OCCURRENCE OF *sym*-HOMOSPERMIDINE IN EXTREMELY THERMOPHILIC BACTERIA

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Summary: Triamines produced by an extreme thermophile, *Thermus thermophilus*, were isolated and their chemical structures were determined. It was found that two novel triamines, norspermidine (1,7-diamino-4-azaheptane, $NH_2(CH_2)_3 \cdot NH(CH_2)_3NH_2$) and *sym*-homospermidine (1,9-diamino-5-azanonane, $NH_2(CH_2)_4NH \cdot (CH_2)_4NH_2$) are present in the thermophile cells in addition to spermidine (1,8-diamino-4-azaoctane, $NH_2(CH_2)_3NH(CH_2)_4NH_2$).

INTRODUCTION

An extreme thermophile, *Thermus thermophilus*, produces two new tetra-amines, thermine (1,11-diamino-4,7-diazaundecane, $NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_3 \cdot NH_2$) (1) and thermospermine (1,12-diamino-4,7-diazadodecane, $NH_2(CH_2)_3NH \cdot (CH_2)_3NH(CH_2)_4NH_2$) (2) as major polyamine components. Besides these new tetraamines, the bacterial cells contain three triamines as minor polyamines. In this study, these triamines were extracted and identified to be norspermidine (1,7-diamino-4-azaheptane, $NH_2(CH_2)_3NH(CH_2)_3NH_2$), spermidine (1,8-diamino-4-azaoctane, $NH_2(CH_2)_3NH(CH_2)_4NH_2$), and *sym*-homospermidine (1,9-diamino-5-azanonane, $NH_2(CH_2)_4NH(CH_2)_4NH_2$), respectively. The presence of *sym*-homospermidine in microorganisms has not been reported so far.

MATERIALS AND METHODS

Authentic Polyamines. Authentic spermidine (Sigma Co.) and norspermidine (Tokyo Kasei Kogyo Co.) were commercial products. Their hydrochloride salts were recrystallized followed by drying at 60°C. *sym*-Homospermidine was synthesized according to a literature (3), and crystallized from a hot ethanol solution

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Microbial Growth. *Thermus thermophilus* HB8(=ATCC 27634) was grown in a nutrient broth (4) or a synthetic medium. One liter of the synthetic medium contained 20 g sucrose, 20 g monosodium glutamate, 2 g NaCl, 0.25 g KH_2PO_4, 0.5 g K_2HPO_4, 0.5 g $(NH_4)_2SO_4$, 0.125 g $MgCl_2 \cdot 6H_2O$, 0.025 g $CaCl_2 \cdot 2H_2O$, 6 mg $FeSO_4 \cdot 7H_2O$, 0.8 mg $CoCl_2 \cdot 6H_2O$, 20 μg $NiCl_2 \cdot 6H_2O$, 1.2 mg $NaMoO_2 \cdot 2H_2O$, 0.1 mg $VOSO_4$, 0.5 mg $MnCl_2 \cdot 4H_2O$, 60 μg $ZnSO_4 \cdot 7H_2O$, 15 μg $CuSO_4 \cdot 5H_2O$, 100 μg biotin, and 1 mg thiamine. The final pH was adjusted to 7.0∿7.2 with NaOH.

Polyamine Analysis. Polyamines were analyzed as reported in a previous paper (2). Triamines were colorimetrically determined by the ninhydrin reaction using spermidine as a standard.

Spectral Measurements. Proton-NMR spectra were recorded on Hitachi R-24 NMR spectrometer using trimethylsilylpropanesulfonate as an internal standard. Proton-decoupled ^{14}C-NMR spectra and infrared spectra were recorded on JEOL FX-100 and Hitachi 260-50, respectively.

Isolation of Triamines. The thermophile cells contained three triamines, *c*, *d* and *e*, as shown in Fig. 1.

Polyamine *c* was isolated from the cells at middle-log phase grown in the synthetic medium at 60°C, because polyamine *d* was poor in these cells and polyamine *c* from these cells were easily separated from polyamine *d*. The cells (460 g wet) were suspended in 1 ℓ of 5% trichloroacetic acid. After centrifugation at 6,000 rpm for 20 min, the debris was re-suspended in 1 ℓ of 5% trichloroacetic acid. The supernatants were combined (containing 540 μmoles of polyamine *c*) and applied on a Dowex 50W-X4 ion exchange resin column (H^+ form, 1.6 cm × 22 cm). After the column was washed with 100 ml of water, 100 ml of 0.1 M sodium phosphate buffer containing 0.7 M NaCl (5), and 100 ml of 1 N HCl, the polyamines were then eluted with 6 N HCl. The fractions containing polyamines were combined and dried under reduced pressure. The residue was dissolved in 200 ml of H_2O and applied on a CK-10S (Mitsubishi Chemical Industries, Ltd., Tokyo) cation exchange resin column (H^+ form, 1.8 cm × 25 cm). The column was eluted with 0.28 M sodium acetate buffer, pH 5.0, containing 2.22 M NaCl (final sodium concentration was 2.5 M) at 65°C. The collected fractions (total 141 ml, containing 480 μmoles of polyamine *c*) were diluted with 3 times volume of water, and applied on a Dowex 50W-X4 column (H^+ form, 1.0 cm × 10 cm). After the column was washed with water, polyamine *c* was eluted with 6 N HCl. The collected fractions were evaporated under reduced pressure at 40°C. The residue was dissolved in the minitest amount of water (about 0.1 ml). Hydrochloride salt of polyamine *c* was precipitated from the solution by adding an excess amount of ethanol:methanol mixture (1:1 w/w). The precipitate was washed with cold ethanol:methanol mixture (1:1, -20°C), and dried at 60°C. The yield was 40 mg (150 μmoles) from 460 g wet cells.

Polyamine *d* and *e* were isolated in similar manners from cells grown in the nutrient broth at 60°C.

RESULTS

Identification of Polyamine *c*. Proton-NMR spectrum (D_2O) of the isolated polyamine *c* revealed two multiplets centered at δ 1.8 ppm and 3.1 ppm (ratio 1:1), and was compared to those of spermidine and spermine. It was suggested that butyl group(s) but no propyl group lies between the amino and/or imino groups. ^{13}C-NMR spectrum, (D_2O, TMS) δ 46.5, 38.4, 23.6, 22.4 ppm, indicated

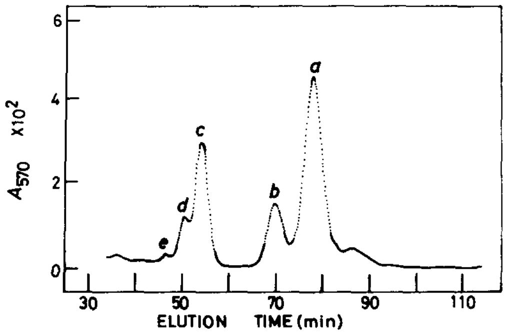

Fig. 1 Polyamine composition of *T. thermophilus*. The cells were grown in a nutrient broth at 60°C. Polyamines were extracted with 5% trichloroacetic acid and an aliquot was analyzed using an automatic amino acid analyzer, JEOL JLC-6AH, with a column of CK-10S ion exchange resin (7 cm long) as reported in a previous paper (2). Polyamine *a* and *b* have been identified to be thermospermine (2) and thermine (1), respectively.

that the molecule consists of four different carbon atoms in terms of the shielding effect of the amino and imino groups. Since the basicity of the compound is similar to that of spermidine (Fig. 1), a structure $NH_2(CH_2)_4 \cdot NH(CH_2)_4NH_2$ can be proposed for polyamine *c* based on these data.

The structure was confirmed by comparing IR spectrum of polyamine *c* with that of the authentic *sym*-homospermidine. IR (KBr disc); 1590, 1480(b), 1440, 1400, 1350, 1280, 1255, 1200, 1175, 1155, 1080, 1055, 1025, 1010(w), 945, 865, 760 cm^{-1}. The IR spectrum was also similar to one reported in a literature (6).

Identification of Polyamine *d* and *e*. The isolated polyamine *d* and *e* were studied in similar manners, and their structures were confirmed to be spermidine and norspermidine, respectively, by comparing IR spectra of these polyamines with those of the authentic samples. IR (KBr disc) for polyamine *d*; 1590, 1490, 1440, 1405, 1355, 1340(w), 1310(w), 1295(w), 1285(w), 1265, 1240, 1150, 1110, 1075(w), 1055, 1030(w), 995, 950, 880, 780, 760 cm^{-1}. IR (KBr disc) for polyamine *e*; 1610(w), 1600, 1570, 1510, 1495, 1480, 1465(w), 1420, 1410(w), 1360, 1340(w), 1315, 1290, 1210, 1190, 1155, 1060, 1030, 1015, 995, 960, 935, 890, 750 cm^{-1}.

DISCUSSION

In the present study, the triamines produced by *T. thermophilus* were identified to be norspermidine, spermidine and *sym*-homospermidine. The triamine contents of the thermophile cell depended on the growth temperature. The cells grown at low temperature such as 60°C contained more triamines, especially *sym*-homospermidine (polyamine *c*) (see Fig. 1), as compared with the cells grown at the optimum temperature of the growth (75°C).

The presence of norspermidine in the living world was firstly reported for a plant virus (7), although a later study did not confirm the observation (8). The presence of this polyamine in thermophilic bacteria was reported by DeRosa et al (9) for *Calderiella acidophila.* The authors found this polyamine in other extreme thermophiles, *T. thermophilus* (including other strains), *T. aquaticus* and *Sulfolobus acidocaldarius*. Norspermidine seems to distribute generally in extreme thermophiles which are capable of growing at 75°C or higher temperatures.

The presence of *sym*-homospermidine has been known so far only in sandal wood (6), newt (10), and green algae (11). Among the extreme thermophiles analyzed, *sym*-homospermidine was found in cells of *T. thermophilus* and *T. aquaticus*, but not in those of *S. acidocaldarius*.

The cells of *T. thermophilus* grown at 75°C contained thermine (= N-aminopropyl norspermidine) and thermospermine (= N^1-aminopropyl spermidine) as major polyamine components (1,2) along with three minor components, norspermidine, spermidine and *sym*-homospermidine. No significant amount of spermine (= N^8-aminopropyl spermidine) was present in the cells (2). Aminopropyl derivative of *sym*-homospermidine was also not detected in the cells. The distribution of polyamines in the cells suggests the presence of an aminopropyltransferase highly specific for a terminal aminopropyl group as the acceptor, which produces thermine from norspermidine, and thermospermine from spermidine, respectively. The enzyme might not be able to act on *sym*-homospermidine which has no aminopropyl terminal. Bio-synthesis of these unusual polyamines in *T. thermophilus* including *sym*-homospermidine is a future subject to be studied (12,13).

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