Induction of the *ara* Operon of *Escherichia coli* B/r

M. ELLIN DOYLE, CANDICE BROWN, ROBERT W. HOGG, AND ROBERT B. HELLING

Department of Botany, University of Michigan, Ann Arbor, Michigan 48104, and Department of Microbiology, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106

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The inducer specificity and kinetics of induction of the *ara* operon were examined in *Escherichia coli* B/r. A difference in the kinetics of induction was found between our B/r strains and the K-12 strain previously described by Schleif. The roles of active transport and metabolism of inducer, and of cell density, in induction were studied. D-Fucose and β-methyl-L-arabinoside were competitive inhibitors of induction. No inducer of the wild-type strain other than L-arabinose was found. However, a procedure for selecting mutants with altered inducer affinity or specificity was developed. The properties of one such mutant (inducible by D-fucose) are described.

*Escherichia coli* can use the pentose sugar L-arabinose as its sole source of carbon and energy. The genes and enzymes specifically involved in the metabolism of this sugar have been extensively studied. [The arabinose (*ara*) genes and the pathway of arabinose catabolism are shown in Fig. 1.] These studies have been of considerable interest because it has become clear that control of arabinose-specific enzyme synthesis differs from that of such well-studied operons as *lac*, *gal*, and *glp*. In those operons, the product of the regulatory gene is a repressor which prevents enzyme synthesis. Induction results from the removal of that repressor. On the other hand, the product of the regulatory gene *araC* is required for synthesis of the arabinose enzymes. This was originally shown by complementation studies (18), but is most simply demonstrated by mutants in which part of the *araC* gene appears to be deleted (10, 19). Such mutants cannot form any of the *ara* enzymes even in the presence of the inducer L-arabinose, nor can they form the arabinose-specific active transport systems (see Fig. 1).

More recently, the following model has been proposed to explain control in this system (11, 12, 32). The *araC* product forms a repressor which prevents *ara* enzyme synthesis by binding to the operator O. In the presence of arabinose, the conformation of the repressor is altered so as to form an "activator" which must bind to the "initiator" I in order to initiate enzyme synthesis. Indirect evidence suggested that these controls affect transcription (4, 16), and recently it has been shown that *ara* messenger ribonucleic acid (mRNA) is absent in uninduced cells and present in induced cells (33). Although most of this work has been done with *E. coli* B/r, the results have been generally supported by the studies of Schleif using *E. coli* K-12 (15, 31). He has developed an in vitro system for the synthesis of L-ribulokinase (the product of the *araB* gene) which appears to be responsive to the controls operative in vivo (15).

Despite the substantial effort which has been made to understand the regulation of arabinose metabolism, no one has made a careful study of the inducer specificity of *ara* enzyme synthesis, nor has anyone made such a study of the kinetics of enzyme induction, in which the effects of active transport and of active metabolism of the inducer on the induction process have been demonstrated. [Schleif has shown the inducer concentration dependence of L-arabinose isomerase induction in a derivative of *E. coli* K-12 lacking both specific transport and the ability to catabolize arabinose (30), but no such studies have been reported with *E. coli* B/r.] Such studies are meaningful in terms of understanding the regulation of *ara* enzyme synthesis. An understanding of the induction kinetics and specificity has provided a starting point for the isolation of mutants with altered regulatory behavior, e.g., mutants which are induced at a lower concentration of arabinose than the wild type, or which are induced by sugars other than arabinose. The properties of one of these mutants are de-
scribed here.

MATERIALS AND METHODS

Strains. Derivatives of *E. coli* B/r are described in Table 1.

Media. Cells were grown in medium R plus 1% Casamino Acids (Difco). Medium R contains (per liter): K₂HPO₄, 7 g; KH₂PO₄, 3 g; (NH₄)₂SO₄, 1 g; MgSO₄·7H₂O, 0.05 g; and MnCl₂·4H₂O, 0.03 g. Mn⁺⁺ prevents inactivation of L-arabinose isomerase in vivo (27). Doubling time in this medium is about 32 to 34 min at cell densities below 3 x 10⁶/ml.

Chemicals. L-Ribulose was made and purified by paper chromatography (9). β-Methyl-L-arabinoside and α-methyl-L-arabinoside were synthesized according to standard procedures (5). Other compounds used were commercial preparations. D-Galactose and, in some cases, L-arabinose, were recrystallized from ethanol before use.

Chromatography. All compounds tested for induction were examined for the presence of impurities by paper and thin-layer chromatography. No lots used showed impurities, under conditions in which 2% contamination by D-glucose or L-arabinose would have been detected. Commercial preparations frequently contain contaminating glucose, and such lots were not used. The D-xylene was probably contaminated with about 0.5 to 1% L-arabinose as will be shown, but this was not detected chromatographically. The following solvent systems were used: (i) 1-butanol-acetic acid-water, 4:1:5; (ii) t-amyl alcohol-1-propanol-water, 4:1:1.5 (6); and (iii) 1-butanol-benzene-formic acid-water, 10:1.9:1:2.5 (13). Compounds were detected with alkaline triphenyltetrazolium chloride (9), aniline hydrogen phthalate (26), and periodate-benzidine (6).

L-Arabinose isomerase (EC 5.3.1.4) assay. A sensitive whole-cell assay was developed. Cells were harvested at culture densities below 3 x 10⁶ cells/ml by collection on 2.5-cm membrane filters (pore size, 0.45 μm) and were washed three times with 10-ml amounts of cold water. The washed filters were kept in plastic dishes on ice until assay. (No change in isomerase activity was detectable during a period of over 2 hr.) The filters were then rapidly agitated (with a Labline Super Mixer) in tubes (16 by 200

![Diagram](http://journals.asm.org/)

**Fig. 1.** L-Arabinose gene-enzyme complex. The *araE* (permease) gene is not linked to this region of the chromosome but, instead, maps at about 56 min (10).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Phenotype</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>UP 1664</td>
<td>araA2 araE1</td>
<td>Isomeraseless, permeaseless</td>
<td>Issacson and Englesberg (Bacteriol. Proc., p. 113–114, 1964); 19</td>
</tr>
<tr>
<td>BH11</td>
<td>araE1 leuB1</td>
<td>Permeaseless, leucine-requiring (Leu⁻)</td>
<td>Transduction: <em>araA</em>⁺ leuB1 into UP 1664</td>
</tr>
<tr>
<td>BH12</td>
<td>araE1 leuB1 xyl-1</td>
<td>As BH11; also unable to ferment D-xylene (Xyl⁻)</td>
<td>Mutagenesis of BH11 with nitroso-guanidine</td>
</tr>
<tr>
<td>BH13</td>
<td>araB23 araE1 xyl-1</td>
<td>Kinaseless, Xyl⁻, permeaseless</td>
<td>Transduction: <em>araB23</em> leuB⁺ into BH12</td>
</tr>
<tr>
<td>BH14</td>
<td>leuB1 xyl-1</td>
<td>Leu⁺, Xyl⁻</td>
<td>From BH12 by transduction</td>
</tr>
<tr>
<td>BH15</td>
<td>araB23 xyl-1</td>
<td>Kinaseless, Xyl⁻</td>
<td>Transduction: <em>araB23</em> leu⁺ into BH14</td>
</tr>
<tr>
<td>BH16</td>
<td>araA2 araE1 xyl-1</td>
<td>Isomeraseless, Xyl⁻, permeaseless</td>
<td>Transduction: <em>araA</em>2 leu⁺ into BH12</td>
</tr>
<tr>
<td>BH17</td>
<td>Dara-2 araE1 leuB1 xyl-1</td>
<td>As BH12 but faster growth on D-arabinose</td>
<td>Mutagenesis of BH12 with nitroso-guanidine</td>
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<tr>
<td>BH18</td>
<td>Dara-2 Dara-1 araE1 leuB1 xyl-1</td>
<td>As BH17 but deficient in D-ribulokinase</td>
<td>Mutagenesis of BH17 with nitroso-guanidine</td>
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<tr>
<td>BH19</td>
<td>Dara-2 Dara-1 araA2 araE1 xyl-1</td>
<td>As BH18 but isomeraseless, Leu⁺</td>
<td>Transduction, <em>araA</em>2 leu⁺ into BH18</td>
</tr>
<tr>
<td>BH20</td>
<td>Dara-2 Dara-1 araA2 araC9001 araE1 xyl-1</td>
<td>As BH19 but <em>ara</em> enzymes inducible by D-fucose</td>
<td>Mutagenesis of BH19 with nitroso-guanidine</td>
</tr>
<tr>
<td>BH21</td>
<td>Dara-2 Dara-1 araC9001 araE1 xyl-1</td>
<td>As BH20 but <em>araA</em>⁺</td>
<td>Transduction, araC9001, Leu⁺ into BH18</td>
</tr>
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</table>

*In addition, all strains lacked a second arabinose permease which has not yet been mapped but which is not linked to the other known *ara* genes by transduction (Brown and Hogg, Bacteriol. Proc., p. 126, 1971, and unpublished data).*
mm) containing three drops of toluene and 2 ml of warm reaction mixture (200 μmoles of glycolyl glycine, pH 7.6, 300 μmoles of L-arabinose, and 5 μmoles of MnCl₂ per 2 ml). The filters were pushed below the surface of the reaction mixture with a polyethylene rod; however, control experiments showed that at least 95% of the enzyme activity was in the reaction mixture and no longer associated with the filter. After 30 min (up to 60 min for very low activity) at 37 C, 2 ml of 0.2 N HCl was added to stop the reaction. The amount of ribulose produced was determined with 0.1- to 0.4-ml samples of the reaction mixture. One unit of L-arabinose isomerase is the amount of enzyme that will convert 1 μmole of L-arabinose to ribulose per hr.

Control experiments indicated that the specific activity determined by use of the whole-cell assay was about 65% of the specific activity of a supernatant solution obtained after centrifuging a sonically treated suspension of the same cells, but slightly more total activity was measured in the whole-cell assay. Isomerase specific activity is expressed as units of isomerase per milligram of total cellular protein.

1-Ribulose determination. The cysteine-carbazole procedure was used (8). D-Ribulose-o-nitrophenyl hydrazone (A grade, Calbiochem) was used as a standard with each set of assays; 0.01 μmole of the hydrazone gives a Klett reading of 22.3 with a 540 filter (25). The assay was highly sensitive and reproducible, and only a low background of color due to arabinose was seen if the following precautions were observed. (i) The reaction was carried out in a room or water bath at 25 C or less and cold 70% H₂SO₄ was used. (High temperature generated on addition of H₂SO₄ will increase background color from arabinose.) (ii) Each lot of H₂SO₄ was checked before use. Some impurities give unacceptably high blank readings.

1-Ribulokinase (EC 2.7.1.16) assay. The kinase was assayed by the method of Lee and Englesberg (22), with the use of sonic extracts. One unit of L-ribulokinase is the amount of enzyme that will convert 1 μmole of L-ribulose to L-ribulose-5-phosphate per hr. Kinase specific activity is expressed as units of kinase per milligram of protein extract.

Protein determination. Protein was determined by measuring the turbidity of the culture at the time samples were removed for whole-cell enzyme assay. With the growth conditions employed, 1 Klett unit (420 blue filter) corresponds to 1.28 μg of protein/ml as determined by the Folin phenol method (23). Protein was determined in extracts by the Folin phenol method (23).

Induction experiments. In all experiments, cells had been growing exponentially at a constant rate for at least two generations prior to addition of inducer. Compounds were tested as inducers at 0.02 M, and as inhibitors at 0.02 M in the presence of 0.01 M L-arabinose. Single-point measurements of enzyme were made after the cells had grown for 3 hr at 37 C in the presence of inducer, to a density of 2 x 10⁷ to 3 x 10⁹ cells/ml. Each datum in this paper represents the average of at least three independent experiments unless otherwise noted. Initial-rate measurements were made by determining enzyme activity in samples removed from a culture periodically after the addition of inducer. The cultures were diluted as necessary with warm medium to prevent the density from exceeding 3 x 10⁹ cells/ml. The slope of a plot of units of enzyme per milliliter versus milligrams of protein per milliliter gives units of enzyme per milligram of protein, or specific activity (see Fig. 2). Slopes were determined by the method of least squares. If rate of enzyme synthesis is constant, this value should equal that found by single-point measurement of fully induced cells.

Diffusion of arabinose. Cells were induced by growth in medium containing 0.02 M arabinose for 70 min. After they were washed twice, the cells were resuspended in warm medium and shaken for 10 min. L-Arabinose-1-¹⁴C was added. Samples (1 ml) were collected periodically on 2.5-cm membrane filters (pore size, 0.45 μm), and were washed twice with 2 ml of warm R salts. Certain samples were washed extensively with cold water to remove label in small-molecule pools.

The following results demonstrate that the diffusion rate is sufficient to nearly equalize the arabinose concentration on both sides of the cell membrane (after 30). Cells of strain BH12 lack the specific transport but are otherwise sufficient for arabinose metabolism. Since the uptake of arabinose depends on diffusion in this strain, the rate of incorporation of radioactive arabinose into the cell should provide a minimal estimate of the diffusion rate under these conditions. When such an experiment was performed, it was found that at an external concentration of 1.7 x 10⁻³ M, L-¹⁴C-arabinose was incorporated at a rate of 5 x 10⁻¹⁸ mole per cell per hr. Most of the label was in the macromolecular material. In contrast, when the same experiment was done with BH13 (kinaseless), label was taken up at about one-tenth to one-fifth of that rate, and almost all of the ´¹⁴C label could be washed from the cell with water.

With a 32-min doubling time, and assuming an internal volume of 10⁻¹³ cm³, 3.2 x 10⁻¹⁶ mole of arabinose per cell per hr must diffuse into the cell to maintain an internal concentration of 1.7 x 10⁻⁴ M. Since the measured rate of arabinose entry was greater than this, the intracellular and extracellular arabinose concentration must be nearly equal. A similar experiment, differing only in that the external concentration of arabinose was 10⁻³ M, showed that L-¹⁴C-arabinose was incorporated into BH12 at a rate of 1.2 x 10⁻¹⁷ mole per cell per hr, a rate much greater than that required to maintain an internal pool of arabinose at 10⁻⁴ M (1.7 x 10⁻¹⁸ mole per cell per hr).

Selection of a D-fucose-inducible mutant. Lee and Bendet have shown that L-ribulokinase can phosphorylate D-ribulose as well as L-ribulose (21). Thus, a cell lacking D-ribulokinase should still be able to metabolize D-arabinose (which is converted to D-ribulose), if L-ribulokinase is synthesized. D-arabinose is not an inducer of the ara genes, but, as expected, cells which constitutively synthesize the ara enzymes or which are induced by L-arabinose, can catabolize D-arabinose, even though deficient in D-
ribulokinase. This was first shown by N. Lee (unpublished data).

We have used d-ribulokinase-deficient cells to select for mutants able to grow on d-arabinose plus d-fucose but not on d-arabinose alone. As will be shown, the ara operon is induced by d-fucose in these mutants, thus providing the L-ribulokinase which allows metabolism of d-arabinose. In theory, this method can be used to select for mutants which are inducible by a very low concentration of L-arabinose, or by other compounds which normally do not induce (such as fucose).

The araC 9009 mutation was selected by plating cells of BH19 (Table 1) treated with the mutagen nitrosoguanidine (17) on solid R medium containing 10^{-2} M d-arabinose plus 10^{-4} M d-fucose. [BH19 cannot grow on d-arabinose because of the mutation Dara-1, and d-fucose is not metabolized by E. coli (1).] Mutant colonies were streaked to R medium containing glucose, and were tested by replica plating for growth on R plus d-arabinose with and without fucose. In the first experiment, 1 of 122 colonies picked grew on d-arabinose plus fucose but failed to grow on d-arabinose alone. The mutation has been shown to map very close to or within the araC gene, but unambiguous assignment to the araC gene deletions.

RESULTS

Induction kinetics. Induced synthesis of isomerase can be detected within 5 min after the addition of arabinose to BH13, a strain deficient in both L-ribulokinase and active transport of arabinose (Table 1), and proceeds at a constant rate of 51 units of enzyme synthesized per mg of total protein synthesized (Fig. 2). This is the maximal level of isomerase in the fully induced cell under our conditions (compare with Fig. 3). Synthesis ceases upon addition of d-fucose, a specific inhibitor of induction of the ara operon (as shown in this paper). The same effect is produced by removal of inducer.

The dependence of enzyme level on inducer concentration is shown in Fig. 3. The single-point measurements and initial-rate measurements are in agreement, which is consistent with a constant rate of enzyme synthesis (as already shown in Fig. 2). Half-maximal induction (Ka) is seen at an external arabinose concentration of about 6 \times 10^{-2} M. (Note that the experiments were done in mutant strains lacking active transport of arabinose.) What we would like to know, however, is the effective inducer concentration (within the cell).

In most of these experiments, the induction of the enzyme converting arabinose to ribulose was measured as an estimate of ara operon induction, because this enzyme was convenient to assay. The cells were therefore capable of converting the inducer (arabinose) to ribulose. However, this conversion results in only a small drain on the internal arabinose pool because an equilibrium mixture contains 80 to 90% arabinose (as inferred from 9 and 30; R. Helling, unpublished data), similar to the d-arabinose-d-ribulose ratio at equilibrium (7). (Ribulose could not be metabolized further because the cells contained the mutation araB23.)

To confirm that the internal conversion of arabinose to ribulose had relatively little effect on induction, the inducer dependence of kinase synthesis was measured in an araB+ strain (UP1664) unable to synthesize isomerase because of the mutation araA2, and otherwise
similar to the strain with which the previous experiments were done. It can be seen that kinase synthesis shows about the same dependence on inducer concentration as isomerase synthesis (Fig. 3). Because the diffusion rate is sufficient to nearly equalize the arabinose concentration on both sides of the cell membrane (see Materials and Methods), we conclude that $6 \times 10^{-3}$ M is a good estimate of the effective inducer concentration at half-maximal induction.

We also examined the role of the araE permease on induction in vivo, and the effect of constantly removing internal arabinose through metabolism (Fig. 4). Cells which can both transport and metabolize arabinose (BH14) show a constant rate of enzyme synthesis similar to that of cells lacking both permease and kinase, at high inducer concentration. The differential rate of isomerase synthesis is initially the same in cells which cannot transport arabinose actively but which can metabolize it (BH12). However, within less than one generation of growth, the differential rate decreases in such transportless cells, presumably because as the newly induced enzymes begin to catabolize arabinose the internal pool of arabinose can no longer be maintained at its initial level by diffusion.

The differential synthesis is also almost the same initially in cells which cannot metabolize arabinose (kinaseless), but which actively accumulate it (BH15). Again, within less than one generation the rate changes, this time increasing over twofold, concurrently with an increase in doubling time. Because the secondary rate is only seen in cells accumulating arabinose but unable to metabolize it beyond ribulose, its appearance depends on the formation of a very high internal pool of arabinose (see 20).

The inducer concentration dependence of induction in these strains is shown in Fig. 5. The ability to concentrate inducer (araE) allows enzyme induction at external inducer concentrations almost 100-fold lower than in cells lacking active transport.

All of the experiments had been done with cultures growing at densities below $3 \times 10^6$ cells/ml. We found that the growth rate decreased and the rate of isomerase synthesis increased at higher cell densities. This is demonstrated by the middle curve of Fig. 5. In this experiment, single-point measurements were made on cells of BH13 which were at much higher densities, and the displacement of the "standard" low-density curve upward is obvious. The differential rates were also higher in cells at higher densities, reflecting the decreased catabolite repression (20).

Schleif has shown the induction of isomerase as a function of inducer concentration in a kinaseless, permeaseless derivative of E. coli

![Fig. 3. Specific activity of L-arabinose isomerase and L-ribulokinase as a function of inducer concentration. Isomerase activities were measured in BH13 (lacks kinase and permease), and kinase activities were measured in BH16 (lacks isomerase and permease). Symbols: O, single-point measurements; Δ, initial rates of synthesis. I, isomerase; K, kinase.](image)

![Fig. 4. Initial rates of isomerase synthesis in strains with and without permease or kinase. Symbols: □, BH12 (lacks permease); Δ, BH14; O, BH15 (lacks kinase). Arabinose (0.05 μ) was added to induce enzyme synthesis.](image)
K-12 (30). Because his results differed somewhat from those we obtained with BH13 (slope steeper and displaced to the left when plotted as in Fig. 3), we compared the two strains directly. Using his conditions, we obtained the same results with his strain as he had reported ($K_m$ for isomerase induction about 10^{-4} M). Under the same conditions, isomerase induction in our strain (BH13) was quite different ($K_m$ about 6 × 10^{-2}), and essentially the same as shown in Fig. 3. (We could not examine induction in his strain under our conditions because it fails to grow on Casamino Acids as sole carbon and energy source.) We transduced the araB6 leu+ genes from his K-12 strain into BH12. Five independent transductants showed isomerase induction similar to BH13. Therefore, the difference in response of the two strains is not due to a mutation in the ara genes linked to leu+.

**Inducer specificity.** The following compounds failed to induce synthesis of L-arabinose isomerase when tested in strain BH13 at a concentration of 0.02 M (single-point measurement): L-arabitol, α-methyl-L-arabinose, β-methyl-L-arabinoside, L-xylose, L-rhamnose, D-fucose, D-galactose, D-glucose, D-arabinose, and L-lyxose. L-Ribulose failed to induce kinase synthesis in UP1664, a strain lacking isomerase.

D-Xylose (from several sources) induced a low but significant level of isomerase. The induction was probably due to contaminating L-arabinose in the xylose. This was shown by an experiment based on the following rationale. Strains which are xyl+ grow well on D-xylose regardless of whether they can concentrate arabinose (araE+ or araE), so at least the part of the arabinose transport system affected by the araE gene is not required for xylose transport. If the xylose-stimulated synthesis of isomerase was in fact due to xylose, then this stimulation should not depend on arabinose transport. On the other hand, if induction resulted from contaminating arabinose in the xylose, the induction should be much greater in a strain which concentrates arabinose (araE+) than in one which cannot (araEl). The latter was found to be the case (Table 2). Isomerase induction by the “xylose” was affected greatly by the ability of the cell to transport arabinose. Also, strains blocked in further metabolism of arabinose were more inducible by the “xylose,” as expected if induction resulted from contaminating arabinose because the internal arabinose pool should be higher in such strains. If xylose was actually an inducer, strains differing in ability to metabolize internal arabinose should not differ in their inducibility by xylose. We therefore concluded that D-xylose does not induce the ara operon.

The following compounds (at a concentration of 0.02 M) failed to inhibit induction by arabinose at 0.01 M (single-point measure-
ment): L-arabitol, α-methyl-L-arabinoside, D-xylose, D-xylose, D-ribulose, and L-lyxose.

D-Fucose inhibited isomerase synthesis markedly. The inhibition appears to be competitive with arabinose (Fig. 6 and 7). Beverin, Sheppard, and Park (3) described the apparent competitive inhibition of ara operon induction by fucose, and others have noted inhibition of induction by fucose in E. coli (10) and Salmonella typhimurium (4). However, it was not possible in their studies to rule out the possibility that the inhibition of induction was an indirect result of inhibition of the active transport of arabinose. All pertinent studies reported here were done with mutant strains which could not actively transport arabinose, thus eliminating this possibility. Fucose has now been shown to inhibit ara enzyme synthesis in vitro (15), which is direct proof that the fucose inhibition of enzyme induction is completely distinct from any effect it may have on arabinose transport.

β-Methyl-L-arabinoside also inhibited isomerase induction in a manner which appeared to be competitive with arabinose (Fig. 8). The weak inhibition observed with D-galactose (tested with a Gal− derivative of BH13) appeared to be competitive with arabinose, but because of variability in the extent of inhibition, and because of growth inhibition by galactose, the results are not shown.

Fucose-inducible mutant. We have developed a method to select for mutants in which the ara operon can be induced by compounds other than arabinose (Materials and Methods). The induction of the ara operon in one such mutant, BH21, by arabinose and by D-fucose, is shown in Fig. 9. This mutant appears to be normally inducible by arabinose. However, it is now inducible by fucose as well, instead of being inhibited. We cannot compare the relative affinities of arabinose and fucose for the inducing site (presumably araC protein) because we do not know the internal fucose concentration. Fucose is transported into the cell by the methylgalactoside permease which it induces (14, 28), so its internal concentration is higher than the external concentration plotted in Fig. 9 (half-maximal induction near 10−4 M), and therefore the affinities of arabinose and fucose for araC protein may be nearly the same. We can compare these results with those of Beverin, Sheppard, and Park (3), who studied an araC constitutive mutant selected for growth on arabinose plus fucose and found

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<th>Strain</th>
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<td>BH15</td>
<td>Kinase</td>
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</tr>
<tr>
<td>BH14</td>
<td></td>
<td>10.8</td>
</tr>
<tr>
<td>BH13</td>
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<tr>
<td>BH12</td>
<td>Permease</td>
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* Isomerase produced after 2.5 hr of induction with 0.03 M D-xylose.

Fig. 6. Double reciprocal plot showing d-fucose inhibition of isomerase synthesis in BH13. Specific activity of isomerase determined by initial-rate measurements. Symbols: △, no fucose; ○, 9 x 10−4 M fucose; ●, 9 x 10−4 M fucose; ×, 5 x 10−4 M fucose.

Fig. 7. Inhibition of isomerase synthesis by d-fucose. Symbols: ○, 10−4 M fucose; ●, 2 x 10−4 M fucose; △, 4 x 10−4 M fucose; , 10−5 M fucose; , 5 x 10−5 M fucose. Curve at left shows induction in the absence of fucose (from Fig. 9). Points are from single-point measurements.
to be further inducible by both arabinose and fucose. Their data show that half-maximal induction with either inducer occurred at an external concentration of about $5 \times 10^{-3} \text{ M}$. Because their strain actively transported arabinose [and fucose which is a substrate for the arabinose permease as well as the methylgalactoside permease (24)], the internal concentrations must have been still higher, suggesting that the affinity of the araC protein for both arabinose and fucose in their mutant strain was lower than in the araC$^+$ parent strain.

**DISCUSSION**

In many respects, the induction of the ara operon resembles induction of the lac operon, the gal operon, or the gfp operon, all of which are subject to negative control. Enzyme appearance at a constant rate within a few minutes after addition of inducer. After removal of inducer, enzyme synthesis ceases, probably because no new ara mRNA is formed, and ara mRNA already formed is gradually degraded.

We found the $K_m$ for induction of the ara operon in our derivatives of *E. coli* B/r to be about $6 \times 10^{-3} \text{ M}$. In contrast, Schleif found it to be $10^{-4} \text{ M}$ in his K-12 strain; we have repeated his work with the same results, and have shown that the difference is not in the ara operon itself. Recently, Greenblatt and Schleif found the $K_m$ for in vitro synthesis of kinase to be $10^{-3} \text{ M}$ (15). If $10^{-3} \text{ M}$ is the true arabinose concentration required for half-maximal induction of the ara operon, it is possible that his strain still retains some ability to concentrate arabinose, although he was not able to detect it (29, 30), and that in our strain diffusion really is rate-limiting. Many other explanations (e.g., a difference between the RNA polymerases of the two strains) are also possible for the strain difference (see 15), and it is quite possible that the $K_m$ measured in vitro may not be the same as the true $K_m$ in vivo.

We have shown that the induction of the ara operon is highly specific. No compounds other than arabinose itself were found which could induce the operon in the standard strain, although we were able to select an araC mutant which had become inducible by D-fucose. The site at which arabinose and fucose bind (and possibly β-methyl-L-arabinoside and D-galactoside as well) is almost certainly on the araC protein. This follows from the observation that the araC gene product acts through the cytoplasm and not solely on the ara genes cis to it (10, 18). Mutants of the araC gene can be obtained which either can no longer synthesize any of the ara enzymes (Ara') or exhibit no inducer requirement for induction (constitutive; 10). Despite an intensive search, no other ara regulatory genes which act in the trans position have been found (11). Thus, arabinose probably interacts directly with araC protein.

The wild-type *E. coli* fails to grow on arabinose in the presence of fucose, but mutants have been isolated which do grow on arabinose in the presence of fucose. These mutants result from araC mutations, and are constitutive for the ara enzymes (10) or inducible by fucose (3). However, not all araC constitutive mutants are insensitive to fucose inhibition (*unpublished data*). Since some, but not all, araC constitutive mutants are resistant to the inhibition by fucose, and some araC mutants are inducible by fucose, we conclude that fucose interacts directly with the araC protein. Furthermore, it
probably binds to the same site as arabinose because it shows essentially competitive inhibition of induction and is sterically very similar to arabinose (Fig. 10).

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