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Multivalent Repression of Aspartic Semialdehyde Dehydrogenase in *Escherichia coli* K-12

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Mutants of *Escherichia coli* in which the lysine-sensitive aspartokinase is feedback-resistant are described. In these strains, as well as in the wild type, aspartic semialdehyde dehydrogenase is subject to multivalent repression by lysine, threonine, and methionine. When these amino acids were added to a culture in minimal medium, the differential rate of synthesis of the enzyme dropped to zero and remained there for about one generation.

Several investigators have considered the involvement of the first enzyme of an amino acid biosynthetic pathway in the repression of the other enzymes of that pathway, and the possibility that this regulation may occur at the translational level (3, 13, 18, 23). Some results, though not completely conclusive, are in agreement with such a hypothesis (2, 19, 20, 22-24, 29). To look for a possible relationship between feedback inhibition and repression, we have isolated mutants of *Escherichia coli* in which the lysine-sensitive aspartokinase (EC 2.7.24; aspartokinase III; reference 25) is less sensitive than the parent to inhibition by lysine. Repression of aspartokinase III and aspartic semialdehyde dehydrogenase (EC 1.2.1.10; ASA-dehydrogenase), the second enzyme of the pathway (Fig. 1), has been studied in some of these mutants. The results obtained can be explained without implying any relationship between inhibition and repression. Previous work (5) had shown that only lysine acted as a co-repressor for ASA-dehydrogenase. However, the work presented here suggests that not only lysine but also threonine and methionine may be involved and that the repression pattern may be a multivalent one.

We were also interested in the possibility of regulation at the translational level and have shown that, as demonstrated earlier by Lavallé and De Hauwer for tryptophan synthetase and ornithine transcarbamylase (22, 23), such a mechanism may be involved in the synthesis of ASA-dehydrogenase.

MATERIALS AND METHODS

Bacterial strains. Strains were derived from *E. coli* K-12 Hfr H. Phenotypes and genotypes are given in Table 1. Strain Gif 99 (25) and strain Gif 105 (28) were previously described. Strain PA 505 MB Δ101 C was kindly provided by F. Jacob. Strain OR 11 is derived from strain Gif 106 (J. C. Patte and F. Borne, *in preparation*). Strain M 26-26 is a lysine auxotroph (6) derived from *E. coli* W.

Transduction. Transductions with phage P1kc were performed according to the method of Jacob (16).

Mutagenesis. *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine was used to produce mutagenesis as described by Adelberg, Mandel, and Chen (1).

Growth conditions and preparation of crude extracts. All procedures used have been previously described (21). For desensitized mutants, the standard buffer (buffer A) was modified by the addition of L-lysine and L-aspartate at the concentration of 10 mM each, and is referred to as buffer B.

Proteins. Proteins were determined by the biuret method (11).

Enzyme assays. Aspartokinase (31), threonine-sensitive homoserine dehydrogenase (EC 1.1.1.31; reference 26), and ASA-dehydrogenase (14) were measured according to previously published procedures. For *S*-adenosylmethionine synthetase (EC 2.5.1.6), a modification of the technique of Holloway (12) proposed by H. Cherest (*personal communication*) was used. A 1-ml incubation mixture contained 0.15 M tris(hydroxymethyl)aminomethane (Tris), *p*H 8.5, 0.1 M KCl, 15 mM $MgCl_2$, 8 mM reduced glutathione, 20 mM L-methionine, and 10 mM adenosine triphosphate, sodium salt (ATP). The reaction was started by addition of enzyme and, after 30 min at 37 C, was stopped by addition of 1 ml of 10% (w/v)

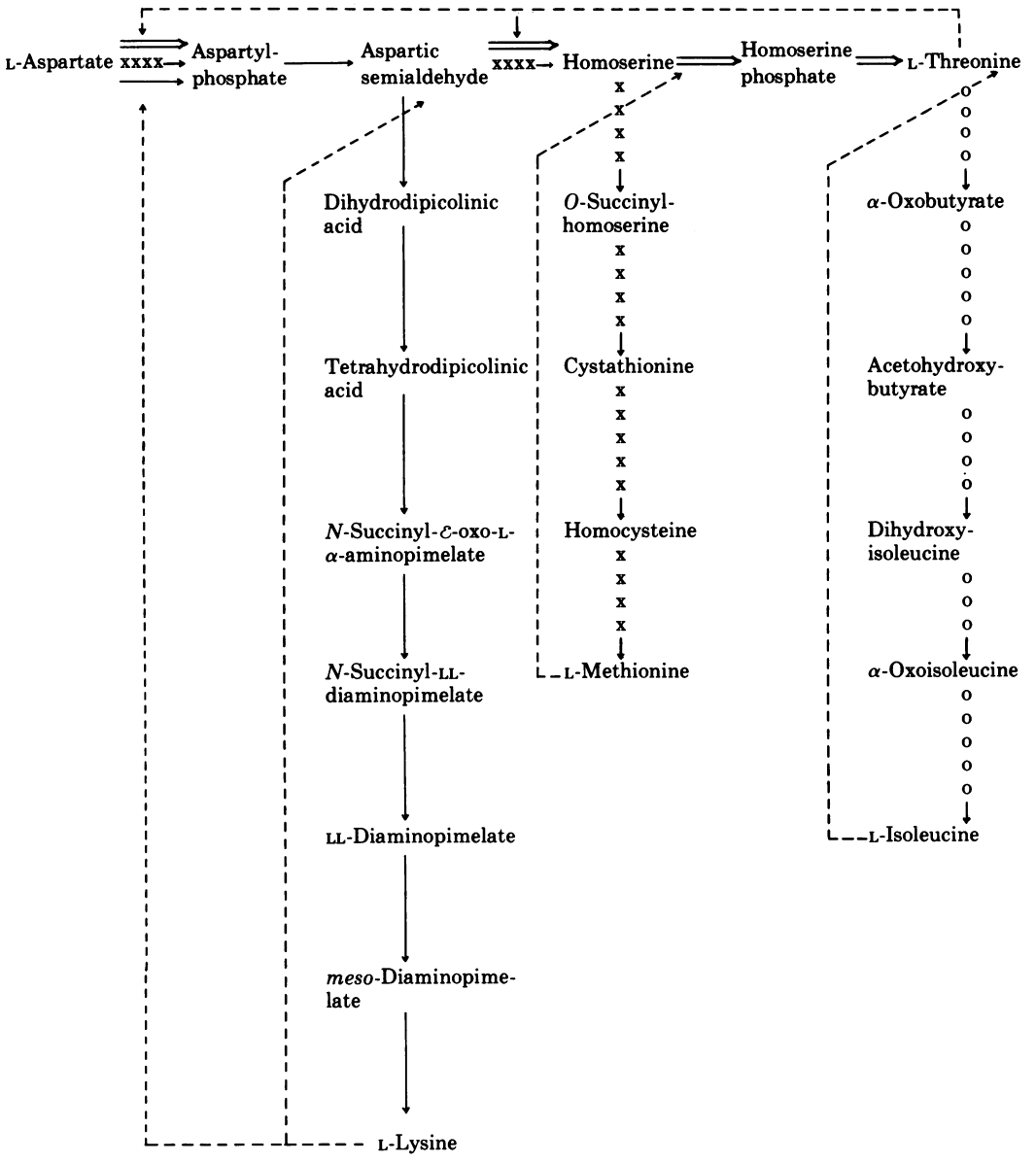

FIG. 1. *Repression of enzymes of the amino acid biosynthetic pathways. Symbols:*→, *enzymes repressed by lysine;* ⇒, *enzymes repressed by threonine plus isoleucine;* xxxxxx→, *enzymes repressed by methionine;* oooooo→, *enzymes repressed by isoleucine, leucine, and valine. This figure is taken from reference 25.*

trichloroacetic acid. After centrifugation at 15,000 rev/min for 10 min, *S*-adenosylmethionine was measured in the supernatant fraction as follows (30): 1.5 ml of supernatant fluid was placed on a Dowex 50 column (200 to 400 mesh; 1 by 3.5 cm). The column was washed with 50 ml of 1 N H_2SO_4 and then eluted with 6 N H_2SO_4. Fractions (1.5 ml) that possessed an absorbancy at 256 nm greater than 0.1 were pooled. The amount of *S*-adenosylmethionine was measured as the total absorption at 256 nm of this pool (extinction coefficient, 1.5×10^{-4} M^{-1} cm^{-1}). The value of a blank reaction mixture without methionine was substracted from all samples.

RESULTS

Selection of mutants in which aspartokinase III is less sensitive to lysine. Strain Gif 105 lacking aspartokinase I, II, and III through

TABLE 1. *Bacterial strains derived from Escherichia coli K-12*

Strain	Phenotype	Genotype
Gif 99	Hs$^-$, Ile$^-$, Arg$^-$	*thr-1100, met-1000, ilvA, argH*
Gif 105	Hs$^-$, Dpm$^-$, Ile$^-$, Arg$^-$	*thr-1101, met-1000, ilvA, argH, lys-1000*
PA 505 MB Δ101 C	Mal$^-$, Arg$^-$, Met$^-$	*malB*(Δ101), *metA, argH*
Gif 106	Ile$^-$, Arg$^-$	*thr-1101, met-1000, ilvA, argH*
OR 11	Met$^-$, Ile$^-$	*thr-1101, met-1000, ilvA, metA*
106 M1	Hs$^-$, Dpm$^-$, Ile$^-$, Arg$^-$	*lys-1001, thr-1101, met-1000, ilvA, argH*
ORE 1	Hs$^-$, Dpm$^-$, Met$^-$, Ile$^-$	*lys-1001, thr-1101, met-1000, ilvA, metA*
106 G21	Ile$^-$, Arg$^-$	*lys-1002, thr-1101, met-1000, ilvA, argH*
ORE 7	Hs$^-$, Dpm$^-$, Met$^-$, Ile$^-$	*lys-1002, lys-1003, thr-1101, met-1000, ilvA*

multiple mutations (homoserine dehydrogenase I activity alone is retained) grows when supplemented with diaminopimelic acid (Dpm) and homoserine (28). Strain Gif 99 (25) lacks both aspartokinase-homoserine dehydrogenase I and II activities and possesses only the lysine-sensitive aspartokinase III. It can grow when supplemented with homoserine. Strains Gif 99 and Gif 105 are isogenic for all characters. After transduction of strain Gif 105 by phage P1kc grown on strain Gif 99, only a recombinant that has retained the homoserine dehydrogenase I gene of strain Gif 105 while gaining the aspartokinase III gene of strain Gif 99 can grow on a minimal medium containing isoleucine and arginine but lacking Dpm and homoserine. Strain Gif 106, isolated in this way, was shown to have aspartokinase III and homoserine dehydrogenase I activities and to lack aspartokinase I and II and homoserine dehydrogenase II activities.

We suspected that strain Gif 106 would be inhibited by high concentrations of lysine, since the lysine-sensitive kinase is the only enzyme present capable of synthesizing aspartyl-phosphate (and homoserine). This synthesis would be stopped if the kinase activity was inhibited, if its synthesis was repressed by lysine or if both occurred. After addition of 10 mM lysine to an exponentially growing culture of strain Gif 106 in minimal medium, the growth curve became immediately linear, suggesting that the major role in growth inhibition is played by feedback inhibition, a type of regulation expected to affect biosynthesis without delay. Growth inhibition was overcome by adding threonine and methionine to the medium.

Among 10^{10} cells of strain Gif 106 plated on agar containing 10 mM lysine, 100 to 200 colonies appeared after 36 hr. Some of these excreted lysine into the growth medium (as shown by cross-feeding of strain M 26-26, a lysine auxotroph). The aspartokinase activity of some of these excretors was measured and found to be partially or entirely insensitive (desensitized) to lysine inhibition. Mutants R46, G21, and G61 were studied in more detail. Table 2 describes the aspartokinase III of these mutants. The enzymes from strains G21 and G61 were much less sensitive to inhibition by lysine than those from the wild type (200 mM for 50% inhibition instead of 0.3 mM for strain Gif 106). The activity in these strains was also insensitive to nonspecific inhibitors (27) such as leucine, isoleucine, and methionine, although the synergistic effect of both leucine and lysine was conserved at high concentrations. The aspartokinase III activity of strain R46 was entirely resistant to either lysine or leucine or a combination of the two (tested at concentrations up to the limit of solubility of these amino acids). In addition, it has been shown that the molecular weight of the R46 enzyme is modified (C. Richaud and J. C. Patte, *in preparation*).

Repression of aspartokinase III and ASA-dehydrogenase in desensitized mutants. Table 3 shows the variation in specific activity of aspartokinase III and ASA-dehydrogenase for strain G21 grown under different conditions. Similar results were obtained for all three mutants under study.

Two generations after addition of 10 mM lysine to a culture of strain G21 in minimal medium, aspartokinase III and ASA-dehydrogenase activities had dropped fourfold. On the other hand, after overnight growth under the same conditions, it became evident that, although aspartokinase III activity was maximally repressed, ASA-dehydrogenase was no longer at the repressed level (compare with levels in strain Hfr H). The generation time of strain G21 in the presence of 10 mM lysine was higher (120 min) than in the absence of this amino acid (90 min). If not only lysine but also threonine and methionine were added in excess during overnight growth, the growth rate was restored to that of the strain grown without any additions. Furthermore, under

TABLE 2. *Inhibition pattern of aspartokinase III of mutants 106 G21, 106 G61, and 106 R46*[a]

Strain	Percent inhibition by the presence in the assay of					
	L-Lysine		L-Leucine		L-Lysine (0.15 mM) + L-leucine (0.5 mM)	L-Lysine (0.1 M) + L-leucine (10 mM)
	0.15 mM	0.1 M	0.5 mM	10 mM		
Gif 106	18	100	21	72	68	100
106 G21	0	12	0	0	0	100
106 G61	0	12	0	0	0	100
106 R46	0	0	0	0	0	0

[a] Experiments were performed with partially purified enzymes (fraction precipitating at 40% saturation with ammonium sulfate). For assay conditions of aspartokinase activity, see Materials and Methods. Protein concentration in the test was about 0.4 mg/ml.

those conditions, aspartokinase III and ASA-dehydrogenase activities were decreased to their repressed levels (Table 3).

One interpretation of these results is that growth in the presence of lysine leads to a decrease in the threonine or methionine pools. To test this hypothesis, the activity of ASA-dehydrogenase was compared with that of homoserine dehydrogenase I (repressed by threonine and isoleucine; 4, 7) and *S*-adenoslymethionine synthetase (repressed by *S*-adenosylmethionine; 15). The size of the intracellular pools of threonine and methionine may be inferred from the specific activities of these latter enzymes. It can be seen in Table 4 that, when strain G21 was grown overnight in the presence of 10 mM lysine, not only did ASA-dehydrogenase show a high specific activity as shown earlier, but homoserine dehydrogenase I and *S*-adenosylmethionine synthetase were also at a derepressed level. For homoserine dehydrogenase, a fourfold derepression (compared to the repressed level) was observed, of the same order of magnitude as the one obtained in severe amino acid restriction (see chemostat experiments, Table 5 and reference 4). In the case of *S*-adenosylmethionine synthetase, a threefold (with lysine alone) to sixfold (lysine plus threonine) derepression is to be compared to the six- to sevenfold increase in the chemostat (Table 5) and the value of ninefold given by Holloway and co-workers (15; these authors used an assay employing radioactive substrate, allowing a more precise estimate of the repressed level than did our experiments). Addition of 5 mM DL-threonine or 2 mM DL-methionine to such a culture decreased the level of homoserine dehydrogenase I and *S*-adenosylmethionine synthetase, respectively, in parallel with the decrease in ASA-dehydrogenase. The simultaneous presence of threonine, methionine, and lysine in the overnight culture led to maximally repressed levels of all three enzymes.

The fact that enzymes repressible by threonine and methionine are, at least partially, derepressed when strain G21 is grown in the presence of lysine suggests that these amino acids are present in the cell at growth-limiting concentrations. The small residual synthesis of these amino acids, leading to a generation time of 120 min, is probably due to the extremely low level (not detectable here) of aspartokinase III during repression by lysine. This enzyme, desensitized to lysine feedback inhibition in strain G21, may catalyze some aspartylphosphate synthesis, but not enough to allow a normal pool of threonine and methionine and a normal growth rate.

These results taken together suggest that lysine alone cannot fully repress ASA-dehydrogenase synthesis under conditions where threonine and (or) methionine are in limiting concentrations.

Multivalent repression of ASA-dehydrogenase. To confirm these findings and to test whether threonine or methionine or both act in a repression mechanism with lysine, chemostat experiments were performed. To achieve a limitation of growth by restricting the rate of synthesis of terminal products, a strain triply auxotrophic for lysine, threonine, and methionine was required. If was also necessary to stop synthesis of intermediate metabolites that might accumulate during growth and modify the results. For these reasons, mutants of strains Gif 106 and G21 that had lost aspartokinase III activity were isolated after mutagenesis with nitrosoguanidine and penicillin selection. These mutants, which require Dpm and homoserine for growth (or Dpm, threonine, and methionine), were called, respectively, strains 106 M1 and G21 M3. When methionine was limiting, it was not possible to maintain the bacterial concentration constant in a chemostat even with a low concentration of this amino acid (it appears that aspartokinase II activity must be extremely leaky but present in those strains as in the parent strain Gif 99). Therefore, we have prepared strains identical to strains 106 M1 and G21 M3, but carrying a *metA* mutation. For this purpose, after transduction of strain 106 M1 (Arg^- aspartokinase III^- Met^+) by phage P1kc grown on strain OR 11 (Arg^+ aspartokinase III^+ Met^-), Arg^+ recombinants were selected on a me-

TABLE 3. *Specific activity of aspartokinase (AK) III and aspartic semialdehyde (ASA) dehydrogenase of mutant 106 G21 under different growth conditions*[a]

Addition to culture medium[b]	Hfr H			Gif 106			106 G21		
	Generation time (min)	Specific activity		Generation time (min)	Specific activity		Generation time (min)	Specific activity	
		AK III	ASA-dehydrogenase		AK III	ASA-dehydrogenase		AK III	ASA-dehydrogenase
None	50	22	690	85	37	1,150	90	16	560
L-Lysine, 10 mM (2 generations)	50	5	430	—[c]	—	—	120	4	400
L-Lysine, 10 mM (after 16 hr of growth)	52	<1.5	360	—	—	—	120	<1.5	1,050
L-Lysine, 10 mM, + DL-threonine, 10 mM, + DL-methionine, 2 mM (about 15 generations)	50	<1.5	350	85	<1.5	375	90	<1.5	385

[a] Extracts of strains Hfr H and Gif 106 were prepared in buffer A; for strain 106 G21, buffer B was used. For measurement of activities, see text. Specific activity is given in nanomoles per minute per milligram.
[b] In each case, 0.5 mM L-isoleucine and 0.3 mM DL-arginine were added to the culture medium.
[c] Strain Gif 106 does not grow exponentially under these conditions (see text).

TABLE 4. *Patterns of enzyme repression and derepression with strain 106 G21 under different growth conditions*[a]

Addition to culture medium	Generation time (min)	Specific activity (nmoles per min per mg)			
		AK III	ASA-dehydrogenase	Homoserine dehydrogenase I	SAM-synthetase
L-Lysine (10 mM)	120	<1.5	1,020	310	1.9
DL-Methionine (2 mM) + L-lysine (10 mM)	120	<1.5	600	285	0.4
DL-Threonine (10 mM) + L-lysine (10 mM)	95	<1.5	545	67	2.8
DL-Methionine (2 mM) + DL-threonine (10 mM) + L-lysine (10 mM)	90	<1.5	385	61	0.55

[a] Additions were made at zero time to a culture of strain 106 G21 grown during 16 hr in the presence of 10 mM L-lysine and diluted in a fresh identical medium so that exponential growth was established. L-Isoleucine (0.5 mM) and DL-arginine (0.3 mM) were added to all cultures. Bacteria were harvested after four generations and extracts were prepared in buffer B. AK III = aspartokinase III; ASA-dehydrogenase = aspartic semialdehyde dehydrogenase; SAM-synthetase = *S*-adenosylmethionine synthetase.

dium containing Dpm, homoserine, methionine, and isoleucine. Among them, approximately 10% were Met^-, and 98% of those 10% have retained the aspartokinase III^- mutation (J. C. Patte, and F. Borne, *in preparation*). One strain that was Arg^+ aspartokinase III^- Met^- Ile^- was called ORE 1. By the same procedure, strain ORE 7 was obtained from strain G21 M3. These strains were grown in methionine-limited chemostats.

Table 5 gives the values of ASA-dehydrogenase activity under limitation of growth by different amino acids. The generation time in the chemostats was about 5 hr. Limitation by lysine led to a 27-fold increase, as described earlier (5). When threonine or methionine was limiting, values (about 600) were higher than with repression conditions (about 400). The increment in specific activity is small (about 1.5-fold) but reproducible; values of Table 5 are the average of at least four independent experiments. Under isoleucine limitation with the same generation time, no variation of ASA-dehydrogenase activity was observed. The same results were obtained with generation times of 7 hr. In contrast, specific activity observed in the case of methionine limitation at a generation time of about 2.5 hr was a little higher (about 800), suggesting that the enzyme activity was more stable under these condi-

TABLE 5. *Effect of threonine and methionine concentration on derepression of aspartic semialdehyde (ASA) dehydrogenase*[a]

Strain	Addition to culture medium	Specific activity (nmoles per min per mg)		
		ASA-dehydrogenase	Homoserine dehydrogenase	SAM-synthetase[b]
106 M1	(a) 10 mM L-lysine + 0.1 mM Dpm + 10 mM DL-threonine + 2 mM DL-methionine + 1 mM L-isoleucine	385	55	0.6
106 M1	(b) Chemostat with same addition as (a) except 40 μM (limiting) L-isoleucine	360	230	
106 M1	(c) Chemostat with same addition as (a) except 50 μM (limiting) Dpm and no lysine	10,500		
106 M1	(d) Chemostat with same addition as (a) except 60 μM (limiting) DL-threonine	585	180	
ORE 1	(e) Chemostat with same addition as (a) except 30 μM (limiting) DL-methionine	515		3.5
G21 M3	Same conditions as (a)	395	46	0.6
G21 M3	Same conditions as (b)	385	295	
G21 M3	Same conditions as (c)	8,000		
ORE 7	Same conditions as (d)	575	186	
ORE 7	Same conditions as (e)	620		4.5

[a] When a limitation in the chemostat is reported, bacteria were grown under limitation conditions for about 3.5 generations during which bacterial concentration (measured by absorbancy at 420 nm) was constant. Generation time was about 5 hr. The chemostat was inoculated with bacteria grown under condition a of the table. The arginine requirement of the strains was met by addition of DL-isomer to 0.3 mM. No or very few (10^{-8}) revertants could be isolated from the cultures after chemostat experiments.

[b] *S*-adenosylmethionine synthetase.

tions. However, methionine and threonine had no effect on ASA-dehydrogenase stability when added to the extraction buffer. The maximal level of derepression was, in fact, obtained in cultures of strain G21 grown in the presence of lysine (Table 3; conditions where pools of threonine and methionine appear to be low) with a generation time of 120 min. It is conceivable that a simultaneous decrease in methionine and threonine pools allows a higher level of synthesis of ASA-dehydrogenase than when only one of these amino acids is growth-limiting.

All of these results indicate that threonine and methionine may participate with lysine in a multivalent repression (8) of ASA-dehydrogenase, though the action of lysine as a co-repressor is the dominant factor. With strain G21 M3 (and other mutants of this type), similar results were obtained.

Kinetics of repression of ASA-dehydrogenase. The principle of this experiment and the mode of presentation of the results are identical to those described by Lavallé and De Hauwer for the repression of tryptophan synthetase by tryptophan (23) and the repression of ornithine transcarbamylase by arginine (22). (In the former paper, Lavallé was able to measure specific messenger ribonucleic acid [mRNA] synthesis; this experiment was impossible in our case.) ASA-dehydrogenase and aspartokinase III activities were determined during the initial generations after addition of excess lysine, threonine, and methionine to a culture of strain Gif 106 in minimal medium. The experiment was performed in such a way that the bacterial concentration and the volume of the culture were practically constant during the experiment to avoid variations in specific activity owing to modifications of growth conditions (see legend of Fig. 2). The results are shown in Fig. 2, 3, and 4, which illustrate, respectively, the variation in specific activity of the two enzymes as a function of the number of generations, the total enzyme content of the culture, and the differential rate of synthesis of ASA-dehydrogenase.

For aspartokinase III, the results are consistent with an exponential dilution of preexisting enzyme; the total enzyme content remains constant during growth. In Fig. 2 and 3 are also represented theoretical curves for the variations of ASA-dehydrogenase activity and enzyme content after addition of co-repressors. They are derived from the assumption that there are two contributing phenomena: first, exponential dilution of preexisting enzyme; and, second, the synthesis of new enzyme at a

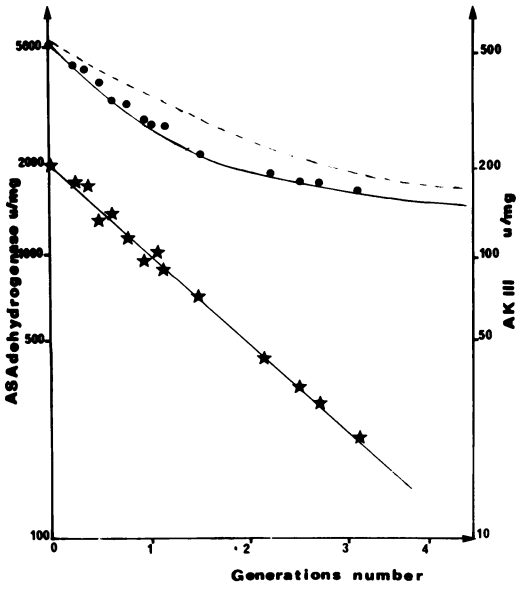

FIG. 2. *Variation in the specific activity of aspartokinase III (AK III) and aspartic semialdehyde dehydrogenase (ASA-dehydrogenase) as a function of the number of generations. At zero time, 1 liter of a culture of strain Gif 106 growing exponentially in minimal medium (plus* L-*isoleucine and* DL-*arginine as described in Table 2) was supplemented with 10 mM* L-*lysine plus 10 mM* DL-*threonine plus 2 mM* DL-*methionine. At each time point corresponding to one-fourth generation, 190 ml of fresh prewarmed medium was added, and an equivalent volume of the culture was removed. Specific activities of AK III and ASA-dehydrogenase were determined for these samples at the times indicated. For theoretical curve, see text and reference 19. Symbols: ★, AK III specific activity (arbitrary units); ●, ASA-dehydrogenase specific activity (arbitrary units); dashed line, theoretical curve of variation of ASA-dehydrogenase specific activity.*

constant rate characteristic of the repressed state, this state being established after addition of amino acids (19). It can be seen that the experimental and theoretical curves for ASA-dehydrogenase specific activity differ from each other (Fig. 2). Clearly, the enzyme content of the culture remains constant for about one generation before increasing (Fig. 3), as is shown by a differential rate of synthesis equal to zero during the first generation. The rate increases later to that of the maximally repressed state (Fig. 4).

DISCUSSION

ASA-dehydrogenase, a biosynthetic enzyme common to the synthesis of lysine, methionine, and threonine, was shown to be repressible by lysine (5) in *E. coli* K-12. At growth-limiting concentration of lysine, the level of ASA-dehydrogenase specific activity increases about 27 times.

The results presented here indicate that, at growth-limiting concentrations of threonine or methionine in the presence of excess lysine, the level of ASA-dehydrogenase increases by a factor of 1.5 over the repressed level. Since these amino acids have no detectable effect in vitro on enzyme stability or activity, we are led to believe that they are acting at the level of enzyme synthesis. An examination of the regulation schemes which have been selected for branched biosynthetic pathways in *E. coli* (25, 32) reveals three possible regulatory mechanisms for controlling the synthesis of enzymes belonging to the common part of the pathway (before the branch points): (i) no repression; (ii) isofunctional enzymes, with each one sensitive to repression by a different end product; and (iii) multivalent repression. Repression of ASA-dehydrogenase by lysine alone does not conform with this general unity. We consider the existence of a second isofunctional enzyme very unlikely. Many mutants lacking ASA-dehydrogenase activity have been isolated by Gilvarg (10) and by us. In all cases, a growth requirement for Dpm plus homoserine was absolute. Experiments to detect

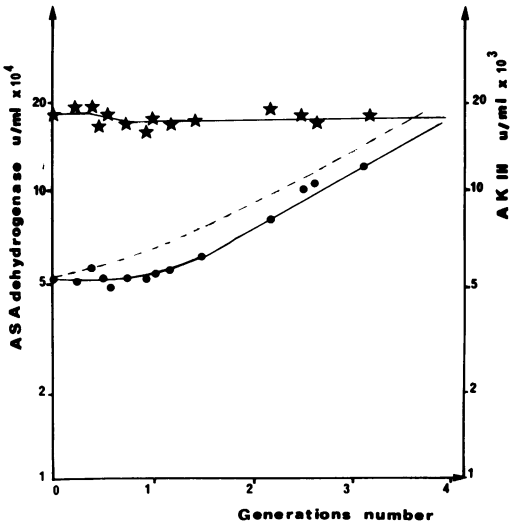

FIG. 3. *Total content of aspartokinase III (AK III) and aspartic semialdehyde dehydrogenase (ASA-dehydrogenase) in the culture. Symbols: ★, total content of AK III in the culture; ●, total content of ASA-dehydrogenase in the culture; dashed line, theoretical content of ASA-dehydrogenase in the culture. For experimental conditions, see Fig. 2.*

ASA-dehydrogenase activity in these mutants after limitation of growth in chemostats by threonine, methionine, or Dpm were always unsuccessful (J. C. Patte and G. N. Cohen, *unpublished data*). Moreover, we have looked at kinetic parameters of ASA-dehydrogenase of strain 106 G21 derepressed by growth in the presence of lysine; the K_m for aspartic semialdehyde and nicotinamide adenine dinucleotide phosphate, and inhibition by aminoethyl cysteine (J. C. Patte and G. N. Cohen, *unpublished data*) were identical to the values of the wild-type enzyme.

Thus, the possibility that ASA-dehydrogenase is subject to multivalent repression by lysine, threonine, and methionine must be considered (a parallel situation may exist for shikimate kinase in the aromatic pathway of *E. coli*, as shown by Pittard [9], even though the level of derepression by threonine and methionine may not be physiologically significant compared to derepression by lysine. The existence of different levels of derepression by each of the amino acids that participate in a multivalent repression mechanism has been previously observed to a slight extent in the isoleucine-valine system (8); in the case of aspartokinase I-homoserine dehydrogenase I enzyme, derepression when isoleucine is growth-limiting is twice as great as the value observed with threonine limitation (4). Any model for multivalent repression must take into account these different efficiencies of repression (17).

From results presented in Table 3 with strain 106 G21, no relationship is indicated between retro-inhibition and repression, because aspartokinase III synthesis is repressed by lysine as effectively in the desensitized mutants as in the parent strain. It may be assumed, on another hand, that aspartokinase III protein, the first enzyme, of the pathway, is involved in the repression of ASA-dehydrogenase; one might have then expected that, in desensitized mutants of aspartokinase III, lysine would no longer behave as a co-repressor of ASA-dehydrogenase. Table 3 shows that the repression pattern of this enzyme by lysine is identical for the mutant and the parent strain. However, since the aspartokinase III mutant enzymes we have isolated may still bind lysine (though they are desensitized) and function in repression, our results do not eliminate the above hypotheses.

The kinetics of repression of ASA-dehydrogenase show that during about one generation the differential rate of synthesis of this enzyme is practically zero. These results are strikingly

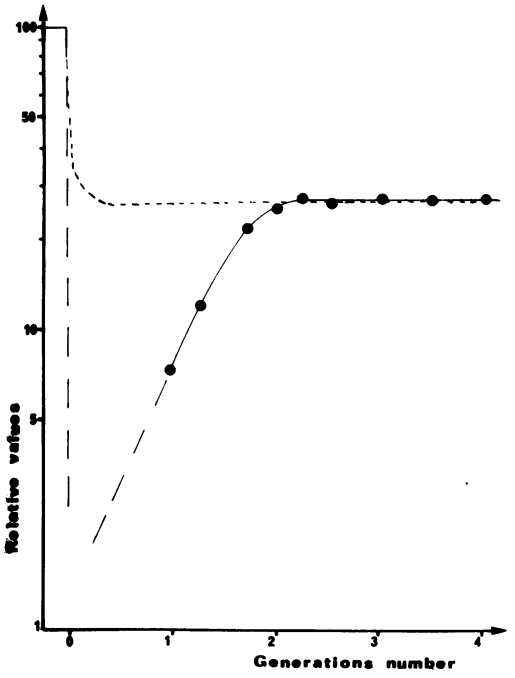

FIG. 4. *Differential rate of synthesis of aspartic semialdehyde dehydrogenase (ASA-dehydrogenase). Relative values are expressed as a percentage of the differential rate of synthesis in minimal medium (before addition) taken as 100%. Symbols: ●, measured differential rate of synthesis for ASA-dehydrogenase; dashed line, theoretical differential rate of synthesis for ASA-dehydrogenase. For experimental conditions, see Fig. 2.*

similar to those of Lavallé with ornithine transcarbamylase (22).

We are therefore strongly tempted to adopt his conclusion that the regulatory mechanism acts at the translation step. (In the case of repression of tryptophan synthetase [23], where specific mRNA synthesis can be measured, it appears that the transcription step does not show this phenomenon.) According to Lavallé (23), one of the best candidates to play a role at the translational level is the enzyme subject to feedback inhibition (aspartokinase III in our case). This molecule has affinity for the regulator molecule (inhibitor or co-repressor), and its cellular concentration is dependent, by direct repression, on the pool of this ligand (which may explain why the arrest of synthesis is transient). According to this view, a difference in the kinetics of ASA-dehydrogenase repression may exist between the wild type and some desensitized mutants of aspartokinase III. Experiments are in progress to see whether such a phenomenon may be observed.

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