A New Naturally Occurring Polyamine Containing a Quaternary Ammonium Nitrogen*

A new polyamine, tetrakis(3-aminopropyl)ammonium, N*(CH$_2$CH$_2$CH$_2$NH$_2$)$_4$, was identified in cells of an extreme thermophile, Thermus thermophilus. This compound was chemically synthesized and its chemical properties were coincident with those of the amine isolated from the thermophile.

Recently it was found that an extremely thermophilic bacterium, Thermus thermophilus, produces a variety of new polyamines (1, 2). Cells of T. thermophilus grown at the optimum temperature contained two novel tetrasamines, thermanine (3) and thermospermine (4), as major polyamines. A considerable amount of a pentaamine, caldopentamine (5), was also present in the thermophilic cell. Eight other polyamines, except for diamine, were also detected as minor polyamines by column chromatographic analyses. Among them, six amines were isolated, purified, and confirmed their chemical structures by comparing with the authentic compounds (1, 6–8). One of the remaining was identified to be homocaldohexamine (1,2-diamino-4,8,12,16-tetraazaneicosane), the longest polyamine so far detected in the thermophilic cell, by comparing its retention time by analytical chromatography with that of the chemically synthesized sample (8). The last one has remained to be identified.

Interesting enough, except for sym-homospermidine and its aminopropylated compound, homospermine, polyamines identified in the thermophile extract are series of compounds which seemed to be synthesized by stepwise addition of an aminopropyl group to an aminopropyl terminal of the corresponding precursory, shorter polyamine starting from either 1,3-diaminopropane, i.e. 1,3-diaminopropane, norspermidine, thermine (norspermine), caldopentamine, and caldohexamine in one series, and spermidine, thermospermine, homocaldopentamine, and homocaldohexamine in the other series. However, the retention time of the above mentioned, unknown polyamine in the cells of T. thermophilus did not coincide with that of any compound in these series. The fact that this compound was eluted from the analytical column immediately after homocaldopentamine, suggests that this unknown polyamine is a pentaamine.

In the present study, this unknown compound was extracted and purified from cells of T. thermophilus grown at 75 °C and determined to be a quaternary polyamine, tetrakis(3-aminopropyl)ammonium, based on its $^1$H, $^{13}$C NMR, and mass spectra. The chemical synthesis of this new amine was achieved.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strain—**T. thermophilus strain HB8 (ATCC27634) was grown at 75 °C in a synthetic medium containing sucrose and L-glutamate as carbon and nitrogen sources (1). The cells were harvested at the middle log phase.

**Reagents—**$3,3',3''$-Nitrotrispropionamide was purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan); N-(3-bromopropyl)phthalimide was from Aldrich. Silica gel powder used for column chromatography, Wako Gel C-300, was from Wako Pure Chemical Co. (Osaka, Japan) and cation exchange resins, CK-10 and CK-10U, were from Mitsubishi Chemical Co. (Tokyo, Japan).

**Polyamine Analysis—**Polyamines were extracted and analyzed as described in the literature (1) with slight modifications. A high performance liquid chromatography (HPLC) column (4.6 mm diameter x 10 cm high) of CK-10S or CK-10U was used and elution of polyamines was monitored by using a spectrophuorometer (Hitachi 204-A) after reacting with a solution of o-phthaldehyde (9).

**Physicochemical Analyses—**$^1$H NMR spectra were measured with a Hitachi R-24B (60 MHz), JEOL FS-100 (100 MHz), JEOL FX-90Q (90 MHz), JEOL JNM FX-200 (200 MHz), or a JEOL JNM GX-400 (400 MHz) NMR spectrometer. Proton-decoupled $^{13}$C NMR and mass spectrometry, HX100. In NMR analyses, samples were dissolved in a suitable solvent such as D$_2$O or CDCl$_3$. Chemical shifts were given in ppm (parts/million) relative to tetramethylsilane or D$_2$O used as an internal standard. Splitting patterns were designated as s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad).

**Infrared spectra (IR)** were recorded on a Hitachi 260-10 spectrophotometer. Solid samples were subjected for analysis as a solution in CHCl$_3$. Data were given in centimeters$^{-1}$ only for the pertinent diagnostic bands.

**Mass spectra** were recorded by secondary ion mass spectrometry (SIMS) on a Hitachi M-80A mass spectrometer. The first acceleration was 9 kV (xenon) and the second was 9 kV using a Pt plate. Samples were mixed in glycerol used as a matrix. A high resolution fast atom bombardment-mass spectrum was recorded by using a JEOL JMS-HX100.

**Melting points** were determined using a Yamato MP-21 Melting Point Apparatus and were uncorrected. Elementary analyses were carried out with a Perkin-Elmer 240 elemental analyzer. Analytical thin layer chromatography (TLC) was carried out using precoated Merck Kieselgel 60F254(SiO$_2$). TLC were visualized by UV light, iodine, or phosphomolybdic acid.

**Isolation of the Unknown Polyamine from T. thermophilus—**Polyamines were extracted as described in a previous paper (5). The unknown polyamine was purified by repeating chromatographic elution from a column (0.9-cm diameter x 40 cm high) of CK-10S.

**Chemical Synthesis of the Quaternary Polyamine—**A quaternary

---

*This work was supported in part by Grants-in-Aid for Scientific Research 60060004, 61010071, 61134026, and 6113001 from the Ministry of Education, Science and Culture and by Special Coordination Funds from the Science and Technology Agency of the Japanese Government. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address: Mitsubishi-kasei Institute of Life Sciences, Machida, Tokyo 194, Japan.

§ To whom correspondence should be addressed.

* The abbreviations used are: HPLC, high performance liquid chromatography; SIMS, secondary ion mass spectrometry.
polyamine, tetrakis(3-aminopropyl)ammonium, was synthesized by reacting tris(3-phthalimidopropyl)amine with N-(3-isocapropropyl)phthalimide. The total scheme of the chemical synthesis is shown in Scheme 1.

Tris(3-aminopropyl)amine (3) was made by reduction of either commercially available 3,3',3'-nitritolotrispropionamide (1) or tris(2-cyanoethyl)amine (2) prepared according to the literature (10) with lithium aluminum hydride in tetrahydrofuran (yield 90%). Three primary amino groups of 3 were then protected by mixing 3 (2.0 g, 5.8 mmol), phthalic anhydride (16.0 g, 107.9 mmol), and anhydrous sodium acetate (2.4 g, 29.0 mmol) followed by heating at 200°C for 20 min. After adding about 500 ml of water, the mixture was heated at 100°C for 10 min. The protected tertiary amine, tris(3-phthalimidopropyl)amine (4), was obtained by extracting the work-up after the solution had been adjusted to pH 8 by adding NaHCO₃ (about 20 g) (11). This compound was purified by a silica gel column chromatography with a mixture of hexane and ethyl acetate (1:1, v/v) (2.4 g, 4.0 mmol, 71% yield).

The quaternary amine was synthesized as follows. First, N-(3-isocapropropyl)phthalimide (5) was prepared by reacting N-(3-bromopropyl)phthalimide with 5 mol eq of NaI in refluxing acetone (96% yield). Then, tris(3-phthalimidopropyl)amine (884 mg, 1.5 mmol) and N-(3-isocapropropyl)phthalimide (578 mg, 1.5 mmol) were dissolved in a minimum volume of dry dioxane (about 1 ml/g of compound 4), and the mixture was refluxed for 3 h under nitrogen. White solid produced was filtered and washed with a minimum volume of dichloromethane. The filtrate and washings were combined and evaporated under reduced pressure. The residue was then dissolved in a minimum volume of dry dioxane, refluxed for 1 h, and then filtered to recover precipitated material. This procedure was repeated several times. The precipitates were saved, combined, and dried to yield tetrakis(3-phthalimidopropyl)ammonium iodide salt (6) as white powders (952 mg, 1.0 mmol, 61% yield).

The phthaloyl protecting groups of this compound (171 mg, 0.2 mmol) were removed by reacting for 2 h with 12 mol eq of hydrazine in ethanol under reflux. The reaction mixture was filtered and the precipitate was washed with ethanol. The supernatant and washings were combined and applied to a small column of Dowex 50W-X4 (H⁺ form). The column was eluted with 6N hydrochloric acid was dried under reduced pressure at 40°C, and the dried residue was used to measure the NMR and mass spectra. This dried powder was extremely hygroscopic.

The molecular ion peak of the unknown polyamine was found at m/z 246 in a SIMS spectrum which corresponds to C₆H₇N₅. Based on the data described here, tetrakis(3-aminopropyl)ammonium ion (Formula 1) was proposed for the chemical structure of the new polyamine.

Properties of the Chemically Synthesized Quaternary Amine—The proposed structure of the new quaternary ammonium was confirmed by chemical synthesis as described under “Experimental Procedures.”

First, the elution time of this compound on the analytical HPLC was exactly the same as that of the unknown polyamine. The molecular ion peak of the unknown polyamine was found at m/z 246 in a SIMS spectrum which corresponds to C₆H₇N₅. Based on the data described here, tetrakis(3-aminopropyl)ammonium ion (Formula 1) was proposed for the chemical structure of the new polyamine.

Second, the NMR data of the chemically synthesized standard are coincident with those (Fig. 1) of the amine extracted from T. thermophilus. The NMR data of this chemically synthesized compound were summarized in Table I. The possible assignments based on our empirical rules (1) are also listed in this table.

The m/z value of major peak in the SIMS spectrum of the synthesized compound was 246. The mass spectrum resembled that of the polyamine isolated from the extreme thermophile.

The final product was so hygroscopic that it was impossible to be subject to conventional elemental analysis. The elementary composition of the synthesized amine was confirmed instead by exact mass measurement. The observed m/z value for the molecular peak in the high resolution fast atom bombardment-mass spectrum was 246.2614; C₆H₇N₅ was calculated 246.2658.

Taken together, the aforementioned data indicate that the structure of the new polyamine isolated from T. thermophilus is tetrakis(3-aminopropyl)ammonium.

Cellular Content—The amount of this new polyamine in T. thermophilus varied depending on the growth conditions. When the cells grown in a synthetic medium at 75°C and harvested at the middle log phase were analyzed, 0.3 µmol of tetrakis(3-aminopropyl)ammonium was detected in a gram of the wet cells.
A New Polyamine Containing Quaternary Ammonium

**Table 1**

<table>
<thead>
<tr>
<th>Chemical shift</th>
<th>Relative intensity</th>
<th>Possible assignmenta</th>
</tr>
</thead>
<tbody>
<tr>
<td>1H NMR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.2</td>
<td>1</td>
<td>b</td>
</tr>
<tr>
<td>3.1</td>
<td>1</td>
<td>c</td>
</tr>
<tr>
<td>3.6</td>
<td>1</td>
<td>a</td>
</tr>
<tr>
<td>13C NMR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20.2</td>
<td></td>
<td>b</td>
</tr>
<tr>
<td>36.3</td>
<td></td>
<td>c</td>
</tr>
<tr>
<td>56.5</td>
<td></td>
<td>a</td>
</tr>
</tbody>
</table>

The chemical synthesis of tetrakis(3-aminopropyl)ammonium was achieved by a reaction of N-(3-iodopropyl)phthalimide with a protected tertiary amine. Quaternary ammonium compound was not formed when N-(3-bromopropyl)phthalimide or acrylonitrile was used. Thus the use of the iodo derivative was crucial. It was also important for this reaction to use a minimum amount of dioxane as a solvent.

It is also worth noting that the molecular ion (but not quasi-molecular ion) of the quaternary ammonium salt of the polyamine family was clearly observed in the SIMS technique, which in turn seems to be a new promising tool for this field.

The physiological functions and metabolism of tetrakis(3-aminopropyl)ammonium should be clarified in future studies. In our preliminary studies, the cellular content of this quaternary ammonium was increased when the thermophile cells were grown at 75°C in the presence of tris(3-aminopropyl)amine. This observation led to a hypothesis that tetrakis(3-aminopropyl)ammonium is synthesized by amination of the tertiary amine and a small amount of this amine may be present in the thermophile cell. Unfortunately, on our analytical HPLC, the elution time of this tertiary tetraamine is the same as that of thermine, one of the major polyamines in the thermophile cell. To determine whether or not this tris(3-aminopropyl)amine is present in the thermophile cell, an analytical procedure is under development in the laboratories.

Acknowledgment—We thank Mitsubishi Chemical Co. for high resolution fast atom bombardment-mass spectrometric measurements.

REFERENCES


**Fig. 1.** NMR spectra of the unknown polyamine extracted from *T. thermophilus*. Top, 1H NMR spectrum; bottom, 13C NMR spectrum. TMS, tetramethylsilane.

**Formula 1**

![Formula 1](image)

**Discussion**

The present study revealed for the first time the presence of tetrakis(3-aminopropyl)ammonium ion, a pentaamine containing a quaternary ammonium nitrogen in nature. The proposed structure was unambiguously confirmed by chemical synthesis together with the spectroscopic data.