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Regulation of Aspartokinase III Synthesis in *Escherichia coli*: Isolation of Mutants Containing *lysC-lac* Fusions

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Mutants containing fusions of the *lac* gene to the *lysC* gene were isolated. In these, the expression of β-galactosidase was regulated by lysine (and arginine), as previously described for aspartokinase III.

The general procedure developed by Casadaban (7) theoretically allows the fusion of the *lac* operon to the promoter of any other gene on the *Escherichia coli* chromosome. In the course of our study of the regulation of lysine biosynthesis in *E. coli*, we decided to use this procedure to obtain fusions of the *lac* operon to the eight genes that compose the lysine regulon and are located separately from each other around the chromosome (21). Such fusion strains could be used for the isolation of mutants altered in their regulatory pattern and as a source of specialized transducing λ phages, as already described in other systems (1-3, 8, 11, 12, 17, 18).

The *lysC* gene codes for the lysine-sensitive aspartokinase (aspartokinase III), the first enzyme of the lysine biosynthetic pathway. We describe in this paper the isolation of mutants containing a fusion of the *lac* gene to the *lysC* gene and some properties of these strains.

As we have previously observed (unpublished data) that the presence of a *relA* mutation limits the derepression of some lysine enzymes, we used strain RM 4102, a Rel^+ derivative (unpublished data of this laboratory) of strain MC 4100 (7), as a starting strain. Standard techniques (6) were used to obtain Mu*cts* lysogens. Enrichment of the Mu lysogens for *lysC* mutants was done with penicillin as previously described (5). *lysC* insertion mutants were identified by the following criteria: (i) growth in minimal medium, but not in the presence of threonine plus methionine (5); (ii) absence of aspartokinase III activity in cell-free crude extracts; and (iii) transduction to $LysC^+$ with phage P1 grown on strain RM 4102, the transductants becoming heat-resistant (owing to the loss of the heat-inducible Mu prophage). Seven independent mutants were thus isolated and identified (designated NC1 to NC7).

These Mu*cts* lysogens were in turn lysogenized with the λp*lac*Mu transducing phage λp1(209) described by Casadaban (7). The selection of strains containing operon fusions was attempted by plating about 5×10^9 bacteria on minimal medium containing 0.4% lactose and incubating overnight at 41 and then 37°C (7). Clones appeared (between 4 and 8 days) in the case of only four strains at a frequency of about 5×10^{-7} per cell plated. A few hundred clones were purified on glucose minimal medium and replica plated on eosin methylene blue lactose minimal medium and on the same medium containing 10 mM L-lysine (14). Only one clone appeared to be Lac^+ on eosin methylene blue lactose minimal medium and Lac^- in the presence of lysine. This clone, derived from strain NC3, was called PAL 1. The low frequency of fusion (compared with values published by other authors, see references 11 and 12) led us to try to obtain quantitative data. From strain NC3, 10 independent λp1(209) lysogens were isolated and plated on lactose agar as described above. In three cases, no clones appeared. With the seven others, Lac^+ clones were isolated at a frequency of 5×10^{-7}. A total of 350 clones, purified on glucose minimal agar containing 40 μg of Xgal (5-bromo-4-chloro-3-indolyl-β-D-galactoside; Sigma) per ml, gave different blue colorations, only seven being dark blue. β-Galactosidase activity was determined on toluene-treated cells (grown either in minimal medium or in minimal medium plus 4 mM L-lysine); 100 strains were studied (the 7 dark-blue strains and others randomly chosen among the different colors). In only two strains (both exhibiting a dark-blue phenotype but derived from different lysogens) did we observe a decrease of β-galactosidase activity when the cells were grown in the presence of lysine. These strains were called PAL 10 and PAL 40. Another strain, PAL 82, that was also dark blue on Xgal agar, was kept because it displayed a peculiar phenotype (see below).

The synthesis of β-galactosidase by strains PAL 1, PAL 10, PAL 40, and PAL 82 was studied more extensively and compared with that of aspartokinase III in the parental strain. The results are given in Table 1. They demonstrate the following. (i) β-Galactosidase activity was similar in each strain, indicating a similar effi-

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TABLE 1. *β-Galactosidase activities in the fusion strains under different growth conditions*[a]

Growth condition	Sp act[b] (nmol/min per mg of protein)				
	Aspartokinase III	β-Galactosidase			
	RM 4102	PAL 1	PAL 10	PAL 40	PAL 82
Minimal medium	11.7	160	155	200	305
4 mM L-Lysine	1.7	42	38	52	300
4 mM L-Lysine + 0.5 mM DL-arginine	4.6	77	62	92	310
20 mM DL-Threonine + 10 mM DL-methionine	—[c]	448	460	510	700

[a] Cell-free crude extracts were prepared on exponentially growing bacteria after an overnight culture in the presence of lysine and arginine when indicated. Threonine and methionine were added to bacteria growing in minimal medium, and linear growth was allowed during 4 h under these conditions. RM 4102: *araD139* Δ*lac169 rpsL thi*; a *rel*$^+$ derivative of MC 4100 (see reference 6). PAL 1, PAL 10, PAL 40, and PAL 82: as RM 4102 but with *lysC-lacZ* fusions; derived from strain NC3 (*lysC1107*::Mu*cts*) as described in the text.

[b] Aspartokinase and β-galactosidase specific activities were determined as described in references 15 and 14, respectively.

[c] —, Not done. There was no lysine limitation of growth under these conditions as RM 4102 possessed a *lysC*$^+$ allele.

ciency of expression after the four fusion events. We have calculated that these fusion strains produce about 650 β-galactosidase polypeptide chains per cell (14). This value can be compared with the value of 1,050 polypeptide chains calculated in a similar manner for aspartokinase III in strain RM 4102 (molar specific activity given in Richaud et al. [16]). (ii) In strains PAL 1, PAL 10, and PAL 40, β-galactosidase synthesis varied in parallel with variations of the lysine pool; an approximately 75% repression was observed when lysine was present in excess during growth; an approximately 2.5-fold derepression was obtained after 4 h of growth in the presence of threonine plus methionine, conditions which led to a growth limitation owing to a decrease of the lysine pool (5). (iii) The extent of repression was similar with these fusion strains and was comparable to the extent of repression of aspartokinase III in strain RM 4102. (iv) We previously observed with a mutant exhibiting constitutive expression of aspartokinase III that the addition of arginine together with lysine during growth led to a higher level of aspartokinase III activity than the addition of lysine alone (9). This possible example of a metabolic interlock (13) could not be observed at that time on the parental strain, Gif 106, because the repressed enzyme level was too low in this strain to be measurable. As shown in Table 1, we could observe this phenomenon both in aspartokinase III synthesis in strain RM 4102 and in β-galactosidase synthesis in the three fusion strains. (v) A different phenomenon was observed in the case of strain PAL 82. Although the addition of lysine did not repress β-galactosidase synthesis, an increase in β-galactosidase activity (to an extent similar to that displayed with the three fusion strains described above) was obtained when the lysine pool was decreased by the presence of threonine plus methionine. This was not due merely to the limiting growth conditions as the inhibition of growth by valine only led to a very slight increase (data not shown).

Although it has been possible to isolate strains containing *lysC-lac* fusions, the frequency of a fusion event in which β-galactosidase is regulated by lysine is apparently much lower than those published by others; from one lysogen, we obtained 2% regulated fusion strains, as compared with 90% in *argA* (11) and 60% in *chlC* (12). The reason for this is unknown, but, if more general, may explain the apparent failure to obtain fusions in some cases.

For three fusion strains (PAL 1, PAL 10, and PAL 40), it appears that the variations in β-galactosidase synthesis parallel those observed in aspartokinase III synthesis, even in the case of the effect of arginine (9, 19).

Different hypotheses may explain the results obtained with strain PAL 82 (the only strain we have found displaying the properties described above). (i) The *Z* gene has been fused to a promoter different from *lysC* that is sensitive to generalized amino acid limitation (more specifically, after lysine limitation), for example, by the guanosine 5′-diphosphate 3′-diphosphate effect (20; see also the review by Umbarger [22]). We have observed that this strain is still Pgi$^+$, the nearest marker on the promoter side (4, 21). (ii) The fusion event has led to a partial loss of the regulatory region that could be highly complex, as in some biosynthetic operons (see review by Umbarger [22]); such a possibility could also

explain the low percentage of lysine-regulated fusions we have obtained. (iii) Strain PAL 82 is a multisite mutant in which the regulation of *lysC* expression is also affected; the isolation of mutants with multisite mutations during the isolation of fusion strains has been observed by others (10). The determination of the structure of the fusion-transducing λ phages might aid in determining which of these hypotheses is correct.

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