THE MECHANISM OF MAINTENANCE OF ELECTRONEUTRALITY DURING THE TRANSPORT OF GLUCONATE BY *E. COLI*

Aline ROBIN and Adam KEPES

Laboratoire des Biomembranes, Institut de Biologie Moléculaire, Tour 43, Université Paris VII, 2, place Jussieu, 75005 Paris, France

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1. Introduction

A gluconate kinase deficient mutant AR13 has been isolated from *E. coli* strain DF1070 [1]. This mutant has an inducible gluconate permease and allows the study of this transport system in the absence of metabolic change of the transport substrate.

Gluconate transport was found to achieve concentration gradients of 200-fold and above. When a plateau of accumulation was reached, the rate of uptake was found unchanged but balanced by an equal rate of exit.

A more detailed report on the isolation of the mutant and on full kinetic description of the gluconate transport is the subject of another article [2], the present paper is devoted to the study of the electrical balance during the uptake of a negatively charged substrate.

2. Experimental

In order to study the ion balance during uptake the following tests have been made:

a) pH dependence of the K_m for uptake to furnish possible indications as to whether undissociated gluconic acid is the real transport substrate;

b) Measurement of uptake in a medium containing no permeant ions other than gluconate and the ions of water;

c) Detection of possible intracellular changes of pH at the beginning of gluconate uptake;

d) Detection and measurement of extracellular pH changes during uptake.

3. Results

3.1. *pH dependence of gluconate uptake*

Fig. 1a shows the double reciprocal plot of initial rates of gluconate uptake versus the concentration of gluconate in the medium at several pH values. The indicated pH values were obtained with 0.066 M sodium potassium phosphate according to Sörensen. Fig. 1b summarizes the K_m values obtained from these data on a logarithmic scale versus pH.

If gluconic acid is the real substrate for transport and if the binding site of the permease did not contain ionizable groups having their pK in the explored pH range, this representation should give a straight line with slope 1 (10-fold increase in K_m for an increase of one unit of pH). At pH values below 7.0, the slope is only 0.4, but between pH 7 and 8.7, the observed slope is 1. Extrapolating this straight line segment to pH 4.05, the pK_a of gluconic acid, an intrinsic K_m of 10^{-7} M could be taken to describe the affinity of gluconate permease for gluconic acid.

On the basis of these results, the possibility of gluconic acid as the true substrate cannot be ruled out.

3.2. *Uptake of gluconate from a medium containing no permeant ions other than gluconate H^+ and OH^-*

Such a medium can be composed from 'ampholines' (LKB, Sweden) which are polyamino-polycarboxylic acids of mol. wt. of the order of 1000. When ampholine 3–10 (pH 5.89) and ampholine 5–8 (pH 6.77) were mixed together in equal parts at 2% final concentration, the resulting pH was 6.35. Washed bacteria were

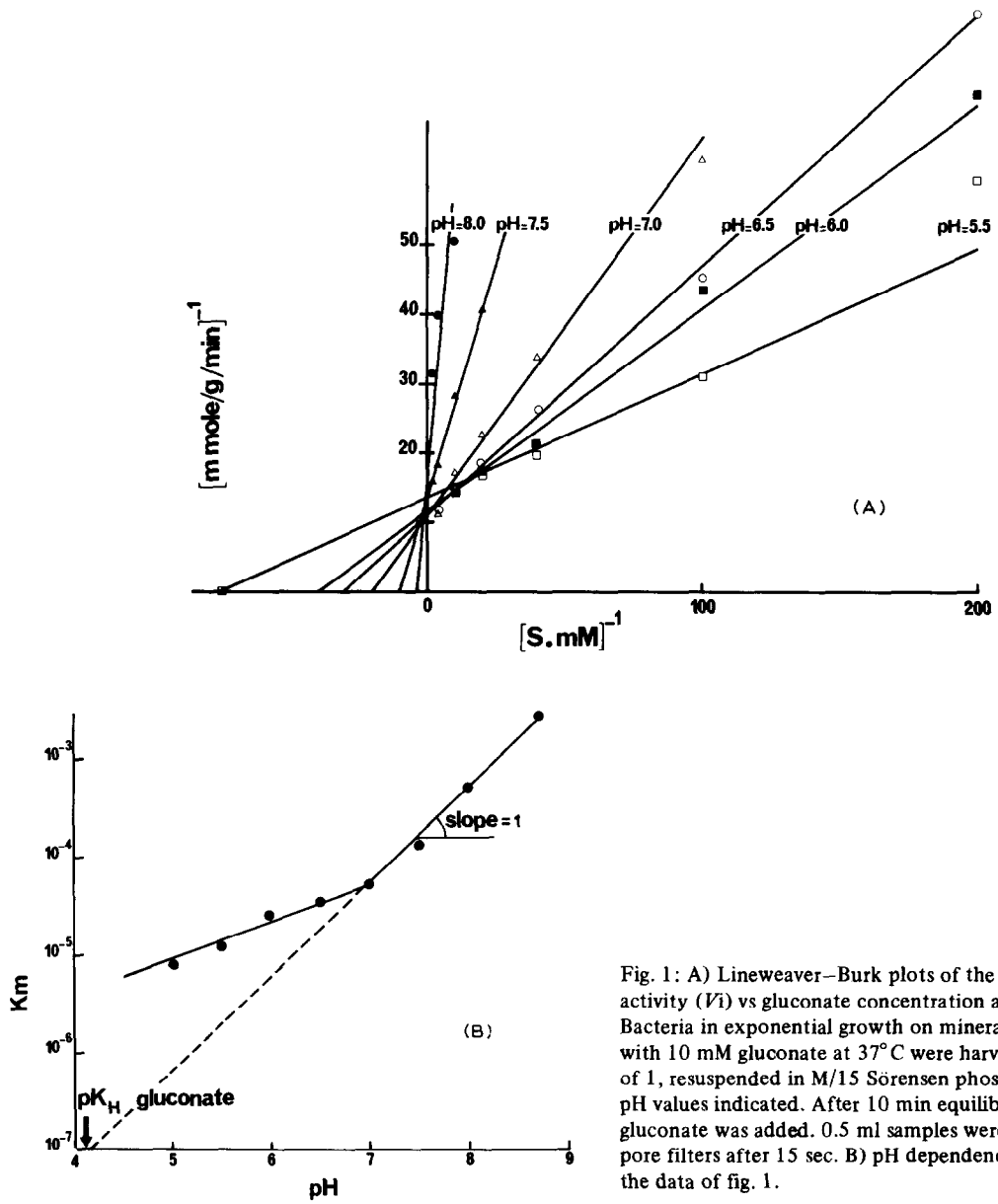

Fig. 1: A) Lineweaver–Burk plots of the gluconate permease activity (Vi) vs gluconate concentration at different pH. Bacteria in exponential growth on mineral medium 63 gly B_1 with 10 mM gluconate at 37° C were harvested at absorbance of 1, resuspended in M/15 Sörensen phosphate buffer at the pH values indicated. After 10 min equilibration at 25° C [^{14}C]-gluconate was added. 0.5 ml samples were filtered on Millipore filters after 15 sec. B) pH dependence of log K_m from the data of fig. 1.

suspended in this solution. An aqueous solution of gluconic acid was diluted in an appropriate mixture of ampholines to give the same final pH. Gluconic acid uptake measured in these conditions (fig. 2) closely resembles the uptake in a phosphate buffer of same pH, where the initial rate of uptake is 100 μmoles/min/g dry weight. The addition of KCl, NaCl or $MgCl_2$ does not result in significant stimulation of the uptake.

Therefore the cotransport of a cation with the negatively charged gluconate ion cannot be an absolute requirement.

3.3. *Detection of intracellular change of pH*

This was based on the very sharp pH dependence

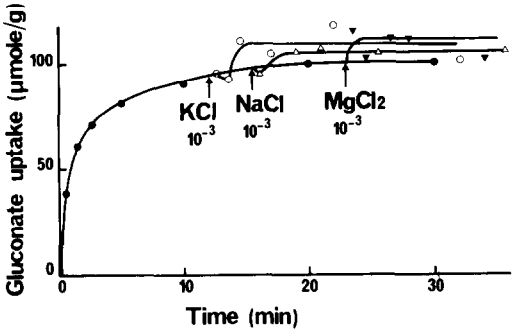

Fig. 2. Effect of Na^+, K^+, Mg^{2+}, Cl^- at the steady state of accumulation of gluconate in ampholine medium at 25°C. 0.2 mM [^{14}C]gluconate was added at zero time.

of fluoride inhibition of gluconate uptake, similar to that reported for the inhibition of phosphoenolpyruvate synthesis and of hexose 6 phosphatase activity [3]. Fluoride inhibition versus pH is pictured (fig. 3) as per cent of the uninhibited rate of gluconate uptake at pH 7.4 and as per cent of the control plateau value when 50 mM sodium fluoride (NaF) was added 10 min before gluconate. The steepest slope is observed when the pH of the medium is varied between pH 6.4 and 6.6. Actually, as shown previously this corresponds to an intracellular pH some 0.7 units more alkaline. When gluconate uptake is started at pH 6.6 with 50 mM NaF added together with substrate (fig. 4), the initial rate

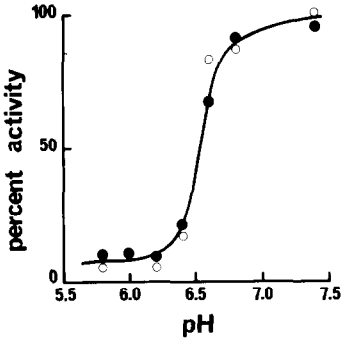

Fig. 3. pH dependence of fluoride inhibition of gluconate uptake. (●—●—●) Initial rate of uptake when 50 mM NaF was added 10 min, before addition of substrate. (○—○—○) Ten min after addition of 50 mM NaF to a bacterial suspension which has reached the steady state of accumulation. Values are expressed as per cent of the control without fluoride.

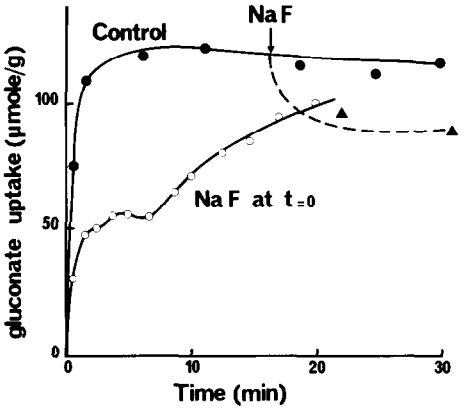

Fig. 4. Effect of NaF added simultaneously with gluconate at zero time of uptake, in medium at pH 6.6. (●—●—●) Uptake in control; (△--△--△) level of chase when NaF was added to control at steady state of accumulation; (○—○—○) time course of uptake when 50 mM NaF was added together with [^{14}C]gluconate.

of uptake is only slightly inhibited, but after 1.5 min transport comes to nearly a complete halt. It starts again after the sixth minute, to reach a plateau which again corresponds to a slight steady state inhibition characteristic of pH 6.6. This two-step time course strongly suggests, that after the first minute of gluconate uptake the intracellular pH was sufficiently acidified, so that the inhibitory activity of NaF was exalted, but due to compensatory mechanisms the pH gradient between medium and cytoplasm was rebuilt in the subsequent minutes, fluoride inhibition decreased and

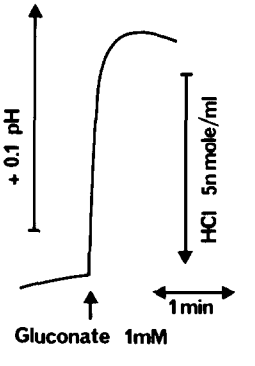

Fig. 5. pH record trace of a suspension of 0.28 mg/ml bacteria in 10 mM KCl at 25°C. 1 mM potassium gluconate provoked the alkalinization of the medium.

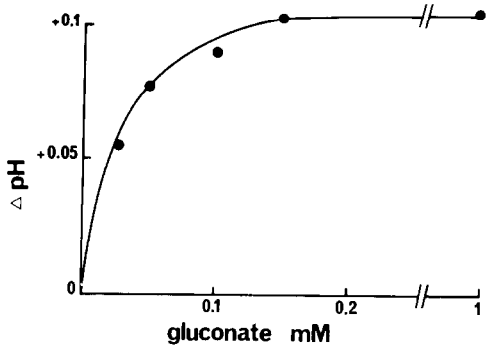

Fig. 6. Gluconate concentration dependence of the pH shift. Conditions as in fig. 3 except that successive amounts of gluconate were added sequentially at close intervals to make up the indicated final concentrations.

uptake recommenced. Such two-step uptake kinetics were observed only with fluoride and only when the starting pH was between 6.5 and 6.65.

3.4. *Changes in extracellular pH*

If the results of the above experiment are interpreted as reflecting a transient acidification of the cytoplasm upon penetration of gluconate due to cotransport of H^+ (or transport of gluconic acid or countertransport of OH^-) a simultaneous alkalinization of the medium is expected. This was explored with unbuffered suspensions of AR13 in KCl. Fig. 5 shows the pH trace obtained upon addition of 1 mM potassium gluconate. The sudden alkalinization of the medium extends over 20–30 sec, followed by a slow return. The amount of H^+ ion subtracted from the medium is approximately equal to the amount of gluconate which disappears from the medium in 20 sec in a parallel experiment made in identical conditions.

When a non-saturating concentration of gluconate caused a pH shift in the medium, additional gluconate caused a further pH shift. Fig. 6 shows the cumulative pH shift versus the final concentration of gluconate added in such a multistep experiment. The K_m calculated from this curve is similar to the K_m of gluconate uptake at pH 6.3 in a control experiment. When AR13 non-induced for the gluconate pathway was used in a similar experiment, the pH shift observed was about 15% of the pH shift of an induced population, and this matches well with the observed basal level of gluconate permease. No pH shift was detected upon addition of gluconate or of TMG to the culture of *E. coli* AR13 or to culture of strains of *E. coli* competent for the uptake of one or the other of these two substrates (not presented). The initial rates of uptake in these systems are comparable to the initial rate of gluconate uptake in induced AR13, namely approx. 100 μmoles/g/min [4, 5]. This is at variance with other results [6, 7] obtained in different conditions with lactose permease, when all energy sources other than the proton gradient have been inhibited.

Specific mechanisms whereby a bacterial ion transport system satisfies the electroneutrality requirement have been explored in very few instances [8].

4. Discussion

From the results described above it can be concluded that uptake of gluconate by the gluconate permease of *E. coli* does not require the presence of a penetrating cation in the medium. During the early stage of net uptake, electronicality seems to be maintained by the ions of water. Three models are possible: 1) transport of undissociated gluconic acid; 2) cotransport of the negatively charged gluconate ion with an H^+ ion or 3) countertransport of gluconate against OH^-. Each of these three hypotheses is compatible with the pH dependence of the K_m of gluconate uptake. After the first 20–30 sec of net gluconate uptake a presumably complex array of ion displacements provide a compensatory mechanism to maintain the pH gradient and osmotic balance across the membrane.

Acknowledgements

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