MODULATION OF THREONINE CONTROL OF ASPARTOKINASE ACTIVITY

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Summary: The control by threonine of the aspartokinase activity of the threonine-sensitive aspartokinase-homoserine dehydrogenase complex isolated from Escherichia coli 9723 is modulated by NADPH and $NADP^+$, cosubstrate and coproduct of the homoserine dehydrogenase. NADPH increases and $NADP^+$ decreases the amount of threonine required for inhibition of the aspartokinase activity. This effect, which is dependent upon the relative concentration of cosubstrate and coproduct of a subsequent step, provides a modulation of the end product control of the initial step of a biosynthetic sequence.

In Escherichia coli the threonine-sensitive aspartokinase and homoserine dehydrogenase activities reside in one enzyme complex (1). An advantage of this complex carrying both activities essential for the first and third steps of the biosynthesis of threonine has not been apparent.

In the present investigation it has been found that NADPH, which is essential for the conversion of aspartic semialdehyde to homoserine by the homoserine dehydrogenase, significantly increases the amount of threonine necessary to inhibit the aspartokinase activity of the complex while $NADP^+$, the coproduct of the homoserine dehydrogenase, greatly diminishes the amount of threonine required for control of the reaction. This modulation of threonine control of the first step by the ratio of the cosubstrate and coproduct for the third sequential step offers a basic advantage of the complex in providing fine regulation of a biosynthetic pathway.

Experimental.--The enzyme complex was prepared by the following method.[1] Cells of E. coli 9723 were cultured for a period of about 12 hours at 30° C in salts-glucose medium (2) supplemented with L-lysine and L-methionine

1. Glenn N. Cunningham, Stephen B. Maul, and William Shive, unpublished data.

(0.5 mM each) and were harvested by continuous centrifugation when the optical density (660 nm) of the culture reached 0.20-0.25 (125-157 mg dry weight of cells/liter of culture). The cells were washed twice, suspended at about 30 mg dry weight of cells/ml in 0.02 M potassium phosphate buffer (pH 6.8) containing 0.03 M β-mercaptoethanol and 2 mM L-threonine, and sonically disrupted at 10 kc for 10 minutes. The resulting extract was purified through high speed centrifugation, streptomycin sulfate precipitation of the nucleic acids, ammonium sulfate fractionation, chromatography on hydroxylapatite (Hypatite-C), ion-exchange on DEAE-Sephadex, and two passages through a Sephadex G-200 column. The protein thus obtained is 80-100 fold purified and appears to be homogenous based on the analysis of this preparation by Sephadex G-200 chromatography, polyacrylamide gel electrophoresis, and sedimentation equilibrium experiments by the high speed method of Yphantis. No spurious peaks were observed in the schlieren pattern during sedimentation velocity experiments. The ratio of specific activity of the aspartokinase to the specific activity of the homoserine dehydrogenase remained essentially constant throughout the stages of purification.

Aspartokinase was assayed during the purification as described by Lee, et al. (3). For the activity studies of this paper, this procedure was modified as outlined in the legends of the figures. Homoserine dehydrogenase activity was measured during purification as described by Cunningham, et al. (4).

Results and Discussion.--The effects of NADPH and $NADP^+$ upon the threonine inhibition of aspartokinase activity of the threonine-sensitive aspartokinase homoserine dehydrogenase complex from E. coli 9723 is shown in Figure 1. The inhibition curve obtained in the absence of supplements of either NADPH or $NADP^+$ is sigmoidal in character as previously reported for the complex from this source (4). Supplements of NADPH increase by about two-fold the amount of threonine necessary for maximal inhibition of the aspartokinase activity. In separate experiments, higher concentrations of NADPH did not significantly enhance this effect. On the other hand, supplements of $NADP^+$ significantly

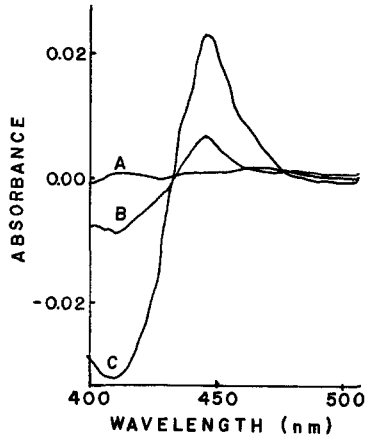

Figure 1. Effect of $NADP^+$ and NADPH on L-threonine inhibition of aspartokinase activity of the threonine-sensitive aspartokinase-homoserine dehydrogenase complex. See Lee, et al. (3) for concentrations of other components. Purified complex (10 μg) was present in each tube of the reaction mixture. Activity is given as percent control with the "no additions, no threonine" point representing 100% activity. (In this experiment 100% activity equals an initial rate of 90 nmoles β-aspartyl hydroxamate/minute based on a spectrophotometric estimation of the Fe^{+++} complex at 540 nm.) Aspartate-containing tubes were compared with the appropriate "no aspartate" controls and the activity given represents that obtained by the difference in optical density of these tubes. Additions are: (a) 0.1 mM $NADP^+$ (b) no additions (c) 0.1 mM $NADP^+$ plus 0.1 mM NADPH, and (d) 0.1 mM NADPH.

decrease the amount of threonine necessary for the inhibition of the aspartokinase activity and also have a slight inhibitory effect in the absence of added threonine.[2] In separate experiments higher concentrations of $NADP^+$ did not alone appreciably enhance the slight inhibitory effect in the absence of threonine and did not further decrease the amount of threonine required for inhibition. In the presence of a mixture of NADPH and $NADP^+$, the amount of threonine required for inhibition was intermediate between the extremes of that obtained with either supplement alone.

As shown in Figure 2, neither NADPH nor $NADP^+$ has an appreciable effect upon the rate of the aspartokinase reaction at different concentrations of

2. It should be pointed out that threonine is present in the reaction mixture at very low levels (0.002 mM) as a result of storage of the enzyme complex in the presence of threonine which stabilizes the aspartokinase activity.

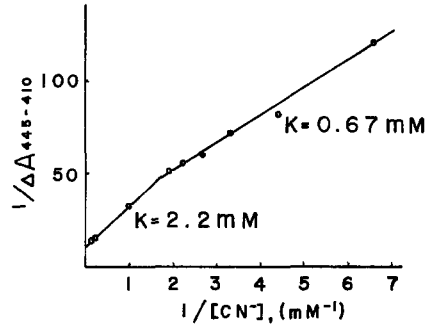

Figure 2. Lineweaver-Burk plot of reciprocal initial velocity versus reciprocal aspartate. All experimental conditions are as in Figure 1 except those indicated, and 5 μg of the purified complex was present in each tube. Additions are: (a) no additions, or 0.04 mM $NADP^+$ alone, or 0.01 mM NADPH alone, (b) 0.4 mM L-threonine plus 0.01 mM NADPH, (c) 0.4 mM L-threonine, and (d) 0.4 mM L-threonine plus 0.04 mM $NADP^+$.

aspartic acid. In separate experiments, similar results were obtained with the other substrate, adenosine triphosphate (ATP). Thus NADPH and $NADP^+$ do not appear to affect the binding of either substrate or the rate of interaction of the substrates. However, the modulation by NADPH and $NADP^+$ alters appreciably the degree of inhibition exerted by threonine. The nature of the interaction between threonine and aspartate remains similar to competitive inhibition, except that sigmoidal responses are obtained upon reversal of the threonine inhibition by increasing concentrations of aspartic acid. In separate experiments the interaction between ATP and threonine appears competitive but is complicated by substrate inhibition by high concentrations of ATP in reversing the effect of threonine. Upon modulation by NADPH and $NADP^+$, the general nature of this interaction is not altered. Hence, the modulation of control results from changes induced by these cofactors in the ability of threonine to interact with the enzyme complex.

Although sigmoidal responses are frequently attributed to cooperative interaction between sites of binding, it is also possible to obtain sigmoidal responses involving single binding sites. For example, the initial binding of threonine could form a threonine-enzyme complex which is readily transformed into a more stable form of the complex which is reversibly dissociable into

an inactive transformed enzyme complex and threonine; and the interaction of the inactive transformed enzyme complex with aspartate forms a complex which readily reverts to the original active form which reversibly associates with aspartate. Since threonine at very low concentrations tends to cause association of subunits of this complex (4,5), further studies are necessary to determine the nature of sigmoidal responses induced by threonine with this enzyme complex.

These results demonstrate that the ratio of cosubstrate to coproduct of the associated homoserine dehydrogenase modulates the threonine control of aspartokinase, the first step in the biosynthesis of threonine. This modulation of the first reaction in relationship to the availability of a subsequent cosubstrate provides the organism with a fine regulation of the end-product inhibition and offers a selective advantage for the retention of this complex by the organism.

REFERENCES

1. Patte, J. C., Truffa-Bachi, P., and Cohen, G. N., Biochim. Biophys. Acta, 128, 426 (1966).
2. Anderson, E. H., Proc. Natl. Acad. Sci. U.S., 32, 120 (1946).
3. Lee, N. M., Lansford, E. M., Jr., and Shive, W., Biochem. Biophys. Res. Commun., 24, 315 (1966).
4. Cunningham, G. N., Maul, S. B., and Shive, W., Biochem. Biophys. Res. Commun., 30, 159 (1968).
5. Wampler, E., and Westhead, E. W., Biochemistry, 7, 1661 (1968).