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Genetic Control of Tyramine Oxidase, Which Is Involved in Derepressed Synthesis of Arylsulfatase in *Klebsiella aerogenes*

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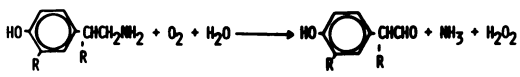
Mutants of *Klebsiella aerogenes* with three types of mutations affecting regulation of tyramine oxidase were isolated by a simple selection method. In the first type, the mutation (*tynP*) was closely linked to the structural gene for tyramine oxidase (*tynA*). The order of mutation sites was *atsA-tynP-tynA*. In the second type, the mutation that relieves catabolite repression of the syntheses of several catabolite repression-sensitive enzymes are not linked to the *tyn* gene by P1 transduction. These strains contained high levels of cyclic adenosine 5'-monophosphate when grown on glucose. The third type of mutation, in which tyramine oxidase was synthesized constitutively, was shown by genetic analysis to involve mutations of *tynP* and *tynR*. The *tynR* gene was not linked to *tynA*. Results using the constitutive mutants showed that the constitutive expression of the *tynA* gene resulted in derepression of arylsulfatase synthesis in the absence of tyramine.

Tyramine oxidase in bacteria, which is found in the cell membrane, is induced by tyramine and related compounds and is highly specific for tyramine, octopamine, dopamine, and norepinephrine (9, 11, 12). The enzyme oxidizes tyramine and catecholamines to hydroxyphenylacetaldehyde compounds and ammonium ions according to the following reaction:



where —R = —H or —OH. Tyramine oxidase in *Klebsiella aerogenes* is specified by the *tynA* gene and subject to catabolite repression by glucose in the presence of ammonium salts. This repression is relieved when the cells are grown on tyramine as the sole source of nitrogen (8, 12). The effect of ammonium ions on tyramine oxidase synthesis is independent of the function of cyclic AMP and glutamine synthetase (12).

Arylsulfate ester is hydrolyzed to an aryl compound and inorganic sulfate by *atsA*-specified arylsulfatase, which is regulated by sulfur compounds and tyramine and related compounds (8). Expression of the *tynA* gene results in derepression of arylsulfatase synthesis, which is repressed by inorganic sulfate or cysteine (8, 13).

In this paper, we describe a simple method for selection of mutants with defects in regulation of tyramine oxidase. By analysis of these mutants, we show that at least two genes regulate the synthesis of tyramine oxidase and that the constitutive synthesis of tyramine oxidase results in derepression of arylsulfatase synthesis.

MATERIALS AND METHODS

Bacterial strains and phage. The bacterial strains used in this study are listed in Table 1. The *K. aerogenes* strains used were P1-sensitive derivatives of *K. aerogenes* MK9000. Transducing phage P1*clr*100KM was obtained from R. A. Bender and B. Magasanik, Massachusetts Institute of Technology, Cambridge.

Media. The rich medium used was LB containing (per liter): tryptone (Difco), 10 g; yeast extract (Difco), 5 g; and NaCl, 5 g. LBKM medium was LB supplemented with 25 μg of kanamycin sulfate per ml. Minimal medium consisted of 0.5% carbon source, 0.1% nitrogen source, 0.05 M potassium phosphate buffer (pH 7.2), 0.01% $MgCl_2 \cdot 6H_2O$, and 1 mM Na_2SO_4. Unless otherwise mentioned, xylose and ammonium chloride were used as the carbon and nitrogen source, respectively. The GT(N) plates used for isolating the regulatory mutants of tyramine oxidase synthesis contained minimal medium with 0.02% tyramine as the nitrogen source, 0.5% glucose as the carbon source, and [for GT(N)I plates] indoxylsulfate (0.25 mg/ml) as an indicator of arylsulfatase; Ats^+ colonies become blue on plates containing indoxylsulfate. The XNI plates used for scoring the arylsulfatase-constitutive phenotype contained minimal medium with 0.5% xylose, 0.1% ammonium chloride, and indoxylsulfate, without tyramine; XT(N) plates contained minimal medium, 0.5% xylose, and 0.1% tyramine.

Isolation of mutants. Mutagenesis with *N*-

TABLE 1. *List of K. aerogenes strains used and their characteristics*

Strain	Relevant genotype	Source or reference
W70	Wild type, PW52[a]	MacPhee (7)
K711	*crr-701*	This laboratory (12)
MK9000	P1[a], PW52[a]	Bender and Magasanik (17)
MKN63	*tynA63*	This laboratory (10)
MK63-1	*tynP4 atsA343*	P1 from MKN343-1 to MKN63
MK63-2	*tynP4 atsA343*	P1 from MKN343-1 to MKN63
MKN301	Constitutive synthesis of tyramine oxidase	P1 from MKN343-1 to MKN63
MKN339	*tynP7*	Mutagenesis of MK9000
MKN343	*tynP4 tynR4*	Mutagenesis of MK9000
MKN344	*tynP5 tynR5*	Mutagenesis of MK9000
MKN345	*tynP6 tynR6*	Mutagenesis of MK9000
MKN360	*tynP8*	Mutagenesis of MK9000
MKN361	*tynP9 crr-361*	Mutagenesis of MK9000
MKN362	*tynP10*	Mutagenesis of MK9000
MKN363	*tynP11*	Mutagenesis of MK9000
MKN364	*tynP12*	Mutagenesis of MK9000
MKN365	*tynP13*	Mutagenesis of MK9000
MKN343-1	*ΔtynPA343 tynR4 atsA343*	Mutagenesis of MKN343
MKN343-1-1	*tynR4*	P1 from MK9000 to MKN343-1
MKN3439	*tynP7 tynR4*	P1 from MKN339 to MKN343-1

methyl-*N'*-nitro-*N*-nitrosoguanidine was achieved as described by Adelberg et al. (2). The mutagenized cells were spread on GT(N) or GT(N)I plates at about 10^8 cells per plate and incubated at 30°C for 3 days. Colonies that grew on GT(N) or GT(N)I plates were picked up and streaked to obtain single colonies. Wild-type cells (strain MK9000) grew very slowly in GT(N) medium because their synthesis of tyramine oxidase, which is required for utilization of tyramine as the nitrogen source, is repressed by glucose.

Growth. Since some strains described here are unstable, it was necessary to maintain selection of the mutation. The mutant strains were therefore grown overnight at 30°C in GT(N) medium. The next morning, the cells were diluted to about 2×10^7 to 5×10^7 cells per ml with fresh minimal medium supplemented with either glucose or xylose. The cells were cultured for three generations and then used for assays.

Transduction. P1 transduction was performed as described previously (10). Scoring of Ats and Tyn phenotypes was done as described previously (8). For detection of catabolite repression-sensitive or -insensitive transductants for tyramine oxidase, each Tyn^+ colony was suspended in 0.5 ml of sterilized saline, and then one loopful of suspension was patched on GT(N) plates. After 2 days of incubation at 30°C, patches that grew rapidly were scored as transductants that were insensitive to catabolite repression of tyramine oxidase. As a control, wild-type strain MK9000 and catabolite repression-insensitive strain MKN339 were patched on the same plate. Results obtained by this method agreed well with those obtained by assay of tyramine oxidase.

Enzyme assay. Arylsulfatase and tyramine oxidase activities were assayed as described previously (8). One unit of arylsulfatase activity was defined as the amount causing formation of 1 nmol of *p*-nitrophenol per min at 30°C. One unit of tyramine oxidase activity was defined as the amount metabolizing 1 nmol of tyramine per min at 30°C. β-Galactosidase was measured as described by Pardee et al. (14). One unit of enzyme activity was defined as the amount catalyzing the release of 1 nmol of *o*-nitrophenol from *o*-nitrophenyl-β-galactoside per min at 30°C. Histidase activity was assayed as described by Smith et al. (16). One unit of histidase activity was defined as the amount producing 1 nmol of urocanate per min at 30°C from histidine.

Assay of intracellular cyclic AMP. Samples for the determination of cyclic AMP were placed in a boiling-water bath for 3 min. They were then cooled, and cell debris was removed by centrifugation. The supernatant fraction was adjusted to pH 4 with HCl (15), and samples were assayed for cyclic AMP by the method of Gilman (4) using a cyclic AMP-assay kit containing cyclic AMP-binding protein (Boehringer Mannheim GmbH, Mannheim, W. Germany).

Protein determination. Protein was determined by the method of Lowry et al. (6) with bovine serum albumin as a standard.

Chemicals. [^{3}H]tyramine hydrochloride was purchased from the Radiochemical Centre, Amersham, England. *p*-Nitrophenylsulfate, obtained from Sigma Chemical Co., St. Louis, Mo., was recrystallized from aqueous ethanol before use. The other compounds used were standard commercial preparations.

RESULTS

Isolation and characterization of mutants insensitive to catabolite repression. *K. aerogenes* normally releases ammonium ion from tyramine through the action of tyramine oxidase. The synthesis of tyramine oxidase is induced by tyramine and is strongly repressed by glucose or other substrates that cause catabolite repression (12). Wild-type strain MK9000 grew very slowly (doubling time, >8 h) on minimal medium with tyramine as the sole source of nitrogen when glucose was used as the carbon source [GT(N) medium]. When *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-treated cells of strain MK9000 were suspended in saline and plated on GT(N) medium, mutants (about 1 to 5 per 10^6 cells plated) arose after 3 days at 30°C. Several mutants isolated in this way were studied. All of them were relieved from catabolite repression of tyramine oxidase and of arylsulfatase synthesis. Insensitivities of catabolite repression varied in different mutant strains. They could be divided into two groups on the basis of the pattern of catabolite repression of their enzymes (Table 2). Strains MKN339, 360, 362, 363, 364, and 365 have a mutation that relieves catabolite repression of tyramine oxidase and arylsulfatase, but

TABLE 2. *Enzyme levels of tyramine oxidase, arylsulfatase, β-galactosidase, and histidase in various mutant strains of K. aerogenes*[a]

Strain	Carbon source (0.5%)	Enzyme activity			
		Tyramine oxidase[b] (U/mg of protein)	Arylsulfatase[b] (U/mg of cells)	β-Galactosidase[c] (U/mg of cells)	Histidase[d] (U/mg of protein)
MK9000	Glucose	<0.1	8	2	23
	Xylose	2.4	70	113	89
K711	Glucose	2.2	166	300	103
	Xylose	1.6	138	313	80
MKN339	Glucose	1.6	40	6	17
	Xylose	3.5	70	101	82
MKN360	Glucose	1.0	14	9	3
	Xylose	2.8	45	114	26
MKN361	Glucose	3.8	51	107	64
	Xylose	1.4	110	273	62
MKN362	Glucose	0.5	17	4	12
	Xylose	1.0	42	132	65
MKN363	Glucose	2.1	24	3	23
	Xylose	3.0	43	128	101
MKN364	Glucose	1.5	19	6	28
	Xylose	1.5	58	147	73
MKN365	Glucose	0.9	16	14	34
	Xylose	2.8	66	165	80

[a] Cells were grown in minimal medium containing glucose or xylose as a carbon source and harvested after approximately three doublings.
[b] Tyramine (3 mM) was added as an inducer.
[c] Isopropyl-β-D-thiogalactopyranoside (1 mM) was added as an inducer.
[d] Histidine (10 mM) was added as an inducer.

not of β-galactosidase or histidase. These enzymes are known to be catabolite repression sensitive, like those in wild-type strain MK9000 (Table 2). This type of mutant, in which catabolite repression of tyramine oxidase is relieved, is called TynP$^-$. Since the derepressed synthesis of arylsulfatase in the presence of tyramine is caused by tyramine oxidase synthesis, the level of arylsulfatase under conditions of catabolite repression is just a reflection of that of tyramine oxidase; that is, synthesis of arylsulfatase itself is not directly subjected to catabolite repression (1, 8, 13).

In the second type of mutation, seen in strain MKN361, catabolite repression not only of tyramine oxidase synthesis, but also of β-galactosidase and histidase syntheses, is relieved, as in strain K711. Strain K711 was isolated as a mutant strain in which synthesis of arylsulfatase was derepressed by tyramine in the presence of glucose and inorganic sulfate (12). This strain also produced high levels of tyramine oxidase, β-galactosidase, and histidase (Table 2). This type of mutant showing carbohydrate repression resistance is called Crr$^-$. This group of mutants may have a mutation in some common gene concerned with catabolite repression, such as that related to the intracellular level of cyclic AMP. Thus, the intracellular level of cyclic AMP was assayed by the method with cyclic AMP-binding protein. The intracellular levels of cyclic AMP in strains K711 and MKN361 were about 0.28 to 1.70 nmol/mg of cells, which is 4 to 60 times higher than the level of wild-type strain W70 (0.03 to 0.07 nmol/mg of cells) when the cells were grown in glucose-NH_4Cl medium. The mutant strains in the first group had cyclic AMP levels similar to that of strain W70. Both types of mutants did not produce tyramine oxidase and arylsulfatase when cells were grown with inorganic sulfate as the sole source of sulfur in the absence of tyramine, irrespective of the carbon source used.

Genetic analysis of the mutations giving insensitivity to catabolite repression. The cotransduction frequency between mutations that gave insensitivity to catabolite repression and the *tynA* gene was determined by P1 transduction. Table 3 shows that these *tynP* mutations were 85 to 95% cotransducible with *tynA*, whereas the *crr* mutation from strain K711 was not linked to *tynA*. Although strain MKN361 shows regulation properties similar to strain K711, 84% of the TynA$^+$ transductants were insensitive to catabolite repression of tyramine oxidase. These transductants from strain MKN361 were purified, and several of their enzyme activities were measured. Catabolite

TABLE 3. *Genetic analysis of mutant strains showing insensitivity to catabolite repression by transduction with strain MK63 (tynA) as recipient*[a]

Donor (genotype)	Tyn$^+$ transductants analyzed (no.)	TynP$^-$ (%)
MKN339 (*tynP7*)	340	319 (94)
MKN360 (*tynP8*)	130	118 (91)
MKN361 (*tynP9*)	147	124 (84)
MKN362 (*tynP10*)	143	134 (94)
MKN363 (*tynP11*)	177	155 (88)
MKN364 (*tynP12*)	101	87 (96)
MKN365 (*tynP13*)	194	164 (85)
K711 (*crr-701*)	200	0 (0)

[a] Tyn$^+$ was selected on XT(N) plates; sensitivity to catabolite repression was analyzed on GT(N) plates.

repression was partially released in these transductants only for tyramine oxidase, not for β-galactosidase or histidase. This result suggests that strain MKN361 has two mutations concerned with catabolite repression, of which one is in the *tynP* gene linked to *tynA* and the other is at *crr*, which is not linked to *tynA*. *tynP* mutants may have a mutation that alters the promoter region for tyramine oxidase (*tynA*).

Isolation of a tyramine oxidase constitutive mutant. Constitutive mutants for tyramine oxidase were isolated by the same procedure using GT(N) plates. Among 20 mutants obtained as regulatory mutants for synthesis of tyramine oxidase, 1 mutant strain, MKN301, apparently synthesized tyramine oxidase constitutively without tyramine as an inducer, although the enzyme synthesis was still stimulated by addition of tyramine. When strain MKN301 was grown with inorganic sulfate, arylsulfatase repression was relieved without tyramine (Table 4). In wild-type strain MK9000, arylsulfatase repression caused by inorganic sulfate was alleviated by the addition of tyramine. The result with the constitutive mutant shows that expression of the tyramine oxidase gene (*tynA*) is essential for derepression of arylsulfatase synthesis and that no tyramine was required for derepression of arylsulfatase other than as inducer of tyramine oxidase synthesis.

Since this mutant strain was too unstable for genetic analysis, we tried to isolate several other mutants in which tyramine oxidase was synthesized constitutively, using another selection method with indoxylsulfate (1). This method is based on our previous finding that derepressed synthesis of arylsulfatase depended on synthesis of tyramine oxidase. Tyramine oxidase constitutive mutants, which also produce arylsulfatase, become blue on xylose-NH_4Cl agar without tyramine in the presence of indoxylsulfate (XNI medium). Three of seven constitutive mutants (MKN343, 344, and 345) thus isolated were stable. All of them synthesized tyramine oxidase constitutively in xylose-NH_4Cl medium in the absence of tyramine, except strain MKN339 (*tynP*), although constitutive levels of tyramine oxidase were lower than we would expect if they had altered repressors (Table 4). This could be due to the selection procedure. However, it seems to be sufficient to cause derepression of arylsulfatase synthesis. Enzyme levels of β-galactosidase and histidase did not differ from the parent strain MK9000 when grown on glucose or xylose with inducer (Table 4). Without inducer, the two enzyme activities were not detected. All these mutants were partially insensitive to catabolite repression of tyramine oxidase synthesis in glucose-NH_4Cl medium in the presence of tyramine (Table 4). These results show that constitutive expression of tyramine oxidase caused derepressed synthesis of arylsulfatase in the absence of tyramine. It is not yet known why the level of tyramine oxidase was so low when these mutant strains were grown in glucose-NH_4Cl medium in the absence of tyramine.

Genetic analysis of constitutive mutants. The linkage of the various constitutive mutations to *tynA* was determined by P1 phage. The constitutive property of strains MKN343, 344, and 345 could not be transferred to the recipient strain MKN63 (*tynA*), whereas 90% of the transductants were insensitive to catabolite repression (Table 5, crosses 2 to 4). These results suggest that for the constitutive synthesis of tyramine oxidase these constitutive mutants have another mutation, not linked to *tynA*, in addition to *tynP*. This mutation is named *tynR*. For more detailed genetic analysis, tyramine oxidase-deficient strain MKN343-1 was isolated from strain MKN343 (*tynP tynR*) by mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. This strain, MKN343-1, probably had an internal deletion of the *tyn* operon from some part of *tynP* to *tynA*, since no revertant of Tyn$^+$ was obtained, and *tynP* was 100% cotransducible with *tynA* (e.g., Table 5, crosses 5 and 6). When wild-type strain MK9000 was crossed with strain MKN343-1 as a recipient, all the TynA$^+$ transductants were insensitive to catabolite repression, but were not constitutive (Table 5, cross 5). When strain MKN339 (*tynP*) was used as a donor, all the TynA$^+$ transductants were both insensitive to catabolite repression and constitutive (Table 5, cross 6). The transductants from cross 6 were purified on GT(N)I plates. Enzyme activities of one of the transductants, MKN3439, are shown in Table 4. These results may be explained by supposing that the *tynR* mutation as well as *tynP* shows the phenotype of catabolite repression resistance. Thus, we constructed

TABLE 4. *Enzyme levels of tyramine oxidase, arylsulfatase, β-galactosidase, and histidase in various mutant strains of K. aerogenes*[a]

Strain	Carbon source (0.5%)	Tyramine (3 mM)	Enzyme activity			
			Tyramine oxidase (U/mg of protein)	Arylsulfatase (U/mg of cells)	β-Galactosidase[b] (U/mg of cells)	Histidase[c] (U/mg of protein)
MK9000	Glucose	−	<0.1	1		
		+	<0.1	6	4	25
	Xylose	−	<0.1	1		
		+	2.4	61	151	113
MKN339	Glucose	−	<0.1	1		
		+	1.6	40	6	19
	Xylose	−	<0.1	2		
		+	3.5	70	101	171
MKN301[us d]	Glucose	−	0.2	11		
		+	2.6	62	6	21
	Xylose	−	0.6	72		
		+	3.2	114	163	124
MKN343	Glucose	−	0.1	2		
		+	2.8	30	3	31
	Xylose	−	0.8	78		
		+	3.1	62	95	111
MKN344	Glucose	−	0.1	5		
		+	2.8	33	7	18
	Xylose	−	1.0	48		
		+	3.4	68	126	98
MKN345	Glucose	−	0.1	2		
		+	3.8	31	5	26
	Xylose	−	0.3	45		
		+	1.4	89	112	107
MKN3439	Glucose	−	0.2	2		
		+	2.5	28		
	Xylose	−	0.9	43		
		+	2.8	57		
MKN343-1-1	Glucose	−	0.1	2		
		+	0.8	21		
	Xylose	−	0.1	2		
		+	2.0	53		

[a] Cells were grown in minimal medium containing glucose or xylose as a carbon source, with or without tyramine as an inducer, and harvested after approximately three doublings.
[b] Isopropyl-β-D-thiogalactopyranoside (1 mM) was added as an inducer.
[c] Histidine (10 mM) was added as an inducer.
[d] us, Unstable.

strain MKN343-1-1, having the *tynP*$^+$ *tynA*$^+$ *tynR* genotype, from strain MKN343-1 (*tynP tynA tynR*) by P1 transduction from wild-type strain MK9000. Strain MKN343-1-1 was partially insensitive to catabolite repression of tyramine oxidase (Table 4). When strain MKN343-1-1 was used as a donor and strain MKN343-1 was the recipient, all the TynA$^+$ transductants were insensitive to catabolite repression, but no transductant with insensitivity to catabolite repression was obtained when strain MKN63 (*tynA*) was used as a recipient (Table 5, cross 7). These results also show that the *tynR* mutation bestows the phenotype of catabolite repression resistance and does not cotransduce with *tynA*. From these findings, we conclude that constitutive strains have *tynP* and *tynR* mutations, that both genes bestow the phenotype of insensitivity to catabolite repression of tyramine oxidase, and that two genes, *tynP* and *tynR*, are required for the constitutive expression of the *tynA* gene. Since strain

MKN343-1-1 (*tynR*) synthesized low levels of tyramine oxidase and arylsulfatase in the absence of tyramine (Table 4), the *tynP* mutation and the *tynR* mutation may have additive effects for full expression of the *tynA* gene constitutively.

Mapping of *tynP* mutation. Previously the *atsA* and *tynA* genes were mapped in *K. aerogenes* by P1 transduction and with F′ episomes from *Escherichia coli* (10). They are linked to *gdhD* and *trp* in the order *atsA-tynA-gdhD-trp-pyrF*. The location of the *tynP* gene was analyzed by three-point crosses in which P1 phage from the AtsA$^-$ TynP$^-$ strain was introduced into the TynA$^-$ strain. The results in Table 6 suggest the order *atsA-tynP-tynA*, 0% representing quadruple crossovers. Figure 1 summarizes the data obtained from these genetic analyses.

DISCUSSION

Tyramine oxidase synthesis in bacteria is of interest because it is controlled by carbon, nitrogen, and aromatic compounds. In particular, the enzyme has been implicated in the regulation of arylsulfatase synthesis.

In the present work we obtained interesting information on the genetic control of tyramine oxidase, which is involved in the derepressed synthesis of arylsulfatase in *K. aerogenes*. We found that mutants that grew rapidly on GT(N) medium were insensitive to catabolite repression of tyramine oxidase, which resulted in derepression of arylsulfatase synthesis. These mutants had a mutation of the *tynP* gene which was 84 to 94% cotransducible with *tynA*. In a second type of mutant, which had *tyn* nonspecific mu-

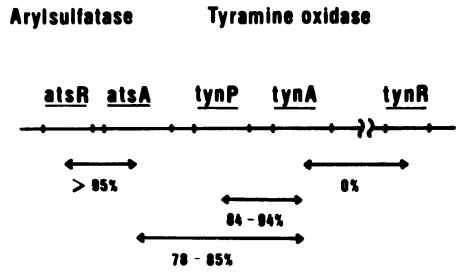


FIG. 1. *Map of the ats and tyn sites of K. aerogenes. Percentages represent cotransduction frequencies by P1 phage.*

TABLE 5. *Genetic analysis of the tynP and tynR genes*[a]

Cross no.	Donor (genotype)	Recipient (genotype)	No. of Tyn$^+$ transductants	Insensitive to catabolite repression	Constitutive synthesis of tyramine oxidase
1	MK9000 (wild type)	MKN63 (*tynA63*)	100	0	0
2	MKN339 (*tynP7*)	MKN63 (*tynA63*)	340	319	0
3	MKN343 (*tynP4 tynR4*)	MKN63 (*tynA63*)	600	527	0
4	MKN344 (*tynP5 tynR5*)	MKN63 (*tynA63*)	500	433	0
5	MK9000 (wild type)	MKN343-1 (Δ*tynPA343 tynR4*)	100	100	0
6	MKN339 (*tynP7*)	MKN343-1 (Δ*tynPA343 tynR4*)	101	101	101
7	MKN343-1-1 (*tynR4*)	MKN63 (*tynA63*)	50	0	
8	MKN343-1-1 (*tynR4*)	MKN343-1 (Δ*tynPA343 tynR4*)	50	50	

[a] Tyn$^+$ selection was done on XT(N) plates; sensitivity to catabolite repression was analyzed on GT(N) plates. Constitutive synthesis of tyramine oxidase was analyzed on XNI plates, or tyramine oxidase activity was assayed in each transductant grown on minimal medium without tyramine.

TABLE 6. *Order of tynP mutation deduced by three-point crosses*

Donor (genotype)	Recipient (genotype)	TynA$^+$ transducts analyzed (no.)	Unselected marker	No.	%
MKN63-1 (*atsA tynP*)	MKN63 (*tynA*)	454	*atsA tynP*	376	83
			atsA tynP$^+$	0	0
			atsA$^+$ *tynP*	19	4
			atsA$^+$ *tynP*$^+$	59	13
MKN63-2 (*atsA tynP*)	MKN63 (*tynA*)	404	*atsA tynP*	346	86
			atsA tynP$^+$	0	0
			atsA$^+$ *tynP*	21	5
			atsA$^+$ *tynP*$^+$	37	9

tations, several catabolite repression-sensitive enzymes, such as β-galactosidase and histidase, were not repressed by glucose, and the level of cyclic AMP was high when cells were grown on glucose. This *crr* mutation was not linked to any genes tested. We also demonstrated that a third type of *tynR* mutation, not linked to *tynA*, partially relieves catabolite repression of tyramine oxidase synthesis, irrespective of the state of *tynP*, and results in the constitutive expression of tyramine oxidase in the presence of the *tynP* mutation.

The constitutive syntheses of tyramine oxidase and arylsulfatase of more than half these mutants were very unstable. Genetic analysis showed that the instability was due to an unstable *tynP* mutation, rather than *tynR* (Oka et al., unpublished data).

It is possible that the product of the *tynR* gene is a negative regulation factor and acts at the *tynP* site, which has a special conformation, and that the *tynR* mutation causes very weak constitutive expression of the *tynA* gene which is increased in the presence of *tynP*. An alternative possibility is that the *tynP* mutation relieves the catabolite repression of the tyramine permease system and the *tynR* mutation results in a positive regulatory element which is more sensitive to tyramine and gives a weak activating ability even in the absence of tyramine. However, the *tynP* gene does not seem to regulate tyramine transport, because tyramine enters *K. aerogenes* cells by passive diffusion, and glucose does not inhibit the uptake of tyramine (1). However, we could not rule out the possibility that the *tynR* mutation results in a positive regulatory factor which interacts more efficiently with an altered *tynP*, is resistant to catabolite repression, and promotes rather weak constitutive expression of tyramine oxidase in the absence of inducer, as has been shown in the D-serine deaminase system (3, 5).

In our model presented previously, the expression of the *tynA* gene induced by tyramine results in derepression of arylsulfatase synthesis, which is repressed by sulfur compounds (8). However, we did not rule out the possibility that for derepression of arylsulfatase synthesis, tyramine itself might be required in addition to the synthesis of tyramine oxidase. The present results obtained with constitutive mutants show that expression of tyramine oxidase is essential for derepression of the *atsA* gene, but that tyramine itself is not required. Tyramine is only required to induce tyramine oxidase synthesis. This study also excludes the possibility that the effect of the carbon source on synthesis of tyramine oxidase and the derepressed synthesis of arylsulfatase may be due to exclusion of inducer from the cells, since the syntheses of both enzymes occurred in the constitutive mutant in the absence of inducer and were still subject to catabolite repression when the mutant cells were grown on glucose without tyramine (Table 4).

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