SELECTION OF ESCHERICHIA COLI MUTANTS DEVOID OF ONE OR OF BOTH THE ACTIVITIES CARRIED BY A MULTIFUNCTIONAL PROTEIN

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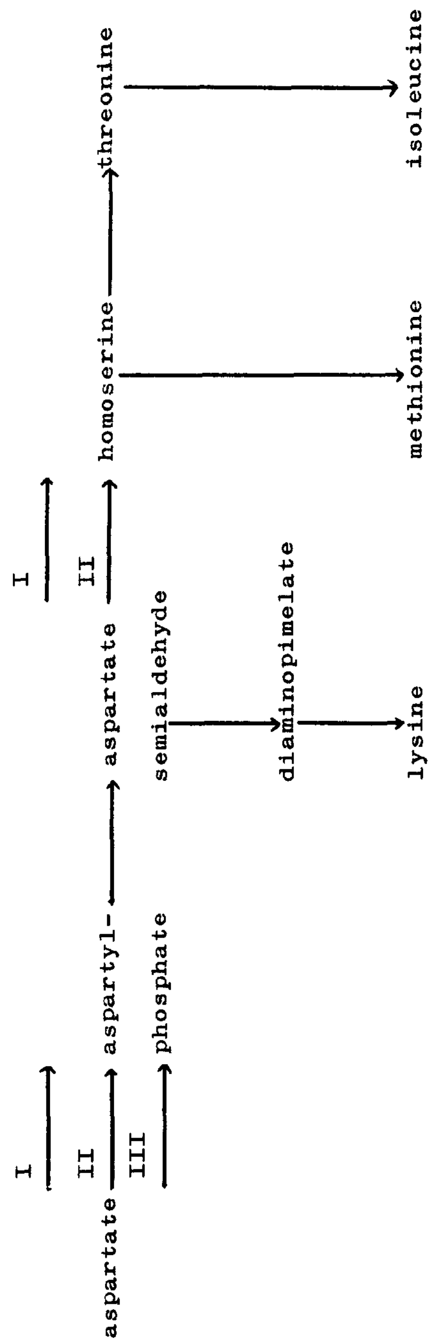
In E.coli K_{12}, the study of the biosynthesis of the amino acids deriving part or all of their carbon atoms from aspartate has revealed the existence of two enzyme complexes, Each of these complexes possesses both aspartokinase and homoserine dehydrogenase activities. Complex I is submitted to a multivalent repression by threonine plus isoleucine (1,2) and the activities carried by it are inhibited by threonine (3,4,5). Methionine represses the synthesis of complex II (6). There exists also in the same cells a third aspartokinase, aspartokinase III, not associated to an homoserine dehydrogenase, the synthesis and the activity of which are respectively repressed and inhibited by lysine (3,7). The following scheme summarizes the above facts and should help the understanding of this paper.

The genetic study of complex I requires the isolation of cells devoid of complex II and of kinase III ; as a matter of fact, the presence of a common activity carried by several different proteins precludes the rational selection of mutants


aspartate
I
II
III
aspartyl-
phosphate
aspartate
semialdehyde
I
II
homoserine
threonine
diaminopimelate
lysine
methionine
isoleucine

in which one or the two activities carried by the complex are absent or modified and hinders their further study by complementation or transduction.

Gif 99, a strain devoid of complex II, had been obtained previously (6). This strain was also devoid of complex I. By crossing it with HfrH, our colleague Jean-Claude Patte has selected a recombinant, Gif 100, which has recovered the activities carried by complex I. The growth of Gif 100 is fully inhibited by threonine : the feedback inhibition exerted by this amino acid on the activities of complex I prevents the synthesis of homoserine, therefore of methionine.

The mutant devoid of kinase III we wished to obtain from Gif 100 should show the following nutritional properties : it should behave as a prototroph in minimal medium, but methionine alone should not reverse the inhibition caused by threonine because of the conditional incapacity to synthesize diaminopimelate and lysine. A culture of Gif 100 is treated by N-methyl-N'-nitro-N-nitrosoguanidine (8). The mutants are allowed to express in minimal medium and the penicillin selection is performed in minimal medium supplemented with 10^{-3}M L-threonine and 5.10^{-4}M L-methionine. The surviving bacteria are isolated on minimal medium ; the clones whose growth is inhibited by a mixture of 5.10^{-3}M L-threonine and 5.10^{-4}M L-methionine are determined by replica plating and purified. Extracts of these different strains are prepared ; one of them, Gif 103, is devoid of aspartokinase III and thus corresponds to the desired strain (Table I).

From strain Gif 103, it is now possible to obtain mutants which have lost one or the other, or both activities carried by complex I. A culture of Gif 103 is treated by nitro-

Strain	Aspartokinase I	Aspartokinase III
	Units/mg	Units/mg
Gif 100	9,2	16,8
Gif 103	17,7	0

Activity is measured by the production of aspartohydroxamate in 2.10^{-1}M Tris buffer, containing 3.10^{-3}M $MgSO_4$, 2.10^{-2}M ATP, 10^{-2}M L-aspartate, 8.10^{-1}M hydroxylamine and 8.10^{-1}M KCl. Final volume : 1 ml ; pH 8.1. Units are expressed as mumoles aspartohydroxamate produced by minute.

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soguanidine ; the expression of the mutants is carried in the presence of 5.10^{-4}M L-homoserine and 5.10^{-4}M diaminopimelate ; the penicillin selection is performed in minimal medium. The clones whose growth depends on the presence of homoserine and diaminopimelate are determined by replica plating. Extracts prepared from different strains thus isolated have shown the existence of two classes of mutants. A first class has lost the two activities carried by complex I (e.g., Gif 104) ; in another class, only aspartokinase I is lost (e.g., Gif 105). Table II illustrates these results and shows that the homoserine dehydrogenase from Gif 105 is inhibited by threonine.

Strain	Aspartokinase I units/mg	Homoserine dehydrogenase I units/mg	Inhibition by 2.10^{-2}M L-threonine (p.cent)
Gif 104	0	0	
Gif 105	0	158	64

Homoserine dehydrogenase activities are measured in 3.10^{-1}M phosphate buffer containing 5.10^{-1}M KCl, 3.10^{-3}M Mg K EDTA (Magnesium titriplex), $2.5.10^{-4}$M NADPH and saturating aspartate semialdehyde. Final volume : 1 ml ; pH 7.2. Units are expressed as mumoles NADPH oxidized per minute.

Since we had described previously (9) mutants which were devoid of homoserine dehydrogenase I, we possess now three types of mutants having lost one or the other or both the activities carried by complex I.

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