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Regulation of D-Xylose and D-Arabitol Catabolism by *Aerobacter aerogenes*

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Aerobacter aerogenes strain PRL-R3 possesses inducible enzyme pathways for the catabolism of D-xylose and D-arabitol. D-Xylose is the apparent inducer for D-xylose isomerase and D-xylulokinase. D-Arabitol is the apparent inducer for D-arabitol dehydrogenase and a separate D-xylulokinase. Both kinases had similar K_m values and substrate specificities, and could not be separated by sucrose gradient centrifugation or polyacrylamide gel electrophoresis. They could be differentiated, however, by their separate regulation, their inhibition by antisera, and by the cold sensitivity of the kinase of the D-arabitol catabolic pathway.

The degradative pathways for C_5 carbohydrates by strains of *Aerobacter aerogenes* constitute a metabolic system which includes a number of converging steps. The regulation of some of these pathways has been elucidated (4, 12). D-Xylose and D-arabitol are metabolized by this organism through the common intermediate D-xylulose (19, 20). D-Xylose isomerase (EC 5.3.1.5) catalyzes the isomerization of D-xylose to the ketopentose D-xylulose (Fig. 1), which is then phosphorylated to D-xylulose 5-phosphate by D-xylulokinase (EC 2.7.1.17). A partial purification of this kinase from cells grown on D-xylose has been reported by several laboratories (3, 17). Using *A. aerogenes* strain PRL-R3, Wood et al., showed that a D-arabitol dehydrogenase (EC 1.1.1.11) mediated the oxidation of this pentitol to D-xylulose (28). Since cell-free extracts prepared from cells grown on D-arabitol contained a D-xylulokinase activity, it was believed that phosphorylation to D-xylulose-5-phosphate represented the second reaction in the pathway of D-arabitol catabolism.

During a study of mutants which had acquired the ability to utilize the uncommon pentitol xylitol, the pathway for xylitol degradation was also shown to involve oxidation to D-xylulose followed by phosphorylation to D-xylulose-5-phosphate (10, 28). The oxidation of xylitol was shown to be catalyzed by ribitol dehydrogenase (EC 1.1.1.56), and the mutation permitting growth on xylitol was found to be a mutation affecting the regulation of the enzymes of the ribitol pathway. Inasmuch as xylitol is not commonly found in nature, growth of *A. aerogenes* on xylitol is permitted by the acquisition of a mutation which results not in the synthesis of a new enzyme or of one specific for xylitol degradation, but in the constitutive synthesis of ribitol dehydrogenase, which catalyzes the oxidation of xylitol to D-xylulose (18). These converging pathways are diagrammed in Fig. 1.

The three enzymes involved in the catabolism of D-xylose and D-arabitol (to D-xylulose-5-phosphate) are inducible (20). These enzymes appeared to be under separate control, since kinase activity could be induced by growth on D-xylose with only trace amounts of dehydrogenase activity detectable in the cell-free extracts, while growth on D-arabitol resulted in induction of kinase activity in the absence of detectable levels of isomerase activity (4). In addition, extracts prepared from cells grown on D-arabitol seemed to possess low levels of kinase activity compared with cells grown on D-xylose, although the high levels of D-arabitol dehydrogenase present in such extracts reduced the sensitivity of the spectrophotometric kinase assay. Kinase activity was very low or undetectable in cell-free extracts of mutants which were constitutive for D-arabitol dehydrogenase (4).

In studies involving the isolation of mutants unable to use D-xylose as a growth substrate, several observations were made. First, some mutants negative for D-xylose isomerase activ-

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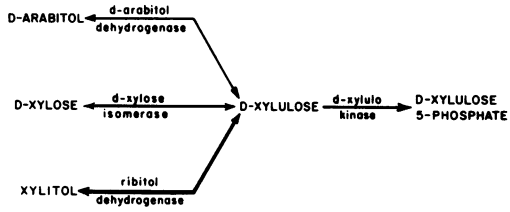

FIG. 1. *Pathways of D-arabitol, D-xylose, and xylitol metabolism by A. aerogenes.*

ity showed normal induction of D-xylulokinase in response to the presence of D-xylose in the medium. Second, mutants with a D-xylose isomerase-positive, D-xylulokinase-negative phenotype could not be isolated directly from the wild-type parent strain. Third, mutants which had lost the ability to synthesize both isomerase and kinase when incubated with D-xylose showed normal growth on D-arabitol. Fourth, the D-xylulokinase present in extracts prepared from D-arabitol-grown cells appeared to be cold sensitive in that activity was rapidly lost upon storage at 0 to 4 C. These data suggested that a separate kinase existed for the catabolism of D-xylulose derived from D-arabitol. Thus, studies were undertaken to explore this possibility and to investigate the regulation of the enzymes of the D-xylose and D-arabitol catabolic pathways. (This work was presented in part at the 70th Annual Meeting of the American Society for Microbiology, Boston, Mass., 26 April to 1 May 1970.)

MATERIALS AND METHODS

Bacterial strains and cultural conditions. *Aerobacter aerogenes* PRL-R3 was the parent strain for the mutants described in Table 1. Unless otherwise indicated, the mutants were isolated after treatment of the parent strain with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, followed by penicillin enrichment (7) and replica plating. The mutant constitutive for D-arabitol dehydrogenase was isolated by the method of Lin, Lerner, and Jorgensen (14).

Cells were grown aerobically at 30 C on a minimal medium (1) supplemented with 0.5% carbohydrate, or 1% vitamin-free casein hydrolysate, or both. The carbohydrates, casein hydrolysate, magnesium sulfate, and uracil (in the case of the uracil-requiring auxotrophs) were autoclaved separately and added to the medium after cooling. Growth was followed by measuring the increase in turbidity on a Klett-Summerson photocolorimeter equipped with a red filter (660 nm; Klett Manufacturing Co., Inc., New York, N.Y.). For determination of enzymatic activities, cultures were grown in 20 ml of the appropriate medium in a 250-ml side-arm flask, and cells were harvested during late exponential phase. For the preparation of cell-free extracts used in the purification of D-xylulokinase activity, cells were harvested from 2 liters of 200-ml aerobic cultures grown to late exponential phase in a medium of minimal salts and either 1% or 0.5% casein hydrolysate. In studies involving the D-arabitol pathway, 0.1% D-arabitol was used as inducer, since higher concentrations were found to be inhibitory to growth.

Preparation of cell-free extracts. Cells were collected by centrifugation at 18,000 $\times$ *g* for 10 min at 4 C (or in later experiments at 15 C when the D-xylulokinase associated with the D-arabitol pathway was to be measured) in an RC-2 refrigerated centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.). Harvested cells were washed once in distilled water and suspended in fractionation buffer consisting of 10^{-2} M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.5), 10^{-4} M tetrasodium-ethylenediaminetetraacetic acid (EDTA), 4×10^{-4} M magnesium chloride, 10^{-3} M reduced mercaptoethanol (MSH), and 10^{-3} M dithiothreitol (DTT). The mercaptoethanol and DTT were added to the buffer just prior to use. The suspended cells were placed in polyethylene tubes, and up to five tubes were placed in the cup of a 10-kc magnetostrictive oscillator (Raytheon Co., South Norwalk, Conn.) containing about 30 ml of distilled water. The cells were disrupted by sonic treatment for 15 min. Cellular debris was removed by centrifugation for 15 min at 27,000 $\times$ *g*. The supernatant fractions, constituting the crude extracts, were collected and stored in an ice bath or at room temperature, depending upon which enzyme activities were to be measured.

Determinations of enzymatic activities. The rate of reduced nicotinamide adenine dinucleotide (NADH) oxidation in the presence of ketopentose was used to assay spectrophotometrically for pentitol dehydrogenase (28). Quantitative kinase assays were based on the continuous spectrophotometric measurement of adenosine-5'-diphosphate (ADP) formation with the pyruvate kinase-lactic acid dehydrogenase system (2). Absorbancy change at 340 nm was measured at 25 C in a DU spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.) and was monitored with a recording attachment (model 2000 Gilford Instrument Laboratories, Inc., Oberlin, Ohio). Control cuvettes lacking ATP or D-xylulose were used to correct measured rates for adenosine-triphosphatase activity and for the oxidation of NADH resulting from the reduction of D-xylulose to D-arabitol catalyzed by D-arabitol dehydrogenase. A qualitative kinase assay, described by Lim and Cohen (13) and involving the measurement of pentulose disappearance, was used when high levels of dehydrogenase in a preparation interfered with the kinase assay system described above. The assay was modified by the omission from the reaction mixture of the 0.5 M of sodium fluoride and the substitution of 0.1 M of magnesium chloride for 0.1 M of magnesium acetate. A unit of enzyme activity was defined as the amount of enzyme necessary to oxidize 1 μmol of NADH in 1 min. Isomerase activity was determined from the rate of pentulose formation by the method of Anderson and Wood (1); at 37 C, one unit of isomerase in 2.0 ml catalyzed the formation of 1 μmol of pentulose per hour. Isomerase activity was also determined by a spectrophotometric assay method which employed partially purified D-arabitol

TABLE 1. *D-Xylose and D-arabitol mutant strains*

Strain no.	Parent strain	Phenotype	Enzyme activities[a]			
			D-Xylose pathway		D-Arabitol pathway	
			Isomerase	Kinase	Dehydrogenase	Kinase
202	PRL-R3	D-Arabitol dehydrogenase constitutive	+[b]	+	Const[c]	Const
252	PRL-R3	No growth on D-xylose	-[d]	+	+	+
281	PRL-R3	No growth on D-arabitol	+	+	-	-
255	281	No growth on D-xylose	+	-	-	-
287	252	No growth on D-arabitol	-	+	-	+
292-3	202	No growth on . . D-arabitol	+	+	-	Const

[a] Crude cell-free extracts prepared following growth of cells on casein hydrolysate medium supplemented with either D-xylose or D-arabitol.
[b] +, Activity present and induced.
[c] Activity constitutive.
[d] -, Activity not detected.

dehydrogenase to catalyze the reduction of D-xylulose, a procedure similar to that described for assay of L-fucose and D-arabinose isomerase activity (EC 5.3.1.3) (24). Ketopentose was measured by the cysteine-carbazole test of Dische and Borenfreund (9). The protein content of the extracts was estimated from the ratio of their absorbancies at 280 and 260 nm (27).

Chemicals. D-Xylulose was prepared chemically by refluxing D-xylose with dry pyridine (21); D-xylulose concentration was determined enzymatically with excess D-xylulokinase and spectrophotometric measurements of NADH oxidation (2). D-Ribulose was synthesized enzymatically by the oxidation of ribitol (23) and prepared for use in enzymatic studies as described previously (12). Alumina c gamma was prepared by the method of Colowick (6). The methods used to obtain partially purified preparations of ribitol dehydrogenase, D-arabitol dehydrogenase, and the D-xylose-induced D-xylulokinase have been reported (11, 17).

The following chemicals were obtained commercially: casein hydrolysate (vitamin-free, salt-free) and protamine sulfate from Nutritional Biochemicals Corp., Cleveland, Ohio; D-arabitol, NADH, 2-phosphoenolypyruvate, and D-xylose from Calbiochem, Los Angeles, Calif.; ATP, DTT, lactic acid dehydrogenase-pyruvate kinase combination (for kinase assays), Trizma base, uracil, and xylitol from Sigma Chemical Co., St. Louis, Mo.; Cyanogum 41 gelling agent for polyacrylamide gels from E-C Apparatus Corp., University City, Philadelphia, Pa.; and *N,N,N',N'*-tetramethylethylenediamine (TEMED) from Distillation Products Industries, Division of Eastman Kodak Co., Rochester, N.Y.

Purification of D-xylulokinase activity associated with the D-arabitol pathway; preparation of cell-free extract. The D-xylulokinase constitutive strain 292-3 was grown in minimal salts containing 1% casein hydrolysate at 30 C. Two liters of culture in the late exponential phase of growth was harvested by centrifugation at 15 C. The cells were washed in distilled water, suspended in fractionation buffer, and disrupted directly in the cup of a 10-kc magnetostrictive oscillator (Raytheon Co., South Norwalk, Conn.), by sonic treatment for 1 min per 10 ml of cell suspension. After removal of cellular debris by centrifugation, the supernatant fluid was maintained at room temperature (22 C). Unless otherwise indicated, all procedures involving the D-xylulokinase associated with the D-arabitol pathway were performed at 22 C, and all reagents so to be used were warmed to approximately 22 C just prior to use.

Protamine sulfate fractionation. The crude extract was diluted to 10 mg of protein per ml by the addition of fractionation buffer. Solid ammonium sulfate was added slowly to a concentration of 13.2 g per liter, followed by the addition of a 1.7% solution of protamine sulfate (1 ml per 10 ml of extract). The precipitate was removed by centrifugation, and DTT was added to the supernatant fluid to a concentration of 10^{-3} M. After a 20-min incubation with DTT at room temperature, the supernatant fluid was assayed for D-xylulokinase activity, and the next purification step was initiated.

Ammonium sulfate fractionation. An additional 235 g of solid ammonium sulfate was added

slowly to each liter of supernatant fluid from the protamine sulfate treatment. The precipitate was removed by centrifugation, and the supernatant fluid was treated with an additional 117.8 g of solid ammonium sulfate. The resulting precipitate, which contained most of the D-xylulokinase activity, was collected by centrifugation and suspended in 15 ml of fractionation buffer. This suspended precipitate was either dialyzed for 4 h against 1 liter of "low-salt buffer" or diluted with low-salt buffer to a salt concentration of 0.02 M as determined with a precalibrated conductivity cell (Barnstead Purity Meter). Low-salt buffer was prepared by diluting the Tris-EDTA-$MgCl_2$-MSH-DTT fractionation buffer previously described, in distilled water (1:100) and adding reduced mercaptoethanol and DTT to 10^{-3} M.

Elution from DEAE cellulose. The desalted enzyme fraction was applied to a diethylaminoethyl (DEAE)-cellulose-phosphatase column (30 by 120 mm) equilibrated with fractionation buffer containing 0.01 M phosphate in addition to Tris. Most of the remaining adenosine triphosphatase activity was removed from the enzyme preparation by allowing 300 ml of 0.065 M potassium phosphate (pH 7.5) prepared in fractionation buffer to run slowly through the column overnight. This elution was followed by a 100-ml portion of 0.08 M phosphate, ten 20-ml portions of 0.095 M phosphate, and three 30-ml portions of 0.1 M phosphate. The fractions were collected and all subsequent steps were performed at 15 C. Most of the D-xylulokinase activity was found in the last seven 0.095 M fractions and the three 0.1 M fractions which were pooled. The pooled enzyme preparations were concentrated by the addition of 549 g of solid ammonium sulfate per liter. The precipitate obtained after centrifugation for 30 min at 27,000 × *g* was suspended in 5 ml of fractionation buffer and stored overnight at 15 C in a tightly closed and filled tube.

Elution from alumina c gamma. The enzyme preparation eluted from DEAE-cellulose was diluted to a salt concentration of 0.01 M in low-salt buffer. Adsorption to alumina c gamma was effected by mixing the two components at a ratio of 3.5 mg of alumina c gamma per mg of protein. Elution of the protein from the gel was accomplished by washing with increasing concentrations of potassium phosphate prepared in the low-salt buffer solution described previously. Most of the D-xylulokinase activity was eluted in 10 ml of 0.03 M phosphate and a subsequent 10-ml portion of 0.05 M phosphate. The procedures outlined so far resulted in a 30 to 60-fold purification of D-xylulokinase and the elimination of any detectable adenosine triphosphatase activity.

Preparation of antisera. Partially purified kinase preparations were used to prepare antisera from rabbits as described previously (17).

Analytical procedures. Apparent Michaelis-Menton constants (K_m) were obtained by plotting kinetic data by the method of Lineweaver and Burk (15).

Analytical polyacrylamide gel electrophoresis utilized the chemical polymerization method of Davis (8), except that a single (running) gel was prepared as described by Tombs and Akroyd (26). Preparations containing the D-xylose-induced D-xylulokinase alone, the D-xylulokinase activity associated with the D-arabitol pathway alone, and mixtures of the two enzymes were subjected to electrophoresis on duplicate gels, one of which was stained with amide black as described by Davis (8). Unstained gels were sliced into 1-mm sections, and each section placed into a Wasserman tube containing 0.5 ml of fractionation buffer. Each gel slice was disrupted with a glass stirring rod to elute enzyme activity. Fractions were maintained at 4 C or at 15 C for elution of kinases.

Sedimentation coefficients were compared by the sucrose density centrifugation method of Martin and Ames with the temperature maintained at 15 C. (16).

RESULTS

Induction of enzymes for D-xylose catabolism. The results of enzyme assays performed on crude cell-free extracts prepared from cells grown with either D-xylose, D-arabitol, or casein hydrolysate are shown in Table 2. It should be noted that the high dehydrogenase levels obtained from D-arabitol-grown cells greatly decreases the sensitivity of the spectrophotometric assay for kinase. Extracts prepared from cells grown on D-arabitol do not contain detectable levels of isomerase activity while extracts prepared from cells grown on D-xylose contain only trace levels of dehydrogenase activity. Since D-xylulose is an intermediate in both of these catabolic pathways it seems likely that the inducer for the dehydrogenase and the inducer for the isomerase are D-arabitol and D-xylose, respectively.

In agreement with previously published work, D-xylulokinase activities were consistently

TABLE 2. *Induction of enzyme activities for the D-xylose and D-arabitol catabolic pathways*

Growth medium	Enzyme activity (units/mg protein)[a]		
	D-Arabitol dehydrogenase	D-Xylose isomerase	D-Xylulokinase
Casein hydrolysate	<0.01[b]	<0.01	<0.02
D-Arabitol	1.13–3.47	<0.01	<0.02–0.27
D-Xylose	0.02–0.10	0.05–0.11	0.36–1.20

[a] Enzyme activities were obtained from crude cell-free extracts of the wild-type strain PRL-R3, prepared and maintained at 0 to 4 C. Activities given represent the highest and lowest values obtained in at least three separate experiments.

[b] Activity not detectable but less than the indicated value.

lower in extracts prepared from cells grown on D-arabitol than in extracts prepared from cells grown on D-xylose. The assay determined kinase activity by measurement of the increase in rate of NADH oxidation caused by the presence of kinase over the rate of oxidation due to dehydrogenase activity. In occasional extracts, prepared from D-arabitol-grown cells, no kinase activity was detectable under the assay conditions employed (Table 2).

Among the possible explanations for these data was the existence of two separate kinases, one induced for D-xylose catabolism and one induced for D-arabitol catabolism. If this were true, it would explain the difficulty in isolating directly from the wild type a mutant with an isomerase-positive, kinase-negative phenotype.

A mutant which had lost the ability to utilize D-arabitol as a growth substrate was isolated and upon examination was found to be unable to produce detectable levels of either dehydrogenase or kinase when incubated with D-arabitol. When this strain (281) was used as the parent strain for the isolation of D-xylose-negative mutants, three of the first six mutants tested showed the isomerase-positive, kinase-negative phenotype. The data for one of these mutants, strain 255, are presented in Table 3. The data in Table 3 indicate that D-xylose is the apparent inducer of both the kinase and isomerase activities involved in D-xylose catabolism.

D-xylulokinase activity associated with the D-arabitol pathway. Since the above kinase appeared to be induced by D-xylose, there remained to be explained the question of the origin and regulation of the kinase activity associated with D-arabitol degradation and the reason for the low activity of kinase in D-arabitol-grown cell-free extracts. These questions were investigated utilizing strain 202, which was isolated as constitutive for D-arabitol dehydrogenase activity (Table 4). Kinase activity, as detected by the spectrophotometric assay, tended to be masked by the high dehydrogenase levels; in most extracts prepared from strain 202 kinase activity was not detectable. When a more sensitive qualitative assay for the ATP-dependent disappearance of D-xylulose was used, this strain was found to be constitutive for D-xylulokinase activity. The enzyme activities of strain PRL-R3, induced and uninduced, were used as controls to show correlation between the spectrophotometric assay and the ATP-dependent D-xylulose disappearance assay. Strain 202 was used to obtain strain 292-3, a D-arabitol-negative strain which had lost its dehydrogenase activity but which was still constitutive for D-xylulokinase. Without the interference of high levels of dehydrogenase, kinase could be accurately assayed using the quantitative spectrophotometric assay. Since isomerase activity remained inducible in strains 202, these data strengthened the concept of two separate D-xylulokinases, one induced by D-xylose and the other coordinately controlled with D-arabitol dehydrogenase,

TABLE 3. *Induction of isomerase and kinase activities by D-xylose*

Strain	Inducer[a]	Enzyme activity (units/mg of protein)	
		D-Xylose isomerase	D-Xylulokinase
PRL-R3	None	<0.02[c]	0.03
PRL-R3	D-Xylose	0.13	1.20
252[b]	None	<0.01	<0.01
252	D-Xylose	<0.01	2.04
255	None	<0.02	<0.02
255	D-Xylose	0.51	0.03

[a] Cells were grown using 1% casein hydrolysate. When indicated 0.5% D-xylose was also added to the medium.

[b] Mutant strains are described in Table 1.

[c] Activity not detectable but less than the indicated value.

Purification and comparison of the D-xylulokinase activities. The D-xylose-induced kinase was purified 50-fold using techniques reported earlier (3, 17, 25) with the temperature maintained at 0 to 4 C. Initial attempts to purify the constitutive kinase (associated with the D-arabitol pathway in strain 292-3) lead to rapid loss of enzyme activity with only 1% of the original activity remaining after the first ammonium sulfate precipitation. Fractionation of the enzyme was successful only when the initial steps were carried out at room temperature (22 C). After treatment with protamine sulfate and precipitation with ammonium sulfate, the temperature was lowered to 15 C for elution from DEAE-cellulose-phosphate and adsorption to and elution from alumina c gamma (see Materials and Methods). A 60-fold purification, representing 14% of the original activity in the crude extract, was obtained with a specific activity of 33 units per mg of protein. When stored at 15 C in an atmosphere of argon, such preparations retained approximately 50% of their activity after 21 days. Both partially purified enzymes were found to have similar K_m values for D-xylulose (3.4×10^{-4} M). Both kinases utilized D-xylulose as substrate but did not use D-fructose, D-ribulose, or L-xylulose.

The two kinases were inseparable by analytical polyacrylamide gel electrophoresis, when detection of kinase was based upon observation of colored bands formed in native gels stained with amido black, or upon spectrophotometric assay of enzyme activity eluted from duplicate unstained gels sliced into 1-mm sections. Variation in gel concentrations (4.0 to 7.5%), pH's (6.0 to 10.0), and time periods of electrophoresis (0.5 to 6.0 h) did not prove successful in separation of the two kinase activities.

Centrifugation of mixtures of the two kinases on sucrose gradients resulted in the observation of only one peak with an estimated sedimentation coefficient of 5.91 and estimated molecular weight of 104,000. The values obtained for the kinase mixtures, using ribitol dehydrogenase as a standard (17), agreed well with those previously published for the D-xylose-induced D-xylulokinase (17) which was reported to have a sedimentation coefficient of 6.1 with an estimated molecular weight of 107,000. When either kinase was centrifuged individually on 5 to 20% (wt/vol) sucrose gradients, a single peak of kinase activity was observed with each preparation. Centrifugation of a mixture of the two kinases on a similar gradient also resulted in a single peak of kinase activity which equalled the sum of the two kinase activities found with the individual gradients.

In the study of the antigenic specificity of certain pentitol dehydrogenases and pentulokinases found in *A. aerogenes*, Mortlock et al. (17) observed that antibodies, produced in response to an injection of purified enzyme preparations into rabbits, were strong inhibitors of the same enzyme when added to the assay system. This same technique was used to examine the extent of immunological similarity of these D-xylulokinases. Table 5 shows the results of an experiment in which each of the partially purified kinases that had been used to prepare antiserum was then incubated with the indicated antiserum prior to measurement of enzyme activity. Antiserum obtained from a rabbit injected with the D-xylose-induced kinase inhibited the activity of this kinase by 96%, but did not inhibit the constitutive kinase associated with the D-arabitol pathway. Neither did this serum inhibit the activity of the enzymes used as controls, D-ribulokinase (EC 2.7.1.47) and D-arabitol dehydrogenase. Antiserum obtained from a rabbit injected with kinase purified from the D-arabitol constitutive mutant was less specific but still differentiated between the two kinases.

Further support for the induction of two D-xylulokinases. The involvement of two kinases in these converging pathways was further indicated by induction studies in which the necessary precautions were taken to maintain proper temperatures (15 C) and DTT concentrations (10^{-3} M) in order to protect both kinase activities. The D-xylose isomerase-negative mutant, strain 252, was used as the parent strain for the isolation of mutants unable to utilize D-arabitol as a growth substrate. One such mutant, strain 287, was found to be deficient in D-arabitol dehydrogenase activity. The results of induction experiments using strain 287 are shown in Table 6. Normal levels of kinase were found upon induction by D-xylose, and comparable levels were induced with D-arabitol, even though strain 287 is negative for the first enzyme in each pathway. Thus, D-xylose and D-arabitol each appear to be inducers of D-xylulokinase activity.

TABLE 4. *D-Arabitol dehydrogenase and D-xylulokinase activities in extracts prepared from cells constitutive for D-arabitol dehydrogenase*

Strain	Growth substrate	Enzyme activity (units/mg of protein)		
		D-Arabitol dehydrogenase	D-Xylulokinase	ATP-dependent D-xylulose utilization
PRL-R3	Casein hydrolysate	<0.01[a]	<0.01	–
PRL-R3	Casein hydrolysate + D-arabitol	3.4	0.27[b]	+
202	Casein hydrolysate	2.6	0.30[b]	+
292-3	Casein hydrolysate	<0.01	0.54	+

[a] Activity not detectable but less than the indicated value.

[b] Activity often not detectable.

The D-arabitol-induced kinase possessed only 9% of its original activity after 2 days of storage at 0 C, whereas the kinase induced by D-xylose had 65% of its original activity after incubation under identical conditions.

In a separate experiment, kinase activities induced in strain 287 were tested for inhibition by antiserum obtained using the partially purified, D-xylose-induced kinase as antigen (Table 7). Such antiserum strongly inhibited the D-xylose-induced kinase activity present in these crude cell-free extracts, but was markedly less active on the D-arabitol-induced kinase activity. When cells had been incubated in the presence of both inducers, inhibition was intermediate between the values for the indi-

TABLE 5. *Inhibition of enzyme activities by antisera prepared against the* D-*xylulokinases*

Enzyme tested[a]	Enzyme substrate	Inhibition of enzyme activity by antiserum (%)	
		Antiserum prepared against D-xylose-induced kinase	Antiserum prepared against strain 292-3 constitutive kinase
D-Xylulokinase (D-xylose induced)	D-Xylulose	96	14
D-Xylulokinase (strain 292-3 constitutive)	D-Xylulose	0	65
D-Arabitol dehydrogenase	D-Xylulose	0	60
D-Ribulokinase	D-Ribulose	0	9

[a] Partially purified enzymes (See Materials and Methods)

TABLE 6. *Induction of kinase activity in mutant strain 287*[a]

Time after addition of inducer (h)	Inducer		
	D-Arabitol	D-Xylose	D-Arabitol + D-xylose
0	<0.01[b]	<0.01	<0.01
2	<0.01	0.47	0.39
4	0.63	0.98	0.76
8	0.76	0.89	1.25

[a] Cells were grown using 1% casein hydrolysate supplemented with 0.1% of the indicated carbohydrate. Crude cell-free extracts were prepared and maintained at 15 C. Enzyme activities are expressed in units per mg of protein.

[b] Activity not detectable but less than the indicated values.

TABLE 7. *D-Xylulokinase antiserum inhibition of D-xylulokinase activity in crude cell-free extracts of strain 287*

Inducer[a]	D-Xylulokinase activity (units/mg protein)		
	Without antiserum	Antiserum treated[b]	Inhibition of activity (%)
D-Xylose	2.8	0.2	93
D-Arabitol	2.2	1.7	23
D-Xylose and D-arabitol	4.5	1.2	73

[a] The growth medium consisted of 1% casein hydrolysate supplemented with 0.5% of the indicated carbohydrates.

[b] The antiserum used was prepared using the D-xylose-induced kinase as antigen.

vidual kinases. Hence, these data also support the concept of two serologically distinct proteins associated with D-xylulokinase activity in *A. aerogenes*.

DISCUSSION

Previous work indicated that the enzymes of the D-arabitol and D-xylose pathways are under separate control in *A. aerogenes* and, furthermore, that there may be more than one species of D-xylulokinase involved in these converging pathways (4). The data presented in this paper support the idea of isozymic D-xylulokinases. Both kinases have similar specificities, K_m values for D-xylulose, sedimentation constants, and mobilities in polyacrylamide gel electrophoresis. Since previous study has shown the other pentulokinases of *A. aerogenes* to be similar in their physical properties (17), such a result is not surprising. The kinase associated with the D-arabitol pathway differs from the kinase of the D-xylose pathway by its cold lability and dependency upon reducing agents. The kinases can also be differentiated by the inhibition of their enzymatic activity using antiserum and by their regulation and induction specificity. The cold sensitivity of the kinase of the D-arabitol catabolic pathway explains why previous investigators normally found much lower levels of D-xylulokinase activity in cells induced by D-arabitol than in cells induced by D-xylose. When the temperature is maintained between 15 and 25 C and the dithiothreitol concentration at 0.001 M during the preparation of cell-free extracts, comparable levels of kinase activity can be found in cells induced with either D-arabitol or D-xylose.

The metabolism of D-arabitol, through D-xylulose as an intermediate, does not result in the induction of detectable levels of D-xylose isomerase. Since the isomerization of D-xylose to D-xylulose does not result in the induction of significant levels of D-arabitol dehydrogenase, the inducer for each of these enzymes probably occurs prior to the formation of D-xylulose during the catabolism of these sugars. Mutants lacking isomerase activity are able to produce kinase when incubated in the presence of D-xylose. D-Xylose, therefore, is the apparent inducer of the isomerase and kinase of its catabolic pathway. Since mutants lacking D-arabitol dehydrogenase activity synthesize kinase activity upon incubation with D-arabitol, D-arabitol is the apparent inducer of the dehydrogenase and kinase of its catabolic pathway. This situation contrasts with the mode of regulation of the ribitol catabolic pathway where the ketopentose intermediate D-ribulose,

rather than ribitol, appears to be the actual inducer (5). If D-xylulose acted as inducer for the D-arabitol pathway, there would be difficulty in maintaining separate control of the D-xylose catabolic route.

D-Xylulose serves as an intermediate in xylitol catabolism (Fig. 1) as well (12, 31), and mutants of *A. aerogenes* which have gained the ability to utilize xylitol can be selected. The mutation results in constitutive synthesis of two enzymes of the ribitol catabolic pathway (ribitol dehydrogenase and D-ribulokinase). The constitutively produced ribitol dehydrogenase catalyzes the oxidation of xylitol to D-xylulose (18); however, the constitutively produced D-ribulokinase (10, 28) has no activity on D-xylulose. If strains lacking either the D-xylulokinase of the D-arabitol pathway (Fig. 2) or the D-xylulokinase of the D-xylose pathway are employed as parent strains, mutants can be selected for growth on xylitol. When a parent strain which could synthesize neither D-xylulokinase was used in similar experiments, mutants capable of growth on xylitol did not appear. This suggests that the D-xylulokinase of either the D-arabitol or the D-xylose pathway can function to permit growth on the uncommon pentitol xylitol.

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