JOURNAL OF BACTERIOLOGY, Jan. 1976, p. 376-378

Vol. 125, No. 1
Printed in U.S.A.

Repression of Diaminopimelic Acid Decarboxylase in *Escherichia coli*: Gene Dosage Effects and Escape Synthesis

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Received for publication 3 October 1975

Gene dosage and escape synthesis experiments support the hypothesis that diaminopimelate decarboxylase repression by lysine involves a repressor molecule in a negative control system.

The conversion of *meso*-2,6-diaminopimelic acid (Dpm) to L-lysine catalyzed by the enzyme diaminopimelic acid decarboxylase (Dpm-DCase; EC 4.1.1.20; *meso*-2,6-diaminopimelate carboxy-lyase) is the last step in the de novo biosynthesis of L-lysine in bacteria. The enzyme in *Escherichia coli* has been purified and characterized (16). Repression of the enzyme by lysine was reported by Patte et al. (9), who observed that 60 to 80% of the Dpm-DCase activity found in cells grown in minimal medium was repressed when cells were grown in minimal medium containing lysine. Similar variations in specific activity with growth conditions were observed by White et al. (17).

The gene that codes for this enzyme, *lysA*, has been mapped in *E. coli* and shown to lie at position 55 min on the chromosome and to be cotransduced with *thyA* at a frequency of 80% (4, 15). The availability of an episome, F'_{15} (7), which carries the *thyA* and *lysA* genes makes it possible to investigate the effect of gene dosage upon this repression of the *lysA* gene in *E. coli*. Additionally, the isolation by Shimada et al. (14) of a transducing phage carrying the *lysA* gene makes it possible to determine whether escape synthesis of this enzyme occurs when the gene is present on a replicating phage genome or whether the repression system can prevent such synthesis. Results presented in this paper support the conclusion that lysine exerts this repressive effect as a corepressor acting in concert with a small number of lysine repressor molecules.

Bacterial strains used in this study are listed in Table 1. The results of experiments in which the effect of gene dosage on repression was studied are summarized in Table 2. The percentage of repression by lysine was a function of the number of copies of the gene present in the cell, since the 50% repression observed in the strain containing one gene copy was reduced to only 26% repression when the number of *lysA* gene copies was increased to three. These results are consistent with the hypothesis that Dpm-DCase synthesis is regulated by a negative Dpm control system consisting of a small number of lysine repressor molecules that are unable to give complete repression of a single copy of the *lysA* gene and are less efficient at repressing additional copies of the gene. These results also suggest that the lysine repressor gene is not closely linked to the *lysA* gene since no such effect of additional gene copies would be expected if the repressor gene copies were increased simultaneously with the *lysA* gene.

Further support for this conclusion was sought by investigating whether escape synthesis of Dpm-DCase occurred after thermoinduction of the lysis-defective, *lysA*-transducing phage. Cells (RK1222) were grown in complex medium at 30 C for several generations, at which time the specific activity of Dpm-DCase was 18% of that observed in minimal medium. This increased repression of 82% in complex medium over that observed in minimal medium plus lysine (40%; see Table 2), has also been reported by White et al. (17). The cells were then shifted to 42 C for 20 min to induce the phage and were returned to 30 C for a total of 2 h after induction. The results of a representative experiment are presented in Fig. 1. An increase of approximately 30-fold in the specific activity of the enzyme was consistently observed during this 2-h incubation period.

It is possible that this increased enzyme synthesis could be an effect of temperature shift or of phage growth rather than the result of escape synthesis after induction of a *lysA*-transducing phage. To rule out this possibility, control experiments were performed by using a strain bearing the *lysA* gene on the bacterial genome and not on the lysogenizing nontransducing phage (RK1021) and a strain having no *lysA* gene at all and lysogenized by a nontransducing phage (RK1221) (Table 3). The large

TABLE 1. *E. coli strains used*

Designation	Relevant genotype	Source
SA270	*his*, [*gal*, *attλ*, *bio*] , *strA*	D. Korn
SA270/F'*gal*	SA270/F'*gal*$^+$, attλ$^+$, *bio*$^+$	D. Korn
W3422 *thy*$^-$	*argA*, *thyA*, *strA*	S. Bourgeois
JC5488	*thr*, *leu*, *thi*, *his*, *pro*, *arg*, *thyA*/F'$_{18}$*lysA*$^+$, *thyA*$^+$	A. J. Clark
1174	[*lysA*]$_\Delta$, *strA*	P. Starlinger
WB41	*ilv*, *gal*	J. Abelson
WB41/F'*gal*	WB41/F'*gal*$^+$, *attλ*$^+$, *bio*$^+$	WB41 × SA270/F'*gal*
RK1021	SA270/F'*gal*$^+$, (λC$_{1857}$*Sam7*), *bio*$^+$	Infection of SA270/F'*gal*[a]
RK1100	SA270 *thyA*	P1 transduction from W3422 *thy*$^-$
RK1110	RK1100/F'$_{18}$*lysA*$^+$, *thyA*$^+$	RK1100 × JC5488
RK1200	SA270 [*lysA*]	P1 transduction from 1174
RK1210	RK1200/F'$_{18}$*lysA*$^+$, *thyA*$^+$	RK1200 × JC5488
RK1220	RK1200/F'*gal*$^+$, *attλ*$^+$, *bio*$^+$	RK1200 × WB41/F'*gal*
RK1221	RK1200/F'*gal*$^+$, (λC$_{1857}$*Sam7*), *bio*$^+$	Infection of RK1220[a]
RK1222	RK1200/F'*gal*$^+$, (λC$_{1857}$*Sam7*, λC$_{1857}$*Sam7*d*lysA*), *bio*$^+$	Transduction of RK1220[b]

[a] The temperature-sensitive, lysis-defective phage used for infection was obtained from J. Miller.

[b] The transducing phage used was a lysis-defective derivative constructed in this laboratory by introducing the Sam7 mutation into a λC$_{1857}$d*lysA* phage derived from strain KS72 {[*gal*, *attλ*, *bio*, *uvrB*]$_\Delta$, (λC$_{1857}$) *lysA*$^+$} obtained from K. Shimada.

TABLE 2. *Effect of gene dosage on repression of Dpm-DCase by lysine*[a]

Strain	Genotype	Gene copies[b]	Repression (%)[c]			Avg
			Expt 1	Expt 2	Expt 3	
RK1100	*lysA*$^+$	1	58	52	49	53
RK1210	[*lysA*] /F' *lysA*$^+$	2		42	44	43
RK1222	[*lysA*] /F'λd*lysA*	2	34	40	45	40
RK1110	*lysA*$^+$/F'*lysA*$^+$	3	31	25	25	26

[a] Based on estimate by Revel (11) of two copies of F' per genome.

[b] Determined as {1.00 − [(Dpm-DCase specific activity in minimal medium + lysine)/(Dpm-DCase specific activity in minimal medium)]} × 100.

[c] Dpm-DCase activity was determined by a modification of the technique of Bukhari and Taylor (3), using *m*-Dpm purified according to White et al. (17) as substrate. Lysine did not inhibit the assay at concentrations used in these studies. Cells were grown in minimal medium (Na_2HPO_4, 7 g; NH_4Cl, 1 g; KH_2PO_4, 3 g; Na_2SO_4, 0.8 g; $MgSO_4$, 2 μmol; sodium citrate, 4 μmol; vitamin B1, 50 μg; glucose, 10 g; 1,000 ml of distilled water) and in minimal medium supplemented with 100 μg of L-lysine/ml. Thymine (400 μg/ml), histidine (100 μg/ml), or biotin (10 μg/ml) was added, where necessary, to satisfy the requirements of strains. Extracts were prepared from logarithmically growing cultures by sonic oscillation in 0.1 M sodium phosphate buffer, pH 6.8, and the lysate was clarified by centrifugation at 24,000 × *g* for 10 min.

increase in Dpm-DCase observed when the *lysA* gene was present on the induced phage genome was not observed when a nontransducing phage was induced either when the strain was *lysA*$^+$ or was deleted for that gene.

Similar escape synthesis has been observed in the *lac* system by Revel et al. with P1*dl* (13) and by Epstein with φ80d*lac* (6), and also in the *gal* operon by Buttin using λd*gal* (5). The most likely explanations for this phenomenon invoke the presence of a repressor which (i) is present in amounts insufficient to repress the newly synthesized copies of the operon on the replicating phage genome (5, 12), (ii) is unable to repress newly synthesized genes owing to topographic isolation of the phage (5), or (iii) does not bind to replicating operators (6). Support for the first of these alternatives has been provided by Epstein et al. (2, 6), who demonstrated that doubling the number of repressor genes decreased the maximal rate of synthesis of β-galactosidase after ultraviolet light induction of φ80d*lac*. More definitive evidence for this first alternative was provided by Pfahl (10), who demonstrated that escape synthesis of β-galactosidase was essentially abolished when the induced transducing phage carried the repressor gene allele (i^q) which results in overproduction of repressor protein.

Recent evidence provided by Adhya et al. (1) implicates read-through synthesis originating in phage promoters rather than at bacterial promoters in the escape synthesis of the galactose operon. However, these studies were performed with an excision-defective phage integrated adjacent to the *gal* operon, and the escape synthesis seen in their studies is not the result of the induction and autonomous replication of a

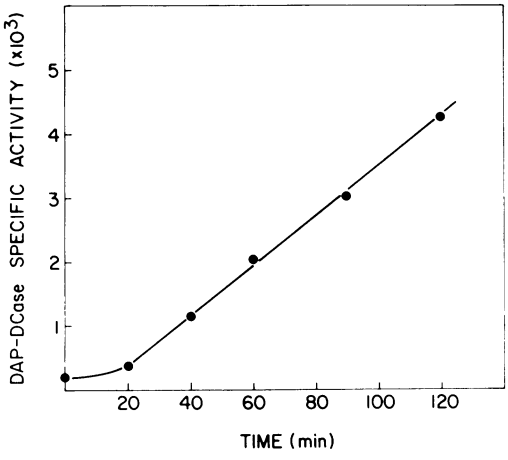

FIG. 1. *Strain RK1222 was grown at 30 C in DYT medium to an optical density at 550 nm of 2.0. The culture was transferred to 42 C and incubated for 20 min, after which it was returned to 30 C for a further 100 min of incubation. Extracts were made and Dpm-DCase assays were performed as in Table 2. Protein determinations and specific activity are as in Table 3. Time is expressed as minutes after transfer to 42 C. (DAP = Dpm.)*

TABLE 3. *Effect of phage induction on Dpm-DCase synthesis*[a]

Strain	Genotype	Treatment	Sp act
RK1222	$[lysA]_{\Delta}$/F'λd*lysA*	Control	139
		Noninduced	190
		Induced	4,431
RK1021	*lysA*⁺(λ)	Control	59
		Noninduced	238
		Induced	101
RK1221	[*lysA*] (λ)	Control	0
		Noninduced	0
		Induced	0

[a] Cells were grown at 30 C in DYT medium (16 g of tryptone, 10 g of yeast extract, 5 g of NaCl per liter of distilled water) to an optical density at 550 nm of 2.0. The culture was split to three equivalent cultures; one was harvested and used as a time zero control, another was returned to 30 C (noninduced), and the third was incubated at 42 C for 20 min and returned to 30 C (induced). The latter two were incubated for a total of 120 min after induction before harvesting. Cell extracts were made and enzyme assays were performed as in Table 2. Protein was determined by a modification of the method of Lowry et al. (8). Dpm-DCase specific activity is expressed as nanomoles of Dpm converted per hour per milligram of protein.

transducing phage. Nevertheless, a final answer regarding the presence of a repressor for *lysA* expression in *E. coli* awaits the isolation of regulatory mutations in the locus specifying such a repressor.

We wish to thank Suzanne Bourgeois and Melvin Cohn for their continued support and Dr. Bourgeois for helpful suggestions regarding this manuscript. We also thank Michael Petru for technical assistance during the preliminary stages of these experiments.

This work was supported by Public Health Service training grant AI00430 to Melvin Cohn from the National Institute of Allergy and Infectious Diseases, by Public Health Service grant GM20868 to Suzanne Bourgeois and training grant GM00702 to the Biology Department at University of California, San Diego, both from the National Institute of General Medical Sciences.

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