Induction of the Galactose Enzymes in *Escherichia coli* Is Independent of the C-1-Hydroxy Optica Configuration of the Inducer \(d\)-Galactose 

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The two optical forms of aldohexose galactose differing at the C-1 position, \(\alpha\)-\(d\)-galactose and \(\beta\)-\(d\)-galactose, are widespread in nature. The two anomers also occur in di- and polysaccharides, as well as in glycoconjugates. The anomic form of \(d\)-galactose, when present in complex carbohydrates, e.g., cell wall, glycoproteins, and glycolipids, is specific. Their interconversion occurs as monomers and is effected by the enzyme mutarotase (aldose-1-epimerase). Mutarotase and other \(d\)-galactose-metabolizing enzymes are coded by genes that constitute an operon in *Escherichia coli*. The operon is repressed by the repressor GalR and induced by \(d\)-galactose. Since, depending on the carbon source during growth, the cell can make only one of the two anomers of \(d\)-galactose, the cell must also convert one anomer to the other for use in specific biosynthetic pathways. Thus, it is imperative that induction of the *gal* operon, specifically the mutarotase, be achievable by either anomer of \(d\)-galactose. Here we report in vivo and in vitro experiments showing that both \(\alpha\)-\(d\)-galactose and \(\beta\)-\(d\)-galactose are capable of inducing transcription of the *gal* operon with equal efficiency and kinetics. Whereas all substitutions at the C-1 position in the \(\alpha\) configuration inactivate the induction capacity of the sugar, the effect of substitutions in the \(\beta\) configuration varies depending upon the nature of the substitution; methyl and phenyl derivatives induce weakly, but the glucosyl derivative does not.

\textbf{MATERIALS AND METHODS}

\(\alpha\)-\(d\)-Galactose, \(\beta\)-\(d\)-galactose, \(d\)-galactosides, and other sugars, \(\alpha\) and \(\beta\) anomers of \(d\)-galactopyranoside were purchased from Omicron Biochemicals, Inc. (South Bend, IN). Methyl-\(d\)-\(d\)-galactopyranoside (catalog no. 66916), methyl-\(d\)-\(d\)-galactopyranoside (catalog no. M0265), \(d\)-\(d\)-galactose-1-phosphate (catalog no. G0380), UDP-\(d\)-\(d\)-galactose (catalog no. U4500), UDP-\(d\)-\(d\)-glucose (catalog no. 94335), \(d\)-melibiose (catalog no. M269-1), \(d\)-fructose (catalog no. 47880), 2-deoxy-\(d\)-galactose (catalog no. D-4407), and 2-nitrophyll-\(d\)-glucuronic acid (catalog no. N1627) were from Sigma-Aldrich Corp. (St. Louis, MO). Lactose (catalog no. L-107) and \(d\)-galactose (catalog no. G-106) were purchased from PharmAid Laboratory, Inc. (Waukegan, IL). Phenyl-\(d\)-\(d\)-galactopyranoside (catalog no. MP29290801) and phenyl-\(d\)-\(d\)-galactopyranoside (catalog no. MP30290801) were purchased from Carboxynth, Ltd. (Berkshire, United Kingdom).

\textbf{Bacteria, phage, and plasmids.} The *E. coli* strains, plasmids, and plasmids used in this study are listed in Table 1. *E. coli* strains with individual *gall*, *galK*, and *galM* open reading frame deletions were obtained from the Keio Collection (2). The promoter regions of the *gall* gene (−115 to +48 from the start codon) were amplified by PCR from plasmid pSL107 and pSL107 with an EcoRI-containing primer at the 5′ end and an NcoI-containing primer at the 3′ end (Fig. 2A). The fragments were cloned into EcoRI and NcoI sites in the backbone of pI24, pSA809, and pSA810 with an EcoRI-containing primer at the 5′ end and an NcoI-containing primer at the 3′ end (Fig. 2A). The fragments were cloned into EcoRI and NcoI sites in the backbone of pSA809 to make a translational fusion of the *gall* segment containing \(\Omega\) and the entire *gusA* gene (Fig. 2A), generating pSL305, pSL306, and pSL307, respectively. In addition, the upstream region (−1540 to −939) of *gusA* and a chloramphenicol marker (+699 to −147 region of the cat gene from plasmid pACYC184 (New England Biolabs, Inc., Beverly, MA) were placed in the upstream region of the *gall* promoters of pSL305, pSL306, and pSL307 to make pSL310 (P2′ *P1′ *gusA*), pSL311 (P2′ *P1′ *gusA*), and pSL312 (P2′ *P1′ *gusA*), respectively (Fig. 2). The two PCR products were mixed, and PCRs were performed to amplify the *galE*-gusA segment containing chloramphenicol marker and homologous regions by using pSL310, pSL311, and pSL312 as templates. The DNA products were electrophoretically separated into *E. coli* BW25113 containing plasmid pKKD46 in which the lambda recombinase was highly expressed by lac-araabinose induction (13). Colonies, which were selected on agar plates containing chloramphenicol (10 \(\mu\)g/ml), were confirmed by chromosomal DNA sequencing and named *E. coli* SL310, SL311, and SL312, respectively (Table 1).

To make a precise deletion of the *galETF* segment, kanamycin resistance cassettes containing chloramphenicol marker and homologous regions by using pSL310, pSL311, and pSL312 as templates. The DNA products were electrophoretically separated into *E. coli* BW25113 containing plasmid pKKD46 in which the lambda recombinase was highly expressed by lac-araabinose induction (13). Colonies, which were selected on agar plates containing chloramphenicol (10 \(\mu\)g/ml), were confirmed by chromosomal DNA sequencing and named *E. coli* SL310, SL311, and SL312, respectively (Table 1).

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mixed, and overlapping PCR was performed. The final PCR products were electro-
products obtained by using chromosomes of JW0742 and JW0739 as templates were
same strategy was employed to make a deletion of the entire

E. coli
lambda recombinase was induced by L-arabinose, to make
Phage P1

FIG. 1. Generation of melR
pJW-4* pJW15 derivative carrying melR(Con) mutations (Y25D, F53Y, N183I, F191S) that is active in the

absence of melibiose; Ap’. 18

pKD46 Temperature-sensitive plasmid containing lambda recombinase inducible by L-arabinose; Ap’
13

pCP20 Temperature-sensitive plasmid having FLP recombinase capable of recognizing FRT sequence; Ap’
9

pSA934 gal P2’ P1 18
pSL1004 gal P2’ P1 18
pi24 pBR322 Δ(EcoRI-Pvull) gal’
S. Adhya
pSA809 p24 gal P2’ P1’ gusA’
22
pSA810 p24 gal P2’ P1’ gusA’
22
pSL310 gal P2’ P1’ gusA Cm’
This study
pSL312 gal P2’ P1’ gusA Cm’
This study

TABLE 1. Bacterial strains, phage, and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain, phage, or plasmid</th>
<th>Relevant genotype or characteristics</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli strains</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MG1655</td>
<td>F’ ihG rfb-50 rph-1</td>
<td>NIH stock</td>
</tr>
<tr>
<td>NM18</td>
<td>lacI::Tn10</td>
<td>26</td>
</tr>
<tr>
<td>BW25113</td>
<td>Δ(arad-arab)677 ∆lacZ4787::(rrnB-3) λ’ rph-1 Δ(rhaD-rhaB)568 hsdR514</td>
<td>13</td>
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<td>JW0742</td>
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<tr>
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<td>SL310</td>
<td>BW25113 gal P2’ P1’ gusA (Cm’)</td>
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</tr>
<tr>
<td>SL311</td>
<td>BW25113 gal P2’ P1’ gusA (Cm’)</td>
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<td>SL312</td>
<td>BW25113 gal P2’ P1’ gusA (Cm’)</td>
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<td>SL307</td>
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<td>This study</td>
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<tr>
<td>SL308</td>
<td>MG1655lacI::Tn10 gal P2’ P1’ gusA (Cm’)</td>
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</tr>
<tr>
<td>SL309</td>
<td>MG1655lacI::Tn10 gal P2’ P1’ gusA (Cm’)</td>
<td>This study</td>
</tr>
</tbody>
</table>

Plasmids

pJW-4* pJW15 derivative carrying melR(Con) mutations (Y25D, F53Y, N183I, F191S) that is active in the

absence of melibiose; Ap’. 18

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22
pSL310 gal P2’ P1’ gusA Cm’
This study
pSL312 gal P2’ P1’ gusA Cm’
This study

Phase P1 vir vir mutations

NIH stock
of gal transcripts (38). To compare the kinetics of inducibility of α- and β-D-galactose, supercoiled plasmid pDL1004 (P2* + P1*) (24) was preincubated at 37°C for 5 min in transcription buffer containing GalR (200 mM). Ten minutes after nucleotides were added, inducers (final concentration, 10 mM) dissolved in DMSO were added. Aliquots were terminated at 0 to 300 s by the addition of loading dye.

RESULTS

The gal operon in E. coli can be expressed from either the P1 or the P2 promoter (29). The promoters are repressed by GalR by two different mechanisms. Either (i) GalR represses P1 by inhibiting open complex formation at the promoter-bound RNA polymerase (33) or (ii) P2 is repressed by a DNA loop that makes the promoter inadequate for transcription initiation (10, 11). The presence of β-D-galactose inactivates GalR to release the repression in each case (25). In the following experiments, we attempted to identify the anomeric configuration (α or β) of D-galactose active as an inducer in vivo and in vitro.

In vivo induction of the gal operon. We constructed gal promoter fusions to a gusA reporter gene to assay the expression of the operon by measuring the level of β-D-glucuronidase activity in the cell (22). We used the wild-type P1* + P2* promoters, as well as two mutants in which one of the two promoters was mutated (P1" + P2" and P1* + P2*) (4). In the three fusions used, P1" + P2" gusA, P1* + P2" gusA, and P1" + P2* gusA, the gusA open reading frame was fused to the 16th codon of galE, the first codon of the gal operon. In essence, the promoter of the gusA gene (at 36.4 min) in the E. coli chromosome was replaced with the above three gal promoter variants by recombineering (13) (see Materials and Methods). We constructed two sets of such reporter fusions, one in which the wild-type gal operon at its normal chromosomal location (17 min) was deleted and another in which galE, but not gusM, was deleted. Expression of the lac operon was rendered constitutive by disruption of the lacI gene, which encodes the Lac repressor (12). The strains were also transformed with a plasmid carrying a mutant allele of the melR gene [melR(Con)] that allows constitutive expression of the mel operon (18). The strains generate β-D-galactose and α-D-galactose intracellularly in the presence of lactose and melibiose, respectively. The ability of the intracellularly generated β-D-galactose and β-D-galactose individually to induce the gal operon was assayed in vivo by assaying the β-D-glucuronidase level. The absence of the mutarotase gene was expected to prevent the interconversion of the β-D-galactose anomers intracellularly. Because the activity of water in cytosol is much less than 1.0, the spontaneous mutarotation in the cytosol is much slower than in an aqueous solution (6). The genes that encode enzymes of D-galactose metabolism, GalK, GalT, and GalE, were deleted to prevent the intracellular generation of any D-galactose by the basal level of galactose enzymes (27). The results are shown in Table 2. When the cells were grown in minimal medium containing succinate as a carbon source, the basal levels of gusA activity from the P1P2, P1, or P2 promoter in exponentially growing cells were very low, as expected. The addition of commercial D-galactose, which is a mixture of 28% α-D-galactose and 72% β-D-galactose, resulted in the synthesis of about 6 and 20 U of β-D-glucuronidase activity from the P2 and P1 promoters, respectively. The reason for lower P2 activity com-
pared to P1 activity is most likely that cells grown in succinate medium accumulate a high level of cyclic AMP (cAMP) (5). The CAMP receptor protein-cAMP complex is known to lower the level of P1 induction in the presence of lactose or melibiose (lane 10) and UDP-β-D-galactopyranoside (lane 3). The inducing activities of other derivatives of D-galactose on P1 transcription were lower than in the presence of the corresponding phenyl-D-galactoside derivatives, suggesting that the inhibitory effect on P1 is likely due to the generation of intracellular glucose.

**Induction in vitro.** We tested the capabilities of various D-galactose analogues to induce the gal operon in an in vitro transcription system. Our assay system consisted of supercoiled plasmid DNA carrying the gal P1 promoter which, when transcribed, produced gal RNA products of a discrete size (125 bp) because of the presence of a transcription termination signal located downstream of the promoter (23). The results are shown in Fig. 3. In the presence of GalR, about 90% of the transcription from P1 was repressed (lanes 1 and 2). The level of P1 transcription was restored by the presence of D-galactose, which lifted P1 repression completely (lane 3). The inducing abilities of other derivatives of D-galactose on P1 transcription are shown in lanes 4 to 14. Derivatives at the C-1 position of α-D-galactose, methyl-α-D-galactoside (lane 4), phenyl-α-D-galactoside (lane 6), and glucosyl-α-D-galactoside (melibiose) (lane 8), did not show any induction in vitro. Derivatives at the C-1 position of β-D-galactose showed mixed results; the methyl (lane 5) and phenyl (lane 7) derivatives showed partial induction, whereas the glucosyl derivative (lactose) (lane 9) did not induce P1. The two D-galactose metabolic intermediates α-D-galactose-1-phosphate (lane 10) and UDP-α-D-galactose (lane 11) did not show any induction, most likely because these

**TABLE 2. In vivo induction of the gal operon with a gusA reporter gene**

<table>
<thead>
<tr>
<th>Inducer</th>
<th>galETK</th>
<th>galM*</th>
<th>galETKM</th>
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<tbody>
<tr>
<td>None</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>β-Galactose</td>
<td>13.2 ± 0.2 (1.00)b</td>
<td>5.7 ± 0.5 (1.00)</td>
<td>21.0 ± 1.0 (1.00)</td>
</tr>
<tr>
<td>D-Lactose</td>
<td>4.5 ± 0.2 (0.34)</td>
<td>5.8 ± 0.9 (1.01)</td>
<td>5.7 ± 0.5 (0.27)</td>
</tr>
<tr>
<td>D-Melibiose</td>
<td>7.1 ± 0.7 (0.54)</td>
<td>6.3 ± 1.6 (1.09)</td>
<td>11.3 ± 0.9 (0.54)</td>
</tr>
<tr>
<td>β-PG(^{c})</td>
<td>16.6 ± 0.7 (1.26)</td>
<td>7.6 ± 0.7 (1.32)</td>
<td>22.3 ± 0.7 (1.06)</td>
</tr>
<tr>
<td>α-PG(^{d})</td>
<td>14.1 ± 1.6 (0.07)</td>
<td>3.7 ± 1.3 (0.65)</td>
<td>18.3 ± 0.6 (0.87)</td>
</tr>
</tbody>
</table>

\(^{a}\) Relevant gal promoters. Shown in parentheses is strain used for GusA assays.

\(^{b}\) The values shown are means ± standard deviations of GusA specific activity. Shown in parentheses is the relative GusA specific activity with the specific activity of each promoter induced by D-galactose set as 1.

\(^{c}\) β-PG, phenyl-β-D-galactoside.

\(^{d}\) α-PG, phenyl-α-D-galactoside.
derivatives of the α anomers at the C-1 position inactivate the inducing property of D-galactose, as was observed for the α derivatives above. UDP-α-D-glucose did not affect promoter repression (lane 12). Whereas 6-deoxy-D-galactose (D-fucose) induced transcription very well (lane 13), 2-deoxy-D-galactose induced transcription poorly (lane 14), suggesting that the C-2 position affects the induction process. The C-1 carbon in the latter two sugars is in equilibrium, as in D-galactose, between the α and β positions.

Since the D-galactose used above is a mixture of the α and β anomers, we also tested the inducibility of gal repression in vitro by using the purified α and β anomers of D-galactose. When the pure anomers are dissolved in water, spontaneous mutarotation starts immediately, with a rate of 0.028 min⁻¹ (21). This was prevented by dissolving the optically pure sugars in DMSO instead of water and immediately using them in an in vitro transcription reaction (3). It has been reported that the half-maximal concentration of D-galactose as an inducer is at least 2 mM in in vitro transcription assays (35). Two different concentrations of anomers of D-galactose (0.2 and 10 mM) were tested, and the reactions were carried out for 10 min. We reasoned that the slow spontaneous mutarotation of the pure anomers of D-galactose in the transcription buffer would not influence the induction process because it has been shown that D-galactose mutarotation takes at least several hours to reach equilibrium under aqueous conditions at room temperature (19, 21, 32). The results showed that both anemic varieties of D-galactose can lift repression well, at least at the higher concentration (Fig. 4).

We followed the kinetics of dissociation of GalR from a gal operator in the presence of optically pure anomers of D-galactose. Binding of GalR to O₁ alone causes a roadblock to transcription elongation from the gal promoter (24). We tested the effect of the galactose anomers on the transcription elongation block by GalR from the P₂ promoter kinetically (Fig. 5). In all cases, the elongation block disappeared with equal kinetics. The rates at which gal RNA was made relative to a control RNAI were 0.069 ± 0.013 min⁻¹ for α-D-galactose, 0.074 ± 0.008 min⁻¹ for β-D-galactose, and 0.072 ± 0.014 min⁻¹ for commercial D-galactose. GalR was inactivated by both anomers even within 1 min (Fig. 5). α- and β-D-galactose had 99.01 and 99.15% anemic purities, respectively (Fig. 6). The rate of spontaneous D-galactose mutarotation is 0.028 min⁻¹. The re-

![FIG. 4. In vitro derepression of the gal P₁ promoter by α- and β-D-galactose.](image)

![FIG. 5. Kinetics of dissociation of GalR from O₁ by α- and β-D-galactose.](image)

![FIG. 6. ¹H NMR spectra of α- and β-D-galactose.](image)
sults demonstrate that both anomers of D-galactose are equally efficient at dissociating GalR from DNA.

**DISCUSSION**

Since the sugar D-galactose can be generated intracellularly in either its α-anomeric or its β-anomeric form, the synthesis of the enzymes of D-galactose metabolism, including the mutarotase that interconverts them, should be available for specific incorporation of each anomer into complex carbohydrates. The expression of the gal operon, which encodes the D-galactose-metabolizing enzymes, is repressed by binding of GalR to its multipartite DNA binding sites in the operon (14, 16, 17). D-Galactose induces transcription of the operon by binding and inactivating the GalR protein (25). By studying the binding to GalR of D-galactose derivatives (methylated at the C-1 position) by fluorescence spectroscopy, it was shown previously that only methyl-β-D-galactose, and not the corresponding α derivative, binds to GalR (7). From these studies, the authors suggested that the β form of D-galactose is the actual inducer (7). Since α- or β-D-galactose can be generated exclusively within the cell by hydrolysis of a disaccharide, we propose that each anomer should be capable of inactivating GalR and inducing the operon to make enzymes of mutarotation and D-galactose metabolism. We attempted to identify whether one or both anomers of D-galactose can derepress gal transcription in vivo and in vitro.

Our in vivo results showed that both α- and β-D-galactose can induce gal transcription in vivo. The results were identical in galM+ and galM mutant cells. This conclusion was further confirmed by assaying the gal induction level in the above-mentioned cells grown in phenyl-α- or phenyl-β-galactoside, which generates α- and β-D-galactose and phenol, respectively. The two galactoside derivatives induced the gal operon with equal efficiency. Both promoters of the operon were inducible. As expected from in vivo results, both α- and β-D-galactose were able to inhibit GalR-mediated repression of P1. Both anomers inactivated the repressor efficiently and with equal kinetics. Interestingly, substitution at C-1 in the α configuration completely inactivated the ability of the sugar to remove repression whereas substitution in the β configuration showed about 80% reduction of the inducing capacity of the sugar. These results agree with the findings of Brown et al. (7), who showed that GalR binds to methyl-β-D-galactoside but not to methyl-α-galactoside. However, the affinity of the β anomer to GalR relative to free D-galactose is not known. We assume that, compared to free D-galactose, the affinity of the methyl-β-D-galactoside is much less and the ability of a galactose-derivative to derepress transcription reflects its affinity for GalR. Substitution at the C-1 position in the α configuration eliminates its binding affinity, while the effect of substitution in the β configuration depends on the nature of the substitution; the methyl or phenyl derivative retains some binding affinity, while the glucosyl derivative completely eliminates it. Incidentally, D-galactose metabolic intermediates, e.g., α-D-galactose-1-phosphate, UDP-α-D-galactose, and UDP-α-D-glucose fail to act as inducers. These results are expected, since both galactose-1-phosphate and UDP-galactose have substitutions at the C-1 position in the α configuration.

In nature, E. coli encounters α- or β-galactosides and generates only one of the two optical anomers of D-galactose. Since both anomers are needed for various biosynthetic reactions, intracellularly generated α- or β-D-galactose must be able to induce the gal regulon members, including the gal operon, to make mutarotase to convert one to the others. Consistently, our results show that this is indeed the case.

**ACKNOWLEDGMENTS**

We thank S. Busby for providing plasmid pJW-4* and B. Wanner for providing pKD46 and pCP20. We are also grateful to the Keio Collection for mutant strains. We thank M. Yarmolinsky for critical reading of the manuscript. We thank our colleagues in the laboratory for discussions and technical help.

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**REFERENCES**


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