Galactose Transport in Escherichia coli

1. GENERAL PROPERTIES AS STUDIED IN A GALACTOKINASELESS MUTANT

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It has been recognized for some time that living cells exhibit a selective behavior toward charged particles. In the absence of specific transport mechanisms, cell membranes tend to be impermeable to such cations as Na⁺ and K⁺ and also to certain organic anions including nucleotides and sugar phosphates. Whereas wild type Escherichia coli is essentially impermeable to citrate, other species can be induced to utilize exogenous citrate (1, 2). On the other hand, it has been generally assumed that cell membranes are freely permeable to uncharged small molecules such as glycerol and glucose. As yet, no satisfactory explanation has been offered for the anomalous behavior of the phosphate ester of glucose; cells which readily utilize both glucose and inorganic phosphate are unable to assimilate these if they are combined as the phosphate ester. It might be concluded from this situation either that replacement of the nondissociated hydroxyl group of phosphoric acid by glucose interferes with the ability of the cell to absorb the ion or that the phosphate ester is excluded because of its greater size. On the basis of recent developments the presence of specific transport mechanisms, cell membranes tend to exhibit a selective behavior toward charged particles. In the absence of such specific transport mechanisms, cell membranes tend to be impermeable to such cations as Na⁺ and K⁺ and also to certain organic anions including nucleotides and sugar phosphates. Whereas wild type E. coli is essentially impermeable to citrate, other species can be induced to utilize exogenous citrate (1, 2). On the other hand, it has been generally assumed that cell membranes are freely permeable to uncharged small molecules such as glycerol and glucose. As yet, no satisfactory explanation has been offered for the anomalous behavior of the phosphate ester of glucose; cells which readily utilize both glucose and inorganic phosphate are unable to assimilate these if they are combined as the phosphate ester. It might be concluded from this situation either that replacement of the nondissociated hydroxyl group of phosphoric acid by glucose interferes with the ability of the cell to absorb the ion or that the phosphate ester is excluded because of its greater size. On the basis of recent developments the former conclusion seems more likely; it is now evident that the uptake of carbohydrates by bacteria is not due to simple diffusion through a semipermeable membrane but to the action of stereospecific active transport systems.

The presence of such specific transport mechanisms for the monosaccharides was first suggested by Doudoroff et al. (3). They isolated a mutant strain of E. coli capable of normal growth on the disaccharide maltose but unable to utilize glucose. The failure to grow on glucose could not be explained in terms of an enzymatic defect, since all of the enzymes required for glucose metabolism, including hexokinase (4), were present in normal quantities. Indeed, free glucose, formed from maltose within the cell by the action of amyloglucosidase, was rapidly and completely utilized. On this basis of these observations Doudoroff postulated that a specific transport system for glucose must exist; only in this way could cells permeable to maltose be impermeable to glucose.

The work of Cohn and Monod (5) and their collaborators has provided a direct demonstration of the existence of such selective bacterial transport systems (permeases) for carbohydrates and glycosides. These are characterized by their great specificity and by their ability to accumulate the substrate within the cell in concentrations far exceeding those found in the external medium. The transport system for methyl-α-d-glucoside is thought to be identical with that responsible for the transport of glucose, since it is absent in the glucose-negative mutant of Doudoroff but appears in glucose-positive isolates obtained by transduction (6). Lactose, o-nitrophenyl galactoside, thiomethyl-β-d-galactoside, and α-thiogalactoside are concentrated by a β-galactoside transport system (7). Rotman (8) has found differences with respect to the uptake of methyl-β-d-galactoside and of thiomethyl-β-d-galactoside which suggest that these may be acted upon by distinct transport systems, one constitutive and the other inducible. This has been confirmed by Osborn,1 who has found methyl-β-d-galactoside to be a substrate for the galactose transport system. Specific permeases are also known for glucuronides (9) and for maltose.

In the case of the thiogalactosides the available evidence supports the view that the bulk of the accumulated substrate is present within the cells as a free solute, chemically identical with that present in the external medium (5). Sistrom (10) has used proteolysis to prove that these substances are concentrated within the cells in an osmotically active form. However, little is known of the detailed mechanism, except that it is energy dependent (5) and exhibits a high degree of specificity. The best evidence for a functional role in bacterial metabolism comes from the study of cryptic mutants whose inability to utilize the substrate in question can be related to the absence of the specific transport mechanism. Such cryptic mutants are now known for a number of substrates, including citrate (2) and lactose (7).

Thus far the study of bacterial transport of monosaccharides has been hampered by the absence of either suitable analogues which might serve as gratuitous substrates or mutants unable to metabolize the monosaccharides themselves. Recently, however, a mutant strain of E. coli ML has been isolated which lacks galactokinase and is therefore incapable of growth on galactose. Resting cells accumulate large quantities of galactose, which appears to be present entirely as the free sugar. The present communication is concerned with the specificity and other properties of the galactose transport system.

EXPERIMENTAL PROCEDURE

The galactose-negative strain employed was a mutant of E. coli ML, isolated at the Pasteur Institute, and designated ML 1. M. J. Osborn, unpublished observations. 2 Herbert Wiesmeyer and Melvin Cohn, personal communication.
The parent type ML 30 was used for purposes of comparison. Growth was on a synthetic salt mixture (medium 63) (10) with the addition of an appropriate carbon source, usually succinate or mannose. Cells were grown at 37° and harvested by centrifugation during the exponential growth phase. Except where otherwise indicated, the sedimented cells were washed once with medium 63 and resuspended in the same medium containing 50 \(\mu\)g per ml of Chloromyein. The density of cells in a suspension was measured optically at 600 nm with the Jean-Constant spectrophotometer, previously calibrated to permit expression of the result in terms of bacterial dry weight. Usually fresh cells were used; in some instances they were further depleted of endogenous metabolites by incubating with aeration at 37°.

Galactose-C\(^{14}\), uniformly labeled, was provided by Dr. Gerard Milhaud, of the Isotope Laboratory of the Pasteur Institute. The stock preparation had a specific activity of 40 \(\mu\)c per mg; this was reduced to appropriate levels by dilution with cold galactose. The isotopic material was chromatographed with several solvent systems and found to contain no detectable radioactive impurities. Thiogalactoside, labeled with S\(^{35}\), was synthesized in this laboratory by Mr. Dietmar Türk.

Other substrates were obtained from commercial sources, except as otherwise indicated.

In general, galactose-C\(^{14}\) accumulated by the cells was measured with a Geiger counter, after the cells had been collected by vacuum filtration on a Millipore HA filter (Millipore Corporation, Waltham, Massachusetts). To insure rapid filtration the total quantity of bacteria in an aliquot to be examined was usually about 125 \(\mu\)g (dry weight). With this quantity, filtration was complete in less than 10 seconds. The cells on the filter were washed 3 times with cold salt solution (medium 63) and dried under infrared lamps before counting. The results are expressed as \(\mu\)moles of galactose accumulated per g dry weight of cells.

Assays for galactokinase were carried out with toluenized cells, prepared from concentrated cell suspensions (approximately 2.5 mg of dry weight per ml) by gentle shaking at 37° with one-twentieth volume of toluene. The time of treatment was usually 30 minutes. The assay method was based on the insolubility of barium galactose phosphate in 80% aqueous ethanol. The reaction mixtures contained 0.2 \(\mu\)mole of galactose-C\(^{14}\) (8 \(\times\) 10\(^4\) c.p.m.), 0.4 \(\mu\)mole each of adenosine triphosphate and MgCl\(_2\), 10 \(\mu\)moles of tris(hydroxymethyl)aminomethane buffer at pH 7.4, 10 \(\mu\)moles each of glutathione and sodium fluoride, and 2 to 16 \(\mu\)l of toluenized bacterial suspension, in a total volume of 0.25 ml. After incubation at 37° for 10 and 20 minutes, respectively, 0.05-ml aliquots were added to 0.4 ml of cold absolute ethanol and treated with 0.05 ml of 1 \(\times\) barium acetate. The suspensions were kept for 15 minutes at 0°, to allow time for flocculation of the barium salts, filtered through a Millipore filter, washed thoroughly with cold 80% ethanol, dried, and counted. The galactose phosphate content was calculated from the counts retained, on the assumption of negligible self-adsorption.

**RESULTS**

**Galactose Uptake and Displacement**—Resting cells of the galactose-negative strain ML 32,400, when incubated with galactose, rapidly accumulate this substrate until a steady state level is reached (Fig. 1). At 37° the plateau level was usually attained within 5 to 10 minutes and maintained for 10 to 30 minutes, depending on the nutritional state of the cells. When no metabolizable substrate was added, incubation beyond 30 to 40 minutes often led to a gradual decline in the quantity of galactose accumulated. As indicated in Fig. 1, the addition of an excess of nonradioactive galactose resulted in rapid and essentially complete displacement of radioactivity from the cells. This would be expected if (a) galactose were present within the cells in chemically unaltered form and (b) the steady state level were the result of a balance between uptake from the medium and return of internal galactose to the medium.

The fulfillment of the first condition was confirmed by examination of the cell contents by paper chromatography. Aliquots of cell suspensions incubated at 37° for varying times with radioactive galactose were centrifuged rapidly and the cell pellets suspended in ice-cold 50% aqueous acetone (volume for volume). Nonradioactive galactose was added and the entire suspension, including all insoluble components, was placed on paper for chromatography. Several solvent systems were used, including acetone-water, phenol-water, and butanol-acetic acid. In each case a single radioactive spot was present which coincided precisely with the carrier galactose spot as revealed by spraying with aniline phthalate (11). No trace of radioactivity was detected at the origin, even when the cells had been incubated with galactose-C\(^{14}\) for some time, indicating the absence of galactose metabolism or incorporation into cellular structure.

Less than 5% conversion would have been detected.

**Absence of Galactokinase**—No trace of galactokinase can be detected in cells of the mutant strain ML 32,400 whether they be grown in the presence or absence of galactose (Fig. 2). Galactokinase in the wild type ML 30 is an inducible enzyme; its activity is increased 20-fold in cells grown on galactose.
Fig. 2. Measurement of galactokinase activity in the wild type and mutant strains. ML 30 was grown on 0.2% glucose (non-induced) or 0.2% galactose (induced). ML 32,400 was grown on 0.4% succinate or 0.4% succinate containing $10^{-4}$ M galactose. Extracts were obtained by shaking for 30 minutes at 37° with 1 drop per ml of toluene. Galactokinase was assayed as described in "Methods."

![Graph showing Galactose Concentration vs. Time](image)

**FIG. 2.** Measurement of galactokinase activity in the wild type and mutant strains. ML 30 was grown on 0.2% glucose (non-induced) or 0.2% galactose (induced). ML 32,400 was grown on 0.4% succinate or 0.4% succinate containing $10^{-4}$ M galactose. Extracts were obtained by shaking for 30 minutes at 37° with 1 drop per ml of toluene. Galactokinase was assayed as described in "Methods."

**TABLE I**

<table>
<thead>
<tr>
<th>Concentration of galactose by ML 32,400</th>
</tr>
</thead>
<tbody>
<tr>
<td>The experimental conditions are given in the legend to Fig. 4</td>
</tr>
<tr>
<td>External concentration</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>$2.1 \times 10^{-4}$</td>
</tr>
<tr>
<td>$2.1 \times 10^{-4}$</td>
</tr>
<tr>
<td>$4.2 \times 10^{-7}$</td>
</tr>
</tbody>
</table>

Similar extracts of ML 32,400 are devoid of activity. That this lack of demonstrable activity is not due to the presence of an inhibitor is clear from the experiment with mixed extracts; the activity in extracts of ML 30 is not depressed by addition of the inactive extracts obtained from ML 32,400.

**Effect of External Galactose Concentration**—The transport system possesses a high affinity for galactose and at low external concentration most of the sugar added to the medium will be taken up by the cells. As the external concentration is increased, the steady state internal galactose concentration is found to increase until saturating levels are reached (Fig. 3). Saturation occurs when the external concentration is about $2 \times 10^{-4}$ M, at which point the internal concentration is about $6 \times 10^{-4}$ M, and galactose accounts for nearly 5% of the total dry weight (Table I). At lower galactose concentrations less galactose is accumulated but the concentration factor is greater.

In the last experiment in Table I the initial external galactose concentration was $10^{-4}$ M; more than half of the total galactose was taken up by the cells and the final internal concentration exceeded 10,000-fold that remaining in the external medium.

**Energy Requirement for Galactose Transport**—From this concentration factor, which may be exceeded at lower levels of galactose, it can be calculated that the free energy requirement for galactose transport (or accumulation) is in excess of +5500 calories per mole. As with the galactosides (7), the transport of galactose is completely inhibited by dinitrophenol; when this substance is added to cells which have accumulated galactose to the plateau level, a rapid and essentially complete loss of galactose is observed (Fig. 4).

The addition of energy sources, such as glucose, affects the level of galactose uptake in a complex manner. At relatively high concentrations glucose strongly inhibits the uptake of galactose. Thus, when it is added to the cells which have previously been allowed to equilibrate with galactose-$^{14}$C in the absence of glucose, it produces a rapid and complete loss of galactose (Fig. 4). At lower concentrations, the effect of glucose is to increase the steady state level of galactose accumulation (Fig. 5).

A similar stimulation of galactose accumulation is observed with succinate. When this substrate is added to washed, starved...
cells, the initial rate of galactose accumulation is little altered, but there is not the slow decline in internal concentration often observed in cells incubated without added succinate (Fig. 6). These observations would tend to support the hypothesis that energy provided by a metabolizable substrate is necessary for optimal accumulation of galactose, except that identical effects have been observed after the addition of compounds which are not metabolized, such as α-methylglucoside (Fig. 6). A possible alternate explanation for this effect is discussed in Paper II of this series.

Specificity of Galactose Transport System—The uptake of galactose-C\textsubscript{14} appears to be catalyzed by a highly specific system. It is little affected by the addition of a number of analogues or possible competitors, even when these are added at a concentration 100-fold greater than that of galactose (Table II). The two levels of galactose employed, \(10^{-4}\) M and \(10^{-6}\) M, represent concentrations which are saturating and approximately one tenth saturating, respectively. When tested with the lower level of galactose, a few compounds, such as methyl-β-D-galactoside and L-arabinose, produced significant inhibition; in the case of L-arabinose, the inhibition has been analyzed kinetically by the method of Dixon (12). This compound appears to be a true competitive inhibitor with a \(K_i\) of \(1.7 \times 10^{-3}\) M, 250 times larger than the \(K_m\) for galactose.

Since no sugar or derivative has been found to compete effectively with galactose at comparable concentrations, it must be concluded that the transport of galactose involves a mechanism possessing a high degree of specificity.

Effect of Growth on Various Substrates—Galactose transport is clearly distinct from the transport of galactosides, since in the mutant strain ML 32,400 galactose accumulation is a constitutive property whereas galactosides are accumulated only by induced cells (Table III). Thus with cells grown on succinate, galactose reaches an intracellular level 25 times greater than that of thiogalactoside. On the other hand, with lactose-grown cells or with cells grown in the presence of isopropyl...
The transport and accumulation of galactose by E. coli exhibit properties similar to those described for galactosides and glucosides. The parallelism with enzymatic mechanisms is striking, particularly the high degree of stereospecificity and the kinetic behavior typical of systems in which enzyme-substrate complexes are presumed to be found. The properties of the system are, as in the case of thiogalactosides, best explained in terms of a catalytic transport mechanism, rather than a binding of substrate to specific sites within the cell. The quantity of galactose accumulated, as much as 1 mole for each 3000 g of dry weight, defies explanation in terms of specific binding sites (5), and no evidence for chemical alteration of the galactose molecule has been obtained. Extraction with cold aqueous acetone and chromatography with the same solvent or with aqueous phenol indicate that all of the accumulated material is free galactose. Furthermore, the rapid loss of intracellular galactose on addition of dinitrophenol is difficult to explain except in terms of an energy-requiring mechanism for the entry of substrate which is not involved in its exit. This is further discussed in Paper II of this series.

The absence of galactokinase in an organism which possesses a very active specific permeation mechanism for galactose proves that the latter system is distinct from and independent of the former. This affords one more example which appears to justify the view (5) that permeation mechanisms are functionally specialized and distinct from the metabolic enzymes involved in the degradation of the substrate. It does not, however, preclude the possibility that phosphorylation either of the permease protein itself or of the transported galactose may play a part in the permeation mechanism. Further work is needed to clarify the nature of energy coupling in the action of the transport enzymes. A clear requirement for an external energy source has not been obtained, but this may be due to the presence of adequate endogenous respiration. The effects of added glucose, augmentation of galactose uptake at low concentrations, and inhibition at high concentrations remain to be explained. Identical observations have made with the uptake of thiogalactosides.

The galactose transport system represents one of the most specific yet studied. None of the aldohexoses available for test appears to be acted upon by the system, since none inhibits the uptake of galactose when added at comparable concentrations. Two substances, L-arabinose and β-methylgalactoside, appear to inhibit competitively but the affinity for these substrates is far less than that for galactose.

**Summary**

A galactokinase-less mutant of *Escherichia coli* ML accumulates galactose against a concentration gradient in a readily reversible manner. Galactose is displaced by high concentrations of glucose or by 2,4-dinitrophenol. The system is highly specific for galactose and exhibits a high affinity for the sugar, with a *Kₐ* of approximately 10⁻³ M. At saturation the internal galactose concentration is nearly 10⁻³ M.

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**Fig. 7.** A comparison of galactose-Cl⁴ uptake and displacement by the galactose-negative mutant ML 32,400 and the wild type ML 30. ML 32,400 was grown on 0.2% mannose; ML 30 was grown on 0.2% galactose, in order to adapt the cells to this sugar. Cells were washed with medium 63 two times and suspended in medium 63 plus Chloromycetin (0.05 mg per ml). The galactose-Cl⁴ concentration was 2 × 10⁻⁷ M. Galactose-Cl⁴ (10⁻⁵ M) was added at 21 minutes.

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**Graph**

- **Wild Type**
- **E. coli**
- **Mutant**

- **µ moles galactose/µm cells**

- **Excess cold galactose**

- **Minutes**

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**Table**

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>µ moles galactose/µm cells</th>
<th>Excess cold galactose</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>150</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>200</td>
<td>20</td>
</tr>
<tr>
<td>20</td>
<td>250</td>
<td>10</td>
</tr>
<tr>
<td>30</td>
<td>200</td>
<td>5</td>
</tr>
<tr>
<td>40</td>
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<td>0</td>
</tr>
<tr>
<td>50</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>60</td>
<td>50</td>
<td>0</td>
</tr>
</tbody>
</table>
Evidence has been obtained indicating that this transport mechanism is distinct from that involved in the transport of β-galactosides or α-glucosides.

The initial rate of galactose uptake is identical in the wild type and in the mutant strain, but in the wild type no free galactose accumulates. This suggests that permeation is the limiting step in galactose utilization by *E. coli*.

REFERENCES

Galactose Transport in *Escherichia coli*: I. GENERAL PROPERTIES AS STUDIED IN A GALACTOKINASELESS MUTANT
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