Control of Lysine-Sensitive Aspartokinase Production in Escherichia coli B*

W. Grady Smith and V. Anne Smith
Department of Biochemistry
University of Arkansas School of Medicine
Little Rock, Arkansas

Received March 1, 1967

E. coli contains an aspartokinase (ATP-L-aspartate-4-phosphotransferase, E.C. 2.7.2.4) which is sensitive to both end product inhibition and repression by lysine (Stadtman et al., 1961). In most reports concerning the aspartokinase of E. coli, the cells were grown at 37°C with 0.2% glucose as the carbon source (Stadtman et al., 1961, Patte et al., 1965, Truffa-Bachi and Cohen, 1966, Cohen and Patte, 1963, Lee et al., 1966). The lysine-sensitive aspartokinase is the predominant aspartokinase activity in the organism used in this investigation. We have observed an increase in specific activity of the lysine-sensitive aspartokinase of about six-fold when the cells are grown at 39° with 0.5% glucose as opposed to cells grown at 25°. At 25° the specific activity is constant throughout the growth curve. At 39° the specific activity of the enzyme increases with the growth of the culture. This increase in specific activity does not appear to be a result of activation of the enzyme and appears to require protein synthesis. In contrast, when cells are grown at 39° with 0.5% glycerol

*Supported by National Science Foundation Grant GB-3968

as the carbon source, the specific activity of the enzyme is constant throughout the growth curve as it is when glucose is the carbon source at 25°.

Experimental: E. coli B was grown in aerated fifteen liter cultures at either 25° or 39° in the salt mixture of Kornberg et al. (1960) which also contained 0.1 mg/100 ml of $ZnSO_4 \cdot 7H_2O$. The carbon source was either 0.5% glucose or 0.5% glycerol. Growth of the cells was estimated by measuring the absorbance of the culture at 620 mμ with a Bausch and Lomb "Spectronic 20." One liter aliquots were removed from the cultures at appropriate points in the growth curve. The cells were harvested by centrifugation and crude extracts prepared as described by Stadtman et al. (1961). The lysine-sensitive aspartokinase activity was assayed essentially as described by Stadtman et al. (1961) except that the incubation volume was 1.5 ml and the reaction was terminated with 1.5 ml of ferric chloride reagent. One unit of enzyme is defined as that amount producing an absorbance change due to hydroxamate formation of 0.001 in 30 min. at 540 mμ at 25°C. Protein was determined by the biuret procedure (Gornall et al., 1949).

Results: Figure 1 indicates the change in specific activity as a function of growth of the culture. As can be seen, with glucose as the carbon source at 25° the specific activity of the lysine-sensitive enzyme is constant throughout the growth of the culture, indicating that the enzyme is produced in a constant ratio to other cellular proteins. However, with glucose as the carbon source at 39°, the specific activity of the enzyme increases with growth of the culture, suggesting that it is produced at an increasingly disproportionate ratio to other cellular proteins. Similar results were obtained at various glucose concentrations, ranging

from 0.1% to 5.0%. After the stationary phase is reached, the enzyme activity decreases rapidly. A boiled extract from the crude sonic extract of 39° cells does not activate the enzyme from 25° cells either when present in the assay or when present during a 1 hour preincubation period prior to assay.

In addition, this increase in specific activity observed as a result of culture temperature is also dependent on carbon source. If glycerol is substituted for glucose at 39°, the specific activity is relatively constant throughout the growth curve but is somewhat higher than is observed when glucose or glycerol is the carbon source at lower temperatures (Fig. 1). In addition, no activation by heat has been observed in crude extracts or partially purified enzyme preparations from cells grown at 25°.

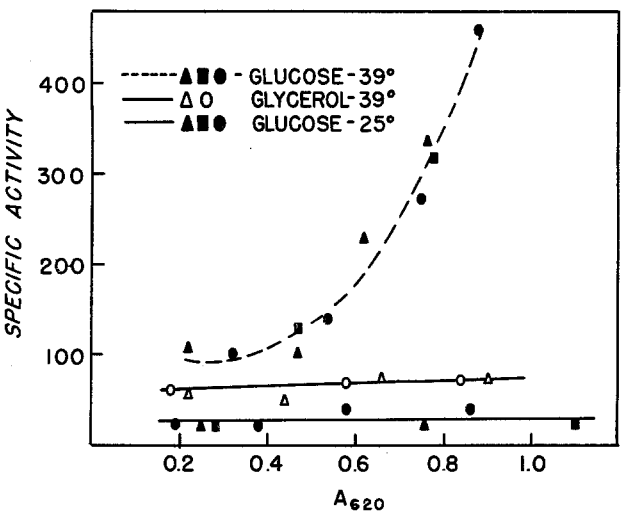

Fig. 1. Effect of Temperature and Carbon Source on the Specific Activity of Aspartokinase. E. coli was grown with vigorous aeration at either 25° or 39° with either 0.5% glucose or 0.5% glycerol as the carbon source in fifteen liter cultures. The cultures were sampled at various times and the absorbance at 620 mμ recorded. Crude sonic extracts were prepared from these samples and the lysine-sensitive aspartokinase and protein content determined as described in the text. The different shaped points represent separate experiments. The specific activity of the lysine-sensitive aspartokinase is plotted versus the absorbance of the culture.

When cells growing at 25° are switched to a 45° temperature for 15 min. and enzyme activity assayed before and after the temperature treatment, no significant increase in specific activity is noted (Table 1). This indicates that pre-existing enzyme is not activated in vivo by heat, however continued incubation at 39° results in a 200% increase in specific activity of the enzyme. This increase in specific activity does not occur if chloroamphenicol is present, indicating that the process depends on

Table 1

Effect of Shifting Temperatures of Culture on Aspartokinase Production

A_{620} before treatment	Treatment	A_{620} after treatment	Duration of treatment min.	Aspartokinase units/L	Protein mg/L	Specific activity
0.29	none	-	-	2,504	90	27.8
0.29	H	0.36	15	3,130	121	25.8
0.32	H, I	0.80	100	30,150	346	87.2
0.32	H, I, C	0.82	130	9,890	281	35.2
0.90	none	-	-	10,650	350	30.4

Table 1. An aerated culture of E. coli growing at 25°C on 0.5% glucose was sampled (1L) at the indicated absorbance (absorbance before treatment) and exposed to one or more of the following treatments: 1) placed in a 45° water bath for 15 min. with continued aeration (H); 2) incubation continued at 39° with aeration (I); 3) chloroamphenicol (70 μg/ml) added to sample (C). At the end of the treatment the absorbance of the culture was again measured (absorbance after treatment), the cells were harvested, and extracts were prepared and assayed for lysine sensitive aspartokinase and protein as described in the text. Aspartokinase units and protein values given are for the total amount in the sonic extract from one liter of culture.

protein synthesis and most probably reflects de novo synthesis of asparto-

kinase. A similar increase in specific activity is noted if the incubation, after heat treatment at 45°, is carried out at 25°.

The lysine-sensitive enzyme has been partially purified by ammonium sulfate fractionation and heat treatment as described by Patte et al. (1965) from cultures grown at 39° and 25° with glucose as carbon source in each case. The enzymes prepared from cells grown under these two conditions are identical in two respects, both lose about 15% of their activity on heating at 45°C for 1 hr. and both are completely inhibited by 10 mM L-lysine.

Lysine at a concentration of 100 μg/ml almost completely represses the formation of the enzyme at either 25° or 39° with glucose as the carbon source.

Assuming that the rate of production of an enzyme is controlled by the concentration of its specific repressor as has been proposed (Jacob and Monod, 1961), the data presented in Fig. 1 indicate a lower repressor concentration when cells are grown at 39°. In fact, the concentration may be so low as to be non-functional since a steady state concentration of enzyme is apparently not obtained under these conditions. Thermolabile repressors and thermolabile repressor forming systems have been described for β-galactosidase (Horiuchi and Novick, 1961, Horiuchi and Novick, 1965, Novick et al., 1963). In the control of aspartokinase production the enzyme is repressed at both temperatures by lysine and derepression occurs at 39° with glucose as the carbon source but not to such a great extent with glycerol as the carbon source. Thus, the carbon source or a metabolite thereof as well as the end product (or co-repressor which in this case is lysine) must influence the thermal stability or synthesis of the repressor. Inducers for β-galactosidase have been reported to alter the thermal stability of the galactosidase repressor (Sadler and Novick,

1965).

On the other hand, the products of glucose catabolism may differ at 39° from those obtained at 25° and the observed effect may be related to catabolite repression. Thermolabile enzymes have been reported in E. coli mutants which result in altered patterns of glucose metabolism at high temperatures (Bock and Niedhardt, 1966a, 1966b) and allow the induction of β-galactosidase in the presence of glucose.

There are no doubt other explanations for the effect observed but the above mentioned ones appear most likely in view of existing knowledge.

These data also emphasize that caution should be exercised in interpreting the effects of culture conditions on enzyme production without considering the population of both control and experimental cultures.

References

Bock, A. and Niedhardt, F. C. (1966a). J. Bacteriol. 92, 464.
Bock, A. and Niedhardt, F. C. (1966b). J. Bacteriol. 92, 470.
Cohen, G. N. and Patte, J. C. (1963). Cold Spring Harbor Symp. Quant. Biol. 28, 513.
Gornall, A. C., Bordawill, J. and David, M. M. (1949). J. Biol. Chem. 177, 751.
Horiuchi, T. and Novick, A. (1961). Cold Spring Harbor Symp. Quant. Biol. 26, 247.
Horiuchi, T. and Novick, A. (1965). Biochem. Biophys. Acta 108, 687.
Jacob, F. and Monod, J. (1961). J. Mol. Biol. 3, 318.
Kornberg, H. L., Phizuckerley, P. J. R. and Sadler, J. R. (1960). Biochem. J. 77, 438.
Lee, N. M., Lansford, E. M. and Shive, W. (1966). Biochem. Biophys. Res. Comm. 24, 315.
Novick, A., Lennox, E. S. and Jacob, F. (1963). Cold Spring Harbor Symp. Quant. Biol. 28, 397.
Patte, J. C., Loviny, T. and Cohen, G. N. (1965). Biochem. Biophys. Acta 99, 523.
Sadler, J. R. and Novick, A. (1965). J. Mol. Biol. 12, 345.
Stadtman, E. R., Cohen, G. N., Le Bras, G. and de Robichon-Szulmajster, H. (1961). J. Biol. Chem. 236, 2033.
Truffa-Bachi, P. and Cohen, G. N. (1966). Biochem. Biophys. Acta 113, 531.