PARALLEL MODIFICATIONS CAUSED BY MUTATIONS IN TWO ENZYMES CONCERNED WITH THE BIOSYNTHESIS OF THREONINE IN ESCHERICHIA COLI.

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We have recently described a mutant of Escherichia coli K 12, Gif 54, lacking the lysine-sensitive aspartokinase (1). As a result of this mutation, only the threonine-sensitive aspartokinase activity is detectable.

In E. coli K 12, threonine plus isoleucine cause a multivalent repression of the threonine-sensitive kinase and the homoserine dehydrogenase (2)(3). In addition, these enzymes are subject to an allosteric inhibition by threonine (4)(5). As a result, the addition of these two amino acids to a wild-type culture creates a phenotypic requirement for methionine. In Gif 54 cultures, threonine plus isoleucine creates in addition a need for diaminopimelic acid. We have obtained revertants of Gif 54, defined by the capacity to grow on threonine plus isoleucine plus methionine, without the necessary addition of diaminopimelate.

Among the spontaneous revertants of strain Gif 54, we have selected three which were threonine excretors : Gif 80, Gif 81, Gif 82. None had reacquired the lysine-sensitive aspartokinase. Because of the overproduction of threonine, the levels of the threonine-sensitive aspartokinase and of homoserine dehydrogenase were very low, precluding the study of these enzymes. Therefore, we have transduced the excretion character into an $Isol^-$ strain with phage P1Kc. With an isoleucine limitation in the chemostat (2)(3), it was then possible to derepress the synthesis of the two enzymes in these mutants, and to study their properties.

In the three mutants, both enzymes are modified in their allosteric inhibition by threonine and the modifications ob-

served are parallel for both enzymes. If one plots the activity against the concentration of inhibitor, the curves obtained are displaced in a comparable fashion with regard to the wild-type curves. Figures 1a and 1b show this clearly.

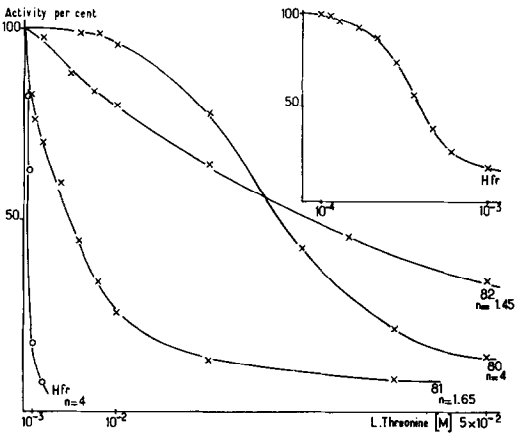

Figure 1a - Inhibition by L-threonine of threonine-sensitive kinases.

After disruption in a 10-Kc Raytheon sonic oscillator and centrifugation of the debris, the extract was freed of nucleic acids by streptomycin precipitation. The supernatant liquid was fractionated by ammonium sulfate precipitation. The activity was located in the fraction precipitating between 35 p.100 and 51 p.100 saturation; the precipitate was resuspended in the buffer used throughout for this enzyme : phosphate 0.02 M, pH 6.75 containing 2 x 10^{-3}M Mg Titriplex, 0,01 M 2-mercaptoethanol, 5 x 10^{-4} M L-lysine and DL-threonine. The concentration of this amino acid in the buffer varied from strain to strain : Hfr H, Gif 80, Gif 81 : 10^{-3}M; Gif 82 : 5 x 10^{-3}M. The assays were performed essentially as described in (4) in the presence of 10^{-2}M L-lysine. The inlet is an enlargement of the Hfr curve.

If one treats the data by plotting $\log \frac{V_i}{V_{max} - V_i}$ against $\log[I]$ (6), one obtains straight lines, the slopes n of which are a measure of the cooperativity between inhibitor molecules (7). The values of n found for the two enzymes under study in the wild type and in the three mutants are shown in figures 1a and 1b. It is interesting to note that the enzymes of Gif 80 have an unchanged cooperativity coefficient,

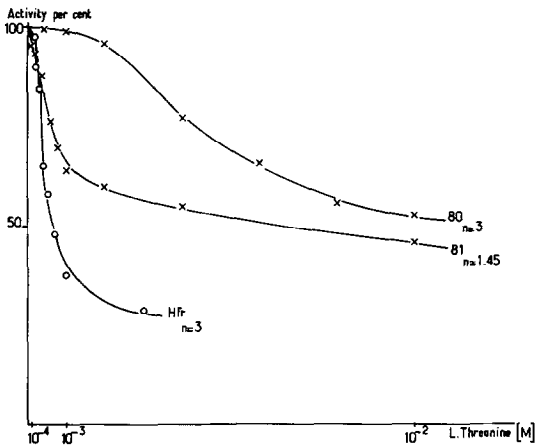

Figure 1b - Inhibition by L-threonine of homoserine dehydrogenases.

Same procedure as above, with the difference that the following buffer was used : 0.02 M Tris, pH 7.6, containing the same additions as 1a. The activity was located below 45 p.100 saturation of ammonium sulfate. The concentrations of L-threonine in the buffers were as in 1a. The assays were performed as described in (5) with aspartic semialdehyde as the substrate. The enzyme of Gif 82 isunstable in the absence of threonine, and no precise inhibition curve can be presented.

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while those of Gif 81 and Gif 82 show much less cooperativity. The enzymes of Gif 80 differ from the enzymes of the wild type by a mere displacement of the curves along the abscissa.

An attempt to determine whether the mutations had brought a change in the molecular mass of the enzymes of the mutant strains was then made. This was accomplished by the gel filtration method described by several authors (8)(9). Sephadex G200 was used throughout (Figure 2).

Glutamic dehydrogenase was not excluded, since it came out after the excluded volume as determined with Blue Dextran 2000 (Pharmacia). Whereas both enzymes came out under the peak of glutamic dehydrogenase when they originated from the wild type, Gif 80 or Gif 81, the behaviour of the Gif 82 enzymes was entirely different : they were eluted at a volume corresponding in the calibration curve (9) to a molecular mass of about 180,000.

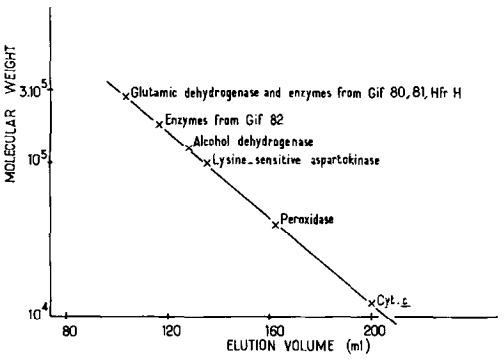

Figure 2 - Elution pattern of the threonine-sensitive kinase and the homoserine dehydrogenase of E. coli K 12, Hfr H and of mutants affected in their allosteric inhibition.

Sephadex G200 was used throughout. The columns (25cm x 18cm) were prepared according to (9). The markers used were glutamic dehydrogenase of beef liver (Sigma, MW under debate) alcohol dehydrogenase from yeast (Worthington, MW 126,000); lysine-sensitive aspartokinase from E. coli (MW about 100,000, unpublished results from this laboratory), horseradish peroxidase (Worthington, MW 40,000) and cytochrome c from horse heart (MW 12,400). The extracts used were those of Figs 1a and 1b. The elution buffer was 0.02 M phosphate, pH 7.2, containing Mg Titriplex 2 x 10^{-3}M, L-lysine 5 x 10^{-4}M and DL-threonine 10^{-3}M. The recovery of the enzymes varied between 70 and 100 p.100, except for the unstable homoserine dehydrogenase of Gif 82, where it was 50 P.100.
"Enzymes" refer to threonine-sensitive kinase and homoserine dehydrogenase.

The fact that, following a single mutation, both threonine-sensitive aspartokinase and homoserine dehydrogenase are affected in the same way leads to the inescapable conclusion that the proteins carrying the two activities share at least a common chain, somehow involved in the allosteric inhibition by threonine. Preliminary results suggest that the two activities are carried by a single enzyme complex. We have studied some mutants which, following a single mutational event, have lost simultaneously the threonine-sensitive kinase and the homoserine dehydrogenase. By reversion, both activities are regained simultaneously.

One should note (see Gif 81) that the diminished cooperativity of inhibitor molecules is not necessarily associated with a decrease in molecular mass. It is highly proba-

ble that the Gif 82 enzymes represent subunits of the wild type enzymes.

The three mutants under study map near the Thr locus of E. coli : transduction by phage P1Kc on a Thr^- (PA 330) recipient cell and selection of the Thr^+ transductants, followed by counting of the threonine excretors, allows an estimation of the relative distance of the three mutants from Thr.

Work is under progress with these mutants and with others of the same type, both from the genetic and the biochemical points of view.

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