REGULATION BY LYSINE OF PRODUCTION OF THREONINE-SENSITIVE ASPARTOKINASE

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Of the two aspartokinases known to occur in Escherichia coli, the synthesis and activity of one enzyme is regulated by lysine, and threonine controls the activity of the other enzyme (1). In E. coli 9723, only lysine-sensitive aspartokinase was detected in extracts from cells grown in inorganic salts-glucose medium, and this enzyme is synergistically inhibited by a combination of lysine and leucine, the inhibitory effects of which are prevented in a competitive manner by Mg^{++} (2).

In the present study, it has been found that lysine, in addition to controlling the synthesis and activity of the lysine-sensitive aspartokinase, induces the formation of the threonine-sensitive enzyme in E. coli 9723. During a nine-hour incubation period in the presence of lysine, the production of the lysine-sensitive enzyme was almost completely suppressed, and at the same time, an appreciable amount of the threonine-sensitive enzyme appeared. The aspartokinase level found in these cells cultured in the presence of lysine was reduced to about one-third of that found in cells grown in salts-glucose medium without lysine; qualitatively, the aspartokinase in the former case was sensitive to inhibition by threonine but not by lysine or leucine, while the aspartokinase in the latter case was inhibited more than 85% by lysine plus leucine. It is suggested that these E. coli cells manifest a biological control mechanism whereby excess production of lysine (one of the products of the aspartokinase pathway) not only controls lysine production, but also produces a change-over in cellular synthesis from the lysine-sensitive enzyme (repressed by lysine) to the threonine-sensitive enzyme (induced by lysine), the latter aspartokinase then being regulated by excess production of threonine, another end-product of aspartokinase.

RESULTS AND DISCUSSION

Cells of E. coli 9723 cultured for 13 hours in salts-glucose medium yielded extracts containing aspartokinase activity that was, as previously

reported (2), not detectably sensitive to inhibition by threonine. However, assays of aspartokinase in extracts from cells cultured for shorter periods (9, 11, or 12 hours incubation at 30°) in medium containing L-lysine (0.5 mM), showed that aspartokinase from the 9-hour culture was inhibited by threonine, and not appreciably by lysine or leucine, nor by combinations of lysine and leucine. Continuance of incubation to 11 or 12 hours was accompanied by a relative decline in the threonine-sensitive enzyme, and an increase in the lysine-leucine sensitive aspartokinase.

The above results suggested that lysine in the culture medium may induce the formation of a threonine-sensitive aspartokinase. A more direct study of effects of exposure of the growing cells to lysine (or other supplements) was made by introducing supplements into a 9-hour culture, and observing subsequent changes in the nature and amount of aspartokinase activity following an additional 4-hour incubation period, to 13 hours total. Table 1 shows a balance study of cell protein, total aspartokinase activity, and the sensitivity of the aspartokinase activity to inhibition by a combination of lysine and leucine, and to inhibition by threonine, in such cultures to which various supplements had been added 9 hours after inoculation. A supplement of 0.1 mM L-lysine reduced the resulting (at 13 hours) lysine-leucine sensitive component of aspartokinase to about one-fourth of that obtained with no supplement, and raised the threonine-sensitive component from less than 5% to about 50% of the activity. In other experiments, lower concentrations of lysine (0.05 mM) produced about the same increase in the threonine-sensitive enzyme, relative to a culture without supplement; supplementation with 0.025 mM lysine led to some increase in the production of enzyme. This effect is primarily produced by lysine, since a supplement of the 19 common protein-derived amino acids (other than lysine) tested in the same manner (Expmt. 2) showed little effect on total aspartokinase specific activity, or on the lysine-leucine sensitive or the threonine-sensitive aspartokinase activity level. Supplementation by lysine plus the 19 other amino acids led to suppression of lysine-leucine sensitive aspartokinase approximately to the same extent as by lysine alone. (Table 1). When added as a supplement during the 9-13 hour incubation interval, D-lysine did not replace L-lysine as inducer of the threonine-sensitive aspartokinase.

The introduction of supplements of individual amino acids (during the 9-13 hour incubation interval) showed that certain amino acids augment or antagonize the activity of lysine as inducer of the threonine-sensitive, or as repressor of the lysine-leucine sensitive, aspartokinase. Thus, methionine augments the induction by lysine, but will not by itself effect the induction, of the threonine-sensitive activity; and threonine

or isoleucine substantially reduces the inductive activity of lysine upon the formation of this enzyme. These effects of other individual amino acids upon induction by lysine of the threonine-sensitive aspartokinase (as well as certain effects upon the repression by lysine of the lysine-leucine sensitive enzyme) will be reported elsewhere. The suppressive effects of threonine or isoleucine appear to account for the low level of threonine-sensitive aspartokinase in the presence of lysine plus 19 other amino acids (Expmt. 2, Table 1). Aspartic acid, the accumulation of which in the culture medium might possibly be promoted during repression of the normally abundant (constitutive) lysine-leucine sensitive aspartokinase, showed no appreciable effect upon either aspartokinase when tested as an individual supplement (0.5-2 mM L-aspartate) during the 9-13 hour incubation interval. In some cases there appeared to be a very small component of enzyme activity which was not sensitive to inhibition by either threonine or lysine; however, further experiments will be needed to determine whether this is related to variation in sensitivity to inhibition by threonine and lysine, or to a third aspartokinase.

In other experiments of the type presented in Table 1, evidence was obtained indicating that the appearance of the threonine-sensitive aspartokinase is in fact a biosynthesis (presumably induced by lysine) of new enzyme, and not a change in conformation of pre-existing molecules of another aspartokinase. Thus, omission of nitrogen sources from the 9-13 hour incubation medium abolished the lysine-induced formation of threonine-sensitive aspartokinase, but left essentially constant and unaffected the level (units per liter of culture in the 9-13 hour interval) of the lysine-leucine sensitive aspartokinase. Similar conclusions resulted from experiments in which chloramphenicol (0.8 μg per ml) was added (rather than omission of nitrogen) for the purpose of preventing synthesis of new protein molecules.

In cultures of E. coli 9723 supplemented with L-lysine the total amount of lysine-leucine sensitive aspartokinase does not rise appreciably in the 9-13 hour interval. An apparent derepression of this aspartokinase was observed in the following experiment: cells cultured for 9 hours in salts-glucose medium containing 0.5 mM L-lysine (added at zero time) showed a low level of the lysine-leucine sensitive enzyme at 9 hours, but after these cells had been resuspended in fresh salts-glucose medium (without lysine) for 4 more hours, the lysine-leucine sensitive enzyme had risen more than 100-fold (accompanying a four-fold rise in total protein). During this 4-hour interval of cell incubation in salts-glucose medium, the level of threonine-sensitive aspartokinase (units per liter culture) remained approximately constant, indicating that the derepressed lysine-leucine sensitive enzyme originates

TABLE 1

Effect of Lysine or Other Supplements upon Aspartokinases in E. coli 9723.

Supplement	Total Incubation Time (hrs)	Total Protein In Extract From 1 Liter Culture (mg)	Aspartokinase Activity [a]			
			Spec. Act. [b]	Total Act. [c]	Sensitivity to: Thr. [d]	Lys-Leu [e]
Expmt. 1:						
None	9	32	0.8	26	<6	84
None	13	160	0.93	149	<5	92
0.1 mM L-lysine	13	175	0.36	63	50	60
0.5 mM L-lysine	13	175	0.32	56	52	47
Expmt. 2:						
None	9	26	0.68	18	<6	81
None	13	168	1.15	193	<4	86
19 Amino acids (1 mM each)	13	257	0.98	250	<3	86
19 Amino acids + 1 mM L-lysine	13	254	0.13	33	20	65
1 mM L-lysine	13	168	0.38	64	47	37

a. Cells were cultured for 9 hours at 30° in salts-glucose medium, then harvested by centrifugation, and resuspended in fresh salts-glucose medium supplemented as tabulated. Incubation was then continued to 13 hours total, the cells were sonically disrupted, and the extracts assayed for aspartokinase activity. The enzyme assay conditions were as previously described (2) except that $MgCl_2$ was 1.6 mM, and no β-mercaptoethanol was added to the mixtures other than that which was introduced with the enzyme solution. Each 1 liter, 9-hour culture had been inoculated at zero time with 1 ml of an 8-hour pilot culture in the same salts-glucose medium.

b. Total aspartokinase activity, expressed as micromoles of β-aspartyl-hydroxamate formed per mg protein during 20 minutes.

c. Total aspartokinase in extract from 1 liter culture.

d. Percent inhibition of hydroxamate formation, exerted by 2 mM L-threonine.

e. Percent inhibition of hydroxamate formation, exerted by 0.5 mM L-lysine plus 5.0 mM L-leucine.

from a source other than an alteration in conformation of threonine-sensitive aspartokinase.

Cells which were cultured for 13 hours in salts-glucose medium without lysine, and which contained as reported (2) only the lysine-leucine sensitive

aspartokinase without detectable amounts of the threonine-sensitive enzyme, were then resuspended in medium containing 0.5 mM L-lysine. Upon further incubation for one hour, threonine-sensitive enzyme appeared in amounts corresponding to half that of the lysine-sensitive enzyme which remained at an essentially constant level during the one hour period.

These results indicate that lysine not only controls the production and activity of lysine-sensitive aspartokinase, but also induces the formation of the threonine-sensitive aspartokinase which does not occur in appreciable amounts in this organism in the absence of supplements of lysine. The enhancement by methionine of the induction of the threonine-sensitive enzyme suggests that additional controls regulating the biogenesis of methionine precursors may occur in this organism.

REFERENCES

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