Galactose Transport in Escherichia coli

II. CHARACTERISTICS OF THE EXIT PROCESS

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Paper I of this series was concerned with the uptake of galactose in a mutant strain of Escherichia coli, ML 32,400. This organism is incapable of growth on galactose because of a complete lack of galactokinase, but it will accumulate this sugar to levels which greatly exceed those found in the external medium. The transport system has a high affinity for galactose, is highly specific, and is sensitive to energy uncoupling agents such as dinitrophenol (1).

Ordinarily, galactose utilization in E. coli is an inducible process (2); in the parent strain ML 30, for example, galactokinase is present only in induced cells (1). Yet the transport of galactose in the mutant strain ML 32,400, derived from ML 30, is not dependent upon previous induction. On the contrary, the capacity of the constitutive transport system is depressed in a striking way when growth occurs in the presence of galactose and certain compounds related to galactose (1) although these compounds are not utilized. Thus, cells grown on glucose or lactose show little activity toward galactose, and the level of galactose accumulation is also depressed by growth in the presence of certain galactosidase inducers, such as isopropyl thiogalactoside and methyl thiogalactoside. Growth of the organism in the presence of traces of galactose, which are not utilized, yields cells which have a much smaller capacity to accumulate galactose, compared with that of cells which have been cultivated on the same carbon source in the absence of galactose. The reduced capacity for galactose can be shown to be caused by an increase in the rate of loss of galactose from the cells, rather than by a decrease in the rate of galactose uptake.

These results are not compatible with exit of sugar by simple diffusion and lend strong support to the "transporter" hypothesis proposed by Kepes (3) to explain galactoside accumulation. Kepes has found the exit of thiogalactosides from E. coli cells to be inhibited by such agents as p-chloromercuribenzoate and accelerated by other galactosides such as α-methyl thiogalactoside. These observations could not be explained in terms of passive diffusion and led Kepes to suggest that it is necessary for the substrate to combine with a hypothetical "transporter" for both entry and exit. The present results obtained with the galactose transport system are consistent with this view.

EXPERIMENTAL PROCEDURE

Cells were grown on synthetic salt medium 63 with a suitable carbon source as described in Paper I of this series (1). The strains employed were wild type E. coli ML 30, and the galactose negative strain ML 32,400. Measurements of galactose uptake and displacement were made as previously described (1). Chloromycetin was added to a final concentration of 50 µg per ml as indicated.

Kinetic Analysis of Galactose-C14 Uptake—The steady state concentration of galactose within the cell can be considered to represent a balance between the rate of uptake of sugar and the rate of its loss to the medium (4). When the external concentration of galactose is beyond the saturating level the rate of entry can be considered to be independent of the external concentration, Gex, and to depend only upon the activity of the transport system, y. Loss of sugar, on the other hand, appears to be a first order function of the internal concentration, Gint, under all conditions thus far examined (see below). The change in internal galactose concentration can be expressed as the difference between the rates of entry and of exit:

\[
\frac{dG_{\text{int}}}{dt} = y - cG_{\text{int}}
\]

where c is the first order rate constant for the exit process. The rate constant for the entry system is contained in the constant y. Upon integration between t = 0 and t = ∞, the latter representing the steady state, this becomes

\[
G_{\text{int}} = \frac{y}{c} \left(1 - e^{-ct}\right)
\]

At t = ∞, the equilibrium value of Gint, designated Geq, is equal to y/c and Equation 2 reduces to:

\[
G_{\text{eq}} = \frac{G_{\text{ex}} - G_{\text{eq}}}{c} = e^{-ct}
\]

\[
2.303 \log \left(\frac{G_{\text{eq}} - G_{\text{ex}}}{G_{\text{eq}}}\right) = -ct
\]

In this form the data can be plotted to yield a straight line whose slope is -c, the rate constant of the exit reaction. From this and the steady state level Geq, the value of y, the activity of the entry process, can be derived.

Effect of Growth in Presence of Galactose—The mutant strain ML 32,400 is incapable of growth on galactose, but the presence of traces of this sugar during growth on another substrate results in a greatly altered capacity to accumulate galactose (Table I). The steady state level of galactose accumulated by resting cells is reduced to one-fifth of the control value when the organ-
TABLE I

<table>
<thead>
<tr>
<th>Growth on</th>
<th>mmoles galactose/g cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinate (0.4%)</td>
<td>15.9</td>
</tr>
<tr>
<td>Succinate (0.4%) + galactose (10^{-4} M)</td>
<td>3.0</td>
</tr>
<tr>
<td>Succinate (0.4%) + galactose (10^{-5} M)</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Galactose accumulation with cells grown in presence of traces of galactose

Cells were harvested, washed by centrifugation in substrate-free salt medium and resuspended in the same medium containing Chloromycetin. The uptake of galactose-C^14 was measured at 37° with the Millipore filter technique described in Paper I of this series (1).

Entry and Exit Rates in Cells Grown in Presence of Galactose—In order to determine whether the presence of galactose during growth affects the transport mechanism directly, i.e. reduces the rate of entry of galactose, kinetic studies were carried out with cells grown in the presence and absence of galactose (Fig. 1). It was evident from a gross inspection of the initial rate of entry that this was not sufficiently altered to explain the 5-fold decrease in the steady state level of galactose-C^14 accumulated. The plot of the data as log (G_w - G_s)/G_w against time yielded straight lines with slopes of -0.11 min^{-1} for the succinate grown cells (G_w = 27.7 mmoles per g dry weight) and -0.52 min^{-1} for cells grown on succinate in the presence of 10^{-5} M galactose (G_w = 5.9 mmoles per g dry weight). The loss of galactose is nearly 5 times more rapid with cells which have been grown on galactose. The value for y, calculated from the relation y = cG_w, is unchanged, 3.0 mmoles X g^{-1} X min^{-1} in cells grown on succinate and 3.1 mmoles X g^{-1} X min^{-1} in cells grown on succinate with galactose. Thus the activity of the entry system is unaffected by growth in the presence of galactose, and the decrease in capacity is the result of an increased rate of galactose exit from the cells.

Induction of Exit System—The increase in exit activity exhibits all the attributes of an inducible process. As already indicated, it is affected maximally by very low concentrations of galactose. It does not occur when cells are incubated with galactose in the absence of growth. Washed cells, incubated at 37° for 2 hours with 10^{-4} M galactose and 50 μg per ml of Chloromycetin, showed a decrease in G_w of 8%, compared with a decrease of 7% in the control culture similarly incubated without galactose. On the other hand when the activity of the exit system was measured in growing cells after addition of galactose, it was found to aug-

Fig. 1. Kinetics of galactose uptake in cells grown with traces of galactose in the growth medium. Growth was on 0.4% succinate in salt medium containing 10^{-5} M galactose. Cells were harvested and washed by centrifugation in salt medium lacking both succinate and galactose. Washed cells were resuspended in salt medium containing Chloromycetin. Incubation was at 25°, with 0.26 mg of bacterial dry weight per ml. Galactose-C^14 (final concentration = 2.7 X 10^{-5} M; 1.1 X 10^{4} c.p.m.) was added at zero time. Aliquots of 1.0 ml were filtered at the indicated intervals. The right hand portion of the figure shows the data plotted according to Equation 4.

Fig. 2. Kinetics of induction of the exit system. Cells were obtained by growth on 0.4% succinate and harvested in the exponential phase. Washed cells were suspended in salt medium containing either 0.4% succinate alone or 0.4% succinate together with 2 X 10^{-4} M galactose. Aliquots were taken at the indicated intervals; growth was stopped by the addition of Chloromycetin. After centrifugation the cells were washed twice with substrate-free salt medium, containing Chloromycetin, resuspended in the same medium and tested for uptake of galactose-C^14. The exit activity c was assumed to be inversely proportional to the steady state level and the values represent the total activity per milliliter of the original culture.

Fig. 3. Kinetics of galactose uptake in cells grown on mannose or glucose. Cells grown overnight on mannose were diluted with 10 volumes of salt medium containing glucose (0.2%) or mannose (0.4%). After 2 hours at 37° each culture was harvested and washed twice with substrate-free salt medium, finally suspended in salt medium containing Chloromycetin. Galactose-C^14 uptake was tested at 25°, as described in the legend to Fig. 1.
The effect of succinate on the accumulation of galactose-C\textsuperscript{14}. Cells were obtained by growth on 0.4\% succinate in the presence of 10\textsuperscript{-4} M galactose, washed twice with substrate-free medium, and resuspended in salt medium plus Chloromycetin. Galactose uptake was measured as described in the legend to Fig. 1, except that one vessel contained 0.025 M succinate whereas the other contained no added substrate other than the galactose-C\textsuperscript{14}.

Experiment No. | Cells grown on | Addition | Equilibrium level (G\textsubscript{in}) | Entry rate $v$ | Exit rate $c$ |
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\mu$moles/g cells</td>
<td>$\mu$moles/g cells $\times$ min $^{-1}$</td>
<td>min $^{-1}$</td>
</tr>
<tr>
<td>1</td>
<td>Succinate</td>
<td>None</td>
<td>27.7</td>
<td>3.0</td>
<td>0.11</td>
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<tr>
<td></td>
<td>Succinate + 10\textsuperscript{-4} M galactose</td>
<td>None</td>
<td>5.9</td>
<td>3.1</td>
<td>0.52</td>
</tr>
<tr>
<td>2</td>
<td>Mannose</td>
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<td>23.8</td>
<td>4.5</td>
<td>0.193</td>
</tr>
<tr>
<td></td>
<td>Mannose then glucose</td>
<td>None</td>
<td>8.4</td>
<td>1.6</td>
<td>0.193</td>
</tr>
<tr>
<td>3</td>
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<td>None</td>
<td>4.2</td>
<td>3.8</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>Succinate + 10\textsuperscript{-4} M galactose</td>
<td>Succinate</td>
<td>28.3</td>
<td>4.1</td>
<td>0.143</td>
</tr>
<tr>
<td>4</td>
<td>Mannose + 10\textsuperscript{-4} M galactose</td>
<td>None</td>
<td>3.6</td>
<td>2.8</td>
<td>0.78</td>
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<tr>
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<td>$\alpha$-Methyl glucoside</td>
<td>6.0</td>
<td>2.4</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Table II

Summary of entry and exit rates under various conditions

The accumulation of galactose in glucose-grown cells also shows a reduced capacity to accumulate galactose, but in this case the rate of exit is unchanged and the rate of entry is reduced (Fig. 3). In this experiment cells were grown first on mannose and then transferred to glucose for several divisions, since the level of galactose-C\textsuperscript{14} accumulation in glucose-grown cells was too low for kinetic studies. In this case the value of $c$ is found to be identical with both types of cells; it is the reduced entry rate which accounts for the lower steady state level.

Inhibitors of Exit Reaction—Under certain conditions it was possible to demonstrate a direct inhibition of the exit process. This was true particularly with cells with a very active exit mechanism, such as those obtained by growth in the presence of succinate and 10\textsuperscript{-5} M galactose. Such cells were able to accumulate far greater quantities of galactose when they were tested in a medium containing succinate (Fig. 4). Kinetic analysis showed the effect of succinate to be due to a decrease in the rate of loss of galactose, rather than to an effect on the rate of entry, which was unchanged by the addition of succinate.

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Galactose Accumulation in Glucose-Grown Cells—Cells grown on glucose, like those grown in the presence of galactose, also show a reduced capacity to accumulate galactose, but in this case the rate of exit is unchanged and the rate of entry is reduced (Fig. 3). In this experiment cells were grown first on mannose and then transferred to glucose for several divisions, since the level of galactose-C\textsuperscript{14} accumulation in glucose-grown cells was too low for kinetic studies. In this case the value of $c$ is found to be identical with both types of cells; it is the reduced entry rate which accounts for the lower steady state level.

Table II

Summary of entry and exit rates under various conditions

\[
\frac{dG_{in}}{dt} = v - cG_{in}
\]
FIG. 6. Displacement of accumulated galactose-$C^{14}$ by 2,4-dinitrophenol in cells grown in presence or absence of traces of galactose. Cells were obtained by growth on 0.2% mannose or 0.2% mannose + $10^{-4}$ M galactose. They were washed and suspended in salt medium plus Chloromycetin as previously described. The experiment was carried out at 37° with 2 X $10^{-4}$ M galactose-$C^{14}$. Dinitrophenol or glucose was added as indicated at 10.25 minutes. The final dinitrophenol concentration was 1.2 X $10^{-5}$ M; glucose was 8 X $10^{-5}$ M. The ordinate is plotted as counts per minute in the cells.

FIG. 7. First order plot of galactose displacement. The data in Fig. 6 are plotted to show the first order reaction velocities. The two upper lines show results obtained with cells grown on mannose alone; the lowest curve is a composite obtained with cells grown on mannose plus galactose. The $\times$ represents displacement by glucose; the $\bullet$, displacement by dinitrophenol. Note that the values do not extrapolate to zero time.

that methyl glucoside produces a similar effect with cells grown on succinate in the absence of galactose.

The entry and exit constants under various conditions are summarized in Table II. The entry reaction is affected by growth on glucose; its activity is inhibited by L-arabinose or methyl-$\beta$-D-galactoside. The activity of the exit system is increased by growth in the presence of traces of galactose. The addition of succinate or $\alpha$-methyl glucoside reduces the rate at which galactose passes out of the cells.

Direct Measurement of Exit Reaction If it be assumed that 2,4-dinitrophenol blocks only the entry of galactose into the cells, and not its exit, then this compound can be employed for a direct measurement of the exit reaction. The results obtained when 2,4-dinitrophenol is added to cells which have accumulated galactose provide a direct confirmation of the difference in exit rates between cells grown in the presence or absence of galactose (Figs. 6 and 7). After addition of dinitrophenol the time required for the internal galactose to reach one-half the steady state level, estimated from the first order plot in Fig. 7, is about 2.5 minutes for cells grown on mannose alone and about 0.3 minute for the corresponding cells obtained from growth on mannose in the presence of galactose. The assumption that dinitrophenol affects only the entry rate is supported by the fact that essentially the same rate of loss is obtained from a determination of $c$ by the kinetic method, with the use of the 0- to 10-minute points in the same experiment. The value of $c$ derived from these data is 0.41 min$^{-1}$; the half-time calculated from this value is 1.7 minutes. This coincidence of exit rates derived from the shape of the entry curve and from the measurements after addition of dinitrophenol lends strong support to the basic assumptions.

The rate of loss of galactose is considerably accelerated by the addition of glucose, in which case the half-time for exit of galactose is about 1 minute (Figs. 6 and 7). A similar stimulation of the exit reaction is noted when galactose-$C^{14}$ is displaced with an excess of cold galactose (1). Kepes (3) has reported such a stimulation of the exit reaction with the galactoside transport system.

**DISCUSSION**

The results reported here offer strong support for the catalytic transport "permease" model proposed by Cohen and Monod (4) for the accumulation of galactosides in *E. coli*. The steady state level is clearly the result of a balance between rate of uptake and rate of exit. That the exit process, to some extent at least, is independent of the entry process is confirmed by the fact that its activity can be increased or decreased without corresponding changes in the activity of the entry system.

Kepes (3) has presented evidence that the loss of galactosides is not caused by simple diffusion, and he has proposed a model involving complex formation with a transporter to account for passage through the osmotic barrier in either direction. The present results with galactose are entirely consistent with such a model, which includes the assumption that the osmotic barrier is completely, or nearly completely, impermeable to galactose.

For the entry of galactose, the first step would be reaction of external galactose with an activated transporter substance ($T^*$), present in the osmotic barrier. This reaction is catalyzed by an enzyme $E_t$, assumed also to be present in the osmotic barrier. The second step would require dissociation of the transporter-galactose complex ($T$-galactose), catalyzed by an enzyme $E_e$, found inside the cell. This would regenerate the transporter $T$.
and liberate free galactose within the cell. Once in the interior of the cell, the substrate would become unavailable to enzyme $E_1$, and would accumulate until the reaction catalyzed by $E_2$ became appreciably reversible, owing to the high concentration of internal galactose. Eventually this reaction would become equal in rate to that catalyzed by $E_1$, and steady state would be reached.

The effect of growth in the presence of galactose might be to increase either the activity of $E_3$, or the quantity of the transporter substance. The acceleration of galactose exit seen in the presence of glucose may be explained if it be assumed that glucose uptake involves an enzyme different from $E_1$, but the same transporter, and that the reaction of internal galactose with free transporter ($T$) is less efficient than an exchange reaction with transporter-bound glucose ($T$-glucose):

$$T$-glucose + galactose $\rightarrow T$-galactose + glucose

To account for the results observed, it is necessary to make the further assumption that this exchange reaction is catalyzed by $E_2$ and that this route cannot therefore be used by external galactose.

This model is consistent with most of the observations reported. It does not explain the stimulating effect of low glucose concentrations (1), nor the effect of succinate in reducing the velocity of the exit reaction. Until further knowledge of the transport mechanism is gained, it may, however, serve to provide some useful guidelines for future research.

**SUMMARY**

Kinetic studies of galactose uptake by *Escherichia coli* ML 32,400, which lacks galactokinase, reveal the presence of an inducible exit mechanism. The uptake of galactose is a constitutive property in this organism. However, growth on mannose or succinate in the presence of traces of galactose reduces the equilibrium internal galactose concentration because of a 5-fold increase in the rate of exit of galactose. Growth in glucose, on the other hand, depresses the rate of entry.

Loss of galactose is a first order reaction. It is inhibited by succinate or methyl-$\alpha$-n-glucoside and both of these increase the steady state level of galactose accumulation.

Direct measurement of galactose exit can be made after the addition of 2,4-dinitrophenol, which blocks the entry reaction. Whether calculated by kinetic methods from the entry curve or directly after the addition of dinitrophenol, identical exit-rate constants are obtained.

The data are consistent with the transporter model of Kepes.

**REFERENCES**

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