ROLE OF THE LYSINE-SENSITIVE ASPARTOKINASE III IN THE REGULATION OF DAP-DECARBOXYLASE SYNTHESIS IN *ESCHERICHIA COLI* K12

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Received 5 April 1974

1. Introduction

In the tryptophan [1] or the arginine [2] biosynthetic pathway, mutants with an altered repressor (in the sense of Jacob and Monod, ref. [3]) have been isolated. With some other amino acids, histidine for example [4], no such mutants could ever be found. This failure would be compatible with some pathways being devoid of such a regulatory molecule, the regulation mechanism remaining unexplained, or with a repressor molecule endowed with some other function, essential to the metabolism of the cell. The first enzyme in the pathway, which is usually sensitive to feed-back inhibition, has long been suspected of playing a role in the repression mechanism. This hypothesis found good experimental support in the case of histidine [5,6] and isoleucine [7–9] biosynthesis, and in the induction of the *hut* operon [10]. We will provide here evidence indicating that in *Escherichia coli* K12 the lysine-sensitive aspartokinase, first enzyme of the lysine biosynthetic pathway, (*lysC* gene, mapping at 80 min on the *E. coli* chromosome, ref. [11]) appears to act as a positive regulator of the synthesis of meso-diaminopimelate decarboxylase, the last enzyme of the pathway (*lysA* gene, mapping at 55 min, ref. [12]).

* *Abbreviations:* AKI: threonine sensitive aspartokinase (EC 2.7.24); AKII: methionine-repressible aspartokinase; AKIII: lysine-sensitive aspartokinase; HDHI: threonine-sensitive homoserine dehydrogenase (EC 1.1.1.31); HDHII: methionine-repressible homoserine dehydrogenase; ASA-dehydrogenase: aspartic semialdehyde dehydrogenase (EC 1.2.1.10); DAP: diaminopimelic acid; DAP-decarboxylase: meso-diaminopimelic decarboxylase (EC 4.1.1.20).

2. Materials and methods

All bacterial strains used (*Escherichia coli* K12) derive from Gif 106 (*thrA1101, metLM1000, arg-1000, ilvA*, refs. [11 and 13]) that has lost AKI*, AKII and HDHII activities, AKIII and HDHI activities being conserved. 106 G21 (same genotype as Gif 106, plus *lysC1002*, refs. [11 and 13]) is a lysine resistant mutant of Gif 106; its AKIII activity is desensitized towards lysine, the feed-back inhibitor. ORA101 is a partially constitutive o^c mutant for AKIII synthesis (same genotype as Gif 106, plus *lys-1100* mutation; J. C. Patte, M. Cassan, E. Boy and F. Borne, in preparation). ORA103 (same genotype as Gif 106, plus *lysC1002* and *lys-1100*) possesses both the o^c mutation of ORA101 and the desensitization mutation of 106 G21. 106 M1 (same genotype as Gif 106, plus *lysC1001*, refs. [11 and 13]) derives from Gif 106 and has lost by mutation the AK III activity. All these strains are also auxotrophic for isoleucine and arginine.

Bacteria were grown and crude extracts (in 20 mM potassium phosphate buffer pH 6.8) were prepared as previously described [13].

Aspartokinase and aspartic semialdehyde dehydrogenase activities were determined as in [13]. DAP-decarboxylase activity was measured by a modification of the manometric method proposed by Work [14]: in order to overcome the non-measurable role of induced lysine-decarboxylase in our cell-free extracts, purified lysine-decarboxylase was added in

excess to the incubation mixture: 2 moles of CO_2 were then produced for 1 mole of DAP. The incubation mixture contained 30 μmol DL-mesodiaminopimelate (Sigma), 0.4 μmol pyridoxal-phosphate (Sigma), 1.4 unit of purified L-lysine decarboxylase (Sigma, type IV), 80 μmol potassium phosphate, pH 6.8, in a final volume of 2.5 ml. Reaction was started by mixing the bacterial crude extract with the reaction mixture and evolving CO_2 was measured at 37°C during 1 hr. Blank reactions in which enzyme or DAP were omitted were run in parallel. Specific activity is expressed in nanomoles of CO_2/min/mg (half of the CO_2 produced in these conditions). Microbiological measurement (by the use of a lysine auxotroph) of the lysine synthesized during incubation was also performed as described in [15]. In this case, no correction was possible for the decarboxylation of lysine by the induced lysine-decarboxylase in the crude extracts.

3. Results and discussion

Table 1 gives the specific activity of AKIII, ASA-dehydrogenase and DAP-decarboxylase of strains Gif 106, 106 G21, ORA101 and ORA103 grown in repression conditions (in the presence of 4 mM L-lysine; as lysine inhibits AKIII activity in strains Gif 106 and ORA103, thus leading to a phenotypic requirement for threonine and methionine, these amino acids were also added in excess to the growth medium for all strains).

It can be seen that when strain Gif 106 is grown in repression conditions all the measured specific activities are at their maximally repressed levels (the AKIII and DAP-decarboxylase activities are undetectable in our experimental conditions). In strain ORA101, carrying only the o^c mutation ('repressed' AKIII specific activity is 23 in this strain), the repressed levels are also observed. In strain 106 G21, carrying only the desensitization mutation, AKIII and ASA-dehydrogenase are normally repressed; DAP-decarboxylase activity is low, but significantly detectable.

In contrast with strain ORA103, carrying both o^c and desensitization mutations, though the repressed level is obtained for ASA-dehydrogenase and AKIII (partially constitutive in this strain), DAP-decarboxyl-

Table 1
DAP-decarboxylase activities of different strains in repression conditions

Strains	Mutations			Growth conditions	Specific activity (nmol/min/mg)		
	1	2	3		AKIII	ASA-dehydrogenase	DAP-decarboxylase
Gif 106	+	+	+	Minimal med. (a)	37	1080	5.8
Gif 106				Repression (b)	< 1.5	350	< 0.3
106 G21	+	d	+	Repression	< 1.5	340	0.9
ORA101	o^c	+	+	Repression	23	330	< 0.3
ORA103	o^c	d	+	Repression	23	340	5.1
106 M1	+	?	(c)–	Repression	0	340	< 0.3
106 M1				lysine limitation (e)	0	4800	5.5

1: o^c Mutation *lys-1100* leading to partially constitutive synthesis of AKIII;
2: *lysC1002* Mutation leading to desensitization (d) of AKIII activity;
3: Mutation leading to the loss of AKIII activity.
(a) The auxotrophic requirements of the strain were met by addition of 0.5 mM L-isoleucine and 0.5 mM L-arginine.
(b) Repression conditions are 4 mM L-lysine (plus 5 mM DL-threonine, 2 mM DL-methionine, 2 mM L-isoleucine and 0.5 mM DL-arginine, see text).
(c) This strain having lost AKIII activity, it is presumed that the allosteric lysine site is not modified by the mutation.
(e) Growth in the presence of 75 μM DAP (plus threonine, methionine, isoleucine and arginine as above). In these conditions, growth is limited by the lysine pool at a concentration of about 10^9 bacteria/ml.

ase specific activity is much higher than the repressed level (approximately at the level obtained with the parental strain Gif 106 grown in minimal medium). Similar variations between the different strains were also observed when the microbiological test was used.

Thus in strain ORA103 the simultaneous presence of the two separately ineffective mutations leads to a partially constitutive synthesis of DAP-decarboxylase, even in the presence of an excess of lysine. The possibility that in this strain 4 mM L-lysine will not lead to the maximal repression has been ruled out: ASA-dehydrogenase is normally repressed at this lysine concentration, and DAP-decarboxylase specific activity does not decrease when 10 mM L-lysine is used.

Intracellular phenotypic variations can be predicted between the different strains, due to the different genotypes. As a matter of fact it appears that, in the case of Gif 106, 106 G21 and ORA101, no (or traces of) aspartylphosphate is synthesized: either because there is no aspartokinase activity after repression (Gif 106, 106 G21) or because the enzyme, when present (ORA101), has its activity inhibited in vivo by lysine, the allosteric ligand; in strain ORA103, the enzyme is not completely repressed and its activity is no longer sensitive to lysine inhibition, thus aspartyl phosphate is always synthesized. The same reasoning can also lead to the following consideration: the AKIII protein, under its non-inhibited allosteric configuration [16], is only present in the cells of strain ORA103; in the others, either the enzyme is absent (due to repression) or it is under its inhibited configuration, by allosteric interaction.

Thus two hypotheses arise: either aspartyl phospnate is the co-inducer of DAP-decarboxylase synthesis, or the non-inhibited AKIII protein itself is required to induce the transcription of the *lysA* gene (the reverse possibility, that inhibited AKIII might repress transcription of the *lysA* gene appears unlikely: the decrease of AKIII synthesis by repression would normally lead in this case to a derepression of DAP-decarboxylase synthesis).

The low, but detectable, DAP-decarboxylase activity observed with strain 106 G21 is in agreement with both hypotheses if one admits that the AKIII synthesis, though undetectable in our experimental conditions, is not completely repressed and that some molecules of enzyme are present in the cell in the non-inhibited form (due to desensitization) allowing a little aspartyl-phosphate synthesis.

The role of aspartyl-phosphate has been studied (table 1) on strain 106 M1, a *lysC* mutant in which AKIII activity is lost [13]. In this strain no aspartokinase activity exists such as allows aspartyl-phosphate synthesis. When this strain is grown on limiting concentration of lysine (as verified by the partial ASA-dehydrogenase derepression [13]) a strong derepression of DAP-decarboxylase is also observed (in repression conditions, the repressed levels are normally obtained for both enzymes). Since DAP-decarboxylase synthesis is observed in the absence of aspartylphosphate synthesis, the role of this metabolite as an inducer is ruled out. The derepression of DAP-decarboxylase in these conditions may then be explained with our second hypothesis, if one admits that the AKIII protein of 106 M1, though catalytically inactive, is normally synthesized in derepression conditions and may then act as an inducer.

These experiments seem to indicate that the AKIII protein, first enzyme of the lysine biosynthetic pathway, plays a role in the regulation of the synthesis of another enzyme of the pathway. This observation is similar to the one described for phosphoribosyl-pyrophosphorylase in the histidine operon [5,6] and threonine deaminase in the isoleucine–leucine–valine cluster [7,9] (however no operon exists in the lysine regulon). The structural configuration of the protein is likely to be involved in the regulatory mechanism, as recently observed for the in vitro induction of the *hut* operon by non-adenylated glutamine synthetase [10]. This hypothesis is in agreement with the previously published observation [15] that an excess of lysine during growth leads to repression of DAP-decarboxylase synthesis; lysine however does not interfere directly, as suggested by this result, but by its action on AKIII synthesis and activity.

Further experiments are required to confirm this hypothesis and to see whether other regulatory mechanisms are involved in parallel. Non-sense mutants in the *lysC* gene could be isolated, proximal enough to the operator region to allow the synthesis of only a very short polypeptide (unfortunately a precise mapping of these mutations would be difficult, as no distal genes exist for demonstrating polar effects); in such mutants, DAP-decarboxylase synthesis should not occur. A more direct confirmation will be obtained

by using in vitro transcription experiments, with a λ*lysA* phage as a source of DNA; such a phage has been recently described by Shimada et al. [17]. These experiments are currently in progress.

Acknowledgements

Financial support came from the Centre National de la Recherche Scientifique, the Délégation Générale à la recherche Scientifique et Technique, and the Fondation pour la Recherche Médicale.

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