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GTP-INSENSITIVE ORNITHINE DECARBOXYLASE IN ACETOBACTERIA ABLE TO SYNTHESIZE SPERMINE

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Several Acetobacteria contained large amounts of spermine in addition to the putrescine and spermidine, which are the polyamines normally found in prokaryotes. A spermine synthase present in cell extracts of these Acetobacteria is the first example of this enzyme in prokaryotes. Dicyclohexylammonium sulphate inhibited both spermidine synthase and spermine synthase activities in Acetobacteria. Their ornithine decarboxylase was not stimulated by GTP nor inhibited by ppGpp and pppGpp (magic spots I and II) in contrast to ornithine decarboxylase of nearly all bacteria studied so far. However, their S-adenosyl-L-methionine decarboxylase resembled other prokaryotic adenosylmethionine decarboxylases in requiring Mg^{2+} ions in vitro for full activity.

The general rule for the distribution of polyamines between prokaryotes and eukaryotes has been that putrescine and spermidine are the only polyamines found in in mesophilic bacteria whereas higher eukaryotes always contain putrescine, spermidine and spermine (1-4).

In prokaryotes putrescine is mainly formed via decarboxylation of ornithine (1-3). This decarboxylation is accomplished by a specific ornithine decarboxylase (ODC). In bacteria that do not contain spermine the activity of ODC is controlled by GTP and its analogues (4-8). On the other hand in higher eukaryotes, which contain spermine, ODC does not need similar low molecular weight effectors (2,9).

The stimulation of adenosylmethionine decarboxylase (ADC) is also different in prokaryotes and eukaryotes. Mg^{2+} ion is needed for full activity all prokaryotes whereas the appearance of spermine in an organism is accompanied by ADC activity dependent on putrescine (2,3). In this paper we report for the first time the occurrence in bacteria of the enzymes needed to synthesize spermine, and show that the control of ODC activity by GTP and its analogues is lost in these spermine-synthesizing bacteria.

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MATERIALS AND METHODS

Chemicals: S-Adenosyl-L-[carboxyl-(1-^{14}C)]methionine (60 mCi/mmol), S-Adenosyl-L-(methyl ^{14}C)methionine (62 mCi/mmol) and L-(1-^{14}C)ornithine (56 mCi/mmol) were purchased from Amersham (Bucks., U.K.). Unlabelled S-adenosyl-L-methionine, putrescine, spermidine and spermine (as their hydrochlorides) were obtained from Sigma Co. (St. Louis, MO., U.S.A.). Unlabelled and (^{14}C)methyl-labelled decarboxylated S-adenosyl-L-methionine (5'-deoxyadenosyl-(5'), 3-aminopropyl-(1), methylsulphonium salt were prepared by decarboxylation with bacterial ADC (10). GTP, ppGpp and pppGpp (magic spots I and II) (11) were from PL Biochemicals (Milwaukee, WI., U.S.A.) and the compounds were stored and used as in (7). Dicyclohexylammonium sulphate was from Sigma Chemical Co.

Bacteria and growth conditions: Acetobacter aceti ATCC 12876 and A. pasteurianus ATCC 7839 and SG-4 were used. SG-4 is a single-cell isolate from submerged spirit vinegar fermentation plant in Rajamäki, Finland (12). The ATCC-strains were maintained on ATCC medium No. 1; A. pasteurianus SG-4 was isolated and maintained on HL-2 agar (13). The cultivation of SG-4 on solid medium was carried out in a water and ethanol saturated atmosphere at 30°C. Single cell isolation of A. pasteurianus SG-4 was done as described by Johnstone (14). For enzyme assays and polyamine measurements, the ATCC-strains were cultivated in ATCC medium No. 1 without agar in Kluyver-flasks to the stationary phase. Temperature was 26°C and aeration 0.67 vvm. The single-cell isolate was grown in HL-2 broth in a 3 litre fermentor (LKB 1601) in a semi-continuous fashion (1/3 of the medium was discharged and the same amount of fresh medium was added at 7 days intervals) under the following standard conditions: temperature, 30°C; aeration 0.67 vvm; stirring speed, 900 rpm. Total concentration of acetic acid (%, wt/v) and ethanol (%, v/v) of the medium varied 8.0-9.0 %. The yield of the bacteria at the end of growth was 2 grams/l (wet weight). Bacillus subtilis (IH 6064, a derivative of strain 1A 289) was cultivated in Penassay broth (Difco). Escherichia coli (ATCC 11303) was obtained as lyophilized powder from Sigma Chemical Co.

Preparation of cell extracts: Acetobacteria and B. subtilis were disintegrated ultrasonically and acetone-treated cells of E. coli were extracted as in (10). The homogenates were centrifuged at 100000g_{max} and dialyzed overnight against 1000 volumes of 25 mM Tris-HCl buffer (pH 7.5) containing 0.1 mM EDTA and 5 mM dithiothreitol and the dialyzed supernatant fractions were used as the source of the enzymes.

Analytical methods: The activity of ODC was measured (at pH 7.5) as in (15) and that of ADC as in (16). Spermidine and spermine synthase was assayed as the production of (^{14}C)methylthioadenosine from decarboxylated (methyl-^{14}C) adenosylmethionine in the presence of putrescine and spermidine, respectively, as aminopropyl acceptors (17). Polyamines were extracted with ice cold 10 % (w/v) trichloroacetic acid and quantitatively analyzed by paper electrophoresis according to Raina and Cohen (18). The presence of spermine was confirmed by gas chromatography-mass spectrometry: The N-trifluoroacetyl derivatives of polyamines were prepared and the mass spectra were measured as described in (19). Protein was determined by the method of Bradford using bovine serum albumin as standard (20).

RESULTS AND DISCUSSION

Direct analysis by paper electrophoresis demonstrated the presence of putrescine, spermidine and spermine in homogenates of Acetobacteria (Table 1). The concentration of spermine did not change when the samples were hydrolyzed in 6 M HCl for 12 h at 105°C. Gas chromatography-mass spectrometry of the material analyzed as spermine from the Acetobacteria (A. pasteurianus, SG-4) confirmed its identity with authentic spermine (results not shown).

Table 1
The concentrations of putrescine, spermidine, and spermine in various bacteria.

Bacteria	Polyamine concentration (nmoles/g wet weight) Putrescine	Spermidine	Spermine
A. aceti	153	1200	1800
A. pasteurianus	90	670	1310
SG-4 (A. pasteurianus)	35	1110	1720
E. coli	1500	1110	< 5[1]
B. subtilis	750	635	< 5[1]

[1]a spot was seen, but the concentration was too low to permit quantitative analyses

To analyze the biosynthetic pathway of polyamines in Acetobacteria the activities of ODC, ADC, spermidine synthase and spermine synthase were measured in the dialyzed supernatant fractions. Table 2 shows that the Acetobacteria had an ODC-activity that was not stimulated by GTP nor inhibited by ppGpp or pppGpp (potent inhibitors of bacterial ODC; 5,7). In this connection it is of great interest that Guirard and Snell (21) have purified to homogeneity from spermine-containing Lactobacillus sp. 30a(22) an inducible ODC that did not need GTP or other NTPs for full activity, thus supporting the idea that the control of ODC is changed in bacteria containing spermine. Table 2 also shows that E. coli (a gram negative organism) and B. subtilis (a gram positive organism), which do not contain spermine (2,3, Table 1), had a GTP-stimulated and Magic spot-inhibited ODC.

We next analyzed ADC activity from Acetobacteria and noted that the enzyme had a stringent requirement for Mg^{2+} ions and was totally insensitive to putrescine thus resembling ADCs from other bacteria studied so far (results not shown).

As shown in Table 3, all bacteria analyzed had a spermidine synthase activity, but Acetobacteria only also had a high spermine synthase activity. This finding strongly suggests that the spermine found in these bacteria is

Table 2

Effect of GTP and magic spot I (ppGpp) and Magic spot II (pppGpp) on ODC activity from various bacteria. The enzyme activity was assayed in the presence of 1 mM GTP, 1 mM GTP plus 1 mM ppGpp, or 1 mM GTP plus pppGpp using dialyzed supernatant fractions as the source of the enzyme. The enzyme activity are expressed as nmoles of CO_2 formed per mg of protein per 30 min. The percentage change (Δ %) from the control activity with no addition is shown in parentheses. The values are a mean of three assays.

Bacteria	ODC activity						
	No additions	GTP	(Δ %)	ppGpp	(Δ %)	ppGpp	(Δ %)
A. aceti	0.86	0.84	(-2)	0.84	(-2)	0.88	(+5)
A. pasteurianus	2.30	2.31	(0)	2.31	(0)	2.38	(+3)
SG-4 (A. pasteurianus)	4.14	4.12	(0)	4.20	(+1)	4.09	(-1)
E. coli	2.34	10.80	(+360)	0.24	(-90)	0.11	(-95)
B. subtilis	0.86	3.20	(+270)	0.11	(-87)	0.07	(-92)

actively synthesized by them and not merely taken up from the medium. Table 4 shows that 2 mM dicyclohexylammonium sulphate, a competetive inhibitor of mammalian spermidine synthase (23), inhibited powerfully bacterial spermidine synthase and also spermine synthase from Acetobacteria. This is in contrast to mammalian spermine synthase which was not inhibited by the drug (23).

Our results show that Acetobacteria can synthesize spermine, although this reaction was earlier thought to occur only in higher eukaryotes (3,24,25). More studies are needed to clarify the reason(s) for the appearance of spermine synthase in these bacteria. One reason could be that the acid growth medium of the bacteria requires a longer polyamine chain to stabilize

Table 3

The activity of spermidine and spermine synthase in extracts of various bacteria. The enzyme activities are expressed as mmoles of methylthioadenosine formed per mg. protein per 30 min. Other details as in Table 2.

Bacteria	Spermidine synthase	Spermine synthase
A. aceti	1.15	1.40
A. pasteurianus	0.92	1.10
SG-4 (A. pasteurianus)	0.80	1.00
E. coli	1.00	< 0.001
B. subtilis	0.35	< 0.001

Table 4

The effect of dicyclohecylammonium sulphate on the activity of spermidine synthase and spermine synthase from various bacteria. Synthase activities are expressed as in Table 3. The concentration of dicyclohexylammonium sulphate (dicy) was 2 mM. Other details as in Table 2.

Bacteria	Spermidine synthase		Spermine synthase	
	No dicy	+ dicy	No dicy	+ dicy
A. aceti	1.00	0.04	1.28	0.05
A. pasteurianus	1.05	0.04	1.15	0.05
SG-4 (A. pasterianus)	0.80	0.03	0.95	0.05
E. coli	1.00	0.10	< 0.001	< 0.001
B. subtilis	0.40	0.04	< 0.001	< 0.001

structures such as membranes or nucleic acids. More studies are also needed to decide whether there is only one enzyme in Acetobacteria synthesizing spermidine and spermine (which is possible since dicyclohexylammonium sulphate inhibited both activities) or two, specific enzymes. Finally, it was interesting to see that the presence of spermine synthase in bacteria is accompanied by a GTP-independent ODC, as in higher eukaryotes.

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