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Regulation of polyamine biosynthesis in *Escherichia coli* by basic proteins

(ornithine decarboxylase/arginine decarboxylase/antizyme/histones)

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ABSTRACT **In *Escherichia coli*, the biosynthetic ornithine and arginine decarboxylases (EC 4.1.1.17 and 4.1.1.19, respectively) are responsible for the biosynthesis of polyamines from ornithine and arginine, respectively. When *E. coli* cells are grown in the presence of increasing amounts of polyamines, a progressive increase in the amount of antizyme 1 and antizyme 2 occurs. The amino acid compositions of antizymes 1 and 2 show them to be basic proteins; antizyme 1 has an amino acid composition similar to that of the *E. coli* histone-like protein HU and of the eukaryotic histone H2B; antizyme 2 is characterized by an unusually high arginine content. We find these proteins to be specific inhibitors of both the biosynthetic ornithine decarboxylase and the biosynthetic arginine decarboxylase. They do not inhibit the corresponding biodegradative ornithine and arginine decarboxylases, nor do they inhibit lysine decarboxylase or *S*-adenosylmethionine decarboxylase. These properties of the antizymes favor their function in the regulation of polyamine biosynthesis in *E. coli*. The ability of the purified antizymes to inhibit the ornithine and arginine decarboxylases is stabilized in acidic buffers and is lost upon prolonged exposure to solutions at neutral or basic pH.**

Putrescine biosynthesis in *Escherichia coli* occurs through two separate pathways (1, 2). One is through the decarboxylation of ornithine to putrescine by the biosynthetic ornithine decarboxylase (EC 4.1.1.17). The other is through the decarboxylation of arginine by the biosynthetic arginine decarboxylase (EC 4.1.1.19) to form agmatine, which is hydrolyzed to putrescine and urea by agmatine ureohydrolase (Fig. 1). These enzymes are normally present in low amounts in *E. coli* grown on minimal medium and fulfill their biosynthetic requirements for polyamines (1, 3, 4).

Polyamines are also synthesized in *E. coli* through the action of the biodegradative ornithine decarboxylase and the biodegradative arginine decarboxylase. These biodegradative enzymes are induced in *E. coli* by growth at low pH in the presence of high concentrations of their respective substrates and are structurally distinct from the biosynthetic ornithine and arginine decarboxylases referred to above (5, 6). The function of the biodegradative enzymes may be related to the maintenance of the intracellular pH; they do not appear to contribute to the intracellular putrescine levels when *E. coli* cells are grown at neutral pH (5, 6).

In many strains of *E. coli* the biosynthetic ornithine decarboxylase provides the predominant route for putrescine biosynthesis (7). The biosynthetic arginine decarboxylase appears to play a complementary role in maintaining intracellular polyamine levels. Such a balance explains why the inhibition of ornithine decarboxylase activity *in vivo* does not lower the putrescine levels, nor does it decrease the growth rate of *E. coli* (8, 9). Only when both the ornithine and the arginine decarboxylase have been completely inhibited does a decrease in polyamine levels and a decrease in cell growth occur (10). Some form of feedback inhibition also occurs, because the addition of polyamines to *E. coli* grown in a chemostat restricts the conversion of ornithine to polyamines (11). This is followed by an apparent repression of both ornithine decarboxylase and arginine decarboxylase activities as determined by the decrease in their respective specific activities (11).

A possible mechanism for such a mode of inhibition of these decarboxylase activities has been provided through the isolation of a macromolecular inhibitor of biosynthetic ornithine decarboxylase from *E. coli* (12). The inhibitory activity increased in response to increased polyamine levels in the growth medium in a manner similar to that in which antizyme activity to ornithine decarboxylase increases in eukaryotic cells when the polyamine levels of the growth medium are increased (13, 14). The complex of ornithine decarboxylase and inhibitor formed *in vitro* can be dissociated with salt, and each component can be recovered in its active form. These characteristics are similar to those of the mammalian antizyme (13, 14). The inhibitory activity has been resolved into three proteins, each of which has been purified to apparent homogeneity (15); an acidic protein of apparent molecular weight 49,000 and two basic proteins with apparent molecular weights 11,000 and 9,000, respectively (15). Although the acidic inhibitor to ornithine decarboxylase was the original antizyme to be identified (12), we now find that the two basic inhibitors account for over 90% of the total inhibitory activity in *E. coli* extracts (15). In this paper we describe the effects of the two basic inhibitors on the biosynthetic ornithine and arginine decarboxylases and compare them to their effects on the corresponding biodegradative ornithine and arginine decarboxylases as well as on the lysine and *S*-adenosylmethionine decarboxylases.

MATERIALS AND METHODS

Materials. DL-[1-^{14}C]Ornithine (50 mCi/mmol; 1 Ci = 3.7 × 10^{10} Bq) was obtained from Moravek Biochemicals (Brea, CA), L-[U-C^{14}]arginine (344 mCi/mmol) was from Amersham, and L-[U-C^{14}]lysine (343 mCi/mmol) and *S*-adenosyl-L-[carboxyl-^{14}C]methionine (7.7 mCi/mmol) were from New England Nuclear. Tris buffer, Hepes buffer, dithiothreitol, and calf thymus histones were from Sigma. All other chemicals were of the highest reagent grade available.

Enzymes. Biosynthetic ornithine decarboxylase was purified approximately 50-fold (19% recovery) from *E. coli* AB1203 as described (12). Biosynthetic arginine decarboxylase was purified approximately 25-fold (25% recovery) according to the pro-

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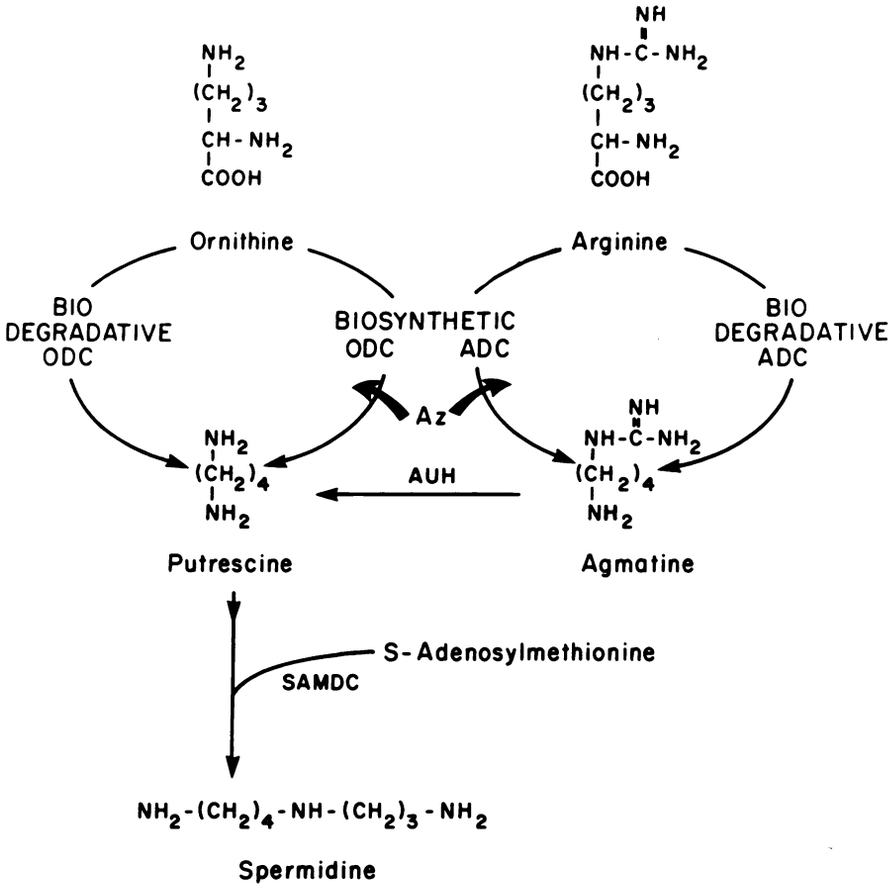

FIG. 1. Outline of the main metabolic reactions leading to the synthesis of the polyamines of *E. coli*. ODC, Ornithine decarboxylase; ADC, arginine decarboxylase; AUH, agmatine ureohydrolase; SAMDC, *S*-adenosylmethionine decarboxylase; Az, antizyme 1 and 2.

cedure of Wu and Morris through the heat treatment step (3). Biodegradative ornithine decarboxylase was purchased from Sigma. Biodegradative arginine decarboxylase was purified 3-fold (95% recovery) according to the procedure of Blethen *et al.* (16). Lysine decarboxylase was obtained from Sigma. *S*-Adenosylmethionine decarboxylase was purified from *E. coli* AB1203 according to the procedure of Wickner *et al.* through the heat treatment step (17).

Basic Antizymes. The basic antizymes were purified from *E. coli* MA255 (*speB, speC*) as described (15).

Enzyme Assays. The concentration of substrate used in the enzyme assays and the reference describing the assay are indicated after each enzyme: biosynthetic ornithine decarboxylase (0.55 mM) (18); biosynthetic arginine decarboxylase (0.075 mM) (3); biodegradative ornithine decarboxylase (0.55 mM) (19); biodegradative arginine decarboxylase (0.075 mM) (16); lysine decarboxylase (0.4 mM) (20); and *S*-adenosylmethionine decarboxylase (0.022 mM) (17).

One unit of enzyme activity was defined as the release of 1 nmol of CO_2 by each decarboxylase from its appropriate substrate at 37°C in 1 hr. One unit of antizyme activity is the amount of antizyme that inhibits 1 unit of enzyme. Protein concentrations were determined by the method of Bradford (21) and by the fluorometric method using *o*-phthaldialdehyde, described by Butcher and Lowry (22).

RESULTS

When MA255 (*speB, speC*) cells are grown in Luria broth (23) in the presence of increasing amounts of putrescine and spermidine, there occurs a progressive increase in the amount of antizyme that can be extracted. A plateau value of antizyme content is attained at about 3- to 6-fold above base line values (Fig. 2). The two basic antizyme proteins purified from *E. coli* MA255 (15) have estimated molecular weights of 11,000 and 9,000 and have been designated antizyme 1 and antizyme 2, or Az 1 and Az 2, respectively (15). A representative titration curve

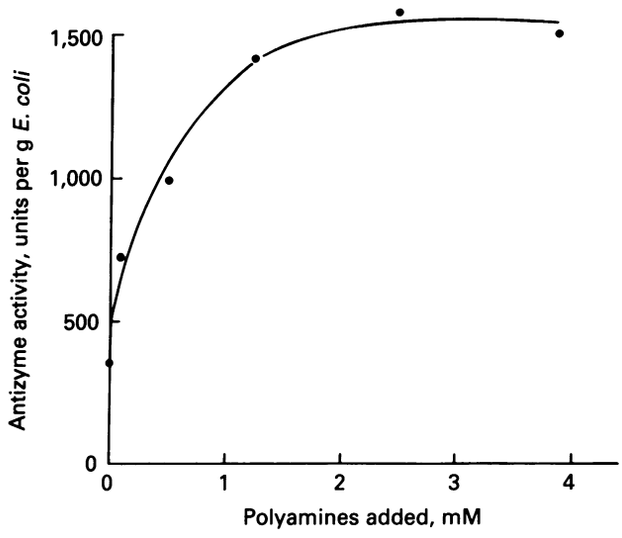

FIG. 2. Effect of increasing putrescine and spermidine concentrations on the activity of antizyme during growth of *E. coli* strain MA255. Cells were grown in 2-liter flasks in 1 liter of Luria broth (23) to which were added the indicated total concentrations of putrescine plus spermidine (1:1) (e.g., on the abscissa, 4 refers to 2 mM of each polyamine). The cells were harvested in late logarithmic phase, and antizyme activity was assayed in the perchloric acid-soluble fraction (15), as described in the legend to Fig. 3.

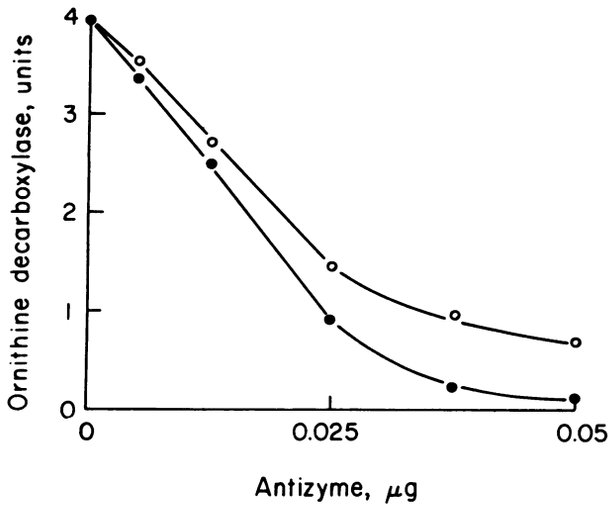

FIG. 3. Inhibition of *E. coli* biosynthetic ornithine decarboxylase by antizyme 1 and antizyme 2. Increasing amounts of either antizyme 1 (○) or antizyme 2 (●) protein were added to approximately 4 units of ornithine decarboxylase.

of the inhibition of the biosynthetic *E. coli* ornithine decarboxylase by increasing concentrations of antizyme 1 and antizyme 2 is shown in Fig. 3. The inhibition of ornithine decarboxylase activity is linear with respect to antizyme protein up to approximately 0.025 μg of protein (61% inhibition for antizyme 1 and 76% inhibition for antizyme 2). Further increases in antizyme protein do not result in a proportional increase in the inhibition of ornithine decarboxylase.

Amino Acid Analysis of Antizymes 1 and 2. Several basic histone-like proteins have been isolated from *E. coli* (24–27). Proteins H (27) and HU (24) are among those whose amino acid compositions are known. In Table 1, we provide the amino acid analyses of antizyme 1 and of antizyme 2 and compare them to the amino acid composition of *E. coli* protein HU. The amino acid composition of *E. coli* protein H is not included in Table 1 because it varies greatly from that of antizyme 1 and antizyme 2. Of the various eukaryotic histones, we have included in Table 1 the amino acid composition of histone H2B (28).

Table 1. Amino acid composition (mol %) of antizymes 1 and 2 and histones HU and H2B

Amino acid residue	Antizyme 1	Antizyme 2	*E. coli* histone-like protein HU*	Eukaryotic histone H2B†
Lys	16.6	11.2	14.0	14.1
His	3.4	2.3	1.5	2.3
Arg	8.4	24.0	5.1	6.9
Glu	9.1	4.3	9.6	8.7
Asp	9.5	4.4	8.1	5.0
Ser	4.7	6.7	4.4	10.4
Thr	2.4	6.4	6.0	6.4
Ala	22.3	11.1	16.3	10.8
Val	1.9	5.5	6.0	7.5
Ile	7.1	—	6.0	5.1
Leu	3.7	5.6	6.6	4.9
Met	3.5	4.5	1.5	1.5
Phe	2.6	4.5	3.0	1.6
Tyr	1.1	—	—	4.0
Pro	1.0	2.5	3.0	4.9
Gly	2.6	6.9	7.4	5.9
Trp	—	—	—	—
Cys	—	—	—	—
Lys/Arg	1.97	0.47	2.7	2.01
Gly × Arg	21.8	165.6	37.7	40.7

*Taken from ref. 24.
†Taken from ref. 28.

The particularly high arginine content of antizyme 2 distinguishes it from the other basic proteins. The amino acid composition of antizyme 1 bears some similarity to that of *E. coli* protein HU and eukaryotic histone H2B. It remains to be determined whether either one or both of these basic proteins have histone-like properties in a variety of nucleic acid-related reactions.

Effect of Eukaryotic Histones and of Basic Polypeptides on *E. coli* Ornithine Decarboxylase. The histone-like composition and basicity of the antizymes 1 and 2 prompted us to determine whether other histones would inhibit *E. coli* ornithine decarboxylase. We tested calf thymus histones H1, H2A, H2B, H3, and H4 for their ability to inhibit the *E. coli* ornithine decarboxylase. No inhibition was detected at 2.5 times the maximal antizyme concentrations tested in Fig. 3—i.e., at histone concentrations of 0.125 μg per assay. Polylysine, at 5.0 μg per assay, was found to inhibit by 50% the activity of *E. coli* ornithine decarboxylase.

Effect of Antizyme 1 and Antizyme 2 on Other Enzymes. The purification scheme for antizymes 1 and 2 includes an initial extraction of the *E. coli* cells, followed by a pH 1.8 precipitation of bulk proteins. The antizymes are then purified from a perchloric acid-soluble fraction, fractionated on a CM Bio-Gel A column (Bio-Rad), and separated from each other by electrophoresis (15). Samples of each fraction corresponding to the last three steps in the purification scheme were tested for their ability to inhibit various *E. coli* decarboxylases related to polyamine synthesis, including lysine decarboxylase, *S*-adenosylmethionine decarboxylase, biosynthetic arginine decarboxylase, biosynthetic ornithine decarboxylase, biodegradative arginine decarboxylase, and biodegradative ornithine decarboxylase. Titrations such as those presented in Fig. 3, of 0.5–1.0 unit of the various decarboxylases by the appropriate antizyme fractions, constitute the basis of the results presented in Table 2. In all cases, the linear portion of the inhibition curve was used to relate one titration to the other.

The individual enzyme preparations were carefully screened and found to be minimally, if at all, contaminated by the other test enzymes. We have especially attempted to differentiate the biodegradative from the biosynthetic ornithine and arginine decarboxylases. Because the K_ms of the ornithine decarboxylases are approximately 100-fold higher than the K_ms of the

Table 2. Titration of the fractions obtained during purification of the *E. coli* antizymes by various *E. coli* decarboxylases

	Units antizyme activity/μg antizyme protein						Inhibition ratio,
			Biodegradative		Biosynthetic		
Antizyme fraction*	Lys-DCase	Ado-Met-DCase	Orn-DCase	Arg-DCase	Orn-DCase	Arg-DCase	Orn-DCase: Arg-DCase
Perchloric acid-soluble	0	0	0	0	0.17	0.01	12
CM Bio-Gel A	—	—	0	0	2.0	0.22	9.2
Antizyme 1	—	—	0	0	45.0	1.7	28
Antizyme 2	—	—	0	0	36.0	4.3	8.1

LysDCase, lysine decarboxylase; AdoMetDCase, *S*-adenosylmethionine decarboxylase; OrnDCase, ornithine decarboxylase; ArgDCase, arginine decarboxylase.
*Antizyme fraction refers to the various fractions obtained during purification of antizymes 1 and 2 (15).

arginine decarboxylases, and in order to have uniform conditions, we assayed these four enzymes at substrate concentrations that were approximately 1/4 their respective K_ms. It can be seen that the only enzymes that are inhibited by the two antizymes are the biosynthetic ornithine and arginine decarboxylases.

DISCUSSION

Antizymes 1 and 2 are induced above their basal level in *E. coli* when the cells are grown in the presence of putrescine and spermidine, the reaction end-products of the two biosynthetic enzymes they inhibit. Although they do not inhibit *S*-adenosylmethionine decarboxylase, the enzyme that is responsible for the biosynthesis of spermidine from putrescine, they inhibit the biosynthesis of putrescine. They do not inhibit the decarboxylation of lysine, which in eukaryotic cells appears to be mediated through ornithine decarboxylase (29), nor do they inhibit the biodegradative ornithine and arginine decarboxylases.

In vitro, the biosynthetic ornithine and arginine decarboxylases differ greatly in their K_ms, and the optimal assay conditions for each enzyme are very different. Furthermore, the activity of arginine decarboxylase is affected by divalent cations (3), whereas ornithine decarboxylase responds greatly to activation and inhibition by a variety of nucleotides, including GTP, AMP, and ppGpp (30, 31). For example, GTP decreases the substrate K_m for ornithine decarboxylase from 1.9 mM to 0.28 mM (30). Because it was not expedient to assay ornithine decarboxylase at saturating ornithine concentrations, we have maintained uniform conditions by assaying the enzymes at substrate concentrations approximately 1/4 their K_m values. We have also used the nonactivated basal form of ornithine decarboxylase, devoid of the multitude of activators and inhibitors, and compared it to the basal form of arginine decarboxylase. It is apparent that antizymes 1 and 2 are more effective against biosynthetic ornithine decarboxylase than against biosynthetic arginine decarboxylase. Such a property is in keeping with the conclusion derived from *in vivo* studies, that ornithine constitutes the main source of polyamines in *E. coli* (1, 2, 7). *In vivo*, however, the relative effectiveness of the antizymes against the intracellular biosynthetic ornithine and arginine decarboxylases may change with the intracellular composition and concentration of nucleotides, as these are affected by the stage of the cell cycle or by the nutritional conditions.

The general properties of antizymes 1 and 2 suggest that they may function intracellularly in the regulation of putrescine biosynthesis in *E. coli*, which would also regulate the size of the spermidine pool. A similar role for antizyme in the regulation of polyamine biosynthesis in eukaryotic cells has been indicated by the inverse relationship that we have found to exist between the activity of ornithine decarboxylase and antizyme in neuroblastoma cells; enhancement of the activity of one leads to the decrease in the activity of the other (18).

As mentioned in the text, on the basis of their amino acid compositions alone, it is possible that one or both antizymes may also be histone-like proteins in a functional sense. This point remains to be clarified. Nevertheless, it should be noted that the inhibition of the biosynthetic ornithine and arginine decarboxylases by antizymes 1 and 2 does not appear to be due only to their basic amino acid composition or to their basic properties. These antizymes have been purified while being assayed for a specific inhibitory enzymatic function. With progressive purification the ability of the antizymes to inhibit these specific reactions is lost if they are stored, even at −20°C, in the absence of Brij 58 or glycerol, or if they are exposed for short periods of time to neutral or slightly basic buffers (15). The extent to which histones in general may have the ability to affect specific enzymatic reactions but lose this function because of the methods of purifying histones remains to be established.

It may be appropriate to speculate whether antizymes 1 and 2 interact with specific regions of *E. coli* DNA and whether, through such an interaction, they may also participate in the structure and the function of DNA and thus relate polyamine synthesis to other cellular functions.

Note Added in Proof. In agreement with the paragraph above, we find that *E. coli* DNA reverses the inhibition of ornithine decarboxylase activity.

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