JOURNAL OF BACTERIOLOGY, July 1988, p. 3131–3135
0021-9193/88/073131-05$02.00/0

Vol. 170, No. 7

Adjustment of Polyamine Contents in *Escherichia coli*

KEIKO KASHIWAGI AND KAZUEI IGARASHI*

Faculty of Pharmaceutical Sciences, Inohana Campus, Chiba University, 1-8-1 Inohana, Chiba 280, Japan

Received 12 January 1988/Accepted 23 April 1988

Adjustment of polyamine contents in *Escherichia coli* was studied with strains of *Escherichia coli* producing normal (DR112) and excessive amounts of ornithine decarboxylase [DR112(pODC)] or *S*-adenosylmethionine decarboxylase [DR112(pSAMDC)]. Although DR112(pODC) produced approximately 70 times more ornithine decarboxylase than DR112 did, the amounts of polyamines in the cells of both strains did not change significantly. The amounts of polyamines in DR112(pODC) were adjusted by excretion of excessive amounts of putrescine to the medium. When ornithine was deficient in cells, polyamine contents in DR112(pODC) were much higher than those in DR112, although polyamine contents were low in both strains. This indicates that large amounts of ornithine decarboxylase increased the utilization of ornithine for putrescine synthesis. During ornithine deficiency, strain DR112 produced 3.4 times more ornithine decarboxylase. Strain DR112(pSAMDC) produced seven times more *S*-adenosylmethionine decarboxylase than DR112 did. In DR112(pSAMDC) an increase (40%) in spermidine content, a decrease (35%) in putrescine content, and no significant excretion of putrescine and spermidine were observed. The amount of ornithine decarboxylase in DR112(pSAMDC) was approximately 30% less than that in DR112. In addition, *S*-adenosylmethionine decarboxylase activity was strongly inhibited by spermidine. A possible regulatory mechanism to maintain polyamine contents in *Escherichia coli* is discussed based on the results.

Polyamines, polycationic compounds, present in all living organisms, have been implicated in a wide variety of biological reactions, including nucleic acid and protein synthesis (20, 21). It is believed that the regulation of ornithine decarboxylase activity is important in the regulation of polyamine biosynthesis in *Escherichia coli*. Ornithine decarboxylase of *E. coli* is stimulated by GTP (1, 6) and inhibited by ppGpp (7, 19), polyamines (1, 23), and antizymes (13, 18). It has been reported that two basic *E. coli* antizyme proteins are ribosomal proteins S20/L26 and L34 (18), but other major ribosomal proteins also show antizyme activity (11). Furthermore, when *E. coli* extracts were separated into ribosomes and supernatant fraction (100,000 × *g*), no significant antizyme activity was observed either in ribosomes or the supernatant fraction. These results suggest that antizymes may not function as inhibitors of ornithine decarboxylase in vivo (11). By using *E. coli* strains producing normal (DR112) and excessive amounts of ornithine decarboxylase [DR112(pODC)] or *S*-adenosylmethionine decarboxylase [DR112(pSAMDC)], we have studied how the amounts of polyamines in *E. coli* are regulated.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *E. coli* DR112 (*speA speB thi*), which lacks the putrescine biosynthetic pathway from agmatine, was kindly provided by D. R. Morris. Plasmids containing the ornithine decarboxylase gene (3) and the *S*-adenosylmethionine decarboxylase gene (22) were kindly supplied by S. M. Boyle and C. W. Tabor, respectively, and transferred into *E. coli* DR112. These strains were cultured in medium containing 4 g of glucose; 6 g of Na_2HPO_4; 3 g of KH_2PO_4; 500 mg of NACl; 1 g of NH_4Cl; 250 mg of $MgSO_4 \cdot 7H_2O$; 13 mg of $CaCl_2 \cdot 2H_2O$; 2 mg of thiamine; 100 mg each of alanine, asparagine, aspartic acid, glutamic acid, glycine, methionine, proline, serine, and threonine; 50 mg each of cysteine, histidine, isoleucine, leucine, phenylalanine, tyrosine, and valine; and 5 mg of tryptophan per liter of water. When growth was sufficient to give an A_{600} of 0.3 (three generations; 6×10^8 cells per ml), the cells were harvested by centrifugation at 15,000 × *g* for 15 min; when indicated, the cells were harvested at an A_{600} of 1.2 (five generations; 2.4×10^9 cells per ml). The cells were washed once with a buffer containing 10 mM Tris hydrochloride (pH 7.5), 10 mM magnesium acetate, 60 mM NH_4Cl, and 6 mM 2-mercaptoethanol, centrifuged as described above, and stored at −80°C until used.

Measurement of polyamine contents. Polyamine levels in *E. coli* were determined by high-performance liquid chromatography as described previously (9), with some modifications. *E. coli* cells (5 mg) were homogenized with 1 ml of 5% trichloroacetic acid, and the supernatant was obtained by centrifugation at 10,000 × *g* for 10 min. Under these conditions, more than 99% of [^{14}C]spermidine was recovered in the supernatant. The polyamines in 10 μl of 5% trichloroacetic acid supernatant was separated on a Toyo Soda high-performance liquid chromatography system on which a TSK gel IEX column (4 by 80 mm) heated to 50°C was mounted. The flow rate of the buffer (0.084 M citric acid buffer [pH 5.15], 2 M NaCl, 20% methanol, 0.01% *N*-caproic acid, 0.1% Brij 35) was 0.65 ml/min. Detection of the polyamines was by fluorescence intensity after reaction of the column effluent at 50°C with an *o*-phthalaldehyde solution containing 0.06% *o*-phthalaldehyde, 0.4 M boric buffer (pH 10.4), 0.1% Brij 35, and 37 mM 2-mercaptoethanol. The flow rate of the *o*-phthalaldehyde solution was 0.5 ml/min, and fluorescence was measured at an excitation wavelength of 388 nm and an emission wavelength of 410 nm. The retention times for putrescine, cadaverine, spermidine, aminopropylcadaverine, and spermine were 7, 10, 14, 20, and 30 min, respectively. Aminopropylcadaverine was a kind gift of D. R. Morris.

Purification of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase. Ornithine decarboxylase was purified from a cell extract of *E. coli* CL510, transformed with a plasmid containing the ornithine decarboxylase gene

* Corresponding author.

TABLE 1. Polyamine contents in *E. coli* producing normal and excessive amounts of ornithine decarboxylase or *S*-adenosylmethionine decarboxylase

Strain	A_{600} at harvest	Amino acid added (mg/ml)	Amt of polyamine (nmol/mg of protein)[a]			
			Putrescine	Cadaverine	Spermidine	Aminopropyl-cadaverine
DR112	0.3		95.7	2.31	14.0	ND[b]
	0.3	Orn (0.1)	104	1.63	11.0	ND
	0.3	Arg (1.0)	0.61	5.12	5.06	0.12
	1.2		66.6	1.56	4.55	ND
DR112(pODC)	0.3		117	2.10	12.3	ND
	0.3	Orn (0.1)	122	0.93	10.3	ND
	0.3	Arg (1.0)	36.5	22.8	14.2	0.24
	1.2		78.4	1.34	5.54	ND
DR112(pSAMDC)	0.3		63.1	2.71	19.6	1.12
	1.2		42.5	1.57	11.3	0.54

[a] Each value is the average of duplicate determinations.
[b] ND, Not detectable.

[CL510(pODC)]. Purification was performed by the method of Applebaum et al. (1), except that Sephadex G-200 column chromatography was omitted and the buffer contained 10 μM FUT-175 (6-amidino-2-naphthyl-4-guanidobenzoate dihydrochloride), a strong proteinase inhibitor (4). The purified enzyme showed about 95% homogeneity, as judged by sodium dodecyl sulfate (SDS)-polyacrylamide slab gel electrophoresis.

S-Adenosylmethionine decarboxylase was purified from a cell extract of *E. coli* CL510(pSAMDC). Purification was performed by the method of Markham et al. (17), except that DEAE-cellulose column chromatography was omitted and the buffer contained 10 μM FUT-175. The purified enzyme showed about 80% homogeneity, as judged by SDS-polyacrylamide slab gel electrophoresis.

Assays for ornithine decarboxylase, lysine decarboxylase, and *S*-adenosylmethionine decarboxylase. *E. coli* wet cells were suspended in 10 volumes of a buffer containing 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)-KOH (pH 7.5), 5 mM magnesium sulfate, 2 mM dithiothreitol, and 40 μM pyridoxal phosphate and then sonicated twice for 1 min each time. After the cell extract was centrifuged at 30,000 × *g* for 15 min, the supernatant was used for enzyme assays. Assays for ornithine decarboxylase, lysine decarboxylase, and *S*-adenosylmethionine decarboxylase in crude extracts or purified preparations were performed as described previously (9).

Measurement of amino acid contents. Amino acid levels in *E. coli* were determined by a Hitachi 835-10 amino acid analyzer after extraction of amino acids from the bacteria with 5% trichloroacetic acid and centrifugation at 30,000 × *g* for 15 min at 4°C. Trichloroacetic acid in the samples was removed by ether extraction before amino acid analysis. The amino acid concentration in *E. coli* was calculated by assuming a 3-μl cell volume per mg of protein (2).

Other procedures and chemicals. Protein was determined by the method of Lowry et al. (15). MGBG-Sepharose, used for the purification of *S*-adenosylmethionine decarboxylase, was prepared by the method of Markham et al. (17). Preparation of *E. coli* Q13 unwashed ribosomes and assay of antizyme activity were performed as described previously (11). The ribosomal proteins from *E. coli* DR112 and DR112(pODC) were prepared as described previously (11). The two-dimensional electrophoresis of ribosomal proteins was performed by the previously described method I (11). Gel electrophoresis and Western blot (immunoblot) analysis were performed by the methods of Laemmli (14) and Towbin et al. (24), respectively, except that 4-chloro-1-naphthol was used as a substrate for the peroxidase (5). The amount of plasmids in *E. coli* was measured after extraction of plasmids by alkaline lysis methods and separation by agarose gel electrophoresis (16). Rabbit anti-ornithine decarboxylase serum was prepared by a method previously described (10). Peroxidase-conjugated AffiniPure goat anti-rabbit immunoglobulin G was obtained from Jackson Immunoresearch Laboratories, Inc. [1-^{14}C]ornithine (49.1 mCi/mmol), [carboxyl-^{14}C]*S*-adenosylmethionine (59.8 mCi/mmol), and [^{14}C]lysine (459 mCi/mmol) were purchased from New England Nuclear Corp. FUT-175 was a kind gift of Torii & Co. Ltd. Other reagents used were of analytical grade.

RESULTS

Adjustment of polyamine contents in *E. coli* producing excessive amounts of ornithine decarboxylase. The polyamine contents in *E. coli* DR112(pODC), which produces excessive ornithine decarboxylase activity, were nearly equal to those in *E. coli* DR112, which produces normal amounts of ornithine decarboxylase, when cells were harvested at the logarithmic phase of growth (Table 1). Only a slight increase in putrescine content was observed in DR112(pODC). When ornithine, a substrate of ornithine decarboxylase, was added to the medium, the polyamine contents in these two *E. coli* cells did not change significantly. The amount of ornithine decarboxylase activity produced in DR112(pODC) was approximately 70 times more than that in DR112 (Table 2). Overproduction of ornithine decarboxylase in DR112 (pODC) was confirmed by Coomassie brilliant blue staining after SDS-polyacrylamide gel electrophoresis and Western blot analysis (data not shown). The amounts of polyamines in the medium were then measured (Table 3). When *E. coli* cells were harvested at an A_{600} of 0.30, cells corresponding to approximately 10 mg of protein were obtained from a 100-ml culture. In DR112, it was calculated that approximately 25% (0.35 μmol/100 ml of medium) or 75% (3.34 μmol/100 ml of medium) of putrescine produced was excreted to the medium by cells grown in the absence or presence, respectively, of ornithine. When DR112(pODC) was grown in the absence or presence of ornithine, 96% (25.1 μmol/100 ml of medium) or 97% (36.1 μmol/100 ml medium), respectively, of putrescine produced in cells was excreted to the medium. These results show that putrescine content in cells was maintained by the excretion of excessive amounts of putrescine to the medium.

TABLE 2. Activities of ornithine decarboxylase, lysine decarboxylase, and *S*-adenosylmethionine decarboxylase in *E. coli* producing normal and excessive amounts of ornithine decarboxylase or *S*-adenosylmethionine decarboxylase

Strain	A_{600} at harvest	Amino acid added (mg/ml)	Activity (nmol/min per mg of protein)[a]		
			Ornithine decarboxylase	Lysine decarboxylase	*S*-adenosylmethionine decarboxylase
DR112	0.3		34.0	0.30	1.15
	0.3	Orn (0.1)	32.4	0.28	1.02
	0.3	Arg (1.0)	116	1.15	1.06
	1.2		31.4	0.27	0.69
DR112(pODC)	0.3		2313	18.7	0.98
	0.3	Orn (0.1)	2519	19.3	0.99
	0.3	Arg (1.0)	2725	21.5	1.02
	1.2		2192	18.2	0.64
DR112(pSAMDC)	0.3		24.1	0.21	7.03
	1.2		21.5	0.19	4.48

[a] Each value is the average of duplicate determinations.

Total putrescine (intracellular plus extracellular) production in DR112 and DR112(pODC) was compared (Tables 1 and 3). Although the amount of ornithine decarboxylase activity was about 70 times more in DR112(pODC) than in DR112, putrescine produced in DR112(pODC) in the presence of ornithine (37.3 μmol/100 ml of medium) was only 8.5 times more than that in DR112 (4.38 μmol/100 ml of medium). Thus, ornithine content in cells was measured by an amino acid analyzer (Table 4). When ornithine deficiency was induced in DR112 by the addition of 1 mg of arginine per ml of medium, ornithine concentration in cells was 4 μM. When DR112(pODC) was grown in the presence of ornithine, ornithine concentration in cells was only 6 μM. These results suggest that low efficiency of putrescine production by overproduction of ornithine decarboxylase may be due to the shortage of ornithine.

When ornithine was deficient in cells, putrescine content in DR112(pODC) was much higher than in DR112, although putrescine content was low in both strains (Table 1). This indicates that large amounts of ornithine decarboxylase increased the utilization of ornithine for putrescine synthesis. In DR112, the amount of ornithine decarboxylase activity increased by 3.4-fold when the strain was made ornithine deficient by arginine supplementation (Table 2). No significant excretion of putrescine to the medium was observed in DR112 and DR112(pODC) under these conditions (Table 3).

TABLE 3. Polyamine contents excreted to the medium by *E. coli* producing normal and excessive amounts of ornithine decarboxylase or *S*-adenosylmethionine decarboxylase

Strain	A_{600} at harvest	Amino acid added (mg/ml)	Amt of polyamine (μmol/100 ml of medium)[a]	
			Putrescine	Cadaverine
DR112	0.3		0.35	0.031
		Orn (0.1)	3.34	0.011
		Arg (1.0)	ND[b]	ND
	1.2		0.51	0.032
DR112(pODC)	0.3		25.1	0.035
		Orn (0.1)	36.1	0.028
		Arg (1.0)	0.075	0.003
	1.2		28.2	0.033
DR112(pSAMDC)	0.3		0.052	0.001
	1.2		0.085	0.002

[a] Each value is the average of duplicate determinations. No spermidine was detectable.

[b] ND, Not detectable.

The polyamine contents during ornithine deficiency were compensated by the increase in the ratio of spermidine to putrescine or by the increased synthesis of cadaverine and aminopropylcadaverine as compensatory polyamines (Table 1).

Adjustment of polyamine contents in *E. coli* producing excessive amounts of *S*-adenosylmethionine decarboxylase. The polyamine contents in DR112(pSAMDC) were measured. Strain DR112(pSAMDC) produced seven times more *S*-adenosylmethionine decarboxylase activity than did DR112 (Table 2). Overproduction of *S*-adenosylmethionine decarboxylase in DR112(pSAMDC) was confirmed by Coomassie brilliant blue staining after SDS-polyacrylamide gel electrophoresis (data not shown). In this strain, the amount of spermidine increased by 40% but that of putrescine decreased by 35% in comparison with DR112 (Table 1). No significant amount of putrescine or spermidine was excreted from the cells (Table 3). The results suggest that the increased amount of spermidine may inhibit the activity of *S*-adenosylmethionine decarboxylase.

The activity of *S*-adenosylmethionine decarboxylase was inhibited slightly by putrescine and strongly by spermidine (Fig. 1). The enzymatic activity was inhibited almost completely by 0.75 mM spermidine, which corresponded to 2.25 nmol/mg of protein. In addition, restoration of the activity of *S*-adenosylmethionine decarboxylase by ribosomes in the presence of spermidine was observed (data not shown). This supports our contention that excessive amounts of spermidine, probably free spermidine, inhibit the activity of *S*-adenosylmethionine decarboxylase.

TABLE 4. Amino acid contents of *E. coli* producing normal and excessive amounts of ornithine decarboxylase or *S*-adenosylmethionine decarboxylase

Strain	Amino acid added (mg/ml)	Amino acid (mM)[a]					
		Orn	Arg	Lys	Glu	Pro	Met
DR112		0.065	0.31	6.97	2.87	0.27	0.035
	Orn (0.1)	0.095	0.28	8.52	2.19	0.25	0.037
	Arg (1.0)	0.004	0.42	6.80	2.98	0.31	0.044
DR112(pODC)		<0.001	0.28	8.13	4.32	0.33	0.028
	Orn (0.1)	0.006	0.27	7.35	3.20	0.30	0.043
	Arg (1.0)	<0.001	0.43	6.83	2.00	0.32	0.043
DR112(pSAMDC)		0.069	0.32	7.82	2.54	0.29	0.027

[a] Each value is the average of duplicate determinations.

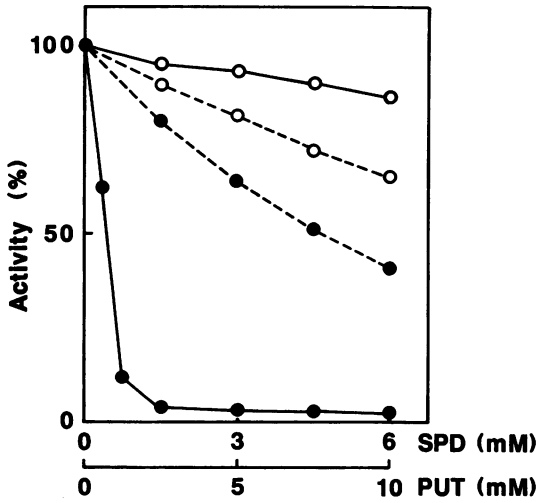

FIG. 1. Effect of polyamines on the activities of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase. The sonicated extract from *E. coli* DR112 was used as the enzyme source. Ornithine decarboxylase (- - -; 100% = 70.4 nmol/min per mg of protein) and *S*-adenosylmethionine decarboxylase (——; 100% = 1.28 nmol/min per mg of protein) were assayed in the presence of various concentrations of putrescine (○) and spermidine (●) added to the reaction mixture. The same results were obtained with purified enzymes.

The amounts of putrescine and ornithine decarboxylase activity in DR112(pSAMDC) were 35 and 30% less, respectively, than those in DR112 (Tables 1 and 2). No significant amount of putrescine was excreted from DR112(pSAMDC) as mentioned above (Table 3). In addition, the in vitro activity of ornithine decarboxylase was inhibited significantly by spermidine (Fig. 1). These results suggest that both synthesis and activity of ornithine decarboxylase may be inhibited by excessive amounts of spermidine.

Analysis of factors which influence polyamine levels in *E. coli*. The concentrations of ornithine and some other amino acids in *E. coli* were measured (Table 4). Although the amount of ornithine in cells increased slightly by the addition of ornithine, enough ornithine to synthesize putrescine seems to exist under normal conditions. However, cells became ornithine deficient when ornithine decarboxylase was overproduced. The amounts of other amino acids which are concerned with ornithine and polyamine metabolism did not change significantly by the addition of either ornithine or arginine to the medium.

We examined whether DR112(pODC) produced more S20/L26 and L34 proteins than did DR112 by use of two-dimensional electrophoresis. The pattern of ribosomal proteins produced in DR112(pODC) was almost the same as that produced in DR112. No antizyme activity was observed in the supernatant and ribosome fractions (data not shown). The results confirmed our previous results (11) that basic antizymes do not function as inhibitors of ornithine decarboxylase.

It is critical to measure the amount of acetyl polyamines for correct interpretation of the results with the overproducing mutants. When polyamine contents in DR112, DR112(pODC), and DR112(pSAMDC) were measured after hydrolysis with 6 N HCl at 110°C for 20 h, the amounts of polyamines were almost the same as the amounts without hydrolysis. This suggests that no significant amount of acetyl polyamines was synthesized in these cells.

DISCUSSION

By using *E. coli* producing excessive amounts of ornithine decarboxylase or *S*-adenosylmethionine decarboxylase, we studied how polyamine contents in *E. coli* are regulated. The following results have been obtained. (i) Putrescine content in cells was maintained by the excretion of excessive amounts of putrescine to the medium. (ii) When ornithine was deficient in cells, the synthesis of ornithine decarboxylase was stimulated. Thus, the efficiency of utilization of ornithine for putrescine synthesis increased. (iii) Spermidine could not be excreted from cells. Spermidine content in cells was maintained through spermidine inhibition of *S*-adenosylmethionine decarboxylase activity. Although the enzyme activity produced in DR112(pSAMDC) was 7 times more than in DR112, DR112(pSAMDC) produced only 1.4 times more spermidine. (iv) Spermidine also inhibited both the synthesis and the activity of ornithine decarboxylase. Inhibition of *S*-adenosylmethionine decarboxylase by spermidine and weak inhibition of ornithine decarboxylase by spermidine were confirmed by in vitro experiments (Fig. 1).

The following findings also suggest that the synthesis of *S*-adenosylmethionine decarboxylase may be regulated by spermidine. (i) The plasmid content of DR112(pSAMDC) was nearly equal to that of DR112(pODC) (data not shown). (ii) Although the amount of ornithine decarboxylase activity produced in DR112(pODC) was 70 times more than that in DR112, DR112(pSAMDC) produced only 7 times more *S*-adenosylmethionine decarboxylase activity. The differences in the two enzyme activities may be associated with a regulatory effect of spermidine at the translational level rather than with their respective promoter strengths.

It is of interest that the amounts of putrescine and spermidine in *E. coli* during the late logarithmic phase of growth were lower than those in *E. coli* during the early logarithmic phase of growth (Table 1). The decrease of *S*-adenosylmethionine decarboxylase paralleled closely the decrease of polyamines in cells with the progress of cell growth (Table 2). This may be explained as follows. Ribosomes are thought to be a major binding site of polyamines. The ribosome content during the late logarithmic phase of growth is much lower than that in the early logarithmic phase of growth (8). The amount of free spermidine may increase as a result of the decrease of ribosomes. The free spermidine inhibits both the synthesis and the activity of *S*-adenosylmethionine decarboxylase and ornithine decarboxylase. Thus, the amounts of polyamines in cells decrease with the progress of cell growth.

We propose that the production of polyamines in *E. coli* is regulated by the *S*-adenosylmethionine decarboxylase activity rather than by ornithine decarboxylase for the following reasons. (i) Putrescine is normally synthesized in excess amounts, and the excess amount of putrescine is excreted from cells. (ii) Antizymes of ODC do not function under our experimental conditions. (iii) The *S*-adenosylmethionine decarboxylase activity paralleled the polyamine contents in cells with the progress of cell growth. (iv) The activity of *S*-adenosylmethionine decarboxylase was more strongly inhibited by spermidine than was that of ornithine decarboxylase. Since spermidine usually is bound to nucleic acids and phospholipids and essentially no free spermidine exists in cells (12), it is rational to conclude that polyamine biosynthesis is inhibited by free spermidine when excess amounts of spermidine are synthesized.

We are very much interested in the mechanism by which putrescine is excreted from *E. coli* cells. It has been reported

that putrescine uptake is dependent on proton motive force (12). It is thus difficult to reconcile that the excretion of putrescine is catalyzed by the same carrier protein. Recently, we have isolated a mutant which lacks a carrier protein for putrescine uptake (unpublished results). In the mutant, transformed with a plasmid containing the ornithine decarboxylase gene, excretion of putrescine has been observed. By using this mutant, we are now studying the characteristics of a carrier protein for putrescine excretion.

ACKNOWLEDGMENTS

We thank F. Ikegami for his kind help in the operation of the amino acid analyzer and S. M. Boyle and C. W. Tabor for the plasmids containing the ornithine decarboxylase gene and the *S*-adenosylmethionine decarboxylase gene, respectively. Thanks are also due to B. K. Joyce for her help in preparing the manuscript.

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan.

LITERATURE CITED

1. **Applebaum, D. M., J. C. Dunlap, and D. R. Morris.** 1977. Comparison of the biosynthetic and biodegradative ornithine decarboxylase of *Escherichia coli*. Biochemistry **16**:1580–1584.
2. **Bakker, E. P., and W. E. Mangerich.** 1981. Interconversion of components of the bacterial proton motive force by electrogenic potassium transport. J. Bacteriol. **147**:820–826.
3. **Boyle, S. M., G. D. Markham, E. W. Hafner, J. M. Wright, H. Tabor, and C. W. Tabor.** 1984. Expression of the cloned genes encoding the putrescine biosynthetic enzymes and methionine adenosyltransferase of *Escherichia coli* (*speA*, *speB*, *speC*, and *MetK*). Gene **30**:129–136.
4. **Fujii, S., and Y. Hitomi.** 1981. New synthetic inhibitors of C1r, C1 esterase, thrombin, plasmin, kallikrein and trypsin. Biochim. Biophys. Acta **661**:342–345.
5. **Hawkes, R., E. Niday, and J. Gordon.** 1982. A dot-immunobinding assay for monoclonal and other antibodies. Anal. Biochem. **119**:142–147.
6. **Hölttä, E., J. Jänne, and J. Pispa.** 1972. Ornithine decarboxylase from *Escherichia coli*: stimulation of the enzyme activity by nucleotides. Biochem. Biophys. Res. Commun. **47**:1165–1171.
7. **Hölttä, E., J. Jänne, and J. Pispa.** 1974. The regulation of polyamine synthesis during the stringent control in *Escherichia coli*. Biochem. Biophys. Res. Commun. **59**:1104–1111.
8. **Igarashi, K., K. Hara, Y. Watanabe, S. Hirose, and Y. Takeda.** 1975. Polyamine and magnesium contents and polypeptide synthesis as a function of cell growth. Biochem. Biophys. Res. Commun. **64**:897–904.
9. **Igarashi, K., K. Kashiwagi, H. Hamasaki, A. Miura, T. Kakegawa, S. Hirose, and S. Matsuzaki.** 1986. Formation of a compensatory polyamine by *Escherichia coli* polyamine-requiring mutants during growth in the absence of polyamines. J. Bacteriol. **166**:128–134.
10. **Igarashi, K., K. Terada, Y. Tango, K. Katakura, and S. Hirose.** 1975. Demonstration by affinity chromatography of the cell-free synthesis of ribonuclease-specific immunoglobulin. J. Biochem. **77**:383–390.
11. **Kashiwagi, K., and K. Igarashi.** 1987. Nonspecific inhibition of *Escherichia coli* ornithine decarboxylase by various ribosomal proteins: detection of a new ribosomal protein possessing strong antizyme activity. Biochim. Biophys. Acta **911**:180–190.
12. **Kashiwagi, K., H. Kobayashi, and K. Igarashi.** 1986. Apparently unidirectional polyamine transport by proton motive force in polyamine-deficient *Escherichia coli*. J. Bacteriol. **165**:972–977.
13. **Kyriakidis, D. A., J. S. Heller, and E. S. Canellakis.** 1978. Modulation of ornithine decarboxylase activity in *Escherichia coli* by positive and negative effectors. Proc. Natl. Acad. Sci. USA **75**:4699–4703.
14. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
15. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
16. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **Markham, G. D., C. W. Tabor, and H. Tabor.** 1983. *S*-Adenosylmethionine decarboxylase (*Escherichia coli*). Methods Enzymol. **94**:228–230.
18. **Panagiotidis, C. A., and E. S. Canellakis.** 1984. Comparison of the basic *Escherichia coli* antizyme 1 and antizyme 2 with the ribosomal proteins S20/L26 and L34. J. Biol. Chem. **259**:15025–15027.
19. **Sakai, T. T., and S. S. Cohen.** 1976. Regulation of ornithine decarboxylase activity by guanine nucleotides. Proc. Natl. Acad. Sci. USA **73**:3502–3505.
20. **Tabor, C. W., and H. Tabor.** 1984. Polyamines. Annu. Rev. Biochem. **53**:749–790.
21. **Tabor, C. W., and H. Tabor.** 1985. Polyamines in microorganisms. Microbiol. Rev. **49**:81–99.
22. **Tabor, C. W., H. Tabor, E. W. Hafner, G. D. Markham, and S. M. Boyle.** 1983. Cloning of the *Escherichia coli* genes for the biosynthetic enzymes for polyamines. Methods Enzymol. **94**: 117–121.
23. **Tabor, H., and C. W. Tabor.** 1969. Formation of 1,4-diaminobutane and of spermidine by an ornithine auxotroph of *Escherichia coli* grown on limiting ornithine or arginine. J. Biol. Chem. **244**:2286–2292.
24. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.