translocation system by treating the cells for 10 min with NEM prior to injection of the TMG pulse practically abolished the time-dependent pH and pK changes. When the membrane was made permeable to H^+ ions with FCCP, not only the pH changes but also the pK changes were abolished, as shown in Fig. 1C. Presumably NEM prevents the pH and pK changes by inhibiting the β-galactoside translocation and consequently inhibiting the effective H^+ ion movements via the β-galactoside porter; whereas FCCP allows re-equilibration of the H^+ ions after they have effectively travelled across the membrane with β-galactoside via the porter.

In Fig. 2, we show the time-course of the effective quantities of H^+ leaving and K^+ entering the outer medium as a result of the TMG addition, calculated from the data of Fig. 1A. The fact that the effective H^+ and K^+ translocations associated with equilibration of the TMG across the membrane were equal and opposite strongly suggests that the

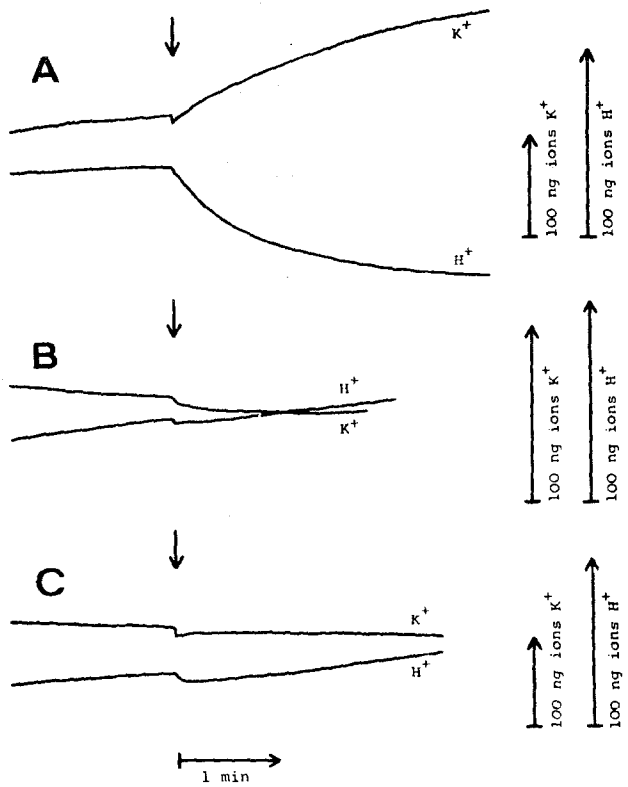


Figure 1. Strip-chart recordings of uncorrected pK and pH electrode responses showing increase in K^+ ion and decrease in H^+ ion activities in the outer medium on adding TMG to anaerobic suspensions of K^+-permeable *E. coli* (prepared as described under Materials and Methods). The temperature-controlled experimental vessel, volume 4.0 ml, contained: 24.3 mg dry wt. of cells, 200 mM sucrose, 30 mM choline chloride, 0.25 mM KCl, 0.1 mg carbonic anhydrase, and further additions as indicated. After anaerobic incubation at 25° at pH 7.0-7.1 for 35 min, 50 μl of anaerobic 0.4 M TMG solution was injected at arrows. A, No further additions; B, NEM, 0.5 mM final concentration; C, FCCP, 2.5 μM final concentration.

translocation of acid equivalents during β-galactoside entry corresponds to the inward translocation of univalent positive charge (compensated by the exit of K^+ ions).

Figure 3 shows the time-course of the effective quantities of H^+ leaving and K^+ entering the outer medium in experiments corresponding to those of Fig. 1A, but using normal cells in a 200 mM sucrose medium, (A) in presence of 30 mM choline chloride, (B) in presence of 30 mM choline thiocyanate. As expected, in A the rate of effective withdrawal of H^+ ions from the medium, and presumably the rate of TMG entry

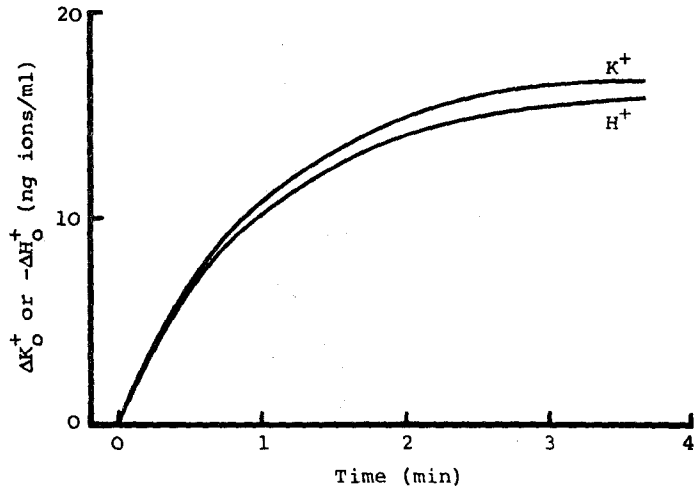


Figure 2. Time-course of appearance of K^+ ion and disappearance of H^+ ion in the outer medium on adding TMG to anaerobic suspensions of K^+-permeable *E. coli*. The continuous recordings of Fig. 1A have been corrected for baseline drift and brought to same scale.

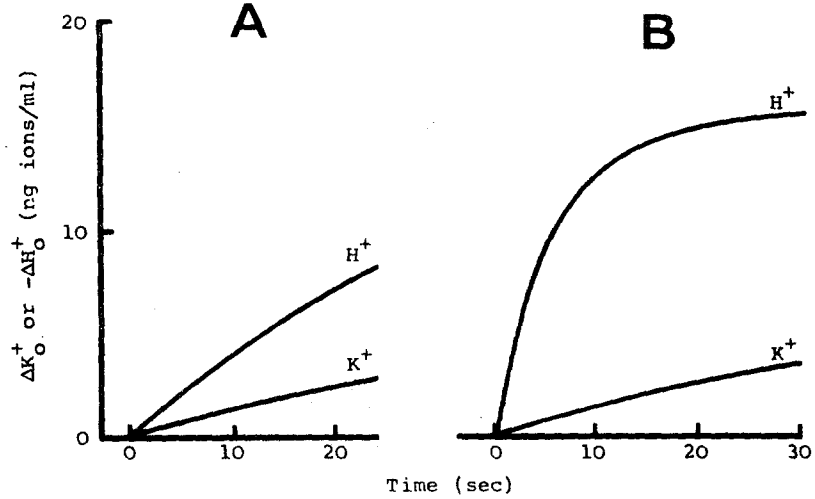


Figure 3. Time-course of appearance of K^+ ion and disappearance of H^+ ion in the outer medium on adding TMG to anaerobic suspensions of normal *E. coli*. Harvested bacteria were washed twice with 120 mM tris/HCl buffer pH 8, and once with 250 mM sucrose. The experimental vessel contained: 21.0 mg dry wt. of cells, 200 mM sucrose, 0.25 mM KCl, 0.1 mg carbonic anhydrase, and either 30 mM choline chloride, or 30 mM choline thiocyanate. After anaerobic incubation for 40 min at 25° at pH 7.0-7.1, 50 μl of anaerobic 0.4 M TMG solution was injected. A, Sucrose-choline chloride medium; B, Sucrose-choline thiocyanate medium.

into the cells, was comparatively slow when the cell membrane was not readily permeated by the major ion species present (K^+, choline$^+$ or Cl^-); and only a small fraction of the shift of charge due to H^+ ions crossing the membrane was evidently neutralized by the K^+ ion movement. However, when the permeant SCN^- ion (Fig. 3B) was present, the presumed rate of TMG entry indicated by the rate of effective H^+ ion withdrawal from the medium was much enhanced, and as before, only a small fraction of the charge-neutralizing current was carried by the K^+ ions.

Experiments corresponding to those of Fig. 1A, in which lactose pulses were injected into suspensions of K^+-permeable cells, gave results such as that shown in Fig. 4. The outward translocation of K^+ closely followed the effective inward translocation of H^+ during lactose equilibration, as in the experiments using TMG. Experiments corresponding to those of Fig. 3, using lactose in place of TMG, showed that in normal cells also, lactose and TMG behaved similarly.

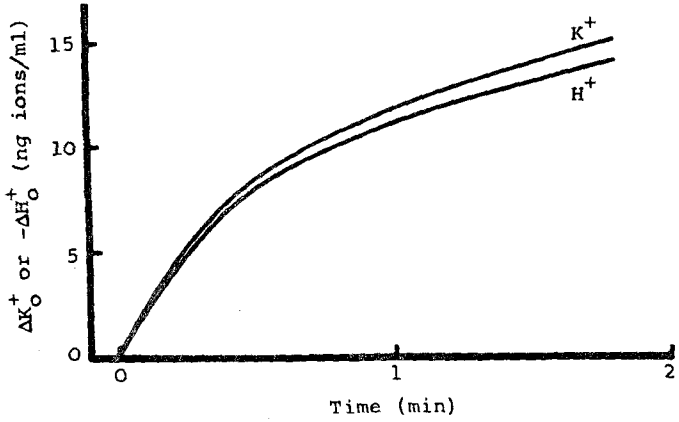


Figure 4. Time-course of appearance of K^+ ion and disappearance of H^+ ion in the outer medium on adding lactose to anaerobic suspensions of K^+-permeable *E. coli*. Details were as in Fig. 1A except that the vessel contained 22.6 mg dry wt. of cells, and 0.4 lactose was substituted for 0.4 M TMG.

These experiments indicate that when acid equivalents are caused to flow across the membrane by added galactoside, they do so with a single charge; thus, the electrogenic process must be either β-galactoside-H^+ symport or β-galactoside/OH^- antiport. The previously investigated proton-coupled porters, such as the phosphoric acid porter of mitochondria [14], and the artificial H^+/Na^+ antiporter, nigericin [15], catalyse net translocations which are electrically neutral. However, the proton-coupled β-galactoside symporter of *E. coli* appears to resemble

the Na^+-coupled glucose symporter (or co-transporter) of animal tissues [16] in catalysing an electrogenic net transport.

Translocation of H^+ Ions via the Proton-β-Galactoside Symporter in Response to a pH Gradient

It has been shown for several bacteria, as for mitochondria from several sources [2, 17, 18], that the reduction of small pulses of oxygen results in the electrogenic ejection of H^+ ions into the medium—and that a pH gradient is developed across the membrane. The extent of the H^+ ion ejection is limited by the charging up of the electrical capacity of the membrane of the cell or mitochondrion, unless a permeant counter-ion is present. In mitochondria, endogenous Ca^{2+} ions are mobile across the membrane via a specific carrier and act as the permeant counter-ion, but in *Micrococcus denitrificans* the specific Ca^{2+}-translocating system is absent, and permeant ion species such as the K^+-valinomycin complex or SCN^- are required for appreciable proton ejection [17]. In this respect *E. coli* has been found to resemble *M. denitrificans*. Figure 5 shows typical strip-chart recordings of the time-course of the pH of the outer medium (pH_o) on adding 50 μl of CO_2-free, air-saturated, KCl solution (23.5 ng atom O at 760 mmHg pressure and 25°) to anaerobic suspensions of cells in media containing 150 mM KCl (A) or 100 mM KCl-50 mM KSCN (B). In the latter case the acidification was much

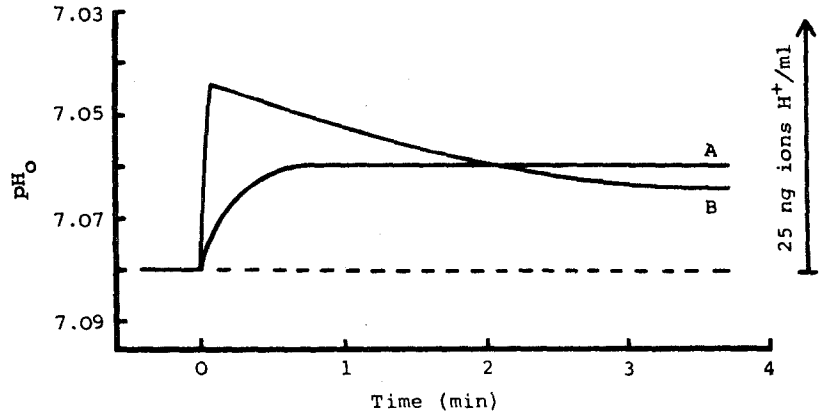


Figure 5. Time-course of change in pH_o on adding air-saturated KCl solution to anaerobic suspensions of normal *E. coli*. Bacteria were harvested and washed in 150 mM KCl-3 mM glycylglycine as described under Materials and Methods. The experimental vessel contained, in 4 ml: 9.8 mg dry wt. of cells, 1.5 mM glycylglycine, 0.1 mg carbonic anhydrase and either 150 mM KCl (A) or 100 mM KCl-50 mM KSCN (B). The suspension was equilibrated anaerobically for 40 min at pH 7.0-7.1 and at 25°, prior to the addition of 50 μl of 150 mM KCl (air-saturated, CO_2-free) at zero time.

more rapid and was followed by an exponential return towards the original pH_0. In the case of experiments such as that of Fig. 5B, the ratio of H^+ ions translocated to atoms of oxygen reduced ($\rightarrow H^+/O$ quotient) was generally near four and seldom less than three, and the half-time of the exponential decay of the acid pulse was 120-200 sec.

Figure 6 describes H^+ ion and K^+ ion movements in a respiratory pulse experiment in a 200 mM sucrose-30 mM choline chloride medium, using K^+-permeable cells. The initial movement of K^+ ions into the cells almost exactly equalled the outward translocation of H^+ ions, showing that the H^+ ion ejection was electrogenic as in *M. denitrificans* [17]. Wimpenny [18] reported that "no proton pulses were ever observed with *E. coli* K12". In his experiments proton ejection may have been restricted by the absence of a mobile counter-ion, or the anaerobic preincubation may have been insufficient to allow dissipation of the respiratory membrane potential. In the present experiments the respiratory proton ejection was used merely as a means of creating a difference of pH across the membrane.

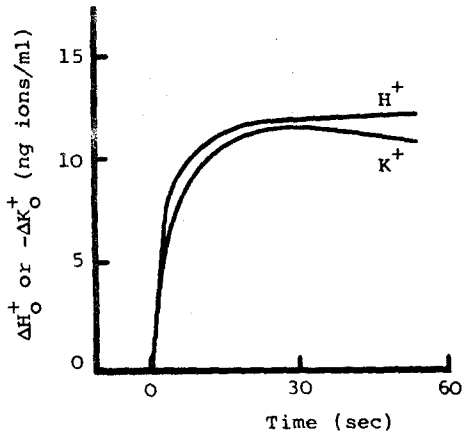


Figure 6. Time-course of appearance of H^+ ion and disappearance of K^+ ion in the outer medium on adding air-saturated KCl solution to an anaerobic suspension of K^+-permeable *E. coli*. Details were as in Fig. 1A except that the vessel contained 11.3 mg dry wt. of cells. At zero time, 50 µl of air-saturated, CO_2-free, solution containing 200 mM sucrose and 30 mM choline chloride was injected.

Figure 7 shows semi-logarithmic plots of the effective quantity of H^+ ions translocated during the decay of the pH gradient induced by injecting 50 µl of air-saturated saline into the anaerobic bacterial suspensions. In the absence of lactose (A) the decay was exponential with a half-time of 170 sec. In the presence of 5 mM lactose (C) there was initially a more rapid decay followed by a return towards the slower

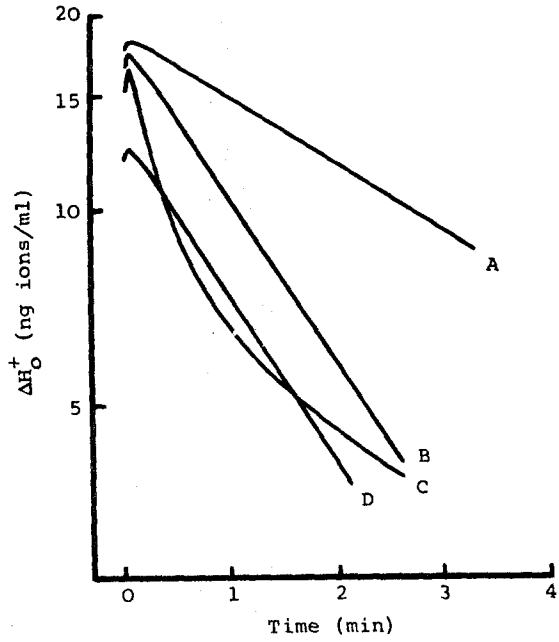


Figure 7. Semi-logarithmic plots of time-course of disappearance of H^+ ion from outer medium after respiratory pulses. Normal bacteria were washed and suspended as in Fig. 5. The 4 ml contained 7.6 mg dry wt. of cells, 100 mM KCl, 50 mM KSN, 1.5 mM glycylglycine, 0.1 mg carbonic anhydrase and 5 mM lactose or 0.2 mM NEM where indicated. In B and D the NEM was added 10 min before injection of 150 mM KCl (50 μl, as in Fig. 5). A, Control; B, 0.2 mM NEM; C, 5 mM lactose; D, 0.2 mM NEM and 5 mM lactose.

decay of the control. Our interpretation is that an inflow of H^+ ions with lactose (or of OH^- ions against lactose) occurred via the porter until the outwardly directed lactose concentration gradient was equal to the remaining inwardly directed proton activity gradient. After that there should be no further effective inflow of H^+ ions with lactose via the porter, and so the remaining effective proton movements should have the characteristic half-time (170 sec) of pH equilibration across the membrane. After preincubation with NEM, to inhibit the β-galactoside porter, curve B shows that, in absence of lactose, the effective proton conductance of the membrane was increased, the half-time of the decay being 71 sec. Allowing for this effect, curve D shows that NEM completely abolished the effective conduction of H^+ ions across the membrane with lactose.

When various concentrations of lactose (5, 20, 40 and 250 mM) were used in experiments corresponding to those of Fig. 7, it was observed

(Fig. 8) that at concentrations up to 40 mM, the effective translocation of H^+ ions with the lactose was faster the higher the concentration of lactose; but at a lactose concentration between 40 and 250 mM this tendency was reversed, as shown by the relatively slow decay of the proton pulse in 250 mM lactose. It is well known that net translocation on a specific carrier is inhibited when the substrate concentration is high enough to saturate the carrier on both sides of the membrane [19]. We attribute the observed inhibition of effective H^+ ion translocation at high lactose concentrations to this effect.

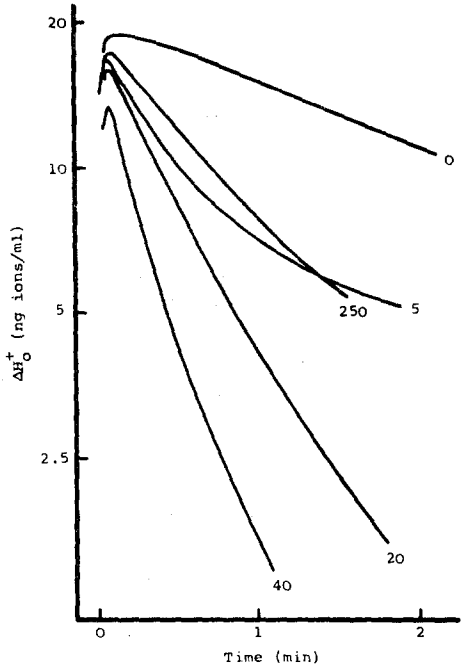


Figure 8. Semi-logarithmic plots of time-course of disappearance of H^+ ion from outer medium after respiratory pulses in presence of various concentrations (0, 5, 20, 40, 250 mM) of lactose. Details were as in Fig. 7 except that there was 10.9 mg dry wt. of cells.

Translocation of Acid Equivalents via the β-Galactoside-H^+ Symporter (or β-Galactoside/OH^- Antiporter) in Response to a Membrane Potential

Since, as confirmed by Fig. 5, SCN^- ions are considerably more mobile in the lipid membrane phase of normal cells than K^+ ions, it was possible to use pulses of anaerobic KSCN solution injected into normal anaerobic cell suspensions to establish a membrane potential, negative inside; and thus, to investigate the effect of the membrane potential on

β-galactoside translocation. In a typical group of experiments, described by the strip-chart recordings of Fig. 9, curve A confirms that, in the absence of TMG, when the membrane was made freely permeable to H^+ ions with 2 μM FCCP, the membrane potential caused by the SCN^- pulse led to a rapid entry of H^+ ions down the electric potential gradient. There was comparatively little H^+ ion movement in the absence of FCCP (curve B); but when 10 mM TMG was present (curve C), effective H^+ ion translocation was significantly catalysed. Inhibition of the β-galactoside porter with NEM completely prevented this catalytic effect, as shown by curve D, with NEM alone and curve E, with NEM + TMG.

These results support the view that the β-galactoside porter catalyses the electrogenic translocation of H^+ ions with (or of OH^- ions against) β-galactoside molecules.

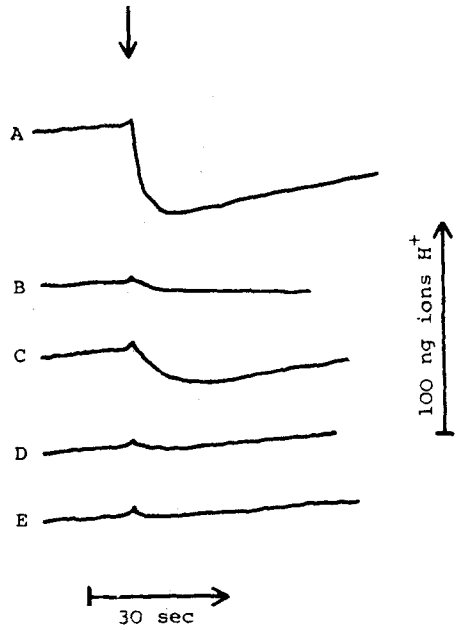


Figure 9. Strip-chart recordings of uncorrected changes in electrode responses showing extracellular H^+ ion activity on adding anaerobic KSCN solution to anaerobic suspensions of normal *E. coli*. Bacteria were washed and suspended as in Fig. 5. The 4 ml contained: 20.2 mg dry wt. of cells, 150 mM KCl, 1.5 mM glycylglycine, 0.1 mg carbonic anhydrase. At arrow, 25 μl of 0.6 M KSCN (freed of O_2) was injected. FCCP, NEM or TMG were added to give the final concentrations indicated. A, 2 μM FCCP; B, No additions; C, 10 mM TMG; D, 0.5 mM NEM; E, 0.5 mM NEM and 10 mM TMG.

Possible Cyclic Involvement of Na^+ or Other Ions in Proton-Coupled β-Galactoside Translocation

According to a recent paper by Stock and Roseman [20], the melibiose (or TMG II) transport system of *Salmonella typhimurium* is a sodium-dependent sugar co-transport system analogous to those of animal cells. Their conclusions were based on the finding that TMG uptake by metabolizing cell suspensions could be accelerated up to 100-fold by adding NaCl to cell suspensions in a medium virtually free of sodium salts. The TMG uptake rate was highly dependent on Na^+ ion concentration in the range 0 to 5 mM, and was about half maximal in 1 mM NaCl. Since Stock and Roseman suggested that considerable activation of the melibiose uptake system could arise from sodium contamination of supposedly sodium-free media, we thought it advisable to investigate the possibility of participation of Na^+ ions in the proton-coupled β-galactoside translocation system according to a mechanism of the type described by equation (2) in the introductory section.

In the experiments described so far in the present paper, sodium salts were not added in the suspension media except as impurities, but the growth medium for the bacteria contained 45 mM sodium salts. It was anticipated that, judging from the known purity of the reagents, and the expected Na^+ ion content of the cells, the total concentration of endogenous Na^+ ions in the incubation media used in our experiments would not have exceeded 0.1 mM. In order to determine whether the rate of entry of β-galactoside into the bacteria via the β-galactoside porter was influenced by the Na^+ ion concentration, as in Stock and Roseman's experiments, we observed the effect of replacing K^+ by choline$^+$ or Na^+ in TMG pulse experiments corresponding to that of Fig. 3B, but employing 50 mM thiocyanate salts. The initial rates of effective H^+ ion inflow in the three different incubation media are shown in Table I together with the Na^+ ion concentrations due either to the added NaSCN or to the Na^+ ion content of the other reagents and the cells. There is no observable stimulation by Na^+ ions of the rate of effective H^+ ion inflow caused by a pulse of TMG in the range 0.05-0.1 mM Na^+ and very little stimulation by 50 mM Na^+. It seems probable that the slight effect of replacing K^+ by choline$^+$ or Na^+ is not due to the cyclic involvement of Na^+ ions represented by equation (2).

In TMG-pulse experiments, if Na^+ ions entered with the β-galactoside, the corresponding change of pNa_0 should, of course, be detectable by a Na^+-sensitive glass electrode under suitably arranged conditions. Figure 10 shows strip-chart recordings of ion-selective electrodes indicating (apparent) pNa_0 and pH_0 in a set of TMG-pulse experiments, using normal bacterial suspensions in media containing 240 mM sucrose and 0.2 mM NaCl. The pNa traces have not been corrected for the significant,

TABLE I. Initial rate of decrease in H^+ ion activity in outer medium on adding TMG to anaerobic suspensions of normal *E. coli*.

Thiocyanate salt (50 mM)	Na^+ concn. (mM)	Initial rate of H^+ ion inflow (ng ions/g dry wt. sec)	
		Expt. 1	Expt. 2
$Choline^+$	0.091	147	163
K^+	0.049	168	187
Na^+	50.0	215	230

Harvested cells were washed twice with 120 mM tris/HCl buffer at pH 8, and twice in 250 mM sucrose, before being suspended in 250 mM sucrose. The experimental vessel contained, in 4 ml: 20 mg dry wt. of cells, 167 mM sucrose, 0.1 mg carbonic anhydrase, and 50 mM thiocyanate ($choline^+$, K^+ or Na^+ salt as indicated). After 35 min anaerobic incubation at 25° at pH 7.0-7.1, 10 µl of anaerobic 1.0 M TMG solution was injected. Contaminating Na^+ concentration was measured as described under Materials and Methods.

but low, reactivity of the Na^+ ion-selective electrode to H^+ ions. In Fig. 10A, 6 mM choline chloride was present: There was a small sluggish change in pH_0 following the TMG pulse, as in Fig. 3A, but the step change in pNa_0 corresponded to the dilution effect observed in D, when the TMG pulse was replaced by a pulse of water. In Fig. 10B, 6mM choline thiocyanate was present: There was a comparatively large and rapid change in pH_0 following the TMG pulse, indicating entry of TMG with H^+ ions or against OH^- ions, as in Fig. 3B, but the step change in pNa_0 corresponded only to the dilution effect, as in A. In Fig. 10C, 6 mM choline chloride and 2.5 µM FCCP were present: The TMG pulse caused no significant change of pH_0 or pNa_0 attributable to H^+ or Na^+ translocation. It was conceivable that in experiment B, as shown by equation (2), Na^+ ions might have entered with the TMG via a hypothetical β-galactoside-Na^+ symporter, but might also have re-equilibrated, practically synchronously, via a Na^+/H^+ antiporter. When the membrane was made freely permeable to H^+ ions by FCCP in experiment C, however, if Na^+ ions had entered with the TMG, the electrogenicity of this process should have caused extrusion of H^+ ions. The fact that there was neither entry of Na^+ nor exit of H^+ in Fig. 10C shows that there is no β-galactoside-Na^+ symporter; but it is entirely consistent with the existence of the postulated β-galactoside-H^+ symporter (or β-galactoside/OH^- antiporter).

It is possible that, as suggested by Stock and Roseman [20], the melibiose accumulation system of *S. typhimurium* differs from the

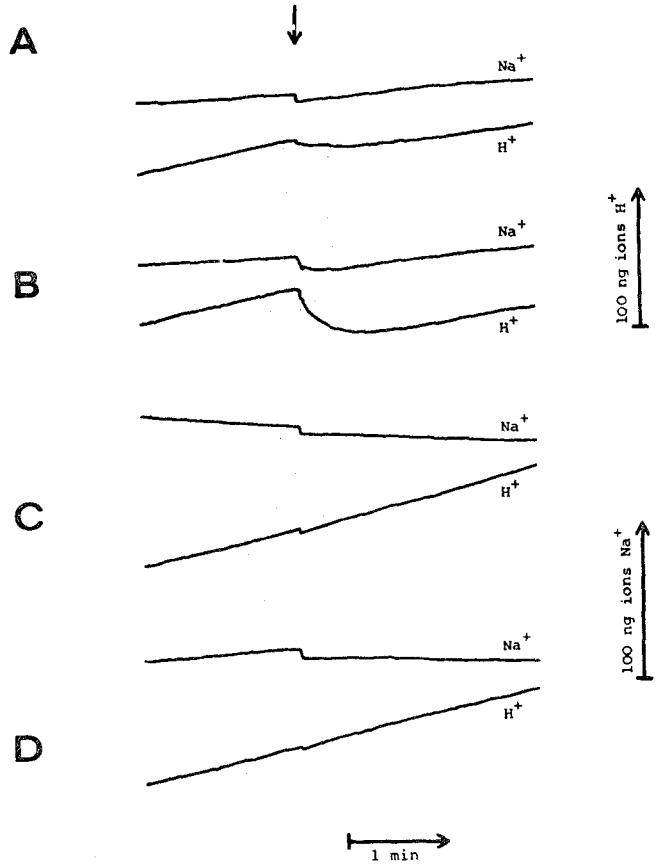


Figure 10. Strip-chart recordings of uncorrected electrode responses showing changes in extracellular Na^+ ion and H^+ ion activities on adding TMG to anaerobic suspensions of normal *E. coli*. Harvested cells were washed twice with 120 mM tris/HCl buffer pH 8, and once in 250 mM sucrose, before being suspended in 250 mM sucrose. The experimental vessel contained, in 4 ml: 22 mg dry wt. of cells, 240 mM sucrose, 6 mM choline chloride or choiine thiocyanate, 0.1 mg carbonic anhydrase, 0.2 mM NaCl. Suspensions were preincubated anaerobically at 25° for 35 min at pH 7.0-7.1. At arrow, 25 μl of anaerobic 1.0 M TMG solution or water was injected. A, Choline chloride medium, TMG injected; B, Choline thiocyanate medium, TMG injected; C, Choline chloride medium containing 2.5 μM FCCP, TMG injected D, Choline chloride medium containing 2.5 μM FCCP, H_2O injected.

β-galactoside transport system of *E. coli* in being a Na^+-coupled rather than a H^+-coupled symporter. But it is also possible, and seems to us more likely, that the striking effect of Na^+ ions observed by Stock and Roseman was due to an effect of Na^+ ions on the metabolism of the actively respiring cells used in their experiments.

Conclusions

The experiments reported in this paper have demonstrated the four related properties of β-galactoside translocation in *E. coli* which, as described in the introduction, are characteristic of a β-galactoside-proton symporter (or a functionally equivalent β-galactoside/OH^- antiporter). We conclude that the β-galactoside translocation in *E. coli* is probably mediated by a β-galactoside-H^+ symporter or by a β-galactoside/OH^- antiporter. The stoichiometry of the β-galactoside-proton symport (or of the β-galactoside/OH^- antiport) has not yet been precisely determined, though preliminary work [7] suggests that there may be a stoichiometry of one H^+ ion (or of one OH^- ion) to one β-galactoside molecule.

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