A STUDY OF THE MECHANISM OF LYSINE-SENSITIVE ASPARTOKINASE CONTROL BY LYSINE

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The control of a single step in a biosynthetic pathway common to several essential metabolites has been demonstrated to involve specific multiple control sites, either on different enzymes catalyzing the same reaction (1-4), or on the same enzyme resulting in a concerted or cooperative regulation by the controlling metabolites (5,6). The conversion of aspartic acid to β-aspartyl phosphate (which is essential for the biosynthesis of lysine, threonine and other metabolites) has been shown to be catalyzed by two enzymes, one sensitive to lysine and the other to threonine, in Escherichia coli Hfr C (1). Recently, it has been shown that the lysine-sensitive aspartokinase of E. coli is subject to cooperative inhibitory effects exerted by lysine in combination with other amino acids including leucine (7).

In the present investigation, it has been found that the aspartokinase of E. coli 9723 is primarily lysine-sensitive, and leucine exerts a synergistic effect in augmenting lysine control of enzyme activity. The dual sites of action of lysine and leucine do not appreciably affect the binding sites of the substrates aspartic acid and adenosine triphosphate; however, the inhibition resulting from either lysine or leucine or a combination of both is competitively reversed by either magnesium or manganese ions.

EXPERIMENTAL METHODS

Preparation of Enzyme.--Cells of E. coli 9723 were grown for thirteen hours at 30° C in a previously described medium (8) supplemented with L-phenylalanine, L-leucine and L-valine (each 2 mM). The cells were harvested by centrifugation, washed twice with 0.02 M potassium phosphate

buffer (pH 6.8) containing 0.03 M β-mercaptoethanol, and were then resuspended (ca. 20 mg cell dry weight per ml) in the same mixture. Cell extracts were obtained by exposing the cells to sonic oscillation followed by centrifugation at 30,000 x g. The crude sonicate was treated with one-tenth its volume of 10% streptomycin sulfate solution, followed by ammonium sulfate to obtain 40% saturation. The precipitated protein was recovered, taken up in the same phosphate-mercaptoethanol mixture (25% of the volume present during sonication), and then heated in the presence of 1.5 mM L-lysine at 50° for 20 minutes. After centrifugation, the supernatant was again treated with ammonium sulfate to obtain 50% saturation. The recovered precipitate was again taken up in phosphate buffer (50% of the volume during sonication) and showed a 40-fold increase in specific activity.

Enzyme assay.--The reaction mixture in a total volume of 1 ml contained: 10 mM ATP; 3.3 mM magnesium chloride; 100 mM Tris free base; 800 mM hydroxylamine·HCl; 10 mM β-mercaptoethanol. The pH was adjusted to 8.0 with 50% potassium hydroxide, and the enzyme was added. L-Aspartate (10 mM) was then added to each tube except the control tubes. After reaction for 20 minutes at 30° C, the reaction was stopped by the addition of 3 ml of $FeCl_3$ reagent (9). The absorption of aspartohydroxamate formed was read at 540 mμ using $FeCl_3$ reagent blank. The amount of product formed was calculated from a standard curve.

RESULTS AND DISCUSSION

Aspartokinase activity of cell-free extracts of E. coli 9723 is affected by lysine, but no appreciable effect of threonine even in the presence of lysine is obtained. Half-maximal inhibition of partially purified aspartokinase is obtained with 0.5 mM L-lysine, and 75% inhibition is observed with a concentration of 2 mM. As in the case of E. coli K12 (Hfr H strain) (7), leucine affects the aspartokinase activity of E. coli 9723. As indicated in Figure 1, L-leucine causes half-maximal inhibition at a concentration of 7 mM, and a synergistic effect is observed with combinations of leucine and lysine necessary for 50% inhibition. This synergism is indicated by the deviation of the response from the dotted line, which represents an additive effect. Such synergistic activity indicates dual sites of action of the two amino acids in control of the activity of lysine-sensitive aspartokinase.

A study of the kinetics of the synergistic action of lysine and leucine indicated that both lysine and leucine individually, or combinations, were non-competitive with respect to the substrates, aspartic acid and adenosine triphosphate; however, it was found that Mg^{++} would competitively

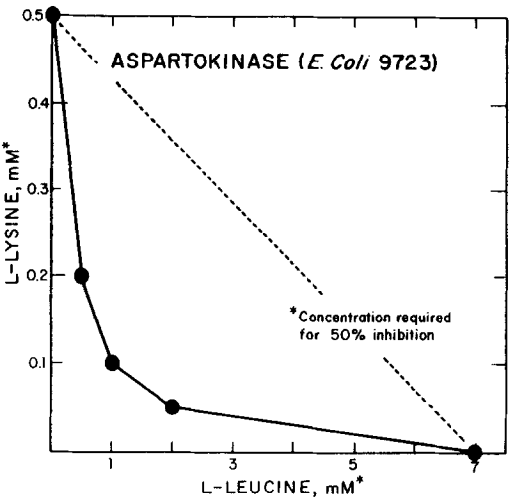

Fig. 1: Synergistic effects of lysine and leucine on aspartokinase activity. Each point represents the concentration of lysine or of leucine, or the concentrations of each in a combination, required to effect 50% inhibition. Each tube contained 670 μg of crude protein from sonically disrupted bacteria.

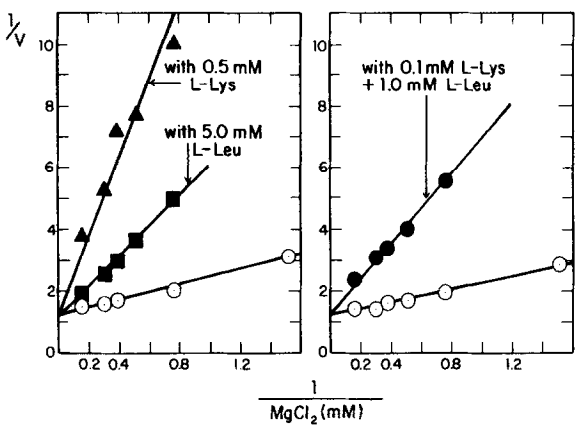

Fig. 2: Plot of reciprocal volocity 1/V (where V = micromoles of β-aspartylhydroxamate formed in 20 minutes) against reciprocal substrate concentration $1/[MgCl_2]$ (where $[MgCl_2]$ is m$\underline{M}$). The assay conditions were as described in "Enzyme Assay." Each tube contained 15 μg of protein.

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reverse the inhibitory effects of lysine and of leucine or of a combination of the two amino acids as indicated in Figure 2. Manganese ions (Mn^{++}) can replace Mg^{++}. Since $\underline{\underline{D}}$-lysine and $\underline{\underline{D}}$-leucine do not replace the corresponding $\underline{\underline{L}}$-isomers, there is a stereospecific site for binding of the

enzyme with the L-isomers, the presence of which prevents binding of Mg^{++} by the enzyme. The synergistic effect of lysine and leucine appears to result from each augmenting the binding of the other to exclude Mg^{++}.

Although the end-product control of aspartokinase by lysine gives an obvious advantage to the organism, the need for the synergistic effect of leucine is not apparent. In another organism, Rhodopseudomonas capsulatus (6), there is a concerted inhibition of aspartokinase by lysine and threonine on a single enzyme, rather than the dual threonine-sensitive and lysine-sensitive aspartokinases of E. coli. The possibility exists that alteration by mutation of the threonine site on a single enzyme controlled synergistically by threonine and lysine may have evolved a new site which binds leucine rather than threonine and necessitated the evolutionary development of a second aspartokinase sensitive to threonine; however, an unknown metabolic relationship of leucine to aspartokinase cannot be excluded.

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