

Catabolite Repression and Derepression of Arylsulfatase Synthesis in *Klebsiella aerogenes*

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When a mutant (Mao⁻) of *Klebsiella aerogenes* lacking an enzyme for tyramine degradation (monoamine oxidase) was grown with D-xylose as a carbon source, arylsulfatase was repressed by inorganic sulfate and repression was relieved by tyramine. When the cells were grown on glucose, tyramine failed to derepress the arylsulfatase synthesis. When grown with methionine as the sole sulfur source, the enzyme was synthesized irrespective of the carbon source used. Addition of cyclic adenosine monophosphate overcame the catabolite repression of synthesis of the derepressed enzyme caused by tyramine. Uptake of tyramine was not affected by the carbon source. We isolated a mutant strain in which derepression of arylsulfatase synthesis by tyramine occurred even in the presence of glucose and inorganic sulfate. This strain also produced β -galactosidase in the presence of an inducer and glucose. These results, and those on other mutant strains in which tyramine cannot derepress enzyme synthesis, strongly suggest that a protein factor regulated by catabolite repression is involved in the derepression of arylsulfatase synthesis by tyramine.

Arylsulfatase synthesis in some bacteria is controlled by the kind of sulfur compound and some aryl compounds in the medium. As shown previously (12), *Aerobacter aerogenes* ATCC 9621 synthesized arylsulfatase when grown on medium containing methionine or taurine as the sulfur source, and enzyme synthesis was repressed when sulfate, sulfide, sulfite, thiosulfate, or cysteine was present as the sole sulfur source or together with methionine. Addition of tyramine, dopamine, DL-octopamine, or L-norepinephrine to the medium relieved the repression of arylsulfatase synthesis (7). Recently, we demonstrated that tyramine itself relieves the repression of enzyme synthesis by inorganic sulfate and that its effect may be due to derepression rather than induction (1).

A useful method for elucidation of problems involving the regulation of enzyme synthesis is genetic analysis of organisms with mutations in appropriate structural and regulatory genes. Unfortunately, it is impossible to use this method to study arylsulfatase in *A. aerogenes* ATCC 9621, since so far no system for the transfer of genetic material has been discovered in this strain. *Escherichia coli* and *Salmonella typhimurium*, the bacteria most widely used in genetic studies, produce little or no arylsulfatase (11). However, recently the taxonomically similar bacterium, *Klebsiella aerogenes* W70, with genetic material which can be transferred

by transduction, has been reported (13). Therefore, we studied the control of arylsulfatase in this organism.

A previous paper (8) showed that arylsulfatase synthesis is repressed in *A. aerogenes* ATCC 9621 in the presence of glucose and certain other rapidly metabolized carbon sources. The present paper indicates that a protein factor sensitive to catabolite repression may be involved in the derepression of arylsulfatase synthesis by tyramine in *K. aerogenes*.

MATERIALS AND METHODS

Cultures and growth conditions. *K. aerogenes* W70, supplied by courtesy of S. Yamagishi, was used as the wild-type strain. The organism was maintained with occasional transfer on slants of nutrient agar (Difco). The cultures were grown aerobically by reciprocal shaking at 30 C in a chemically defined medium containing 0.05 M potassium phosphate buffer (pH 7.2); 0.1% NH₄Cl; 0.05% MgCl₂·6H₂O; 0.001% each of NaCl, MnCl₂·4H₂O, and FeCl₃·6H₂O and carbon and sulfur compounds. Unless otherwise stated, 0.25% D-xylose was used as a carbon source. The sulfur compounds used are mentioned in the individual experiments. Growth was followed in a Klett-Summerson colorimeter (590 to 660 nm), calibrated by dry weight determination.

Strains. K13 is the derivative of *K. aerogenes* strain W70 described by MacPhee, Sutherland, and Wilkinson (13). The derivatives of K13 used are described in Table 1.

Isolation of mutants. *N*-methyl-*N'*-nitro-*N*-

TABLE 1. List of strains and their characteristics

Strain	Relevant phenotype	Derivation
K13	Monoamine oxidase defective (Mao ⁻)	Mutagenesis of <i>K. aerogenes</i> W70
K601	Mao ⁻ , defect of structural gene for arylsulfatase (Ats ⁻)	Mutagenesis of K13
K611	Mao ⁻ , lack of derepression of arylsulfatase synthesis by tyramine	Mutagenesis of K13
K701	Mao ⁻ , insensitive to catabolite repression	Mutagenesis of K13

nitrosoguanidine was used as a mutagenic agent, as described by Adelberg et al. (2). Strain K13 was selected as a monoamine oxidase-defective mutant with similar characteristics to *A. aerogenes* T11, as reported previously (1). Mutants capable of overcoming catabolite repression and incapable of showing derepressed arylsulfatase synthesis with tyramine were selected by the filter paper method developed by Harada (9). In brief, filter paper is dipped into a 0.01 M solution of a chromogenic substrate, such as nitrocatechol sulfate, and then dried at room temperature. The test paper is placed on colonies grown on agar medium. The hydrolase activity of the test organism can be determined by measuring the time required for development of a color due to hydrolysis.

Synthesis of arylsulfatase. Cells growing exponentially with inorganic sulfate as a sulfur source were harvested, washed twice with 0.1 M potassium phosphate buffer (pH 7.2), and resuspended in the same buffer. These cells were inoculated into appropriate medium (10 ml) to give an absorbance of about 10 Klett units (approximately 1.5×10^8 cells per ml) and were incubated with shaking at 30 C.

Determination of arylsulfatase. After three doublings (approximately 80 Klett units) on incubation at 30 C, cells were collected on Whatman glass fiber paper GF/B with an aspirator and washed with 5 ml of 0.1 M tris(hydroxymethyl)aminomethane-hydrochloride buffer, pH 7.2. The filters were then mixed with 0.5 ml of the same buffer, and the reaction mixtures were preincubated for 5 min at 30 C before adding warm *p*-nitrophenylsulfate in 0.1 M tris(hydroxymethyl)aminomethane-hydrochloride, pH 7.2 (1.5 ml) at a final concentration of 2.5 mM. The mixtures were incubated at 30 C for 5 to 60 min, depending on the activity present, and then the reaction was terminated by adding 0.5 ml of 1 N NaOH containing 0.1 M Na₂PO₄, since phosphate ion strongly inhibits arylsulfatase activity (18). The filter paper and cells were removed by centrifugation at 3,000 rpm for 20 min, and the optical density of the color developed was measured in a Hitachi spectrophotometer, type 124, at 400 nm. The amount of *p*-nitrophenol liberated was calculated from a calibration curve. As a control, cells on filter paper and substrate were incubated separately and combined immediately before addition of NaOH plus Na₂PO₄. Results obtained by the filter

paper method agreed well with those obtained using crude cell extracts, and this assay method was much simpler. One unit of activity was taken as the amount causing formation of 1 μmol of *p*-nitrophenol per min at 30 C.

Assay of tyramine uptake. After three doublings, cells were harvested, washed twice with 0.05 M phosphate buffer (pH 7.2) at 5 C, and resuspended in the same volume of cold buffer solution. Uptake of radioactive tyramine was measured at 30 C in 1 ml of reaction mixture containing various amounts of [³H]tyramine, 0.05 M phosphate buffer (pH 7.2), and 0.7 mg (dry weight) of cells. At intervals, cells were filtered through membrane filters (0.45-μm pore size; Toyo Roshi Co.) and quickly washed with 5 ml of 0.05 M phosphate buffer at room temperature. Filters were dissolved in 10 ml of Bray fluid (3), and cellular radioactivity was measured using a scintillation spectrometer (Beckman LS-100). To establish the initial rate of uptake, a sample (0.5 ml) of cell suspension was rapidly pipetted into an equal volume of prewarmed medium containing an appropriate concentration of isotope. The initial rates of uptake were calculated from the linear uptake determined at 30-s intervals for 2 min.

Assay of β-galactosidase activity. β-Galactosidase was assayed by the method of Pardee et al. (16).

Chemicals. [³H]tyramine hydrochloride was obtained from the Radiochemical Centre, Amersham, England. *p*-Nitrophenylsulfate, obtained from Sigma Chemical Co., was recrystallized from aqueous ethanol before use. Tyramine hydrochloride, nitrocatechol sulfate, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, cyclic adenosine 3',5'-monophosphate (cyclic AMP), and the other compounds used were standard commercial preparations.

RESULTS

Effect of the carbon source on arylsulfatase synthesis. The levels of arylsulfatase in cells of the mutant of *K. aerogenes*, K13, deficient in an enzyme (monoamine oxidase) involved in tyramine degradation, were measured under various conditions. Table 2 shows the activities in synthetic media with and without tyramine, with inorganic sulfate or methionine as the sole sulfur source, and with xylose or glucose as the carbon source. Enzyme activity was high in cells grown on xylose in the presence of tyramine, irrespective of the kind of sulfur compound used. In our previous experiments, similar observations were obtained with a monoamine oxidase-deficient mutant, strain T11, derived from *A. aerogenes* ATCC 9621 (1). No enzyme synthesis was observed when cells were grown with glucose and inorganic sulfate, even in the presence of tyramine (Table 2). When cells were grown with methionine as the sulfur source, however, arylsulfatase was synthesized irrespective of the kind of carbon source used, and tyramine had relatively little effect on the

TABLE 2. Levels of arylsulfatase in cells of *K. aerogenes* K13 grown in various media^a

Supplement			Arylsulfatase activity ^b (m units/mg of cells)
Carbon source (0.25%)	Sulfur source (1 mM)	Tyramine (1 mM)	
Xylose	Na ₂ SO ₄	-	0.08
Xylose	Na ₂ SO ₄	+	4.28
Xylose	Methionine	-	3.38
Xylose	Methionine	+	6.14
Glucose	Na ₂ SO ₄	-	0.11
Glucose	Na ₂ SO ₄	+	0.18
Glucose	Methionine	-	9.45
Glucose	Methionine	+	11.82

^a Cells were harvested after approximately three doublings.

^b Values are averages of three independent experiments. See Materials and Methods.

synthesis. The effect of the glucose concentration of arylsulfatase synthesis in the presence of 0.25% xylose, 1 mM Na₂SO₄, and 1 mM tyramine is shown in Fig. 1 as a plot of the arylsulfatase content against the cell density. The results showed that arylsulfatase synthesis occurred after disappearance of glucose, and the delay in enzyme synthesis depended on the glucose concentration, corresponding with the increment of cell density. Thus, glucose seems to inhibit the action of tyramine in relieving repression of arylsulfatase synthesis caused by inorganic sulfate, since glucose does not repress enzyme synthesis in cells grown with methionine as the sole sulfur source.

Another possible explanation of the effect of glucose is that it may inhibit tyramine transport into the cells. To study this, uptake of tyramine into cells grown on glucose or xylose was examined (Fig. 2). No difference was found in tyramine uptake in cells grown with glucose and xylose. The results also showed that tyramine entered the cells by passive diffusion, since tyramine uptake did not follow saturation kinetics. Glucose also did not kinetically interfere with the uptake of tyramine, since no difference was found in tyramine uptake in the presence or absence of glucose or xylose in the reaction mixture.

Effect of cyclic AMP on repressed enzyme synthesis by glucose. There is evidence that in *E. coli* cells cultured in the presence of glucose, many catabolic enzyme systems are repressed (4) and the intracellular level of cyclic AMP is reduced (14). Cyclic AMP has been shown to overcome this repression by glucose (4, 15, 17, 19). Figure 3 illustrates the repression of arylsulfatase synthesis by glucose and its reversal

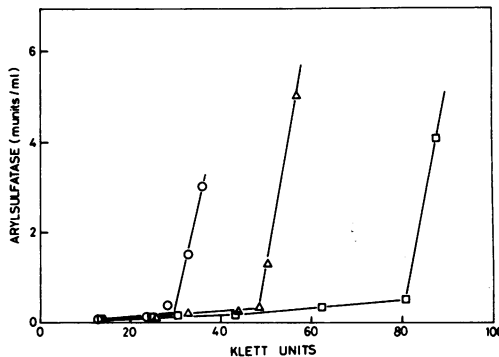


FIG. 1. Effect of glucose on arylsulfatase synthesis in strain K13. Cells from an exponentially growing culture of strain K13 were diluted to about 10 Klett units with fresh medium containing 0.25% xylose, 0.15% NH₄Cl, 1 mM Na₂SO₄, 1 mM tyramine, and various concentrations of glucose and were incubated with shaking at 30 C. Symbols: O, with 0.02% glucose; Δ, with 0.05% glucose; □, with 0.1% glucose.

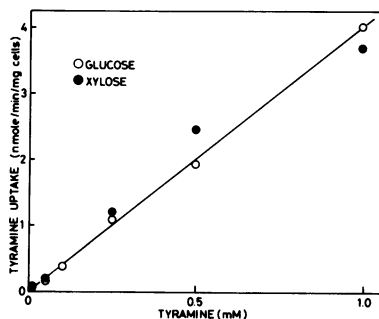


FIG. 2. Kinetics of tyramine uptake into cells. Strain K13 cells were grown in minimal medium with 0.25% glucose (O) or xylose (●). The amount of tyramine taken up at 30 C in 30 s was determined as described in Materials and Methods.

by cyclic AMP during growth of cells in the presence of inorganic sulfate and tyramine. As with repression, its reversal might occur by increasing the rate of enzyme synthesis. Figure 3 shows that addition of high concentrations of cyclic AMP during growth increased the rate of arylsulfatase synthesis. The strain used here required about twice the concentration of cyclic AMP required by *A. aerogenes* ATCC 9621 to reverse repression of enzyme synthesis by glucose (data not shown). These results suggest that glucose represses a protein involved in the action of tyramine in derepressing synthesis of arylsulfatase and that cyclic AMP overcomes its repression. To confirm this, we isolated a mutant strain in which synthesis of arylsulfatase could be derepressed by tyramine in the presence of glucose and inorganic sulfate. The presence of glucose and tyramine in the mutant

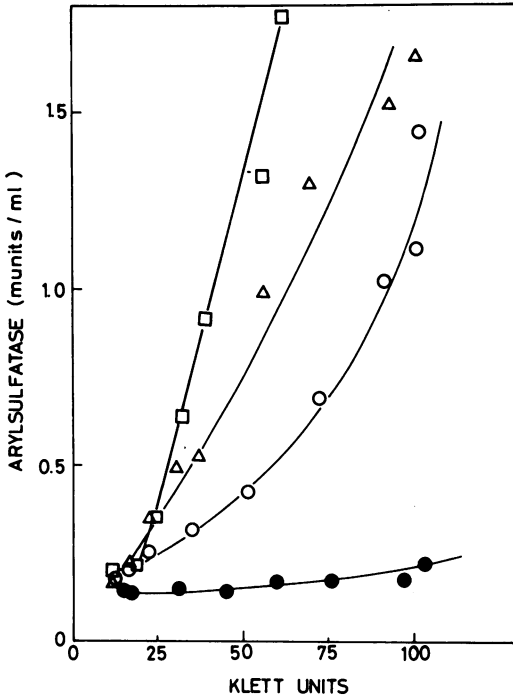


FIG. 3. Effects of glucose and cyclic AMP on arylsulfatase synthesis. Conditions were as described for Fig. 1. The enzyme levels in K13 cells grown with various compounds were assayed. Symbols: \square , with 0.25% xylose; Δ , with 0.25% glucose and 20 mM cyclic AMP; \circ , with 0.25% glucose and 10 mM cyclic AMP; \bullet , with 0.25% glucose and without cyclic AMP.

strain, K701, resulted in a rate of synthesis comparable to that observed in strain K13 grown with xylose and tyramine (Fig. 4). K13 could not synthesize the enzyme when grown in medium with glucose, sodium sulfate, and tyramine or with xylose and sodium sulfate in the absence of tyramine. It was also demonstrated that in the mutant strain K701 β -galactosidase synthesis was induced immediately after addition of isopropyl-thio- β -D-galactopyranoside (IPTG) in the presence of glucose, whereas synthesis of arylsulfatase occurred a relatively long time after addition of tyramine (Fig. 4). We also isolated other mutant strains which are incapable of derepressed synthesis of arylsulfatase, even when grown on xylose and inorganic sulfate in the presence of tyramine. The results obtained with these strains are summarized in Table 3. The mutant strain, K611, in which tyramine cannot relieve repression of arylsulfatase was distinguished from the mutant strain, K601, deficient in the structural gene of arylsulfatase by its high enzyme activity in medium containing methionine as the sulfur source. It can be seen that cyclic AMP stimulated arylsul-

fatase synthesis in K13 cells grown on glucose about 17-fold, whereas it had no effect on enzyme synthesis in the mutant strains K601, K611, and K701. In the mutant strain, K611, repression of arylsulfatase synthesis by sodium sulfate was not relieved in the presence of tyramine, cyclic AMP, or both. However, the enzyme was synthesized in cells grown with methionine as the sulfur source. Thus, arylsulfatase synthesis with nonrepressible sulfur compounds, such as methionine, is independent of catabolite repression. No arylsulfatase activity was detected in K601 cells grown under any conditions. When grown on xylose, β -galactosidase synthesis was induced by IPTG in both mutant strains, K601 and K611 (data not shown).

DISCUSSION

The present results indicate that repression of arylsulfatase synthesis caused by a sulfur compound is relieved by tyramine and that a protein factor regulated by catabolite repression is involved in derepressed synthesis of the enzyme. The protein factor has not yet been identified, but studies on the kinetics of arylsulfatase synthesis after various derepression regimes and experiments using mutant strains should provide clues on its nature.

Previously, Harada reported that when the

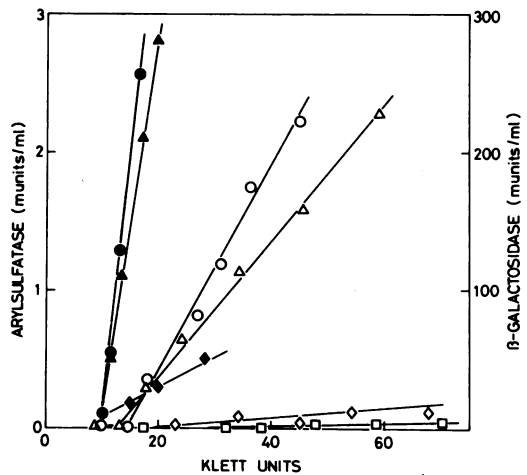


FIG. 4. Kinetics of arylsulfatase and β -galactosidase synthesis in mutant cells grown in various media. Conditions were as described in Fig. 1, except that two types of carbon source (0.25%) were used. For synthesis of β -galactosidase IPTG was added just before incubation. Open symbols represent levels of arylsulfatase and closed ones show β -galactosidase synthesis. Symbols: \circ , K13 grown on xylose; Δ , K701 grown on glucose; \diamond , K13 grown on glucose; \square , K13 grown on xylose in the absence of tyramine.

TABLE 3. Effects of tyramine and cyclic AMP on arylsulfatase synthesis in various mutant strains^a

Supplement			Arylsulfatase activity ^b (m units/mg of cells)			
Carbon source (0.25%)	Tyramine (1 mM)	Cyclic AMP (10 mM)	K13	K601	K611	K701
Xylose	-	-	0.06	0.07	0.04	0.05
Xylose	-	+	0.10	0.07	0.04	0.06
Xylose	+	-	4.03	0.06	0.06	1.14
Xylose	+	+	3.51	0.06	0.03	1.00
Xylose	-	-		0.09 ^c	2.13 ^c	
Glucose	-	-	0.07	0.10	0.08	0.12
Glucose	-	+	0.10	0.07	0.08	0.11
Glucose	+	-	0.12	0.07	0.08	1.55
Glucose	+	+	2.00	0.06	0.06	1.18

^a The medium contained 0.1% NH₄Cl, 1 mM Na₂SO₄, and the supplements shown. Cells were harvested after approximately three doublings.

^b Values are averages of three independent experiments.

^c Cells were grown with 1 mM methionine as the sole sulfur source.

cells of *A. aerogenes* ATCC 9621 were grown on glucose, tyramine failed to cause enzyme synthesis in the presence of inorganic sulfate, which causes repression (8). The present work showed that arylsulfatase synthesis in *K. aerogenes* occurs after the consumption of glucose during growth in the presence of tyramine (Fig. 1). However, the enzyme is synthesized in the cells grown with methionine as the sole source of sulfur, irrespective of the type of carbon source used (Table 2). Like many catabolic enzyme systems (15, 17, 19), the repression by glucose observed here is reversed by cyclic AMP. From these results, glucose seems to repress synthesis of some protein concerned with the action of tyramine in relieving repression of arylsulfatase synthesis. We found that tyramine transport into cells was similar in cells grown on glucose and xylose (Fig. 2). Since a monoamine oxidase-deficient strain K13 was used, tyramine should not be metabolized to any other compounds, even in the presence of glucose, as previously reported by us with *A. aerogenes* T11 (1). Thus, the effect of glucose on arylsulfatase synthesis does not appear to be related to an alteration of tyramine transport or degradation.

Our results with three kinds of mutant strains also support this assumption. One of the mutants, K701, produces arylsulfatase in the presence of glucose, sodium sulfate, and tyramine (Fig. 4). Since K701 also induces β -galactosidase, even in the presence of glucose, this mutant is probably deficient in the common gene concerned with catabolite repression, such as the enzyme systems involved in the formation of catabolite-repressible compounds derived from glucose or in the degradation of cyclic AMP. In this strain, arylsulfatase is

derepressed by tyramine after a relatively long time compared with that for β -galactosidase synthesis. This also suggests that derepressed arylsulfatase synthesis requires some inducible protein factor(s). Synthesis of this protein might be induced by tyramine or an unknown factor affected by tyramine. In the mutant strain, K611, repression of arylsulfatase synthesis by tyramine cannot be relieved even in the presence of cyclic AMP. Furthermore, this mutant strain produces a normal level of β -galactosidase. These results suggest that the fact that repression cannot be relieved is not due to a low level of cyclic AMP or cyclic AMP receptor protein, as reported by Emmer et al. (5). In K611 cells, unlike in the mutant K601 deficient in the structural gene of arylsulfatase (*Ats*⁻), the enzyme is synthesized in medium with methionine as a sulfur source. Thus, K611 cells are probably defective in a protein which is involved in the derepression of enzyme synthesis by tyramine. The genetic bases on these mutations in *K. aerogenes* are now under investigation using a generalized transducing phage, PW52.

In *K. aerogenes*, methionine does not repress arylsulfatase synthesis directly. This compound probably does not cause appreciable accumulation of inorganic sulfate or cysteine, which cause repression.

In previous work, tyramine was found to relieve the complete repression of arylsulfatase formation in *A. aerogenes* due to either sodium sulfate or L-cysteine, causing at least 230- and 450-fold increases in enzyme activity, respectively (12), whereas in *Pseudomonas aeruginosa* arylsulfatase was not formed in response to this compound (10). Fitzgerald et al. showed that

tyramine partially relieved full repression of arylsulfatase synthesis due to L-cysteine in a strain of *Pseudomonas* and caused slight stimulation of arylsulfatase formation in the presence of sodium sulfate (6). In the *K. aerogenes* strain used here, tyramine relieved repression of arylsulfatase synthesis by sodium sulfate, causing at least a 50-fold increase in enzyme activity (Tables 2 and 3). The significance of the different effects of tyramine on enzyme synthesis in various bacteria should be investigated with mutant strains incapable of degrading tyramine. However, we could not find much difference in the rates of arylsulfatase synthesis in the wild-type and mutant strain on derepression by tyramine, although the tyramine was almost consumed by the wild-type strain during growth (1). Therefore, the differences in the effects of tyramine in derepression of arylsulfatase synthesis in different bacteria may be due to complexity in its regulation in these bacteria, rather than in differences in the extent of tyramine degradation. It is not yet known why the aryl compound, tyramine, is required for arylsulfatase synthesis. The complexity of regulation of arylsulfatase synthesis may be a reflection of the complexity of sulfate metabolism, including that of arylsulfate ester. The protein factor regulated by catabolite repression could be included in this metabolism, or some repressor molecule activated by inorganic sulfate may be inactivated through the protein factor with or without tyramine. Recently, using mutant strains, we found that the repressions of arylsulfatase synthesis by inorganic sulfate and by cysteine are different and that there are at least two independent functional co-repressors of arylsulfatase synthesis in *K. aerogenes* (Adachi et al., manuscript in preparation).

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