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Insensitivity of D-Amino Acid Dehydrogenase Synthesis to Catabolic Repression in *dadR* Mutants of *Salmonella typhimurium* *

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Summary. It has been found that synthesis of D-amino acid dehydrogenase in *Salmonella typhimurium* is stimulated by cyclic AMP and *crp* gene product. This indicates that catabolic control of the dehydrogenase resembles other bacterial systems of catabolic repression. We have isolated *S. typhimurium* mutants, *dadR*, which are resistant to L-methionine-interference with D-histidine utilization and are able to utilize D-tryptophan as a precursor of L-tryptophan. Mapping data indicate that the *dadR* locus is closely linked to *dadA* coding for the structure of D-amino acid dehydrogenase. The synthesis of the dehydrogenase in *dadR* mutants is completely insensitive to the repression by glucose, but remains inducible by L-alanine. We conclude thereof that *dadR* mutants have changes in the promoter region which increase the expression of the *dadA* gene in the presence of glucose metabolism. A likely possibility that induction of the *dad* operon by alanine might be under positive control is discussed.

Introduction

In our previous report D-amino acid dehydrogenase of *Salmonella typhimurium* has been described (Wild, Walczak, Krajewska-Grynkiewicz and Kłopotowski, 1974). The enzyme catalyzes oxidative deamination of D-alanine, D-histidine, D-methionine, D-phenylalanine and several other D-amino acids. Mutants *dadA* lacking activity of the dehydrogenase were shown to be unable to utilize D-histidine and D-methionine as precursors of the respective L-amino acids. This fact has implied that D-amino acid dehydrogenase plays an essential role in racemization processes of these two D-amino acids and very likely of the others which are substrates of the enzyme. We have also found that synthesis of D-amino acid dehydrogenase is inducible by L- or D-alanine and repressed by glucose.

Kuhn and Somerville (1971) have isolated in *Escherichia coli* tryptophan auxotroph mutants which are able to utilize D-tryptophan as an L-tryptophan substitute. The *dadR* mutants have increased activity of an enzyme deaminating D-histidine and D-phenylalanine.

In this paper we report on isolation by a different selection procedure of *S. typhimurium dadR* mutants in which synthesis of D-amino acid dehydrogenase is no longer repressed by glucose. The mutations map in close proximity of the structural gene coding for D-amino acid dehydrogenase and very likely make the promoter locus insensitive to an effector of catabolic repression.

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Tabelle 1. Description of strains

Strain	Genotype	Source or isolation
TA812	*hisDC129*	B. N. Ames
TA3302	*crp-403*	B. N. Ames
TK63	*dhuA13 hisDC129*	K. Krajewska-Grynkiewicz
TK630	HfrB2 *dadA1 dhuA1 gal-50 hisCBHAFIE3501 trp-1057*	W. Walczak
TK901	HfrB2 *dhuA1 gal-50 hemA207 hisCBHAFIE3501 trp-1057*	as neomycin resistant in TK657
TK904	HfrB2 *dadA1 dhuA1 gal-50 hemA208 hisCBHAFIE3501 trp-1057*	as neomycin resistant in TK630
TK909	*dadR6 dhuA13 hisDC129*	as L-methionine resistant in TK63
TK925	*dadR6 dhuA13 hisDC129 trp-1060*	by NG mutagenesis of TK909
TK931	*dhuA13 hisDC129 trp-1061*	by NG mutagenesis of TK63
TK938	*dhuA13 trp-1061*	by transduction of TK931 with TK1000
TK939	*dadR6 dhuA13 trp-1060*	by transduction of TK925 with TK1000
TK940	*dadR16 dhuA13 hisDC129 trp-1061*	as L-methionine resistant in TK931
TK941	*dadR17 dhuA13 hisDC129 trp-1061*	,,
TK942	*dadR18 dhuA13 hisDC129 trp-1061*	,,
TK943	*dadR19 dhuA13 hisDC129 trp-1061*	,,
TK944	*dadR20 dhuA13 hisDC129 trp-1061*	,,
TK1000	N. D. Zinder's LT2 strain (wild type)	B. N. Ames

Materials and Methods

Bacterial Strains. All strains used in the present study were derivatives of *Salmonella typhimurium* strain LT-2. They are presented in Table 1. The genetic nomenclature used obeys the general rules proposed by Demerec, Adelberg, Clark and Hartman (1966).

Media and Growth Conditions. Minimal media were prepared using the complete salt mixture C of Vogel and Bonner (1956) or the modified mixture NC which was devoid of citric acid (Berkovitz, Hushon, Whitfield, Roth and Ames, 1968). Rich media were made of nutrient broth, Difco or Biomed. For other details see Wild *et al.* (1974).

Genetic Procedures. The mutant *int-4* of phage P22 isolated by Smith and Levine (1967) was used for transduction. Phage cultures and transductions were performed as previously described (Krajewska-Grynkiewicz, Walczak and Kłopotowski, 1971). Mutants *hemA* requiring δ-aminolevulinic acid for growth were isolated using the neomycin procedure of Sasarman, Sanderson, Surdeanu and Sonea (1970).

Preparation of Toluenized Cells. The toluenization of washed bacterial suspensions was performed in an ice bath as described previously (Wild *et al.*, 1974).

Assay of D-amino Acid Dshydrogenase Activity. Activity of the enzyme was assayed in toluenized cells. The assay was performed as previously (Wild *et al.*, 1974) with some modifications. The reaction mixture contained 15 μmol of pyrophosphate buffer, pH 9.0, 15 μmol

of D-histidine, pH 9.0, an aliquot of toluenized cells in phosphate buffer, pH 7.4 and water added to the final volume of 0.50 ml. The reaction was stopped after 30 min incubation at 37° C by adding 1.0 ml of 1.67 M sodium hydroxide. Controls contained the complete reaction medium, but substrate was added after the alkalization. The alkaline reaction mixtures were incubated at 37° C for 30–40 min before optical density at 310 nm was measured. The molar extinction coefficient of 4000 M^{-1} cm^{-1} was used for calculating amounts of imidazolepyruvate from net optical density values.

Chemicals. All chemicals used were commercial products of reagent grade.

Results

Isolation of dadR Mutants

It was noticed that growth of the strain TK63 (*dhuA1 hisDC129*) on minimal plates supplemented with D- but not L-histidine was inhibited by L-methionine. A series of *S. typhimurium* strains carrying various mutations in the histidine operon and in the *dhuA* locus was examined in spot tests. The latter mutations increase activity of the specific histidine permease and enable thereby utilization of D-histidine as a substitute for the natural L-amino acid (Krajewska-Grynkiewicz *et al.*, 1971). It appeared that only a few other strains were sensitive to the L-methionine inhibitory effect.

The conditional L-methionine-sensitivity was not due to the *dhuA13* allele present in TK63 as its transfer to some other histidine auxotrophs gave strains resistant to L-methionine. On the other hand, strain TK274 constructed by transduction of *dhuA1* from TK51 to *hisDC129* was sensitive to L-methionine. Strains derived from TK63 in which mutations *hisCBHAFIE3501* (TK919) or *hisC630* (TK273), but not *hisF645* (TK912), had been substituted for *hisDC129* were sensitive to L-methionine on D-histidine plates. It was therefore concluded that the sensitivity is due to a non-identified gene allele in the genetic background of TK63 and that it is fully expressed in the absence of *hisC* product, i.e. imidazoleacetol phosphate aminotransferase. Evidence has been presented indicating that the enzyme functions in the second step of D-histidine racemization process (Walczak, 1974).

In routine tests 0.5 μmol of L-methionine or glycyl-L-methionine applied on filter paper discs put on soft agar overlayer with bacteria and 3 μmol of D-histidine produced inhibition zones of about 3.5 cm in diameter. The same amount of D-methionine did not inhibit the growth. There was not any inhibition with either L- or D-methionine on plate containing L-histidine.

L-Methionine did not inhibit D-histidine transport nor the activity or synthesis of D-amino acid dehydrogenase (data not shown). Therefore, biochemical basis of the inhibition remains unknown.

When D-histidine minimal plates inoculated with TK63 on which paper discs with L-methionine were put on, were incubated for three days colonies growing within the inhibition zones could be found. The growing clones were picked and purified by single colony restreaking on nutrient agar plates. Most of the isolated strains appeared to be resistant to L-methionine and glycyl-L-methionine.

Since L-methionine sensitivity was observed only on D-histidine plates, the enzyme involved in its metabolism was assayed in the L-methionine-resistant mutants grown in glucose minimal medium. Some of the mutants had the activity of D-amino acid dehydrogenase increased by a factor of 4 to 30.

Kuhn and Somerville (1971) used the designation *dadR* to *E. coli* mutants isolated as D-tryptophan utilizers and having increased activity of an enzyme

Table 2. Inducibility of D-amino acid dehydrogenase in *dadR* mutants by L-alanine[a]

Strain	Pertinent genotype	Specific activity of D-amino acid dehydrogenase			
		Pyruvate	Pyruvate + L-alanine	Glucose	Glucose + L-alanine
TK931	wild type	7	80	4	8
TK925	*dadR6*	25	101	53	169
TK940	*dadR16*	37	66	128	250
TK941	*dadR17*	46	66	124	174
TK942	*dadR18*	40	91	93	267
TK943	*dadR19*	22	71	17	51
TK944	*dadR20*	75	114	63	166

[a] Cells were grown overnight in NC medium supplemented with 0.1 mM L-histidine, 0.03 mM L-tryptophan and other additions indicated in the Table: sodium pyruvate 10 mM, L-alanine 5 mM or glucose 0.5%. Cells were collected and washed with NC medium on membrane filters. For the assay cells resuspended in 0.1 M phosphate buffer, pH 7.4, and treated with toluene in an ice bath were used.

[b] Specific activity is expressed as nmol of imidazolepyruvate formed per min per mg dry weight cells.

deaminating D-histidine and D-phenylalanine. Because our mutants, though isolated by a different selection procedure, had increased activity of D-amino acid dehydrogenase and were able to utilize D-tryptophan (manuscript in preparation) we named them *dadR*.

The ability to grow on D-tryptophan was used as a screening procedure for mutants with elevated activity of D-amino acid dehydrogenase. For this purpose strain TK931, a double histidine and tryptophan auxotroph was used. It turned out that about 15% of all L-methionine-resistant clones were able to give positive prints on glucose minimal plates supplemented with 0.3 mM D-tryptophan after 4–5 days of incubation at 37°C. Our attempts to isolate on D-tryptophan plates mutants of *S. typhimurium* able to utilize the D-stereoisomer as a substitute for L-tryptophan have failed.

D-Amino Acid Dehydrogenase Activity in dadR Mutants

Specific activity of D-amino acid dehydrogenase was measured in toluenized cells of the *dadR* mutants. D-Histidine was used as substrate. The mutants had been grown in liquid NC medium supplemented with pyruvate or glucose as carbon source. Parallel cultures contained L-alanine as an inducer. As a reference strain TK931 was used. The results are presented in Table 2. Non-induced activities of the *dadR* mutants grown on either carbon source were higher than those of the reference *dadR*$^+$ strain, 3 to 10-fold on pyruvate and 4 to 32-fold on glucose. There was practically no effect of *dadR* mutation on D-amino acid dehydrogenase activity in cells grown in the presence of L-alanine and pyruvate. However, when mutants were induced in the presence of glucose they had activity of the dehydrogenase 6 to 41-fold higher than that of the *dadR*$^+$ strain. When glucose and L-alanine were present in culture media the strain with wild type allele of *dadR*

Table 3. Partial reversal by cyclic AMP of catabolic repression of D-amino acid dehydrogenase by glucose[a]

Strain	Pertinent genotype	Specific activity of D-amino acid dehydrogenase[b]		
		—	Glucose	Glucose+cAMP
TK1000	*crp*+	86	6	57
TA3302	*crp-403*	34	10	17

[a] The bacteria were grown in NC medium containing 10 mM pyruvate as carbon source and 10 mM L-alanine as the inducer. Other supplements indicated in the Table were 0.5% glucose or 5 mM cyclic AMP.

[b] D-Amino acid dehydrogenase was assayed in toluenized cells. Its specific activity is expressed as nmol of imidazolepyruvate formed par min per mg dry weight cells.

gene had D-amino acid dehydrogenase activity reduced by 90%. In the mutant *dadR19* it was reduced only by about 30%. In all other *dadR* mutants the activity was rather increased from 50 to almost 400%. Hence in *dadR* mutants glucose does not repress D-amino acid dehydrogenase synthesis and even, in most cases, apparently increases the extent of the induction by L-alanine.

Because expression of many operons regulated by glucose repression is affected by cyclic AMP and the product of *crp* gene, effectiveness of cyclic AMP in reversing the catabolic repression of D-amino acid dehydrogenase synthesis was examined. As wild type the strain LT-2 (TK1000) was used. The mutant *crp-403* (TA3302) has a defective cyclic AMP receptor protein. Table 3 shows that the presence of cyclic AMP in glucose minimal medium increased the dehydrogenase activity almost 10-fold in the strain with wild type *crp* gene. Therefore catabolic control of D-amino acid dehydrogenase formation involves cyclic AMP as an effector. In the *crp* mutant cyclic AMP could not even double the activity of the enzyme. This is again in line with the accepted model of catabolic control involving *crp* gene protein product in the process of induction by cyclic AMP.

Histidase was chosen as an enzyme under catabolic control to examine whether *dadR* mutations affect only D-amino acid dehydrogenase or all enzymes the induction of which is repressible by glucose. This experiment is shown in Table 4. No significant difference between glucose effects on histidase specific activity in *dadR*+ and *dadR* strains can be seen. Although other enzymes undergoing catabolic control were not examined, it seems likely that the *dadR* locus is involved in catabolic control of only *dadA* product formation.

In several experiments question was asked whether the increased activity of *dadR* mutants, routinely assayed with D-histidine as substrate, was due to the same enzyme, i.e. the inducible D-amino acid dehydrogenase. K_m values for D-histidine were practically the same, 26 and 20 mM, with toluenized cells of strains *dadR*+ and *dadR6*, respectively. At 90 mM D-histidine the effect of another substrate of the enzyme, D-alanine, present at 50 mM concentration was the same, i.e. 65% inhibition. Cyanide, 2 mM, inhibited D-amino acid dehydrogenase of either origin by more than 90%. Therefore, it has been concluded that *dadR*

Table 4. Catabolic repression of histidase in strains carrying wild type or mutant alleles of *dadR* gene[a]

Strain	Pertinent genotype	Specific activity of histidase[b]			
		—	L-Histidine	Glucose	Glucose + L-histidine
TK938	*dadR*$^+$	2.1	6.6	0.1	0.5
TK939	*dadR6*	1.2	5.5	0.1	0.6

[a] The bacteria were grown in NC medium containing 10 mM sodium pyruvate as carbon source and 0.1 mM L-tryptophan. Other supplements added as indicated in the Table were 0.2 mM L-histidine or 0.5% glucose. Cells were collected and washed with NC medium on membrane filters. For the histidase assay they were resuspended in 0.1 M phosphate buffer, pH 7.4 and treated with toluene at room temperature. The reaction mixture contained 100 μmol of diethanolamine-hydrochloric acid buffer, pH 9.6, 20 μmol of L-histidine, toluenized cells and water to the final volume of 1.0 ml. The reaction was started by adding L-histidine solution, run at 37° C for 30 min and stopped by adding 0.1 ml of 36% perchloric acid. Precipitates were removed by centrifugation. Urocanic acid in the supernatants was determined by measuring optical density at 277 nm.

[b] Specific activity of histidase is expressed as the increase of optical density at 277 nm per hour per mg dry weight cells.

mutations increase the activity of D-amino acid dehydrogenase and not of another enzyme of similar substrate specificity.

Genetic Mapping of dadR Mutations

Because *dadR* mutations alter the catabolic repression of D-amino acid dehydrogenase one can expect that they map near to the gene *dadA* which has been shown to code for the structure of D-amino acid dehydrogenase. The *dadA* mutations are 3% linked to *hemA* in P22-mediated transduction (Wild *et al.*, 1974).

To examine this point, the strain TK630 (HfrB2 *dadA1 dhuA1 gal-50 his-CBHAFIE3501 trp-1057*) was used as recipient in crosses on D-histidine-containing minimal agar plates with phage grown on *dadR* donors. For screening the recombinants advantage was taken of the ability of *dadR trp* mutants to utilize D-tryptophan as a source of L-tryptophan. When the strain TK909 (*dadR6 dhuA13 hisDC129*) was used as the donor 85% of *dadA*$^+$ recombinants had the *dadR* marker. The linkage of other *dadR* mutations with *dadA* varied from 85 to 93%.

To check whether the *dadR* locus is cotransducible with *hemA* gene, a cross was made on nutrient broth plates with a *hemA trp* recipient (TK901) and phage grown on the *dadR6* strain (TK909) as donor. Eight percent of *hemA*$^+$ recombinants had the *dadR* marker.

The result of a three-point cross performed to establish order of the genes *hemA*, *dadA* and *dadR* is presented in Table 5. Among 259 *dadA*$^+$ recombinants obtained on D-histidine plus δ-aminolevulinate plates, 196 had the *dadR* character, 21 of those became also *hemA*$^+$. The cotransducibility values with *dadA1* calculated from these data were 75.7% for *dadR6* and 10.4% for *hemA208*. The rarest class of 6 recombinants was *dadR*$^+$ *hemA*$^+$. It would have resulted from a quadruple

Table 5. Genetic mapping of *dadR* locus by three-point cross[a]

Nonselective markers		Numbers of crossovers for the given gene order			Number of recombinants	Percent of recombinants
dadR	hemA	A	B	C		
+	+	4	2	2	6	2.3
+	208	2	2	2	57	22.0
6	+	2	2	2	21	8.1
6	208	2	4	2	175	67.6
				Total	259	100.0

[a] In the transduction cross *dadA*$^+$ was the selective marker. The cross was performed on the plate containing D-histidine plus δ-aminolevulinic acid using phage grown on the strain TK909 (*dadR6 dhuA13 hisDC129*) and the recipient cells of the strain TK904 (HfrB2 *dadA1 dhuA1 gal-50 hemA208 hisCBHAFIE3501 trp-1057*). Designations of possible gene orders are: A = *dadA dadR hemA*, B = *dadA hemA dadR* and C = *dadR dadA hemA*.

crossover if the gene order was *dadA dadR hemA*. We therefore conclude that this is the gene order.

None of the three markers could be cotransduced using phage P22 with *trp* or *purB*. Mojica-a (1974) used phage P1 for transductions in this region. He found that *hemA* was 10 and 2% cotransducible with *trp* and *pyrF*, respectively. Analogous values for *dadA* were 1 and 0%. Fig. 1 shows the localization of *dad* operon in respect to other markers on the genetic map of *S. typhimurium*. The map is drawn to scale. The physical distances were calculated using the equation derived by Kemper (1974). The two bars represent DNA length of phages P22 and P1. One gene length is assumed to be $^1/_{40}$ or $^1/_{88}$ of P22 or P1 DNA, respectively.

Kuhn and Somerville (1971) mapped their *dadR* mutants on the *gal*-proximal side of *trp*. This cannot argue against possible identity of *S. typhimurium* and *E. coli dadR* loci as the *trp* region is inverted in one species in respect to the other (Sanderson, 1972).

Discussion

In this paper we report on isolation by a novel procedure of *dadR* mutants in *S. typhimurium* and their insensitivity to catabolic repression of D-amino acid dehydrogenase. Our selection procedure is feasible in certain strains carrying at the same time *hisC* and *dhuA* mutations and which cannot utilize D-histidine in the presence of L-methionine. The procedure is empirical and no explanation how it works can be proposed at the moment. About one seventh of mutants resistant to L-methionine on D-histidine plates had increased activity of D-amino acid dehydrogenase and were able to utilize D-tryptophan. In both respects they resembled *dadR* mutants isolated previously by Kuhn and Somerville (1971) in *E. coli* as D-tryptophan-growers. We also interprete the genetic localization close to *trp* operon as pointing to a possible identity of *S. typhimurium* and *E. coli dadR* loci.

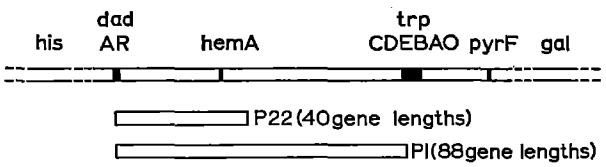

Fig. 1. Localization of *dad* operon on the genetic map of *S. typhimurium*. The Figure represents the segment of the bacterial chromosome at about minute 52 of the standard *S. typhimurium* genetic map (Sanderson, 1972). The intergenic distances are drawn to scale. The physical distances were calculated from percent linkage values obtained with phage either P22 or P1 using the equation developed by Kemper (1974). The distance of 52 average gene lengths for the segment *hemA-trp* and other distances measured with P1 were taken from the data of Mojica-a (1974). The distance *dadA-hemA* was calculated from P1 (45 genes) and pooled P22 cotransduction values (32 genes) of this paper and of the data of Walczak (1974). The distance *trp-pyrF* calculated from P1 data of Mojica-a is 24 genes and from P22 transductions of Wiater and Kłopotowski (1972)—22 genes. The distance *dadA-pyrF* calculated from different combinations of P22 or P1 data is long from 106 to 121 average genes. The two bars represent P22 and P1 DNA lengths drawn to the same scale as the chromosome segment

In wild type *S. typhimurium* synthesis of D-amino acid dehydrogenase is induced by either L- or D-alanine and repressed when glucose is the carbon source (Wild *et al.*, 1974). In this paper we show that cyclic AMP added to growth medium largely reverses the repressive effect in a strain with functional gene *crp* coding for cyclic AMP receptor protein and is much less effective in a *crp* mutant. Experiments with *in vitro* protein synthesizing systems have shown that RNA polymerase attaches to promoter DNA and that this determines initiation of transcription process (Chambers and Zubay, 1969). Cyclic AMP bound to *crp* gene product was demonstrated to function *in vitro* in stimulating the transcription process of *E. coli lac* operon (Emmer, deCrombrugghe, Pastan and Perlman, 1970; Zubay, Schwartz and Beckwith, 1970). Silverstone, Goman and Scaife (1972) have described *E. coli* mutants *alt* in which expression of *lac* operon was independent of the presence of cAMP-CRP complex. This finding allows to presume that a product of *alt* or of another gene must be present in wild-type form for the expression of a catabolic operon depended on the presence of cAMP-CRP complex. Effectiveness of cyclic AMP in stimulating synthesis of D-amino acid dehydrogenase repressed by glucose indicates that expression of *dadA* gene follows the pattern described for catabolic repression of *lac* operon.

In most of the *S. typhimurium dadR* strains induction of D-amino acid dehydrogenase by L-alanine was completely insensitive to catabolic repression by glucose. This has indicated that *dadR* function is involved in catabolic repression. Because the glucose repressive effect on histidase synthesis was unchanged in the mutants and *dadR* mutations were located at a very close distance, one to three average gene lengths, from the structural gene of the dehydrogenase, *dadA*, it is concluded that the two *dad* genes belong to the same operon.

It should be noted that all our *dadR* mutants remained sensitive to the inducing effect of L-alanine on D-amino acid dehydrogenase synthesis. In other words, none of the *dadR* mutations produced constitutivity of *dadA* expression and hence none of them could fulfill the essential element of the definition of an

operator mutant. We conclude thereof that *dadR* mutants have changes in the promoter region which increase expression of the *dadA* gene.

There are several reports on promoter mutations which increase expression of catabolic operons or genes. In *lac* operon promoter-up mutations are clearly distinct from operator mutations. In many *lac* promoter-up mutants β-galactosidase was inducible to practically the same level in the presence of either glycerol or glucose used as carbon source (Arditti, Grodzicker and Beckwith, 1973). Berman-Kurtz, Lin and Richey (1971) isolated in *E. coli* promoter-up mutants with partially reduced sensitivity to catabolic repression of glycerol kinase operon and still inducible by L-α-glycerophosphate. In *S. typhimurium* the best studied promoter-up mutants are those in *hut* operons coding for enzymes of L-histidine degradation (Smith and Magasanik, 1971). Some of the promoter-up mutants were no longer inducible by L-histidine (Brill and Magasanik, 1969). Newell and Brill (1972) isolated catabolic repression-insensitive mutants for *put* operon which codes two enzymes of L-proline degradation. Only partial insensitivity to the glucose effect was observed in the *putR* mutants.

In many respects our *dadR* mutants resemble promoter-up mutants in other catabolic operons. This implies that the *dadR* locus is the site necessary for the initiation of transcription of the structural gene *dadA* and for interactions of regulatory molecules involved in catabolic repression. The inducibility of D-amino acid dehydrogenase by alanine persisting in catabolic-repression-insensitive mutants suggests that an operator locus must exist adjacently to the promoter. However, in spite of our efforts any mutants which would have regulation of *dadA* expression independent from the presence of L-alanine in growth medium could not be isolated, on plates with either glucose or any of several poor carbon sources. In other words, neither operator-constitutive or unlinked regulatory mutants could be found.

The easiness of obtaining *dadR* mutants indicates that their promoter-up character may result from random mutations. This could most likely happen if the indiscriminate changes in the promoter prevent binding of an *alt*-like factor and release thereby transcription of *dadA* from dependence on cyclic AMP-CRP complex rather than increase the effectiveness of RNA polymerase interaction with the changed DNA sequence.

A change of the presumed operator to constitutivity, i.e. to an inability to bind a repressor should not be very stringent, either. A random mutation of a repressor gene, unless essential for another function, should also result in an inducer-independence. Therefore, the difficulty of isolating operator or repressor gene mutants, suggests that the *dad* operon is under positive control. In such a case random mutation in either unlinked regulatory gene or the operator would produce lack of *dadA* expression and inability to grow on the selection medium in which D-histidine was the only possible precursor of L-histidine. More specific changes in structure of the activator or the operator which would allow expression of *dadA* at high constitutive level could be very rare.

Beelen, Feldman and Wijsman (1973) have described *E. coli* mutants lacking alaninase, an enzyme which deaminates D-alanine. They map in two loci distant from each other in the *thr-leu* region of the chromosome. Because the enzyme

is inducible by either L- or D-alanine and repressible by glucose it could be homologous if not identical with D-amino acid dehydrogenase of *S. typhimurium*. In a temperature-sensitive mutant *alnR* alaninase could not be induced by L-alanine in non-permissive conditions. The authors discuss the possibility that alaninase production is regulated positively. Other authors have described D-alanine oxidase, a structure-bound enzyme of *E. coli* inducible by L-alanine (Raunio and Jenkins, 1973; Raunio, D'Ari Straus and Jenkins, 1973). It may well be that D-amino acid dehydrogenase of *S. typhimurium* which is also bound to cell structure (Wild *et al.*, 1974), alaninase and D-alanine oxidase of *E. coli* are different names for the enzyme of the same function and regulation. The genetic data of Beelen *et al.* (1973) will be used in our study aimed at finding more evidence for positive regulation of D-amino acid dehydrogenase in *S. typhimurium*.

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