THE REGULATION OF POLYAMINE SYNTHESIS DURING THE STRINGENT CONTROL IN ESCHERICHIA COLI

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Summary: The activity of ornithine decarboxylase (L-ornithine carboxy--lyase EC 4.1.1.17) and the accumulation of putrescine and spermidine were studied in Escherichia coli CP 78 (rel^+) and CP 79 (rel^-) strains during normal growth and amino acid starvation. Amino acid starvation decreased the activity of ornithine decarboxylase in crude lysates from the stringent strain. Similarly, the accumulation of polyamines was ceased by the deprival of the amino acids in the stringent strain but not in the relaxed one. Partially purified ornithine decarboxylase from Escherichia coli CP 78 was inhibited by guanosine 5'diphosphate, 3'diphosphate in a competitive manner with respect to guanosine triphosphate, the activator of the enzyme. These results suggest that the synthesis of polyamines in Escherichia coli CP 78 is regulated, at least partly by guanosine 5'diphosphate, 3'diphosphate.

Amino acid starvation of the stringent strains of Escherichia coli (rel^+) results, as shown by Cashel et al., in the accumulation of two unusual nucleotides, guanosine 5'diphosphate, 3'diphosphate and guanosine 5'-triphosphate, 3'diphosphate (1,2,3). These nucleotides have been suggested to be responsible for the cessation of the synthesis of stable ribonucleic acids during the stringent control (4,5). Recently, Travers has suggested that the synthesis of ribosomal RNA is regulated by ppGpp and TuTs elongation factor (6). There are also some indications in vitro that these nucleotides may control the synthesis and transport of purine nucleotides (7,8), the synthesis of proteins (8) and of phospholipids (10).

We found recently that the formation of putrescine by L-ornithine decarboxylase (EC 4.1.1.17) in E.coli appears to be controlled by nucleotides GTP being an especially effective activator for the enzyme (11). Because of the earlier reports that stringent response in E.coli brings about profound changes in the concentrations of guanine nucleotides (1) and, in fact, also in the synthesis and accumulation of polyamines during

Abbreviations: guanosine 5'diphosphate, 3'diphosphate, ppGpp; guanosine 5'triphosphate, 3'diphosphate, pppGpp.

amino acid starvation (12), we decided to study the synthesis of polyamines in isogenic strains of E. coli CP 78 (rel^+) and CP 79 (rel^-). The specific inhibition of ornithine decarboxylase by ppGpp suggested that the changes in the accumulation of putrescine and spermidine are partly due to the appearance of guanosine polyphosphate(s).

MATERIAL AND METHODS

Escherichia coli CP 78 (rel^+) and CP 79 (rel^-) requiring threonine, leucine, arginine, histidine and thiamine were kindly provided by Dr. Elsebet Lund. They were grown in the synthetic medium supplemented with the requirements mentioned above as described by Lund and Kjeldgaard (13). Guanosine 5'diphosphate, 3'diphosphate was a generous gift from Dr. Michael Cashel. Guanosine 5', 3'diphosphate, guanosine 5', 2'diphosphate and cyclic guanosine monophosphate were purchased from Boehringer Mannheim. Ornithine decarboxylase was partially purified from Escherichia coli CP 78 and CP 79 and assayed as described by Hölttä et al. (11). The cells and culture media were analyzed for the polyamines as described by Raina et al. (12).

RESULTS AND DISCUSSION

As seen in Table 1 ornithine decarboxylase activity in crude lysates from starved E. coli CP 78 was much lower than in lysates from normal CP 78 cells. In lysates of relaxed strain of E. coli CP 79 the amino acid starvation actually did slightly increase the activity of ornithine decarboxylase. The withdrawal of amino acids did not cause any decrease in the activities of ornithine decarboxylase when assayed in the presence of 1 mM GTP. Although the enzyme activities in crude lysates do not necessarily reflect the situation in vivo, the data suggest that the synthesis of polyamines might be affected through ornithine decarboxylase during stringent control of E. coli CP 78.

Raina et al. have shown that during prolonged amino acid starvation of the stringent strain of E. coli 15 TAU the synthesis of polyamines, especially that of spermidine is ceased (12). Because the response to the amino acid starvation in stringent cells appears to be very rapid, at least as far as the accumulation of ppGpp and the cessation of RNA synthesis are concerned (1, 13), we determined the accumulation of putrescine and spermidine in the cells and culture media during the early period of starvation. As one can see in Fig. 1 amino acid starvation of stringent strain of E. coli CP 78 abolished the accumulation of putrescine and spermidine. In the relaxed strain of E. coli CP 79 there were practically no changes in the accumulation of polyamines after the deprival of amino acids. The

Table I. Activity of ornithine decarboxylase of E.coli CP 78 and CP 79 during normal growth and amino acid starvation

	time (min.)	Enzyme activity (nmoles CO_2 released per mg of protein per 30 min.) minus GTP	plus GTP
CP 78 (rel^+) (complete medium)	0	178	303
	5	198	318
	10	177	361
	20	172	410
(amino acid starvation)	0	118	338
	5	68	296
	10	64	325
	20	44	342
CP 79 (rel^-) (complete medium)	0	115	250
	5	120	246
	10	123	280
	20	136	365
(amino acid starvation)	0	226	298
	5	167	282
	10	155	271
	20	178	302

E.coli CP 78 rel^+ and E.coli CP 79 rel^- were grown exponentially at 37°C to a cell density of 5×10^8 cells ml^{-1}. The cells were collected by centrifugation and resuspended into a complete medium or a medium lacking amino acids and reincubated at 37°C with constant aeration. 20 ml of each culture were removed at various times, chilled by ice and centrifuged (the point zero actually represents about ten seconds, which time is needed to resuspend the cells into warm medium and remove the sample). The cell pellets were washed and resuspended into 1 ml of 10 mM Tris-HCl buffer pH 7.4 containing 10 mM EDTA, 1 mM DTT and 20% glycerol. The cells were desintegrated by sonication, centrifuged and the activity of ornithine decarboxylase of lysates were assayed in the absence or presence of 1 mM GTP as described by Hölttä et al. (11).

media of both cultures were also analyzed for polyamines. Five to 15% of total amounts of putrescine and spermidine were found in culture media. The largest leakage was found in E.coli CP 79 grown in complete medium.

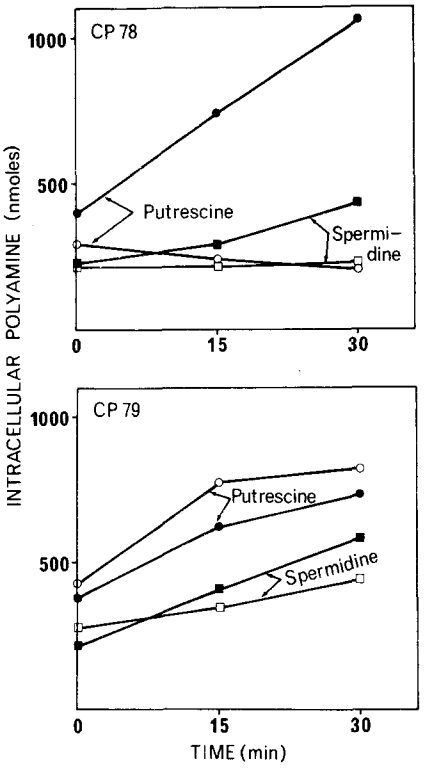

Fig. 1. Intracellular polyamines of E. coli CP 78 and CP 79 during normal growth and amino acid starvation.

The cultures were grown as described in Table I into cell density of 5×10^8 cell ml^{-1}. Each culture was divided into two parts, centrifuged and resuspended into complete medium or into starvation medium lacking amino acids except of L-arginine and L-glutamic acid which were supplemented at original concentration (20 μg/ml). The cultures were incubated at 37°C with constant aeration. Aliquots of 100 ml of culture were taken at various times, chilled by 50 g of crushed ice and centrifuged. The pellets and media were analyzed for polyamines as described by Raina et al. (12). Symbols:

closed circles:	putrescine during normal growth,
open circles:	putrescine during starvation,
closed squares:	spermidine during normal growth,
open squares:	spermidine during starvation.

We have earlier shown that ornithine decarboxylase from E. coli is strongly activated by GTP (11). As already seen in Table I, the activity of ornithine decarboxylase in crude lysates of E. coli CP78 was inhibited during the early period of amino acid starvation. This might be due to the depletion of GTP. However, it is known that the concentration of GTP decreases during amino acid starvation only from 4 mM to 2 mM (personal communication by M. Cashel), which based upon our data

in vitro of the high affinity of ornithine decarboxylase for GTP, hardly explains the observed changes. As another possibility one might think that ppGpp may compete as a structural analog with GTP for ornithine decarboxylase. To explore this possibility ornithine decarboxylase was partially purified from cells of *E. coli* CP 78 through the procedure described earlier (11). The partially purified enzyme was assayed in the presence of 1 mM GTP and of various concentrations of other guanine nucleotides. As it can be seen in Table II the only nucleotide inhibiting the activity of ornithine decarboxylase was ppGpp. The close analogs, guanosine 5', 3' diphosphate, guanosine 5', 2' diphosphate and cyclic guanosine monophosphate did not inhibit the enzyme activity.

It is shown in Fig. 2 the effect of ppGpp on the activity of ornithine decarboxylase of *E. coli* CP 78 when assayed in the presence of 1 mM GTP. Based on these results one can calculate K_i for ppGpp to be 0.5 mM. Similar inhibition by ppGpp was observed when partially purified ornithine decarboxylase from *E. coli* CP 79 was used in the assay. The type of the inhibition by ppGpp in respect to GTP was found to be purely competitive. It is known that the concentrations of GTP and ppGpp change during amino acid starvation of the stringent strain from 4 mM and 0.05 - 0.1 mM to 2 mM and 2-4 mM, respectively (personal communication by Michael Cashel). Consequently, it can be said that the concentrations of nucleotides used in these experiments are in the physiological range.

The regulation of metabolic pathway by modulators is often reflected in changes of the affinity for the substrate of the regulatory enzyme. As shown in Fig. 3 the K_m value for ornithine, the substrate of the first enzyme in polyamine pathway, was 0.36 mM when assayed in the presence of 1 mM GTP. When 0.5 mM ppGpp was also present, K_m of ornithine increased up to 3.5 mM approaching the K_m in the absence of GTP.

When *E. coli* is grown in the absence of exogenous amino acids, putrescine is synthesized from ornithine by a straight decarboxylation(14). It has also been reported that, when bacteria is grown in the presence of arginine, a substantial portion of putrescine is derived from arginine through the formation of agmatine and a subsequent hydrolysis of agmatine to putrescine (15,16). *E. coli* CP 78 and CP 79 require arginine for their growth. It can be seen in Fig. 1, however, that there was no accumulation of putrescine during amino acid starvation although arginine was present in the culture medium. The reason might be that *E. coli* CP 78 and CP 79 actually do not use arginine in used concentration as a source for putres-

Table II The effect of various guanine nucleotides on the activity of partially purified ornithine decarboxylase of E. coli CP 78.

	Addition	Activity of ornithine decarboxylase
1.	—	34.1
2.	0.1 mM ppGpp	29.0
3.	0.5 mM "	17.4
4.	2.0 mM "	8.5
5.	0.5 mM guanosine 5', 2' diphosphate	42.5
6.	3.0 mM - " -	39.0
7.	0.5 mM guanosine 5', 3' diphosphate	40.7
8.	3.0 mM - " -	40.9
9.	0.5 mM cyclic guanosine monophosphate	40.6
10.	3.0 mM - " -	38.0

The partially purified enzyme (0.11 mg of protein) was assayed as described earlier by Hölttä et al. (11) in the presence of 1 mM GTP. The activities are expressed as nmoles of CO_2 released during 30 minutes of incubation.

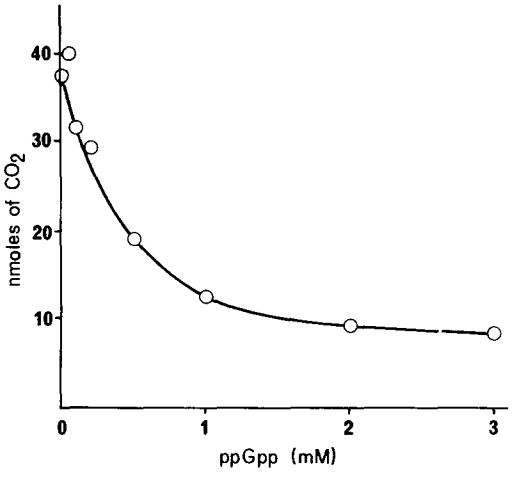

Fig. 2. The effect of guanosine 5' diphosphate, 3' diphosphate on the activity of partially purified ornithine decarboxylase from E. coli CP 78.

The enzyme was purified and assayed as described by Hölttä et al. (11) in the presence of 1 mM GTP. The activities are expressed as nmoles of CO_2 released during 30 minutes of incubation.

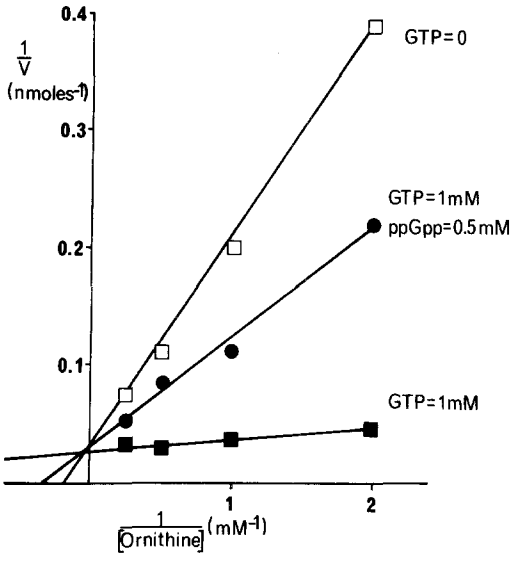

Fig. 3. The effect of L-ornithine on ornithine decarboxylase activity in the absence or presence of guanine nucleotides.

The enzyme was assayed as described by Hölttä et al. (11) in the absence or presence of GTP and ppGpp. The double reciprocal lines were plotted by the least squares method.

cine. Another possibility would be that the formation of putrescine from arginine is also inhibited during the stringent control. In order to determine whether arginine decarboxylase is also inhibited by ppGpp this enzyme was partially purified from E.coli CP 78 and assayed in the presence of various nucleotides (17). It was found out (the results not shown) that not only ppGpp but also all nucleotide triphosphates, in the concentrations of 2 mM, inhibit this enzyme when assayed in the presence of 4 mM Mg^{++} (required for the maximal activity). However, when the concentration of Mg^{++} was increased to 10 mM, the inhibition by nucleotides disappeared. The question, whether this observation in vitro have any physiological significance, remains unanswered.

In conclusion, it can be said that during "stringent control" there is a cessation of polyamine synthesis. The molecular basis for that is, at least partly, a marked inhibition of ornithine decarboxylase activity by ppGpp.

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