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PUTRESCINE AND SPERMIDINE SENSITIVITY OF LYSINE DECARBOXYLASE IN ESCHERICHIA COLI: EVIDENCE FOR A CONSTITUTIVE ENZYME AND ITS MODE OF REGULATION

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Cells of Escherichia coli grown under physiological (noninducing) conditions have a low level of lysine decarboxylase activity. This activity differs from the enzyme found in induced cells in its sensitivity to putrescine (33% of control in the presence of 20 mM putrescine). It is also sensitive to spermidine (20% of control in the presence of 6 mM spermidine). A mixture of putrescine and spermidine completely eliminated lysine decarboxylase activity. This provides evidence for the existence of a biosynthetic enzyme and suggests a mechanism to explain the appearance of cadaverine in polyamine-depleted cells.

Increased polyamine levels have been implicated in a large number of growth related biological processes (1-4). The biosynthesis of these ubiquitous, low molecular weight cations (putrescine, spermidine and spermine) is initiated by the action of amino acid decarboxylases on arginine, ornithine and S-adenosyl methionine (5).

Escherichia coli has been shown to contain two arginine and ornithine decarboxylases. One is induced by low pH and high substrate concentration in a rich medium (6,7). The second is a constitutive enzyme, produced by cells grown in minimal medium at physiological pH (8,9).

Mutants of E. coli that are unable to synthesize putrescine show reduced rates of growth (10-20). Under conditions of putrescine starvation of such mutants grown in minimal medium it has been observed that cadaverine (10,13,17,18,21,22) and its aminopropyl derivative (17,18) appear. It has been suggested that cadaverine provides a diamine substitute for putrescine and can at least partially replace its growth-promoting properties (10,13,

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17,18). It has further been suggested (10,23) that this synthesis is due to the presence of a constitutive lysine decarboxylase (LDC).

An inducible lysine decarboxylase (E.C. 4.1.1.18) has been known for many years and has been well characterized (24,25). In this paper, lysine decarboxylase from cells grown under physiological conditions, in contrast to that from induced cells, is shown to be inhibited by putrescine and spermidine. This provides evidence for the existence of a constitutive enzyme and suggests a mechanism for the regulation of cadaverine biosynthesis.

Materials and Methods

E. coli K12, B_1^-Hfr (obtained from Dr. Dirk Elseviers) was used throughout these experiments. Inocula were prepared by transferring cells from a stock slant to 10 ml of Davis-Mingioli medium (26) supplemented with 0.2% glucose and 1 µg/ml thiamine (minimal medium) in a 50 ml Erlenmeyer flask. This culture was grown with shaking at 37°C until turbid ($O.D._{450}$=0.5)

Noninducing conditions: 1 ml of this suspension was inoculated into 500 ml of minimal medium in a 2-liter Erlenmeyer flask and shaken overnight at 37°C. Inducing conditions: 1 ml of the inoculum was added to 500 ml of Falkow medium (27) supplemented with 0.5% L-lysine. A 500 ml Erlenmeyer flask was completely filled to produce an essentially anaerobic environment. The culture was grown at 37°C without shaking to early stationary phase (9-10 hours) as recommended by Sabo et al. (24).

The cells were washed once in 1.5 M saline and resuspended in 8 ml of 5 mM Tris buffer containing 1 mM $MgSO_4$ and 1 mM EDTA, pH 7.0. The cells were disrupted by sonication in the cold with eight bursts of fifteen seconds each at setting 2 (Branson sonifier, Model 350, long tip). This sonicate was centrifuged for 20 minutes at 8000 rpm, 4°C. The supernatant, containing the enzyme extract, was withdrawn and stored at -4°C.

The assay for lysine decarboxylase was carried out in a standard scintillation vial. Whatman 3MM filter discs, 24 mm diameter, (VWR Scientific) were fitted into the cap and were impregnated with 100 µl of Nuclear Chicago Solubilizer (NCS, Amersham Corp.), a CO_2-trapping agent. To 0.1 ml of enzyme extract (400-600 µg protein), chilled on ice, was added 0.4 ml of a reaction mixture ("mix") containing 12 µmoles $MgSO_4$, 0.0125 µmoles pyridoxal phosphate, 35 µmoles KH_2PO_4, 5 µmoles K_2HPO_4 (phosphate buffer, pH 6.0) and 12 µmoles [^{14}C] L-lysine hydrochloride (New England Nuclear), to give a final volume of 0.5 ml. The specific activity was 0.033 µCi/µmole for enzyme from noninduced cultures and 0.0067 µCi/µmole for enzyme from induced cultures.

This mixture was incubated at 37°C for 20 minutes. The reaction was stopped by adding 6-7 drops of 1 N H_2SO_4. The vials were incubated an additional 30 minutes to trap residual CO_2. The discs were removed and transferred to new vials containing 10 ml Liquiscint (National Diagnostics). They were cooled at 4°C for 24-48 hours. Radioactivity was measured in a liquid scintillation counter (Searle Analytic Inc. Mark IV). The results were expressed as nanomoles of CO_2 produced per minute per mg of protein.

The concentration of protein was determined by the method of Lowry et al. (28) using bovine serum albumin (National Diagnostics) as the standard.

Results and Discussion

The appearance of cadaverine in cells grown in minimal medium (10,13, 17,21,22) suggests the existence of a constitutive lysine decarboxylase. A

study was initiated in this laboratory to provide evidence for such an activity. Cells grown under inducing conditions had a high level of lysine decarboxylase activity, 1123 nmoles/min/mg protein. Cells grown under non-inducing conditions contain a low level of enzyme activity, 4.6 nmoles/min/mg protein, which was found to be linear with respect to time and protein concentration.

The possibility was considered that this activity might represent a basal level of the inducible enzyme. Goldemberg (23) had reported a differentiation between the two based on heat sensitivity - the postulated constitutive enzyme showing a 30% decrease in activity after heating for 4 min at 60°C. This observation could not be reproduced in this laboratory.

The appearance of cadaverine in putrescine-depleted cells suggested that putrescine may have an inhibitory effect on lysine decarboxylase activity. The effect of this diamine was studied as a possible method of distinguishing between the two enzymes.

Enzyme extracts were prepared from cells grown under inducing and noninducing conditions. Putrescine was added to the reaction vial at final concentrations of 20, 25 and 30 mM. It was observed that putrescine has essentially no effect on the enzyme from induced cultures. On the other hand, lysine decarboxylase activity from uninduced cultures was reduced to 33% of the control value at 20 mM (physiological levels; 29). See Table 1. This difference in putrescine sensitivity provides evidence that the enzyme activity found in noninduced cells is different from that found in induced cells, i.e. it is the postulated biosynthetic lysine decarboxylase.

The effect of spermidine on LDC activity was also investigated. Spermidine was added to the reaction vials at final concentrations of 2, 4, 6 and 8 mM. It was observed that spermidine had essentially no effect on the enzyme from induced cultures. When added to extracts from cells grown under physiological conditions, lysine decarboxylase activity was reduced to 20% of the control value at 6 mM (physiological levels; 29). See Table 2.

Table 1
Putrescine Sensitivity
of
Lysine Decarboxylase
from Cells Grown Under Inducing and Noninducing Conditions

GROWTH CONDITIONS	PUTRESCINE CONCENTRATION (mM)	% ACTIVITY REMAINING
Inducing	0	100
	20	100
	25	89
	30	92
Noninducing	0	100
	20	33
	25	23
	30	18

A stock solution of putrescine dihydrochloride (Fisher Scientific) was prepared at a concentration of 100 mg/ml. The initial addition contained 30 µl of enzyme extract and 70 µl of phosphate buffer. 16, 20 or 24 µl of the putrescine stock solution were added after the "mix", replacing some of the above buffer, such that the final volume was retained at 0.5 ml. The incubation was carried out as described in Materials and Methods.

This finding again indicates that the enzyme found in uninduced cultures represents an activity distinct from that found in induced cultures.

Furthermore, when putrescine (25 mM) was added together with spermidine (6 mM), the induced enzyme activity was unaffected. However, the LDC activity from cells grown under noninducing conditions was totally inhibited (Table 2).

These observations suggest a model (10,30) for the regulation of lysine decarboxylase activity. According to this model, the biosynthetic enzyme is normally present but is inhibited by physiological levels of putrescine and spermidine. When starved of polyamines, the inhibition is relieved and cadaverine appears. Consistent with this view is the observation by Popkin and Maas (30) that exponentially growing cells of a mutant of E. coli which has a depressed level of lysine decarboxylase has no cadaverine pool. Furthermore, cadaverine was detected in stationary cells of the mutant. This might be explained by the conversion of spermidine to its glutathione derivative (31), thus making it unavailable for LDC inhibition.

Table 2

Spermidine Sensitivity
of
Lysine Decarboxylase
from Cells Grown Under Inducing and Noninducing Conditions

GROWTH CONDITIONS	SPERMIDINE CONCENTRATION (mM)	% ACTIVITY REMAINING
Inducing	0	100
	2	119
	4	103
	6	96
	8	93
	6 (+25 mM Putrescine)	101
Noninducing	0	100
	2	64
	4	18
	6	20
	8	11
	6 (+25 mM Putrescine)	0

A stock solution of spermidine trihydrochloride (Fisher Scientific) was prepared at a concentration of 100 mg/ml. The initial addition contained 30 µl of enzyme extract and 70 µl of phosphate buffer. 6.6, 13.2, 20 or 26.6 µl of the spermidine stock solution were added after the "mix", replacing some of the above buffer, such that the final volume was retained at 0.5 ml. The incubation was carried out as described in Materials and Methods.

There are a number of reports in the literature describing growth stimulation by the addition of cadaverine (10,16,17,18,20) or aminopropyl cadaverine (20,32) to polyamine-depleted cells. It has been suggested that cadaverine or aminopropyl cadaverine can substitute, at least partially, for putrescine or spermidine to restore growth rate (10,13,17,18).* The precise mechanism of this growth promotion has not been elucidated, although Morris has reported an effect of aminopropyl cadaverine on protein and mRNA chain elongation rates (32). The presence of cadaverine in the peptidoglycan

*The finding of Tabor et al. (33) that a cadA (lysine decarboxylase) mutant grew at the same rate as the parental strain may reflect the unusually low cadaverine pools in the parent (a Δ(SpeA-SpeB) ΔSpeC ΔSpeD quadruple mutant), 10-fold lower than that found by others (10,21).

layer of S. ruminantium (34) and the detection of elevated serum levels of cadaverine derivatives in patients with schizophrenia (35) suggest that cadaverine may have additional biological roles. The study of the regulation of cadaverine biosynthesis is an important step towards an understanding of the role(s) of this molecule.

Towards this end, a mutant has been isolated (to be described elsewhere) which is deficient in the inducible lysine decarboxylase. The residual activity found in cells grown under inducing and noninducing conditions has been identified as the biosynthetic enzyme, based on its sensitivity to putrescine. This strain can serve as the starting point in a search for a mutant deficient in the biosynthetic enzyme. In addition, by using this (inducible LDC) mutant and/or by taking advantage of putrescine and spermidine sensitivity to monitor the desired activity, the biosynthetic enzyme can be purified and characterized so as to further elucidate its biological role.

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References

1. Cohen, S.S. (1972) Introduction to the Polyamines, Prentice Hall, Englewood Cliffs, NJ, 179 pp.
2. Bachrach, U. (1973) Function of Naturally Occurring Polyamines, Academic Press, NY, 211 pp.
3. Tabor, H. and Tabor, C.W. (1972) Adv. Enzymol. 36, 203-268.
4. Jänne, J., Pöso, H. and Raina, A. (1978) Biochim. Biophys. Acta 473, 241-293.
5. Morris, D.R. and Fillingame, R.H. (1974) Annu. Rev. Biochem. 43, 303-325.
6. Gale, E.F. (1946) Adv. Enzymol. 6, 1-32.
7. Boeker, E.A. and Snell, E.E. (1972) in The Enzymes (P.D. Boyer, ed.). 3rd Edition, 6, 217-253.
8. Morris, D.R. and Pardee, A.B. (1965) Biochem. Biophys. Res. Commun. 20, 697-702.
9. Morris, D.R. and Pardee, A.B. (1966) J. Biol. Chem. 241, 3128-3135.
10. Leifer, Z. (1972) Isolation and Characterization of Mutants of Escherichia coli Conditionally Deficient in the Biosynthesis of Putrescine and Cadaverine, University Microfilms, Ann Arbor, MI, 157 pp.
11. Hirschfield, I., Rosenfeld, H.J., Leifer, Z. and Maas, W.K. (1970) J. Bacteriol. 101, 725-730.
12. Maas, W.K., Leifer, Z. and Poindexter, J. (1970) Ann. N. Y. Acad. Sci. 171, 957-967.
13. Cunningham-Rundles, S. and Maas, W.K. (1975) J. Bacteriol. 124, 791-799.
14. Morris, D.R. and Jorstad, C.M. (1970) J. Bacteriol. 101, 731-737.
15. Morris, D.R. and Jorstad, C.M. (1973) J. Bacteriol. 113, 271-277.

16. Hafner, E.W., Tabor, C.W. and Tabor, H. (1979) J. Biol. Chem. 254, 12419-12426.
17. Dion, A.S. and Cohen, S.S. (1972) Proc. Nat. Acad. Sci. U.S.A. 69, 213-217.
18. Dion, A.S. and Cohen, S.S. (1972) J. Virol. 9, 423-430.
19. Young, D.V. and Srinivasan, P.R. (1972) J. Bacteriol. 112, 30-39.
20. Munro, G.F. and Bell, C.A. (1973) J. Bacteriol. 115, 469-475.
21. Srinivasan, P.R., Young, D.V. and Maas, W.K. (1973) J. Bacteriol. 116, 648-655.
22. Goldemberg, S.H. and Algranati, I.D. (1972) Mol. Cell. Biochem. 16, 71-77.
23. Goldemberg, S.H. (1980) J. Bacteriol. 141, 1428-1431.
24. Sabo, D.L., Boeker, E.A., Byers, B., Waron, H. and Fischer, E.H. (1974) Biochemistry 13, 662-670.
25. Sabo, D.L. and Fischer, E.H. (1974) Biochemistry 13, 670-676.
26. Davis, B.D. and Mingioli, E.S. (1950) J. Bacteriol. 60, 17-28.
27. Falkow, S. (1958) Am. J. Clin. Path. 29, 598-600.
28. Lowry, O.H., Rosebrough, N., Farr, A. and Randall, R. (1951) J. Biol. Chem. 193, 265-275.
29. Tabor, H. and Tabor, C.W. (1969) J. Biol. Chem. 244, 2286-2292.
30. Popkin, P.S. and Maas, W.K. (1980) J. Bacteriol. 141, 485-492.
31. Tabor, C.W. and Tabor, H. (1970) Biochem. Biophys. Res. Commun. 41, 232-238.
32. Jorstad, C.M., Harada, J.J. and Morris, D.R. (1980) J. Bacteriol. 141, 456-463.
33. Tabor, H., Hafner, E.W. and Tabor, C.W. (1980) J. Bacteriol. 144, 952-956.
34. Kamiyo, Y., Itoh, Y., Terawaki, Y. and Kusano, T. (1981) J. Bacteriol. 145, 122-128.
35. Dolezalova, H., Stepita-Klanco, M., van der Velde, C. and Cassone, V. (1979) Biol. Psych. 14, 27-35.