INHIBITION OF ASPARTOKINASE AND N-ACETYLGLUTAMOKINASE OF ESCHERICHIA COLI BY HEXOSEMONOPHOSPHATES AND AMP

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Summary: Fructose-6-phosphate, glucose-6-phosphate and AMP have been found to inhibit the lysine-sensitive aspartokinase and N-acetylglutamokinase in extracts of Escherichia coli W. Neither fructose 1,6-diphosphate nor ribose-5-phosphate was effective in these system. Aspartokinase shows competitive inhibition by the hexosemonophosphates and AMP with aspartic acid, and apparent competition of AMP with ATP as well. N-Acetylglutamokinase shows competitive inhibition of the hexosemonophosphates and AMP with both the amino acid substrate, N-acetylglutamic acid and with ATP. Control of amino acid biosynthesis by carbohydrates may be a fairly general phenomenon.

Control of amino acid biosynthesis has been previously described as resulting from various combinations of end-product inhibition and repression mechanisms (1-3). Aspartokinase III (E.C. 2.7.2.4) of E. coli is inhibited by lysine, and its synthesis is repressed by lysine and methionine (4-6). The reaction catalyzed is (7):

$$\text{Aspartic acid} + \text{ATP} \xrightarrow{Mg^{++}} \beta\text{-aspartyl phosphate} + \text{ADP}$$

Control of N-acetylglutamokinase activity is exerted by repression of this enzyme, and of the entire arginine pathway, by arginine (8). The enzyme is not subject to end-product inhibition by arginine. The reaction catalyzed by this enzyme is (8):

$$\text{N-Acetylglutamic acid} + \text{ATP} \xrightarrow{Mg^{++}} \text{N-acetyl-}\gamma\text{ - glutamyl phosphate} + \text{ADP}$$

The reactions catalyzed by aspartokinase and N-acetylglutamokinase are formally similar, in that they are kinases which act upon the terminal carboxyl group of dicarboxylic amino acids. They differ in that aspartokinase, and the previously reported proline-sensitive glutamokinase (9) are the first enzymes in their respective pathways, and thus subject to end-product inhibition, while N-acetylglutamokinase functions in the middle of the pathway, and is not sensitive to end-product inhibition.

All of these amino acid kinases are inhibited by hexosemonophosphates and AMP.

Materials and Methods

Aspartokinase activity was measured by the method of Black and Wright (8), using a 0-30% ammonium sulfate fraction of extracts of bacteria prepared as described previously (10). The bacteria were grown in minimal medium, and the observed aspartokinase activity was inhibited more than 95% by 5×10^{-3} M lysine. N-Acetylglutamokinase was estimated in a similar system, except that the substrate was N-acetylglutamic acid rather than aspartic acid, and a 30-40% ammonium sulfate fraction was used. All other procedures were described previously (10), except that spectophotometric measurements were made on a Bausch and Lomb Spectronic 20.

Results and Discussion

As is shown in Table 1, glucose-6-phosphate and fructose-6-phosphate appear to be equally effective in inhibiting aspartokinase and N-acetyl-glutamokinase while glucose-1-phosphate is less active. Since crude extracts

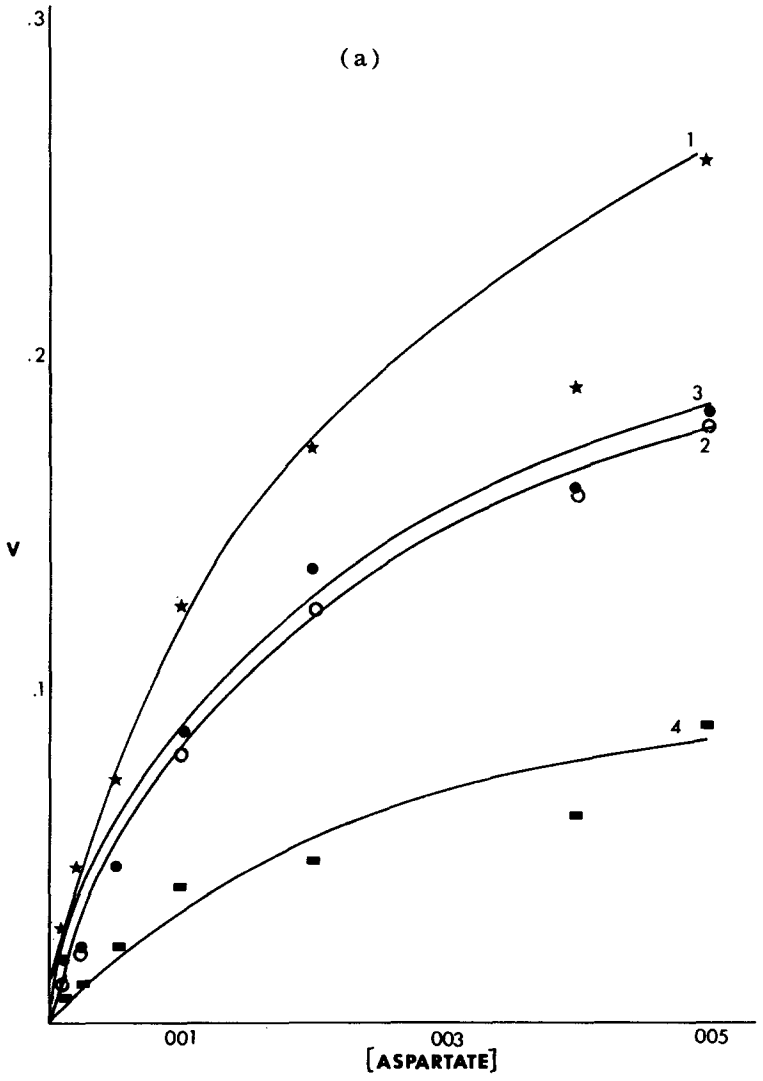
Table 1

Inhibition of amino acid kinases by sugar and nucleotide phosphates. All inhibitors, 5×10^{-3} M.

Inhibitor	% Inhibition Enzyme	
	Aspartokinase	N-Acetylglutamokinase
Fructose-6-phosphate	61	80
Glucose-6-phosphate	63	73
5'AMP	81	88
3'AMP	0	0
Fructose 1,6-diphosphate	0	0
Ribose-5-phosphate	0	0
Glucose-1-phosphate	12	57
Glucosamine-6-phosphate	0	0

were used for these assays, phosphoglucoseisomerase activity would make glucose-6-, and fructose-6-phosphate readily interconvertible (11), and phosphoglucomutase could form glucose-1-phosphate from glucose-6-phosphate (12). Neither fructose 1,6-diphosphate nor ribose-5-phosphate acted as an inhibitor, but 5'-AMP is an inhibitor of both of these reactions. This last effect may be a specific response of this strain, since Stadtman, et al (6) found no effect of AMP in strain Hfr C. 3'-AMP is inactive.

Both of the hexose 6-monophosphates and AMP show apparent competitive



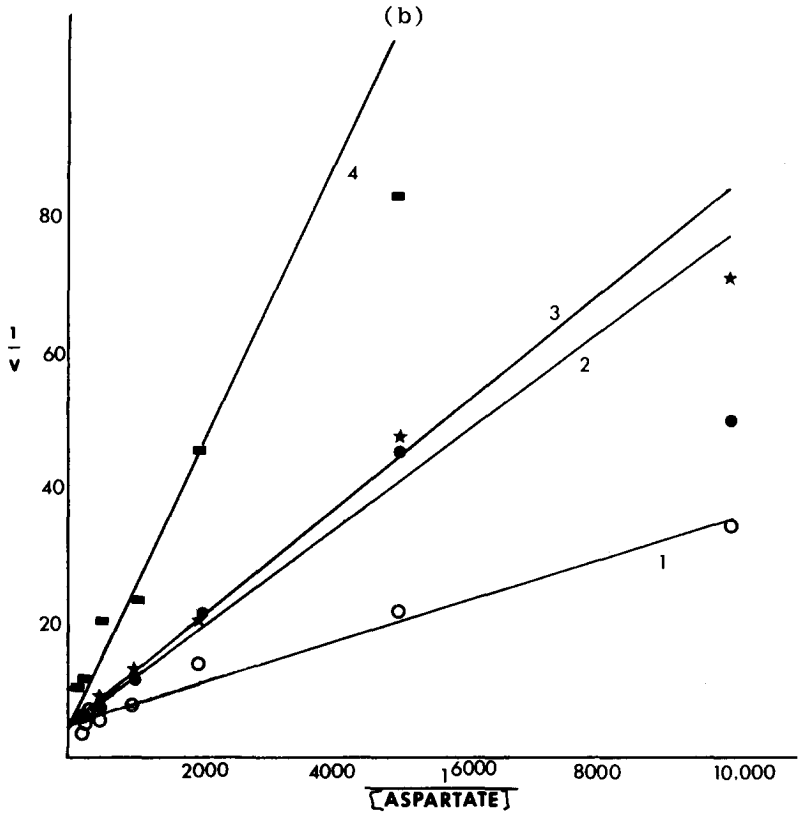


Figure 1a. Activity of aspartokinase III in absence (★) and presence of 5×10^{-3} M glucose-6-phosphate (●), fructose-6-phosphate (o) and 5×10^{-4} M AMP (■), as aspartic acid concentration is varied. b. Lineweaver-Burk analysis of effect of fructose-6-phosphate (★), glucose-6-phosphate (●), and AMP (■) on aspartokinase reaction (o).

inhibition of aspartokinase when the aspartate concentration is varied (Figure 1,a,b). The behavior is non-competitive with respect to the ATP concentration except for AMP, which appears competitive (Figure 2). In these experiments the ratio of ATP/MgCl was kept at 1:1. The sigmoidal kinetics displayed by aspartokinase in the presence of AMP and the hexose monophosphates (Figure 2) suggests that the binding is cooperative.

N-Acetylglutamokinase shows unusual kinetic behavior, since variation of either substrate in the presence of the hexosemonophosphates or AMP gives apparent competitive inhibition (Figures 3 and 4). The reaction is activated at high concentrations of N-acetylglutamic acid (Figure 3).

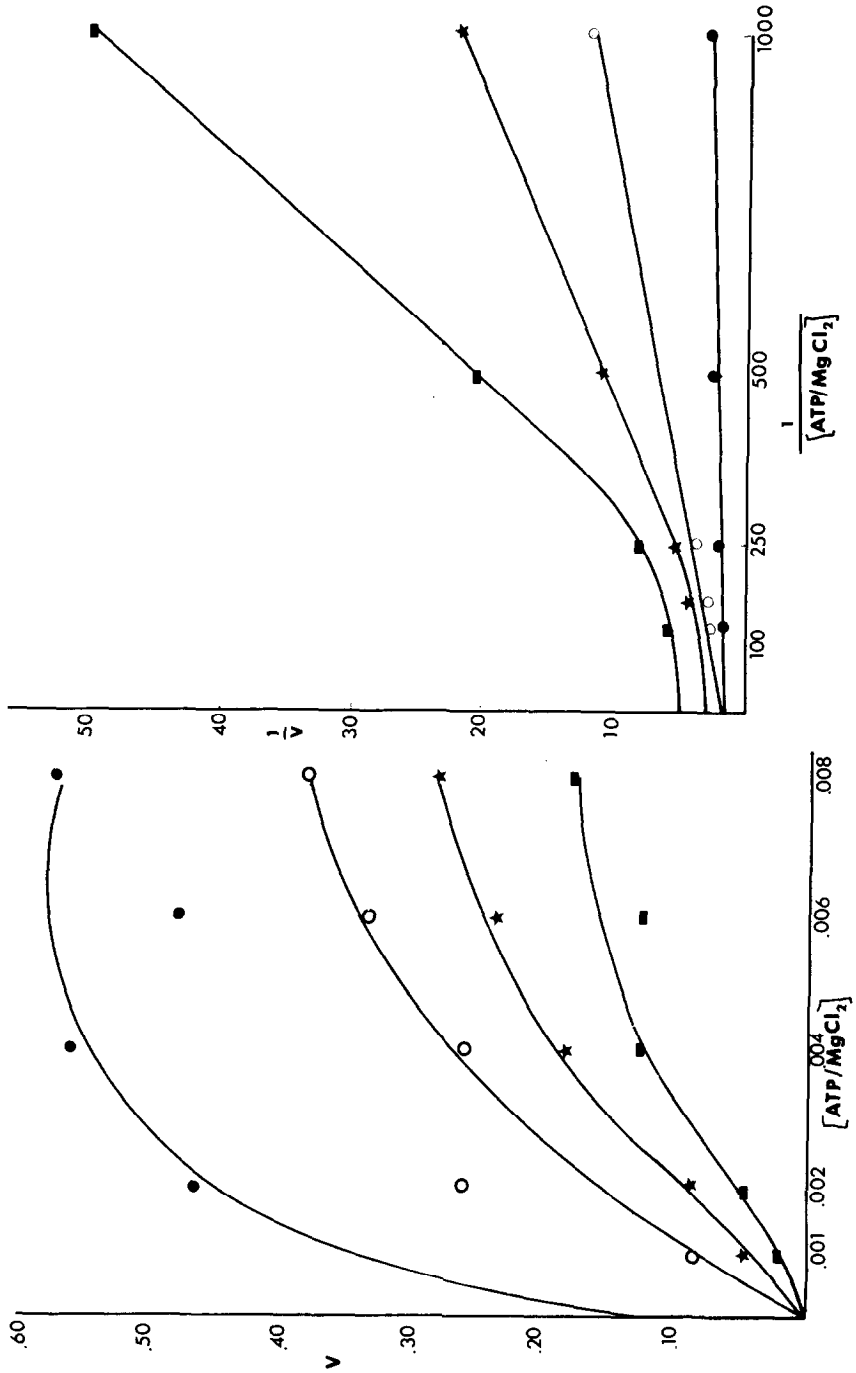


Figure 2. Activity of aspartokinase III in the absence (●) and presence of 5×10^{-3} M glucose-6-phosphate (○), fructose-6-phosphate (★) and AMP (■) as the ATP concentration is varied. Lineweaver-Burk analysis of the effect of glucose-6-phosphate (○), fructose-6-phosphate (★), and AMP (■) on aspartokinase reaction (●).

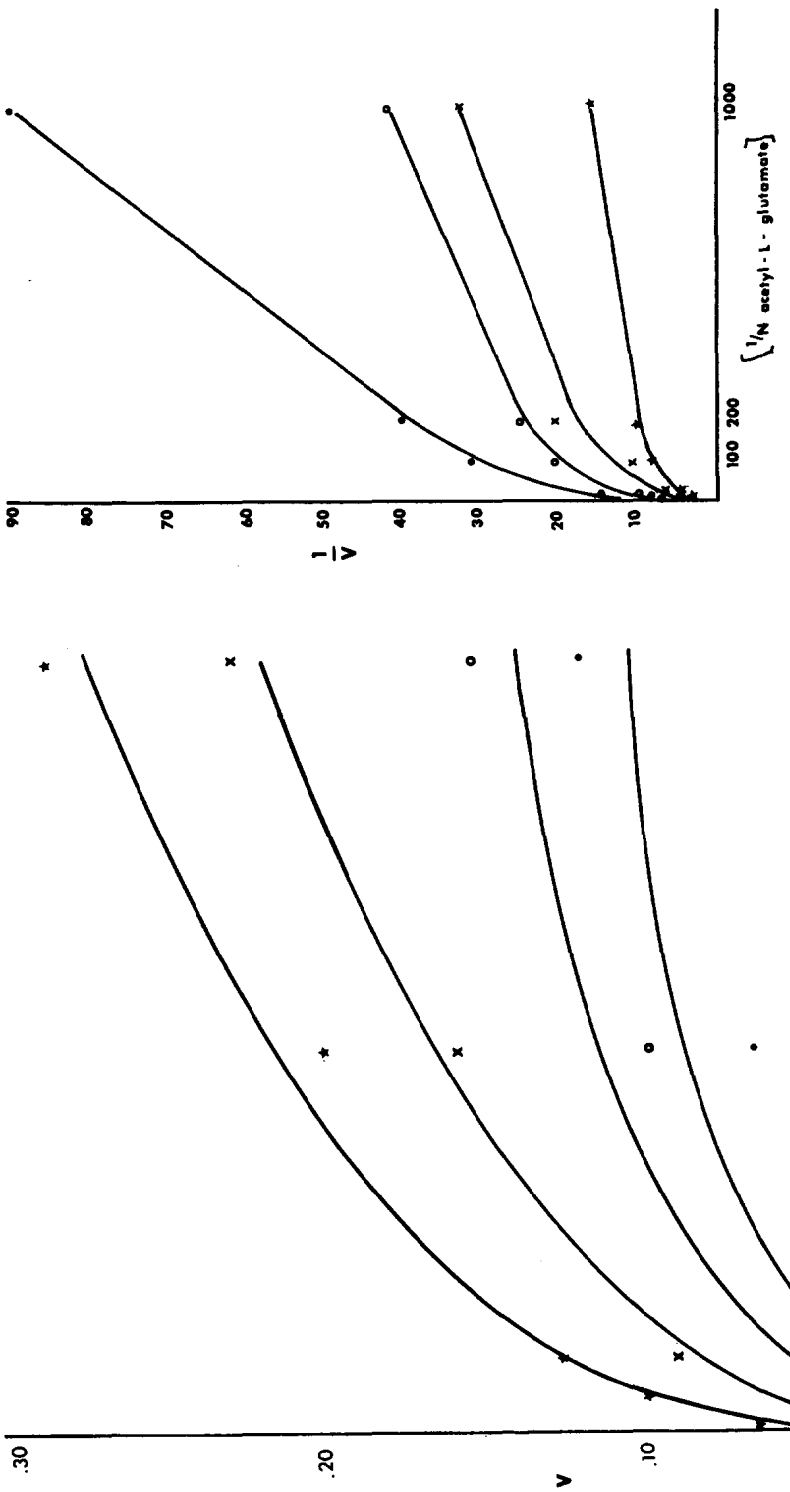


Figure 3. Activity of N-acetylglutamokinase in the absence (★) and presence of 5×10^{-3} M glucose-6-phosphate (o), fructose-6-phosphate (×) and 5×10^{-4} M AMP (●) as N-acetylglutamic acid concentration is varied. Lineweaver-Burk analysis of the effect of glucose-6-phosphate (o), fructose-6-phosphate (×), and AMP (●) on N-acetylglutamokinase reaction (★).

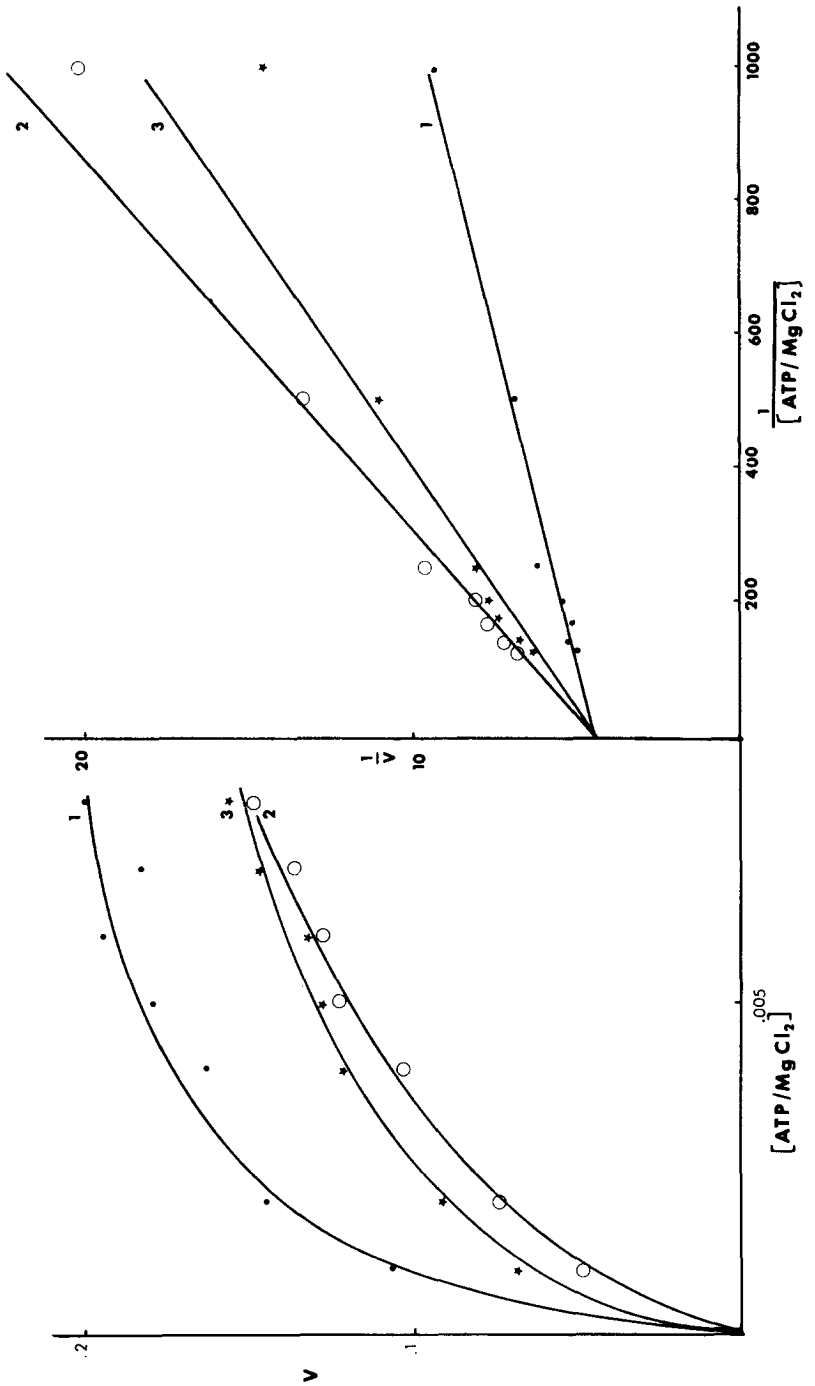


Figure 4. Activity of N-acetylglutamokinase in the absence (●) and presence of 5×10^{-3} M glucose-6-phosphate (★) and AMP (○) as the ATP concentration is varied. Lineweaver-Burk analysis of the effect of glucose-6-phosphate (★) and AMP (○) on N-acetylglutamokinase (●).

N-Acetylglutamic acid was inactive when tested with glutamokinase (9), and the activity of N-acetylglutamokinase was not inhibited by 10^{-2}M proline (10), indicating that the substrate had not been deacylated to glutamic acid.

Conclusions

Since the hexosemonophosphates and AMP are structurally dissimilar to each other and to the amino acid substrates, it appears that they bind to aspartokinase and N-acetylglutamokinase at sites separate from the active sites on the enzymes. Each of these enzymes has, then, at least one, and possibly two more, control sites than those previously reported (4-6).

AMP inhibition of amino acid biosynthesis can be understood in terms of the energy charge of the system (12), since under conditions where the energy charge is low, that is, when the concentration of AMP is high, inhibition of amino acid biosynthesis would tend to conserve the energy of the system, and the rate of protein synthesis would decrease. Inhibition of amino acid biosynthesis by hexosemonophosphates provides a more subtle control linking carbohydrate metabolism and protein synthesis, since accumulation of these monophosphates may reflect temporary disturbances of energy metabolism. N-Acetylglutamokinase inhibition by the hexosemonophosphates is overcome at high ATP concentrations, but this does not occur in aspartokinase inhibition. The activities observed in relatively crude exttracts may not be found in more purified systems.

The previous report of proline-sensitive glutamokinase (9) and the data reported here indicate that the phenomenon of carbohydrate control of amino acid biosynthesis may be fairly widespread. The three reactions are similar in that they are all dicarboxylic amino acid kinases, although they differ in the end-products produced, namely, arginine, proline, and lysine. The similarities in the activities of these kinases, and of their responses to metabolic inhibitors, may reflect divergent evolution of a primitive enzyme system, which retains the original control mechanisms.

Acknowledgments

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