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Natural and Altered Induction of the L-Fucose Catabolic Enzymes in *Klebsiella aerogenes*

E. J. ST. MARTIN[1]* AND R. P. MORTLOCK

Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01002

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Mutants of *Klebsiella aerogenes* W70 were isolated that had gained the ability to utilize the uncommon pentose D-arabinose as their sole source of carbon and energy. In contrast to the D-arabinose-negative, parent strain, these mutants were found to be either constitutive for certain enzymes of the L-fucose catabolic pathway or inducible for such enzymes when incubated in the presence of D-arabinose. The mutants used L-fucose isomerase to convert D-arabinose to D-ribulose, which is an intermediate and inducer of the ribitol catabolic pathway. The D-ribulokinase of the ribitol pathway was then induced. This enzyme catalyzed the phosphorylation of D-ribulose at the 5-carbon position. Mutants that were negative for D-ribulokinase could still dissimilate D-arabinose slowly by using all three enzymes, the isomerase, kinase, and aldolase, of the L-fucose pathway. Using conditional negative mutants, we were able to demonstrate that the natural induction of the L-fucose pathway enzymes by L-fucose required the activity of a functional L-fucose isomerase and a functional L-fuculokinase but not an L-fuculose-1-phosphate aldolase. A metabolic intermediate, L-fuculose-1-phosphate, was thereby shown to be a probable inducer of at least the isomerase and kinase of the L-fucose catabolic pathway. Similar experiments, with D-arabinose-positive mutants, which were induced for the L-fucose pathway enzymes upon incubation with D-arabinose, revealed that the activities of the L-fucose isomerase and the L-fuculokinase were also required for the induction of the L-fucose enzymes. These D-arabinose-positive mutants apparently produced an altered regulatory protein that accepted both L-fuculose-1-phosphate and D-ribulose-1-phosphate as inducers. Examination of constitutive mutants revealed that L-fucose isomerase and L-fuculokinase were both synthesized constitutively, with the aldolase apparently under separate control.

Previous studies with *Klebsiella aerogenes* PRLR3 and *Escherichia coli* K-12 have shown that, although the wild-type strains were incapable of utilizing the uncommon pentose D-arabinose as sole source of carbon and energy, mutants capable of growth on D-arabinose could be isolated (8, 10). Both *K. aerogenes* PRLR3 and *E. coli* K-12 gained the ability to metabolize D-arabinose by virtue of control mutations that permitted, in the first case, the constitutive synthesis of enzymes in the L-fucose pathway and, in the second case, the induction of these enzymes in the presence of D-arabinose.

Three inducible enzymes are utilized for the catabolism of L-fucose to common metabolic intermediates (Fig. 1). L-Fucose isomerase (EC 5.3.1.3) converts L-fucose to L-fuculose. L-Fuculokinase (EC 2.7.1.51) phosphorylates L-fuculose at the 1-carbon position to yield L-fuculose-1-phosphate, which is then cleaved by L-fuculose-1-phosphate aldolase (EC 4.1.2.17) into dihydroxyacetone phosphate and L-lactaldehyde.

The *K. aerogenes* PRLR3 mutants were capable of using a constitutively produced L-fucose isomerase to isomerize the structurally similar D-arabinose molecule to D-ribulose. D-Ribulose is an intermediate and the inducer of the ribitol catabolic pathway of *K. aerogenes* and is phosphorylated by D-ribulokinase (EC 2.7.1.47) at the 5-carbon position to yield D-ribulose-5-phosphate. D-Ribulose-5-phosphate is then epimerized by D-ribulose-5-phosphate epimerase (EC 5.1.3.1) to D-xylulose-5-phosphate, a key intermediate in pentose metabolism (3).

The *E. coli* K-12 D-arabinose-positive mutants had become capable of inducing L-fucose isomerase when grown in the presence of D-arabinose and also used this isomerase to convert D-arabinose to D-ribulose. Since *E. coli* K-12 did not have a ribitol catabolic pathway, it did not possess a D-ribulokinase to convert D-

[1] Present address: Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Mass. 02115.

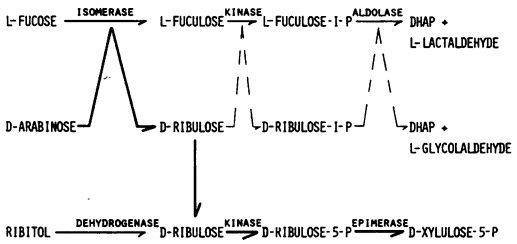

FIG. 1. *Metabolism of D-arabinose-utilizing enzymes of the L-fucose and ribitol pathways. DHAP, Dihydroxyacetone phosphate.*

ribulose to D-ribulose-5-phosphate. These *E. coli* mutants could, however, induce and alternately use L-fuculokinase to phosphorylate D-ribulose at the 1-carbon position to yield D-ribulose-1-phosphate. The mutants then used L-fuculose-1-phosphate aldolase to cleave D-ribulose-1-phosphate into dihydroxyacetone phosphate and L-glycolaldehyde (8).

We have recently isolated a number of D-arabinose-utilizing mutants of *K. aerogenes* strain W70. These mutants likewise utilized L-fucose isomerase for converting D-arabinose to D-ribulose and possessed an inducible ribitol pathway, making possible the conversion of D-ribulose to D-ribulose-5-phosphate. Unlike *K. aerogenes* PRLR3, however, *K. aerogenes* strain W70 had a genetic exchange system mediated by the generalized transducing phage PW52. This made it possible to introduce D-ribulokinase-negative mutations into D-arabinose-positive mutants by co-transduction with the closely linked D-arabitol pathway (2). Although these transductants lacked a functional D-ribulokinase, they were still capable of metabolizing D-arabinose via D-ribulose-1-phosphate by using all three L-fucose pathway enzymes, as was described for *E. coli* K-12. Growth of the cells was more rapid, however, when D-arabinose could be metabolized through the more efficient pathway that led to D-ribulose-5-phosphate.

Some of these D-arabinose-positive mutants were constitutive for the synthesis of L-fucose isomerase, whereas other mutants were capable of inducing the synthesis of isomerase when grown in the presence of D-arabinose, in a manner similar to that reported for *E. coli* K-12.

In an attempt to study the natural mechanism of induction of the L-fucose pathway enzymes as well as the mutation in the second class of D-arabinose-positive mutants, where the metabolism of D-arabinose resulted in the induction of the L-fucose isomerase, a number of L-fucose-negative mutants were isolated. Most of these mutants were found to be missing all three L-fucose pathway enzyme activities. Mutants that lacked only isomerase or kinase activity were not found, even though mutants that lacked only aldolase activity could be isolated. One possible explanation for these results would be that the enzymes of the L-fucose pathway were under coordinate control and induced not by L-fucose itself but by a metabolic intermediate of L-fucose dissimulation, such as L-fuculose or L-fuculose-1-phosphate. A negative mutation in any step leading to the synthesis of the intermediate inducer would prevent induction and subsequent assay for all three L-fucose pathway enzyme activities. The feeding of pathway intermediates to these pleotrophically negative mutants, in an attempt to bypass their metabolic lesions and thereby identify the inducer, was not possible, since substrate levels of purified L-fuculose and L-fuculose-1-phosphate were not available. Another approach to this problem was provided by constitutive control mutants, which permitted the identification of specific L-fucose isomerase- or kinase-negative mutations. These mutations could then be transferred by transduction into strains with wild-type-inducible regulation. In this manner we could then determine which enzyme activities were required for induction of the pathway and thereby attempt to identify the probable intermediate-inducing molecules. (This paper was presented in part at the 74th Annual Meeting of the American Society for Microbiology, Chicago, Ill., 12-17 May 1974.)

MATERIALS AND METHODS

Bacterial strains and cultural conditions. *K. aerogenes* W70 and transducing phage PW52 were obtained from J. F. Wilkinson, Department of General Microbiology, University of Edinburgh. The parent strain used for this study had a uracil requirement *(ura-1)* and was L-ribulokinase negative *(arak4)* (Table 1). An additional L-rhamulokinase-negative mutation *(rhaK3)* was introduced into this strain prior to L-fuculokinase-negative mutant selection. Growth of cells, negative mutant selection, transduction methods, and preparation of extracts have been described previously (3).

Selection of control mutants. Spontaneous L-fucose pathway control mutants were obtained by selection for growth, utilizing D-arabinose as sole source of carbon and energy. Approximately 5×10^8 cells were inoculated into 5 ml of basic salts medium containing 0.5% D-arabinose and incubated at 37 C until growth was observed (7 to 10 days). Mutants of either type, those constitutive for synthesis of the L-fucose pathway enzymes or those with which incubation with D-arabinose resulted in induction of the enzymes, were found in approximately equal numbers.

Enzymatic assays. L-Fucose isomerase activity was determined spectrophotometrically (10). Quan-

TABLE 1. *K. aerogenes W70 mutants*

Mutant	Phenotype
ura-1, araK4	Uracil requirement, L-ribulokinase negative
araK4, fucC6	Constitutive L-fucose enzymes
araK4, fucI66	Temperature-sensitive L-fucose isomerase
rhaK3, fucP6	L-Rhamulokinase negative, L-fuculokinase negative
fucP6, fucC10	Constitutive L-fucose enzymes
araK4, fucA26	L-Fuculose-1-phosphate aldolase negative
fucI66, fucC30	D-Arabinose induces L-fucose enzymes
fucP6, sup-1	Suppressed L-fuculokinase
sup-1, fucC31	D-Arabinose induces L-fucose enzymes
fucP6, fucC31	L-Fuculokinase negative; D-arabinose induces L-fucose enzymes
fucA26, fucC25	Aldolase negative; D-arabinose induces L-fucose enzymes

titative L-fuculokinase assays were based on the continuous spectrophotometric measurement of adenosine 5'-diphosphate formation with the pyruvate kinase-lactic acid dehydrogenase system (1). When high ribitol dehydrogenase levels interfered with the above assay, a qualitative kinase assay involving the measurement of L-fuculose disappearance was used (9). A continuous spectrophotometric assay, which measured dihydroxyacetone phosphate production using glycerophosphate dehydrogenase, was used to assay L-fuculose-1-phosphate aldolase activity (5).

Chemicals. L-Fucose, D-arabinose, a lactic acid dehydrogenase-pyruvate kinase mixture, and α-glycerophosphate dehydrogenase were purchased from Sigma Chemical Co., St. Louis, Mo. L-Fuculose was prepared using whole cells of an L-fuculose-1-phosphate aldolase-negative strain of *K. aerogenes (fucA1)*, which was capable of excreting L-fuculose into the medium when incubated in a 0.1% L-fucose solution. Examination of the above cell-free media by paper chromatography in a 5:7:1, butanol-ethanol-water solvent system revealed only two spots corresponding to L-fucose and L-fuculose standards. Conversion of L-fucose to L-fuculose varied from 60 to 80%, and no further purification was employed prior to its use in assays. L-Fuculose-1-phosphate was prepared enzymatically from L-fuculose (6) and purified as the barium salt (4).

RESULTS

Isolation of an isomerase mutant. If the actual inducer of the L-fucose pathway was an intermediate of the pathway, we would expect a lesion in the first enzyme (L-fucose isomerase) to prevent induction and subsequent assay for the remaining pathway enzymes. We therefore first selected an L-fucose pathway constitutive control mutant *(fucC6)*, which permitted us to subsequently isolate and identify mutants that were missing L-fucose isomerase activity but still constitutively produced L-fuculokinase.

One such L-fucose-negative mutant was mutagenized, and revertants were selected at 30 C on agar plates containing L-fucose. Revertant colonies were picked to agar plates containing L-fucose and incubated at 40 C. Using this procedure, we were successful in isolating a mutant *(fucI66)* that was capable of growth on L-fucose at the permissive temperature (30 C) but not at the restrictive temperature (40 C).

Growth of the parent strain *(araK4)* versus the temperature-sensitive mutant *fucI66* was compared with L-fucose as substrate during a shift from a permissive to a nonpermissive temperature (Fig. 2). Both strains were grown at the permissive temperature (30 C) to equal optical densities and then transferred to the nonpermissive temperature (40 C). Growth of the parent strain was uninterrupted by the transfer to 40 C, whereas growth of strain *fucI66* ceased completely 1 h after transfer. Strain *fucI66* was also tested with L-rhamnose as substrate during a shift from 30 to 40 C, and no interruption of growth was observed. L-Rhamnose is a methyl pentose that parallels L-fucose in its dissimulation and converges with it by also yielding dihydroxyacetone phosphate and L-lactaldehyde. The temperature-sensitive lesion in L-fucose metabolism must therefore be in or before the aldolase cleavage reaction.

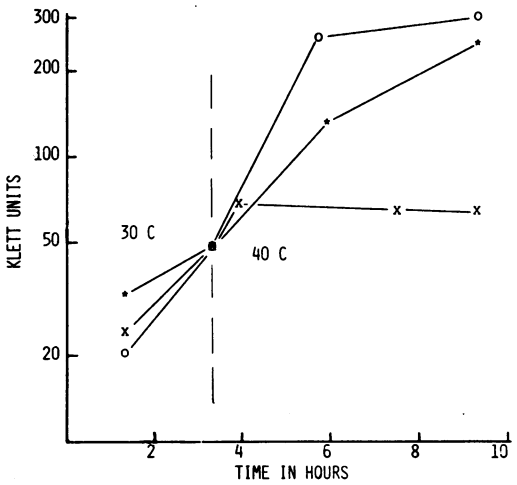

FIG. 2. *Growth of parent strain araK4 (○) on L-fucose, temperature-sensitive mutant fucI66 (×) on L-fucose, and fucI66 on L-rhamnose (*) during a shift from 30 to 40 C.*

Cell extracts were prepared from both the parent and mutant strains grown on L-fucose at the permissive temperature (Fig. 3). When these extracts were incubated at 40 C, the L-fucose isomerase activity in extracts prepared from strain *fucI66* was inactivated after 30 min, whereas the isomerase activity in extracts prepared from the parent strain was stable during this time period. This result confirmed the assumption that strain *fucI66* contained a temperature-sensitive L-fucose isomerase mutation.

Although a constitutive strain was required for identification of isomerase-negative mutants, it could not be used for induction studies. This temperature-sensitive isomerase mutation *(fucI66)* was therefore co-transduced into a recipient strain that was L-fuculose-1-phosphate aldolase negative but inducible for the L-fucose pathway enzymes. Transductants were isolated that could grow on L-fucose at 30 C but not at 40 C (temperature-sensitive isomerase) and were negative for growth on D-arabinose at either temperature (wild-type-inducible control). These latter strains could be used in experiments to determine if isomerization of L-fucose to L-fuculose was required for induction of the L-fucose pathway enzymes.

Identification of a kinase-negative mutant. Since L-rhamnulokinase has been reported to possess activity for the phosphorylation of L-fuculose (4), an L-rhamnulokinase-negative mutant *(rhaK3)* was used as the parent strain in an attempt to isolate and identify an L-fuculokinase-deficient mutant. A number of L-fucose-negative mutants were then selected from this parent strain, and, upon examination, most were found to be pleotrophically negative for the L-fucose pathway enzymes. Each of these pleotrophically negative strains was then used as a parent strain in an attempt to select an additional mutation that would permit growth on D-arabinose. A mutation for the constitutive synthesis of the L-fucose pathway enzymes would be predicted to permit growth on D-arabinose providing the cells were capable of synthesizing a functional L-fucose isomerase.

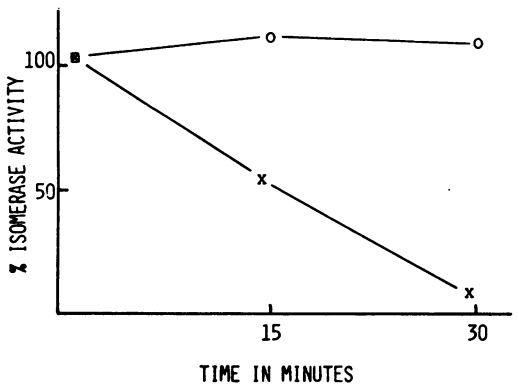

FIG. 3. *L-Fucose isomerase activity in crude extracts of parent strain araK4 (○) and temperature-sensitive mutant fucI66 (×) after incubation at 40 C.*

Three of 11 pleotrophically negative L-fucose mutants were able to mutate to gain the ability for growth on D-arabinose while remaining negative for growth of L-fucose. Cell extracts prepared from these isolates after growth on casein hydrolysate showed them to be constitutive for L-fucose isomerase activity, but they possessed no detectable L-fuculokinase activity. Thus, the subsequent introduction of a constitutive control mutation identified the L-fucose lesion in several of these pleotrophically negative mutants as being in the kinase structural gene rather than in an isomerase or control gene.

Induction in the presence of L-fucose. Induction of the L-fucose pathway enzymes in the parent and three mutant strains is described in Table 2. The parent strain *(araK4)* was capable of inducing all three L-fucose pathway enzymes when grown in the presence of L-fucose at 40 C. Mutant *fucI66*, which contains a temperature-sensitive isomerase, was capable of inducing all three enzymes at the permissive temperature (30 C), but none of the enzymatic activities were detectable at the nonpermissive temperature (40 C). The inducer of the kinase and aldolase must, therefore, be a metabolic intermediate beyond the isomerase-catalyzed reaction. A kinase-negative mutant *(fucP6)* was unable to induce the two remaining L-fucose enzymes (isomerase and aldolase) when grown in the presence of L-fucose. Conversion of L-fuculose to L-fuculose-1-phosphate, therefore, appears necessary for induction of these enzymes to take place. Mutant *fucC10* is a constitutive mutant derived from strain *fucP6* and confirms that the mutation in strain *fucP6* was in the kinase rather than the isomerase structural gene, since a functional isomerase but no kinase activity can be measured in this constitutive mutant. Mutant *fucA26* is an L-fuculose-1-phosphate aldolase-negative mutant, and it is capable of inducing the isomerase and kinase when grown in the presence of L-fucose (Table 2). Since the metabolism of L-fucose through the isomerase- and kinase-catalyzed reactions, but not the aldolase-catalyzed reaction, appears to be required for induction to occur, the intermediate L-fuculose-1-phosphate is the apparent inducer of at least the isomerase and kinase of the L-fucose catabolic pathway.

Separate control for the aldolase. The above data also indicate that the inducer of L-fucu-

TABLE 2. *Induction in the presence of L-fucose*

Strain	Substrate[a]	Temp (C)	Sp act (μmol/mg of protein per min)		
			Isomerase	Kinase	Aldolase
araK4	CH	40	<0.07	0.07	<0.005
araK4	CHF	40	1.19	1.00	0.063
fucI66	CHF	40	<0.06	0.07	<0.005
fucI66	CHF	30	0.26	0.53	0.086
fucP6	CHF	37	<0.03	0.06	<0.005
fucC10	CH	37	0.78	0.07	<0.005
fucA26	CH	37	<0.03	0.07	<0.005
fucA26	CHF	37	0.62	0.71	<0.005

[a] CH, 1% casein hydrolysate; CHF, 1% casein hydrolysate + 0.5% L-fucose.

lose-1-phosphate aldolase is formed after the reaction catalyzed by L-fuculokinase and could be L-fuculose-1-phosphate or some metabolite derived from it. The aldolase is apparently controlled separately from the isomerase and kinase of the L-fucose pathway (Table 3).

The parent strain *(araK4)* was capable of inducing all three L-fucose pathway enzymes when grown in the presence of L-fucose. However, a constitutive mutant *(fucC6)*, derived from *araK4*, synthesized only isomerase and kinase when grown on casein hydrolysate alone. Aldolase activity was still inducible in this strain when cells were grown in the presence of L-fucose. Since the constitutive mutant was selected for by its ability to grow on D-arabinose, which requires only the constitutive synthesis of the isomerase, these results suggest that the isomerase and kinase were under coordinate control. Constitutive mutant *fucC10*, derived from L-fuculokinase-negative strain *fucP6*, synthesized only the isomerase when grown on casein hydrolysate alone. When grown in the presence of L-fucose, strain *fucC10* was still incapable of inducing the aldolase. Since it was possible to readily select L-fucose-positive revertants of *fucC10*, it is not likely that *fucC10* had mutations in both the kinase and aldolase genes. Therefore, the L-fucose aldolase appeared to be under separate control and induced by an intermediate of L-fucose metabolism beyond the kinase-catalyzed reaction.

Isolation of a conditional kinase-negative mutant. As mentioned previously, one class of mutants could be isolated from wild-type *K. aerogenes* W70 that was capable of growth on D-arabinose and was not constitutive for L-fucose isomerase and kinase but could be induced for these activities by the metabolism of D-arabinose. We were equally successful in isolating D-arabinose-inducible control mutants from the temperature-sensitive isomerase mutant *(fucI66, fucC30)* and the L-fuculose-1-phosphate aldolase-negative mutant *(fucA26, fucC25)*. However, when kinase-negative strains were used, all the D-arabinose-positive isolates tested were constitutive for L-fucose isomerase activity.

TABLE 3. *Control of L-fuculose-1-phosphate aldolase*

Strain	Substrate[a]	Sp act (μmol/mg of protein per min)		
		Isomerase	Kinase	Aldolase
araK4	CH	<0.07	0.07	<0.005
araK4	CHF	0.60	0.30	0.052
fucC6	CH	0.36	0.13	<0.005
fucC6	CHF	0.96	0.60	0.056
fucC10	CH	0.78	0.07	<0.005
fucC10	CHF	0.60	0.03	<0.005

[a] CH, 1% casein hydrolysate; CHF, 1% casein hydrolysate + 0.5% L-fucose.

If L-fuculose-1-phosphate functioned as the inducer of L-fucose isomerase and kinase in the wild-type strain, then D-arabinose-positive mutants, in which the metabolism of D-arabinose resulted in induction of the isomerase and kinase of the L-fucose catabolic pathway, should have an altered regulatory protein capable of recognizing a product of D-arabinose metabolism as an alternate inducer. It seemed likely that such mutants might be capable of recognizing the analogue of L-fuculose-1-phosphate, D-ribulose-1-phosphate, as an inducer. If this hypothesis were correct, D-arabinose-positive mutants, where metabolism of D-arabinose induced the L-fucose enzymes, would require a functional L-fuculokinase to catalyze the phosphorylation of D-ribulose to D-ribulose-1-phosphate in order for induction to occur. In contrast, D-arabinose-positive mutants that were constitutive for the isomerase activity could metabolize D-arabinose without a requirement for L-fuculokinase activity.

To confirm the requirement for a functional kinase in the D-arabinose-mediated induction, a conditional kinase-negative mutant was isolated. Revertants of the kinase-negative mutant that could grow on L-fucose at 30 C but not at 37 C were selected, but these revertants

were found to be also incapable of growth on nutrient broth at 37 C. When this inability to grow on rich medium at 37 C was repaired by transduction, the original L-fucose-negative phenotype was restored. No genetic linkage between this temperature-sensitive suppressor mutation and the L-fucose pathway mutations could be detected. The L-ribulokinase, L-rhamnulokinase, and uracil mutations present in this strain were not suppressed by this mutation.

Using this suppressed kinase mutant, we were now able, by selection for growth on D-arabinose, to obtain a D-arabinose-inducible control mutant. Subsequent removal of the suppressor mutation from this strain, by transduction and selection for growth on nutrient broth media at 37 C, restored the original kinase-negative lesion. In this manner a D-arabinose-inducible-kinase-negative mutant was constructed to be used in induction studies.

Induction in the presence of D-arabinose. Induction experiments with D-arabinose-inducible control mutants, derived from either L-fucose isomerase, L-fuculokinase, or L-fuculose-1-phosphate aldolase-negative strains, are described in Table 4. The D-arabinose-inducible mutant *(fucI66, fucC30)* with a temperature-sensitive isomerase was capable of inducing the L-fucose pathway enzymes only when grown in the presence of D-arabinose at the permissive temperature (30 C). Therefore, the isomerization of D-arabinose to D-ribulose appeared to be required for induction to occur. The D-arabinose-inducible mutant *(sup-1, fucC31)*, which carried the suppressed kinase lesion, was capable of inducing the L-fucose enzymes when grown in the presence of D-arabinose. After removal of the suppressor from this kinase-negative mutant *(fucP6, fucC31)*, induction in the presence of D-arabinose no longer occurred. Thus, a functional L-fuculokinase also appeared to be required for induction of the L-fucose pathway enzymes in D-arabinose-inducible control mutants. In contrast, the D-arabinose-inducible and aldolase-negative mutant *(fucA26, fucC25)* was capable of inducing isomerase and kinase when grown in the presence of D-arabinose.

Therefore, in these mutants in which the incubation of cells with D-arabinose leads to the induction of enzymes of the L-fucose pathway, the metabolism of D-arabinose to D-ribulose-1-phosphate appears to be required for such induction to occur.

DISCUSSION

Several bacterial mutants that have gained the ability to metabolize uncommon substrates have been described (7). Many of these mutants gained their new catabolic activity by virtue of a control mutation that permitted constitutive synthesis of existing enzymes, which possessed some catalytic ability for converting the novel substrate into a common metabolic intermediate. Uncontrolled synthesis of enzymes, however, is not a practical metabolic strategy. If constitutive mutations represent a first step in the evolution of new metabolic activities by appropriation of existing enzymes, secondary mutations that turn over control of these borrowed enzymes to the new substrate might have selective advantage.

The D-arabinose-inducible control mutants we have isolated have gained both the use and control of L-fucose pathway enzymes in establishing their D-arabinose catabolic activity. Using strains with mutations in each of the three individual L-fucose pathway enzymes, we were able to demonstrate that the normal induction of the L-fucose pathway in the presence of L-fucose requires both isomerase and kinase, but not aldolase, enzyme activity. A metabolic intermediate, L-fuculose-1-phosphate, is therefore the probable inducer of at least the isomerase and kinase of the L-fucose pathway. When

TABLE 4. *Alternate induction in the presence of D-arabinose*

Strain	Substrate[a]	Temp (C)	Sp act (μmol/mg of protein per min)		
			Isomerase	Kinase[b]	Aldolase
fucI66, fucC30	CHA	40	<0.02	−	<0.005
fucI66, fucC30	A	30	0.15	+	0.149
sup-1, fucC31	CH	30	0.05	−	<0.005
sup-1, fucC31	A	30	0.64	+	0.027
fucP6, fucC31	CH	30	0.07	−	<0.005
fucP6, fucC31	CHA	30	0.04	−	<0.005
fucA26, fucC25	CH	37	<0.02	−	<0.005
fucA26, fucC25	A	37	0.21	+	<0.005

[a] CH, 1% casein hydrolysate; A, 0.5% D-arabinose.
[b] A positive assay represents ≥0.15 μmol/mg of protein per min.

similar studies were conducted using D-arabinose-inducible control mutants grown in the presence of D-arabinose, D-ribulose-1-phosphate was identified as the probable alternate inducing molecule. These D-arabinose-positive mutants apparently produce an altered regulatory protein, which accepts both L-fuculose-1-phosphate and D-ribulose-1-phosphate as inducer. Although D-arabinose-inducible control mutants are capable of inducing and using all three L-fucose pathway enzymes for growth in the presence of D-arabinose, they normally borrow only the isomerase to convert D-arabinose to D-ribulose. Further dissimulation of D-ribulose is primarily through phosphorylation to D-ribulose-5-phosphate catalyzed by the D-ribulokinase of the ribitol pathway.

The requirement of only the isomerase for catabolism presents an interesting problem for the future evolution of a D-arabinose catabolic activity in this strain. Although L-fuculokinase is not directly involved in the dissimulation of D-arabinose, it is required for induction of both the isomerase and itself. The aldolase, however, is not required for either dissimulation or induction. In fact, continuous cultivation of a D-arabinose-inducible control mutant on D-arabinose has been found to lead to the selection of mutants unable to grow with L-fucose as the substrate (Rosenberg and Mortlock, unpublished data). A close examination of one of these mutants revealed that it had lost its L-fuculose-1-phosphate aldolase activity. This mutant was also capable of a more rapid induction of the L-fucose enzymes when grown on D-arabinose. Whether this enhanced D-arabinose induction is only due to accumulation of D-ribulose-1-phosphate in the aldolase-negative mutant or to a separate regulatory gene mutation is not yet known.

With the acquisition of one regulatory mutation, *K. aerogenes* W70 has acquired an inducible D-arabinose catabolic pathway in which a side product of D-arabinose dissimulation, D-ribulose-1-phosphate, serves as an inducer. Basal levels of L-fucose isomerase and L-fuculokinase convert D-arabinose to D-ribulose-1-phosphate, which results in the induction of both enzymes. The D-ribulose formed by the isomerization of D-arabinose induces the enzymes of the ribitol pathway, and the majority of the D-ribulose is phosphorylated to D-ribulose-5-phosphate by the D-ribulokinase of the ribitol pathway.

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