THERMINE: A NEW POLYAMINE FROM AN EXTREME THERMOPHILE

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SUMMARY: A new polyamine compound was isolated from an extreme thermophile, Thermus thermophilus HB8, and named thermine. Chemical structure of thermine was determined to be 1,11-diamino-4,8-diazaundecane, $NH_2CH_2CH_2CH_2NHCH_2CH_2CH_2NHCH_2CH_2CH_2NH_2$.

In the course of study(1,2) on protein synthesis by cell-free extract from an extreme thermophile, Thermus thermophilus HB8, it was found that the addition of some polyamines was required for in vitro protein synthesis at high temperature. Polyphenylalanine synthesis directed by polyuridylic acid at 65°C did not proceed unless such polyamine as spermine was added to the reaction mixture(2). The effect of spermine could not be replaced by other cationic compounds such as putrescine or magnesium. Somewhat more detailed study revealed that spermine played an essential role in the formation of initial ternary complex between ribosomes, m-RNA and amino acyl-tRNA at high temperature.

To investigate the role of polyamines in vivo, polyamine compounds in the thermophile cells were analyzed and two polyamines were detected as major components; one was identified to be spermine and the other was a new tetraamine, of which presence in living world has not been reported so far. The new polyamine compound named thermine, was determined to be 1,11-diamino-4,8-diazaundecane(=N,N'-bis(3-aminopropyl)propane-

diamine). This paper describes the isolation and identification of the new biological polyamine.

EXPERIMENTAL AND RESULTS

Reagents Commercial 1,11-diamino-4,8-diazaundecane (Eastman Kodak Co.) was dissolved in 0.1 N HCl. After evaporation under reduced pressure at 40°C, the residue was dissolved in the minute quantity of water, followed by addition of an excess amount of methanol : ethanol (1:1) mixture. The resulting precipitate, the authentic 1,11-diamino-4,8-diazaundecane hydrochloride, was dried in an oven at 60°C.

Cation exchange resins, Diaion CK-10 and CK-10S were products of Mitsubishi Chemical Industry Ltd., Japan.

Analytical equipments Automatic amino acid analyzer was model JLC-6AH, JEOL Ltd., Tokyo, Japan. Polyamines were analyzed on a column of CK-10S ion exchange resin (0.8 cm diameter × 7 cm long) equipped with the amino acid analyzer. The column was eluted with 0.4 M acetate buffer, pH 4.9, containing 0.25 M sodium chloride and 5%(v/v) n-propanol at the rate of 0.51 ml/min at 60°C. Amines were analyzed with ninhydrin as used for amino acid analysis.

Nuclear magnetic resonance spectra were recorded at 100 MHz using a JEOL JNM-SP-100 Spectrometer.

Extraction of polyamines The thermophile used was Thermus thermophilus HB8(=ATCC 27634, DSM 579, formerly designated as Flavobacterium thermophilum)(3). The cells were grown at 75°C and harvested at the late log-phase. Polyamines were extracted by suspending 100 g wet cells in 200 ml 5% trichloroacetic acid. The extraction was repeated twice. The combined extracts (roughly 420 ml) were applied on a Dowex 50 × 2 ion exchange

resin column (1.7 cm × 24 cm) chromatography according to Dubin and Rosenthal(4) with a slight modification. The column was eluted with a linear gradient elution of HCl concentration 0 to 4 N, after washed with 30 ml of 0.1 M sodium phosphate buffer, pH 8.0, containing 0.7 M NaCl(5). The major component eluted at 2.6-2.7 N HCl as shown in Fig. 1, was precipitated by adding

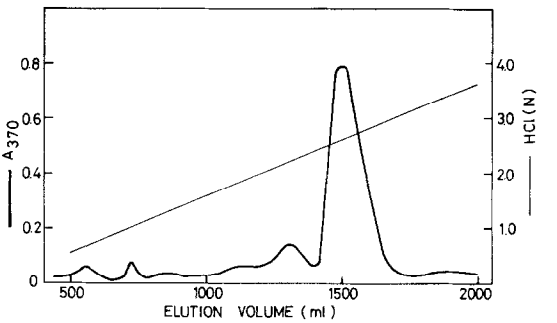

Fig. 1. Elution profile of polyamines from T. thermophilus. Polyamines extracted in 5% trichloroacetic acid were applied on a Dowex 50×2 column according to ref. 4. Polyamines were detected with 2,4-dinitrofluorobenzene(4).

an excess amount of methanol. The resulting precipitate was analyzed by the analytical chromatography described above. The elution profile shown in Fig. 2, indicated that the preparation consisted of two compounds, A and B. Compound B was identified to be spermine by co-chromatography on the analytical column and on a thin layer plate with the authentic spermine hydrochloride.

Isolation of compound A The methanol precipitate containing spermine and compound A was applied on a Diaion CK-10 ion exchange resin column(0.8 cm × 26 cm) and the column was eluted with 0.4 M acetate buffer, pH 4.9, containing 0.25 M NaCl and 3% (v/v) n-propanol at the rate of 22 ml per hour at 60°C. The fractions containing compound A were collected. Compound A was

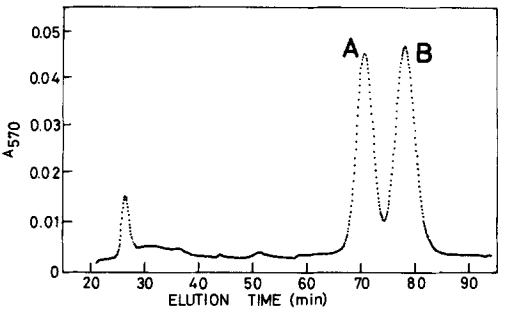

Fig. 2. Analysis of the major polyamine fraction using a Diaion CK-10S column chromatography. An aliquot(contained about 100 nmoles of tetraamine) of the major polyamine fraction obtained by the Dowex 50 chromatography shown in Fig. 1, was applied on an analytical column equipped with an automatic amino acid analyzer. Amines were detected by ninhydrin reaction. Peak B corresponds with that of authentic spermine.

further purified by rechromatography on the CK-10 column, and finally concentrated by adsorption on a Dowex 50 column (0.8 × 7 cm) and elution with 3 N HCl. The polyamine compound was precipitated by adding excess methanol-ethanol mixture (1:1 v/v), washed with methanol, then with ether, and dried at 60°C. Compound B(=spermine) was isolated by a similar manner.

Identification of compound A The NMR spectrum of compound A shown in Fig. 3a can be interpretable that the structure is $NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH_2$, and this consideration was confirmed by comparison of the NMR spectrum with that of the authentic sample (Fig. 3b). Infrared spectrum of compound A closely resembled that of the authentic preparation. Compound A could also be identified to be 1,11-diamino-4,8-diazaundecane by co-chromatography with the authentic sample on the analytical column and on a thin layer plate.

The author proposes to give a trivial name thermine to the new biological polyamine found in T. thermophilus.

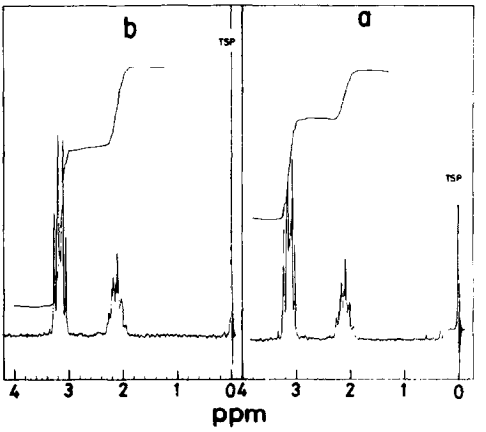

Fig. 3. NMR spectra of thermine (a) and authentic 1,11-diamino-4,8-diazaundecane (b). Samples were dissolved in D_2O and sodium trimethylsilylpropanesulfonate(TSP) was used as a reference.

DISCUSSION

Polyamine compounds are known to be present ubiquitously in nature. Bisides three major polyamines, putrescine, spermidine and spermine, some other diamines and such triamines as N-3-aminopropyl-1,5-diaminopentane or homospermidine(=1,9-diamino 5-azanonane) have been found in bacteria, higher animals and plants(6). There has, however, been no report of the occurrence of thermine, the spermine analog. The presence of bis(3-amino-propyl)amine, a triamine which is presumably a precursor of thermine, in a plant virus had been reported(7), but the later study could not confirm the results(8). The occurrence of this triamine in the extreme thermophile has not yet been proved.

The addition of thermine (final 2 mM) instead of spermine supported the cell-free synthesis of polyphenylalanine catalyzed by the thermophile extract at 65°C. A preliminary study revealed that spermine and thermine were rich in the cells of early

log-phase and the ratio of thermine content to spermine increased in the later stage of growth. These results suggest that these polyamines may play a role in metabolic regulation in the thermophile cell. Thermine was also found in other extreme thermophiles belonging to the genus Thermus, such as T. aquaticus YT-1 and T. flavus AT-62. A moderate thermophile, Bacillus stearothermophilus, contained no detectable amount of thermine.

Further studies on distribution and action mechanism of thermine will contribute to the elucidation of thermophily as well as that of physiological roles of polyamines.

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