AUGMENTATION BY β-ASPARTYL PHOSPHATE OF THREONINE CONTROL OF THE DEHYDROGENASE ACTIVITY OF THE ASPARTOKINASE-HOMOSERINE DEHYDROGENASE COMPLEX

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Summary.--In the presence of low levels of magnesium adenosine triphosphate and moderate levels of potassium ion, the amount of threonine required for control of the dehydrogenase activity of the aspartokinase-homoserine dehydrogenase complex of Escherichia coli can be reduced about six-fold by the presence of very low amounts of β-L-aspartyl phosphate. β-Aspartyl phosphate appears to enhance the binding of threonine to the complex with retention of a competitive-like but sigmoidal response of homoserine in overcoming the inhibitory effect of threonine. Enhancement of control of the dehydrogenation activity by the product of the kinase activity of the enzyme complex offers another basis for an advantage of the dual enzyme system over separate enzymes.

In Escherichia coli, the threonine sensitive aspartokinase-homoserine dehydrogenase complex catalyzes the first and third steps in the biosynthesis of homoserine, a precursor of threonine and methionine (1). Advantages to the organism in having this bifunctional complex include the modulation of the threonine control of aspartokinase activity by the co-substrate, NADPH, and co-product, $NADP^+$, of the homoserine dehydrogenase activity (2) and the enhancement of the rate of the dehydrogenase reaction by the substrates of the aspartokinase reaction, $ATP\text{-}Mg^{++}$ or aspartic acid at normal potassium ion concentrations (3).

In the present investigation, it has been found that the product of the first reaction, β-L-aspartyl phosphate, affects the dehydrogenase activity in a manner which could provide an additional advantage of the dual enzyme complex over single, independent activities. The present study

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shows that low levels of β-L-aspartyl phosphate augment the inhibition by threonine in the regulation of the dehydrogenase activity, apparently by aiding the binding of threonine.

Experimental.--The method of growing the cells of E. coli 9723 and the method of purification of the complex has been previously reported (4). Although partially purified preparations (50-70%) were used in these studies, the threonine control and activities of these preparations were observed to be similar in all respects to those of the purified complex. Homoserine dehydrogenase activity was measured using a spectrophotometric assay (5) at 27.0 ± 0.5°C with the initial reaction conditions described in the legends of the figures. Because the enzyme was stored in threonine containing buffer (2) a low amount of threonine (0.4 μM) was present in all assays. To avoid addition of monovalent cations all solutions were carefully prepared, and Tris-ATP (Sigma) was used. β-L-Aspartyl phosphate was prepared according to the method of Black and Wright (6). As a result of this procedure an undetermined concentration of K^+ was present in the β-L-aspartyl phosphate solution. To insure that the effects reported were due to β-L-aspartyl phosphate and not to the K^+, controls were run using β-L-aspartyl phosphate which had been autoclaved 15 minutes. All effects reported here as being due to β-L-aspartyl phosphate were not observed after additions of autoclaved β-L-aspartyl phosphate solutions.

Results and Discussion.--The augmentation by β-L-aspartyl phosphate of the threonine inhibition of the homoserine dehydrogenase activity is shown in Figure 1. For maximum activity 4 mM ATP-Mg^{++} is required at 23 mM K^+ (3). It has been shown (2) that high levels of ATP-Mg^{++} increase the requirement for threonine, but even at levels as low as 0.5 mM Mg-ATP the augmentation by β-L-aspartyl phosphate is apparent. The higher level of ATP-Mg^{++} (4 mM) was used in these assays because this level was necessary to stabilize the enzyme at the lower concentrations of L-homoserine shown in Figure 3. ADP-Mg^{++} does not replace the requirement for ATP-Mg^{++} under

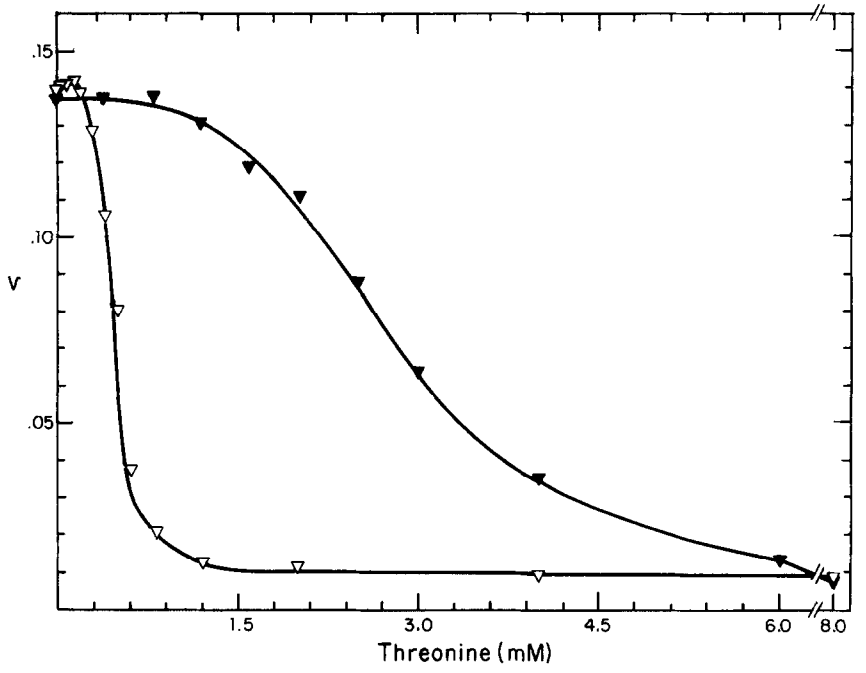

Figure 1. Effect of β-L-aspartyl phosphate on the level of threonine required for control of homoserine dehydrogenase. Initial velocity (V) expressed as the change in absorbance per minute at 340 nm. Initial reaction conditions: 0.1 M Tris, pH 8.8; 23 mM KCl; 50 mM L-homoserine; 30 mM 2-mercaptoethanol; 0.4 mM $NADP^+$; 4.0 mM ATP-Mg^{++} and about 0.022 units of enzyme where one unit is defined as the amount of enzyme that would produce 1.0 μmoles NADPH/minute in the presence of 800 mM KCl at 27°C. The following additions were made: (▼) no additions; (▽) 0.92 mM β-L-aspartyl phosphate.

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these conditions. This augmentation by β-L-aspartyl phosphate is not observed at high levels of K^+ (200 mM or higher) which would be difficult to maintain physiologically. This is not too surprising since it is known that high concentrations of K^+ displace the threonine inhibition curve of both the homoserine dehydrogenase (7) and the aspartokinase (8).

β-L-Aspartyl phosphate is effective in augmenting threonine inhibition at levels as low as 0.5 mM as shown in Figure 2. The same level of inhibition shown in Figure 2 (~85%) can be obtained at levels of β-L-aspartyl-phosphate as low as 0.2 mM and of threonine as low as 0.4 mM if the ATP-Mg^{++} level is lowered from 4 mM to 0.5 mM.

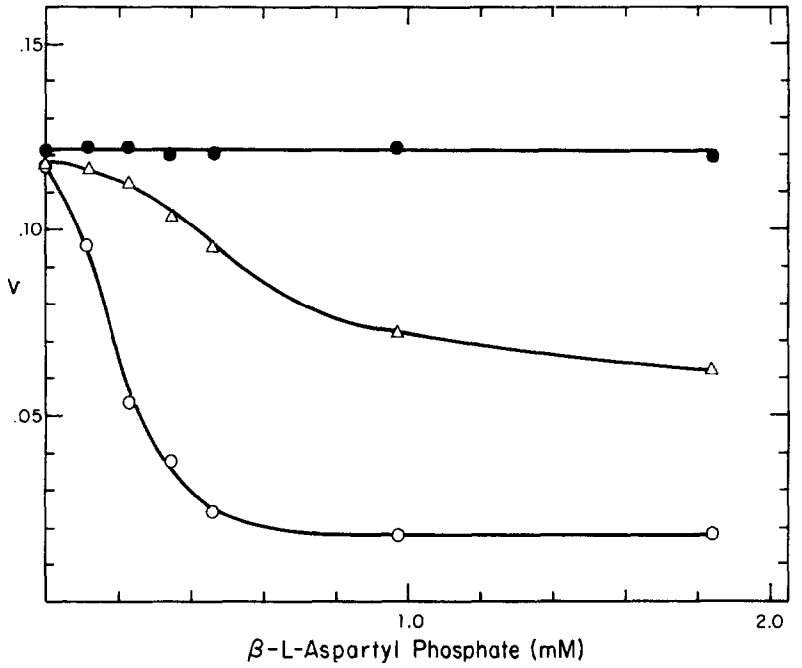
.15
.10
.05
V
1.0
2.0
β-L-Aspartyl Phosphate (mM)

Fig. 2.

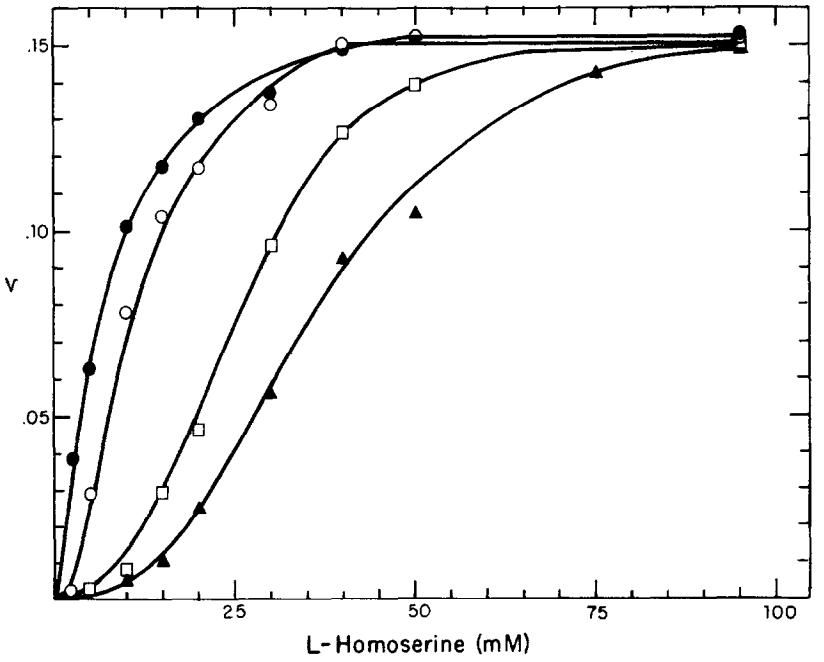
.15
.10
.05
V
25
50
75
100
L-Homoserine (mM)

Fig. 3.

Homoserine at increased concentrations can overcome the inhibitory effect of threonine on the dehydrogenase activity. As shown in Figure III, a level of threonine which causes slight inhibition is greatly augmented by the two levels of β-L-aspartyl phosphate, but the augmented inhibition is reversed by correspondingly higher concentrations of homoserine. These results indicate that β-L-aspartyl phosphate enhances the binding of threonine to the complex without altering the competitive-like but sigmoidal response of homoserine in overcoming the inhibitory effects of threonine.

If β-L-aspartyl phosphate exerts this augmentation of threonine binding at the product site of the aspartokinase activity and if this is also the aspartate binding site, the free carboxyl of aspartate acts in an opposite manner from the acyl phosphate group since aspartate displaces threonine from the enzyme complex while β-L-aspartyl phosphate enhances the threonine interaction. These interactions may offer some insight into the conformational changes which occur during these processes.

Figure 2. Level of β-L-aspartyl phosphate required for augmentation of threonine control of homoserine dehydrogenase. The reaction conditions are the same as in Figure 1 except that the following additions were made: (●) no additions; (▲) 0.4 mM threonine; (○) 0.8 mM threonine.

Figure 3. Effect of homoserine on the β-L-aspartyl phosphate augmented control by threonine of homoserine dehydrogenase. The reaction conditions were the same as in Figure 1 except that the following additions were made: (●) no additions; (○) 0.8 mM threonine; (□) 0.077 mM β-L-aspartyl phosphate and 0.8 mM threonine; (▲) 0.153 mM β-L-aspartyl phosphate and 0.8 mM threonine.

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