Lactose Permeation Via the Arabinose Transport System in Escherichia coli K-12

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This paper describes the isolation and characterization of a mutant of Escherichia coli that transports lactose and its analog thiomethylgalactoside via the arabinose permeation system. Unlike transport via the lactose permease, this transport is not inhibited by thiogalactoside, but was inhibited by arabinose, xylose, and fucose. The site of the mutation was in the arabinose C gene and confers constitutivity on the entire arabinose operon. Furthermore, this transport was found in 24 independently isolated arabinose-constitutive strains, and in strains which had been induced with arabinose and then starved to remove all traces of it. It was therefore concluded that lactose and thiogalactoside are low-affinity substrates of at least one component of the normal arabinose permeation system.

The mechanism by which bacterial permeases interact with their substrates in the course of substrate transport remains poorly understood. It has been proposed in the case of the most thoroughly studied system, the lactose permease (1, 3, 4, 7), that the binding site for an inhibitor substrate analog, thiodigalactoside (TDG), is distinct from the site for substrate binding. In the present study, as a test of this proposal, a mutant was sought in which lactose transport is not subject to TDG inhibition. Such mutants were sought among revertants from a y strain. In the mutant studied, the genetic alteration was not as expected in the lactose permease nor were the properties consistent with any of the galactoside transport systems described by Rotman et al. (14). On the basis of genetic and other experiments, it is concluded that lactose transport in this mutant in the presence of TDG is mediated by a totally distinct transport system, that for the pentose arabinose.

The arabinose operon (Fig. 1) has been examined mainly as an example of a positive genetic control system, both in Escherichia coli B/r (5) and in E. coli K-12 (15). Brown and Hogg (2) report that E. coli B/r has two active transport systems for arabinose, both under the control of araC. They have identified two genes, one associated with low-affinity uptake and the other with both high-affinity uptake and the arabinose binding protein. Schleif (15) has demonstrated two kinetically distinguishable arabi-

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Company) were inoculated with approximately $10^7$ mutagenized AM7 bacteria. In those cases where visible growth occurred, samples were streaked on tetrazolium plates. L-broth cultures were prepared from single colonies and then inoculated into supple-mented G-glycerol plus $5 \times 10^{-4}$ M isopropyl-$\beta$- d-thiogalactopyranoside (IPTG) for testing.

**Selection of Lac** $^+$ **AM37.** AM37 was streaked on tetrazolium lactose, and colonies were allowed to grow until papillae formed on their surfaces. The papillae were restreaked on tetrazolium lactose plates. Lactose-positive colonies were then streaked on MacCon- key lactose. Colonies which were clearly positive were picked into L broth and grown for stocks and testing.

**Transduction.** A P1 stock (Lennox [12]) made on AM37 was used to transduce RFS 3 and RFS 162 (multiplicity of infection of about 0.1) from Ara$^-$ to Ara$^-$. Transductants were selected by their ability to form colonies on G-arabinose plates. Cultures were prepared from single colonies for testing for thiomethylgalactoside (TMG) uptake and arabinose isomer-ase activity.

**Selection of arabinoise constitutives.** AM7 (after mutagenesis as indicated) was spread on arabinose fucose minimal plates. After 2 days of incubation, the larger colonies were streaked on tetrazolium arabinose plates. Two colonies from each plate were picked, grown in L broth, and then inoculated 1:300 into G-arabinose fucose medium. One tube from each pair was made into a stock and used for further tests.

**Test of $[^{14}C]TMG$ uptake (steady-state level).** Five microliters of $[^{14}C]TMG$ (New England Nuclear, 2.2 mCi/mmol at 50 $\mu$Ci/ml) were added to 0.5 ml of cells (at 28 $^\circ$C) that had been filtered, washed, and suspended in G buffer containing other additions as noted. After 6.25 min at 28 $^\circ$C without shaking, the samples were diluted with 3 to 5 ml of G buffer (room temperature), were rapidly filtered, and were washed with two more volumes of buffer. The time was chosen for convenience in assay. Uptake did not increase significantly after the first 5 min.) Filters were then transferred to vials and dried. They were counted in a liquid scintillation counter.

**Beta-Galactose (EC 3.2.1.23) assay.** The assay was done at 28 $^\circ$C by the method of Horiiuchi et al. (10).

**$K_a$ values for TMG uptake.** Bacteria were grown in G-glycerol plus histidine, arginine, and thiamine for at least six doublings. Assay tubes were prepared with the appropriate volumes of $[^{14}C]TMG$ to give the desired concentrations, and the volumes were brought up to 0.25 ml with M9. After equilibration at 28 or 37 $^\circ$C, 0.25 ml of bacteria at about $2.5 \times 10^8$ cells per ml were added to start the assay. After 2 min, the assay mixture was diluted with 3-ml volumes of M9. Filters
were dried and counted as described above. $K_m$ was determined from a plot of substrate concentration per velocity versus substrate concentration.

**Growth curves.** Growth was observed in G buffer containing the required amino acids plus 1% of the desired sugar by following optical density in a Beckman DB spectrophotometer. Cells which had been starved for 4 h in buffer plus requirements were diluted to an optical density at 350 nm of 0.1 to 0.2 (3 to 6 x 10^4 cells per ml) as necessary, and carbon source was added at zero time.

**Osmotic shock.** The procedure of Heppel (9) was used. Fifty microcuries of $\alpha$-methylglucoside ($\alpha$MG; Calbiochem, 12.3 mCi/mmol) was diluted in 1.0 ml of 25 mM cold $\alpha$MG for use.

**Growth test for constitutivity.** The ability of strains which are constitutive for the arabinose system to grow on L-arabinose in the presence of $\alpha$-fucose was utilized to screen for constitutive mutants. A dilution of at least 1:200 from dense L-broth culture was made into G buffer supplemented with the required amino acids, 0.1% arabinose, and 0.2% fucose. Inducible strains would not grow overnight under these conditions; constitutive strains would.

**Arabinose isomerase (EC 5.3.1.4) assay.** The procedure used was that of Schleif (15), but the toluenized cultures were incubated for 5 min at 28 C rather than at 37 C. In addition, the 20% arabinose was steam sterilized, which resulted in exceptionally high blank and basal values due to overflow of arabinose during sterilization (R. Schleif, personal communication). Units were calculated according to the following formula to facilitate comparison with Schleif’s data: $2 \times 10^{14} \times$ (optical density at 350 nm)/5 min x (number of cells).

**RESULTS**

**Isolation of the TDG-resistant mutant.** Since the desired mutant was expected to be able to grow on low concentrations of lactose in the presence of TDG, tubes containing such medium were inoculated with mutagenized AM7 as described above. After 6 days, one of the inoculum tubes was turbid. Following platting of this culture, isolated colonies were tested for their ability to concentrate $[^{14}C]$TMG in the presence of 10 mM TDG (Table 2).

One of these mutant colonies, AM37, grew slowly on normal concentrations of lactose with or without TDG. Doubling time at 37 C in G-lactose plus histidine, arginine, and thiamine was about 8 h, and there was an 8-h lag before growth ensued unless the culture was pre-induced with IPTG. (Doubling time of the wild-type AM5 under the same conditions was 75 min, with less than a 30-min lag.)

**Characterization of the TMG transport activity: (i) induction.** TMG uptake and $\beta$-galactosidase levels of induced and uninhibited cultures of AM5 and AM37 are compared in Table 3. In the case of AM5, the levels of lactose

<table>
<thead>
<tr>
<th>Strain</th>
<th>Addition</th>
<th>$[^{14}C]$TMG accumulated (counts/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM5</td>
<td>5 ml of buffer</td>
<td>14,000</td>
</tr>
<tr>
<td></td>
<td>5 ml of 1 M TDG</td>
<td>150</td>
</tr>
<tr>
<td>AM7</td>
<td>5 ml of buffer</td>
<td>150</td>
</tr>
<tr>
<td>AM37</td>
<td>5 ml of buffer</td>
<td>7,000</td>
</tr>
<tr>
<td></td>
<td>5 ml of 1 M TDG</td>
<td>7,200</td>
</tr>
</tbody>
</table>

* Bacteria were grown at least six doublings in G-glycerol plus histidine, arginine, and thiamine and $5 \times 10^{-4}$ M IPTG. They were filtered, washed, and assayed as described. TDG final concentration was 10 mM. Counts represent an average of at least two separate uptakes. Values were corrected for background and normalized to standard cell density, optical density at 350 nm = 1.0 (14,000 counts/min represents 2.800 $\mu$mol per g [dry weight]).

<table>
<thead>
<tr>
<th>Induction</th>
<th>Strain</th>
<th>$[^{14}C]$TMG accumulated (counts/min)</th>
<th>$\beta$-Galactosidase (mM per ml per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>TDG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Induced</td>
<td>AM5</td>
<td>15,000 &lt;250</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>Uninduced</td>
<td>AM5</td>
<td>&lt;250 &lt;250</td>
</tr>
<tr>
<td>Induced</td>
<td>AM37</td>
<td>6,200 6,500</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>Uninduced</td>
<td>AM37</td>
<td>5,900 6,500</td>
</tr>
<tr>
<td>Induced</td>
<td>AM37R</td>
<td>15,200 6,300</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>Uninduced</td>
<td>AM37R</td>
<td>8,100 6,100</td>
</tr>
</tbody>
</table>

* Growth and TMG uptake were done as described in Table 2, except that uninhibited cultures had no IPTG added. $\beta$-galactosidase was assayed on the same cultures used to measure uptake.

permease and $\beta$-galactosidase were coordinate, whereas in AM37, TMG permease activity was not induced by IPTG under conditions where $\beta$-galactosidase activity was, suggesting that transport in AM37 may be by some other permease.

That TMG transport in AM37 was not via the lactose permease is supported by studies of AM37R, a revertant of AM37 selected for faster growth on lactose. AM37R behaved as if the normal lactose permease activity was superimposed on that of AM37. In uninhibited cultures, only the TDG-uninhibited component of the TMG transport was seen, whereas the uptake characteristic of the y gene product was seen when the assay was done on induced cells (in the absence of TDG).

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**Table 2. $[^{14}C]$TMG uptake with and without 10 mM TDG**

**Table 3. Uptake of $[^{14}C]$TMG by induced and uninhibited cultures**
(ii) Inhibition of TMG uptake. Various inhibitors of TMG uptake by strain AM37 are shown in Table 4. Maximal inhibition was obtained either with substances most resembling TMG or arabinose, or with known competitors of the arabinose permease.

Induction of the arabinose transport system in AM37. If the arabinose permease was responsible for the lactose and TMG permeation properties of AM37, the presence of the permease in the absence of any of its known inducers must be accounted for. A possibility tested below was that AM37 formed the arabinose permease constitutively.

(i) Growth tests. The possibility that AM37 was constitutive for the arabinose enzymes was tested by comparing growth on G-arabinose plus fucose of AM37 with that of its parent, AM7. Fucose is an anti-inducer of the arabinose system; therefore, only strains which synthesize the arabinose enzymes constitutively can grow on arabinose in the presence of fucose (5). AM37 had a doubling time of 70 min; AM7 showed no growth after 15 h.

(ii) Arabinose isomerase assay. AM37 had over four times the arabinose isomerase activity of AM7 when the strains were grown on glycerol, but about the same enzyme level when both strains were grown on arabinose. This difference for uninduced conditions is much smaller than normal, and seems to be due to very high values for basal levels as noted above. Despite the lower temperatures used here, AM37 showed 800 U of isomerase activity after growth on glycerol, which is among the highest values seen by Greenblatt and Schleif (8; personal communication) in a series of assays of inducible and constitutive strains. Their basal level, however, was 5 to 7 U, whereas AM7 gave almost 200 U.

Several other uninduced (araC*) strains tested, as well as RFS 3 (which is isomerase-negative), also gave values in the range of 100 to 200 U, suggesting that the problem was in the assay rather than the strains.

Throughout this study, strains which appeared constitutive by the growth test on arabinose plus fucose (and which took up TMG constitutively) showed isomerase levels of three to five times those of inducible strains when all were grown without inducer. The strains included those arising spontaneously, after N'-methyl-N' -nitro-N-nitrosoguanidine or ethyl methane sulfonate mutagenesis and after transductions. The consistency of the correlations among strains with a variety of histories plus the high level of isomerase comparable to those noted in Greenblatt and Schleif (8) suggest that the low ratios seen are characteristic of full constitutives under these assay conditions.

(iii) Induction of TMG uptake by arabinose. When AM5 (y+) was grown on arabinose, only a small amount of TMG uptake could be observed. A portion (10 to 20%) of this uptake was not inhibitable by TDG (data not shown).

Co-transduction with araC and araA: (i) araC. To test the likely hypothesis that the constitutive synthesis of the arabinose enzymes by AM37 is the result of a mutation at the locus (C) known to produce constitutivity for the arabinose operon, genetic studies were carried out. A P1 stock made from AM37 was used to transduce strain RFS 162 (F* C*/Δlac Δara) to the ability to utilize arabinose. Since the C gene product was required for synthesis of arabinose utilization enzymes, RFS 162 would not grow on arabinose. Arabinose-positive transductants were tested for constitutivity for the arabinose system and for TMG uptake. Of twenty tested, seven were neither constitutive nor able to take up TMG (presumably having mutated to C*). All of the others were C*, being constitutive and, with TMG uptake, inhibited by arabinose but not by TDG. It appears, therefore, that AM37 TMG permease activity is closely linked to araC.

(ii) araA. To see whether the TMG permease activity in AM37 was co-transduced with the arabinose isomerase gene (araA), strain RFS 3 (araA*) was transduced with P1 grown in AM37. Arabinose-positive transductants were tested for TMG uptake and constitutively for the arabinose system. All 20 transductants tested were presumably A*, since they were able to grow on arabinose at wild-type rates.

<table>
<thead>
<tr>
<th>Table 4. Inhibitors of TMG uptake in AM37*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibitor</td>
</tr>
<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>L-Arabinose</td>
</tr>
<tr>
<td>Fucose</td>
</tr>
<tr>
<td>TMG</td>
</tr>
<tr>
<td>Galactose</td>
</tr>
<tr>
<td>Lactose</td>
</tr>
<tr>
<td>Xylose</td>
</tr>
<tr>
<td>Glucose</td>
</tr>
<tr>
<td>Phenylgalactoside</td>
</tr>
<tr>
<td>Malrose</td>
</tr>
<tr>
<td>IPTG</td>
</tr>
<tr>
<td>Melibiose</td>
</tr>
<tr>
<td>TDG</td>
</tr>
<tr>
<td>Fructose</td>
</tr>
</tbody>
</table>

* Growth and uptake were the same as in Table 2, except without IPTG. Each percentage is an average of at least two determinations. TMG final concentration approximately 10⁻⁴ M.
Nine were also arabinose constitutives. All nine constitutives but none of the eleven inducible strains could transport TMG. Again the transport was inhibited by arabinose but not by TDG. These data confirm that the mutation in AM37 was closely linked to araC, and that it was closer to araC than araA (Fig. 1).

Tests of other arabinose constitutives. To see whether the mutation to constitutivity for the arabinose system was a sufficient condition to produce uptake of TMG, constitutive mutants were isolated from the parental strain AM7. By using an ethyl methane sulfonate-mutagenized culture, mutants were selected for their ability to grow on arabinose in the presence of fucose, a property of constitutive mutants.

Of 22 independent colonies isolated following ethyl methane sulfonate mutagenesis and selection of arabinose-fucose plates, all could grow in arabinose plus fucose, but only 21 were able to accumulate TMG. The remaining one turned out to be fucose-inducible rather than a true constitutive (8).

All of the 10 spontaneous mutants isolated likewise grew on arabinose plus fucose and all took up TMG.

Test of starved cultures. Since most arabinose constitutives seemed to be able to take up TMG, it seemed possible that only the presence of residual arabinose in bacteria induced by the sugar prevented TMG uptake in inducible strains (as suggested by Schleif [15]). This was tested by comparing TMG uptake in starved and unstarved arabinose-grown cultures of both constitutive and inducible strains (Table 5). Cultures growing exponentially on arabinose were filtered and washed without prior starvation. When these were tested, neither the constitutive nor the inducible strain took up significant amounts of TMG. After starvation, AM7 (inducible) accumulated TMG to exactly the same level as AM37 (constitutive). If the strains were grown on glycerol and then starved, only the constitutive could take up TMG.

Affinity of the arabinose permeation system for TMG. The rate of TMG uptake as a function of TMG concentration fits Michaelis-Menten kinetics (Fig. 2). Cultures were grown in G medium with histidine, arginine, and thiamine. From these data, the affinity of the arabinose permeation system for TMG was $5.6 \times 10^{-4}$ M at 37 C and $5.8 \times 10^{-8}$ M at 28 C. Schleif (15) measured the $K_m$ of the system for arabinose itself, using both a strain constitutive for synthesis of the arabinose enzymes and one which required induction. Both had no arabinose isomerase so that arabinose, once it was transported, could not be metabolized. The inducible strain showed first-order kinetics of uptake with a $K_m$ of $5 \times 10^{-8}$ M at 37 C. The constitutive strain, however, did not exhibit first-order kinetics of arabinose uptake, and two $K_m$ values measured at 37 C were $5 \times 10^{-8}$ M and $3 \times 10^{-4}$ M. Thus, the $K_m$ for arabinose was at least 10-fold lower than that for TMG.

Osmotic shock. The transport of lactose via the y gene product was unaffected by prior osmotic shock to the cells, whereas transport of $\alpha$MG via the phosphotransferase system, which contains a shockable component, is reported to be reduced (11). Since Schleif reported a shockable binding protein related to arabinose transport (15), cultures of AM5 and AM37 were tested for both $\alpha$MG and TMG uptake before and after osmotic shock. Although the reduction in $\alpha$MG uptake after osmotic shock was comparable in the two cultures (70% for AM5; 65% for AM37), there was less than a 20% reduction of AM5 TMG counts compared to a 65% reduction of AM37 counts. This implies that there was a component released by osmotic shock which was necessary for the TMG transport via the arabinose system and not for TMG transport via the lactose permease.

DISCUSSION

The major conclusion to be drawn from this work is that lactose and some of its analogs can be transported via the arabinose transport system. The transport was found in 24 independently isolated strains in which the arabinose enzymes are synthesized constitutively, and in strains which were induced with arabinose and then starved to remove all traces of it.

There seem to be many transport systems for...
galactosides in E. coli. Rotman et al. (14) have reported four separate galactose and galactoside transport systems in E. coli K-12 with a wide range of substrate and inducer specificities. Two of the permeases (MG and galactose) do not transport TMG or lactose, and require fucose for induction. The two TMG permeases both transport TDG and require either IPTG (TMG I) or galactose (TMG II) for induction. Thus, all of these differ in at least two separate characteristics of substrate or inducer from the activity described here and would, therefore, require two mutations to account for the observed transport. Such a double mutant could hardly have occurred as frequently as was observed here, with TMG-transporting strains selected spontaneously and after ethyl methane sulfonate or N-methyl-N'-nitro-N-nitrosoguanidine mutagenesis. Singer and Englesberg (16) observed arabinose transport via what appeared to be the MG permease in late log phase E. coli B/r. Unlike the E. coli K-12 MG permease, this one is not inducible by fucose, and it is expressed in an araC- mutant. The TMG transport activity described here was fully controlled by the C gene, and, as noted earlier, the K-12 MG permease (if the substrate part of the analogy between the two strains was more valid than the inducer comparison for this permease) did not transport TMG or lactose.

Brown and Hogg (2) report two very distinct arabinose transport systems in E. coli B/r. The high-affinity system (K_m = 8.3 × 10^{-4} M) is distinguished from the low-affinity system (K_m = 1.0 × 10^{-4} M) by its inhibition by galactose and its many similarities to the arabinose binding protein. The TMG uptake is inhibited >95% by galactose and is sensitive to osmotic shock (a characteristic of transport mediated via a binding protein). This suggests that the high-affinity permease was responsible for most of the TMG uptake described here.

The observation that constitutive and inducible strains for the arabinose system will not transport TMG after growth on arabinose followed by washing of the cells, but will transport TMG after growth followed by starvation, supports but does not prove the hypothesis that the cells retain small amounts of arabinose that can interfere with transport measurements. Since an uninduced strain grown in the absence of arabinose cannot gain the capacity to transport TMG by means of starvation, the starvation process itself is not sufficient to establish transport. That starvation specifically eliminates arabinose, which is otherwise present in cells grown on it, could be tested by measuring the exit rate of arabinose from unstarved cells during dilution and washing. (The exit experiments reported by Novotny and Englesberg [13], which measured arabinose exit in E. coli B/r, are probably not applicable since their strain seems to contain only one of the two arabinose transport systems.)

The above can be generalized to note that testing the ability of an inducer for a transport system must be done under conditions which will prevent competition of a low-affinity substrate by the inducer being tested.

From the observation that lactose can be transported via the arabinose permeation system, it follows that any further attempts to isolate a mutant of the lactose permease with altered substrate specificity must be made by using a strain with a nonreverting C^- mutation.

ACKNOWLEDGMENTS

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LITERATURE CITED


