SUBUNITS OF THE COMPLEX PROTEIN CARRYING THE THREONINE-SENSITIVE ASPARTOKINASE ACTIVITY IN A MUTANT OF ESCHERICHIA COLI K 12

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We wish to report the characterization of a small molecular weight component of the complex protein which carries in the wild type of E.coli K_{12} the threonine-sensitive aspartokinase I and homoserine dehydrogenase I activities. From a mutant described here, a protein can be characterized which possesses only the threonine-sensitive aspartokinase I activity. This protein can be dissociated to a threonine-insensitive low molecular weight component.

During a survey of the revertants of Gif 54, a mutant lacking the lysine-sensitive aspartokinase (AK III)*, some threonine excretors have been obtained and their study has led to the finding that the threonine-sensitive activities, AK I and HDH I, are carried by the same complex protein (2,3,4,5).

There existed another class of revertants which were heavy lysine excretors. The growth of some was inhibited by methionine, this inhibition being relieved by threonine. We wish to report here the properties of one of these mutants, Gif 101 and

* AK I and HDH I represent the threonine-sensitive aspartokinase and homoserine deshydrogenase activities, AK II and HDH II the enzymes under the repressive regulation by methionine and AK III the lysine sensitive kinase.

TABLE I

Enzyme pattern in E.coli wild-type and in several mutants derived from it.

Extracts were prepared according to (2), except that a concentration of 2×10^{-3}M L-thréonine was used in the extraction buffer for Gif 101 and Gif 102. Enzyme assays were performed according to (2).

Strain	AK I	HDH I	AK II	HDH II	AK III
	Specific Activity mμ moles/min/mg		Specific Activity mμ moles/min/mg		Specific Activity mμ moles/min/mg
E.coli K 12	21	109	undetectable		23
Gif 54	22	112	undetectable		absent
Gif 101	12	absent	5,5	11	absent
Gif 102	6	absent	absent		absent

of one of its derivatives, Gif 102.

Table 1 summarizes the pattern of the various AK and HDH involved in the biosynthesis of the amino acids derived from aspartic acid in the several strains considered. Gif 101 differs from Gif 54 by the absence of HDH I activity. Gif 102 is derived from Gif 101 by UV mutagenesis and selected as an absolute homoserine-requiring auxotroph ; it has lost the methionine-repressible activities, AK II and HDH II (carried also by a single protein complex, 6).

Gif 101 has only small amounts of AK II and HDH II. These activities are however at a higher level than the one observed in the wild-type, due to their partial derepression. The amount of HDH II is sufficient to ensure methionine and threonine biosyntheses and the relative excess of aspartate semialdehyde is diverted towards lysine biosynthesis, lysine being excreted. The addition of methionine to cultures of Gif 101 represses HDH II and causes an absolute threonine requirement. The enzyme pattern of Gif 101 thus explains its phenotypic characteristics in a satisfactory manner.

Gif 102 presents the advantage of being more amenable to biochemical analysis, the presence of AK I not being obscured by the presence of AK II ; on the other hand, the level of this enzyme is reduced since its synthesis is repressed by the growth factors.

The inhibition by L-threonine of the AK I of Gif 102 presents the same characteristics as the one from the wild-type (50 p.100 inhibition with 2.2×10^{-4} M, cooperativity coefficient $\underline{n} = 3,6$). In Table II, the apparent affinity constants for L-aspartate and ATP are given.

Two characteristics are widely different, namely a) the inhibition by L-homoserine reaches completion, whereas the wild-type activity is only partially inhibited (2) b) the apparent molecular weight as determined by filtration on Sephadex G-200, is of 1.8×10^5, as opposed to more than 3×10^5

TABLE II

Comparison of the aspartokinase I from Gif 102 and from the wild-type.

Strain	Molecular weight	Inhibition by L-thréonine 2×10^{-2}M per cent	Inhibition by L-homosérine 5×10^{-2}M per cent	Km ATP M	Km ASP M
E.coli K 12	3×10^{5}	100	30	3×10^{-3}	2×10^{-3}
Gif 102	1.8×10^{5}	95	86	3×10^{-3}	6×10^{-3}
Gif 102 desensitized	$4\text{-}5 \times 10^{4}$	0	40	2×10^{-3}	$1,6 \times 10^{-2}$

for the wild-type (1, 2).

The extraction of the enzyme is made in the presence of a concentration of L-threonine ($2x10^{-3}M$) sufficient to protect the enzyme. If the crude extracts of Gif 101 or Gif 102 are freed of threonine either by dialysis or by filtration on Sephadex G-25, one obtains readily the desensitization of AK I activity : whereas the enzyme obtained has entirely lost its sensitivity to L-threonine, it has retained most of its homoserine sensitivity. The apparent molecular weight of the desensitized enzyme is of the order of 4×10^4. Figure 1 shows the filtration patterns of the native AK I from Gif 102 and of the desensitized enzyme on Sephadex G 200.

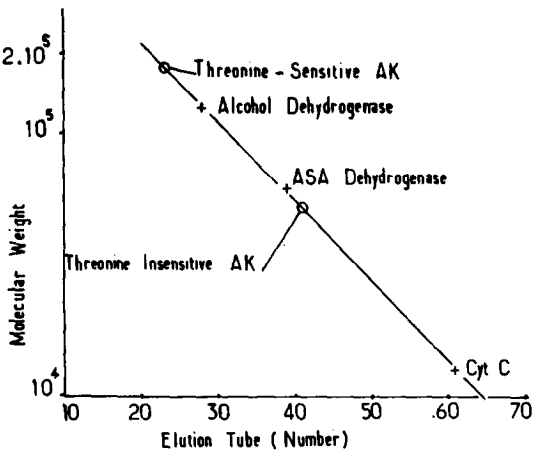

Figure 1. Elution pattern of threonine-sensitive and threonine-insensitive aspartokinases I from Gif 102. A column of Sephadex G 200 was used (80 cm x 2,5 cm). Tube volume : 1.05ml. For conditions, see (12).
AK = aspartokinase
ASA = aspartate semialdehyde

It appears that the mutation of Gif 101 results in the synthesis of a protein devoid of homoserine dehydrogenase I activity*. Although this

* A modified enzyme, also of a molecular weight of 1.8 x 105, but possessing the two associated activities, has already been found in mutant Gif 84 (1). This enzyme does not break down into smaller subunits upon removal of threonine.

protein has lost the catalytic activity of HDH I, it has retained the affinity for its substrates as evidenced by the inhibition of AK I by homoserine and TPN. The allosteric effector, L-threonine, is involved in the stability of the 1.8×10^5 unit, since in its absence, the latter breaks down into a smaller subunit still showing AK I activity, but having lost the capacity to be inhibited by L-threonine.

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