

The crystallographic study of left-handed Z-DNA d(CGCGCG)₂ and thermine complexes crystallized at various temperatures and at various concentration of cations

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Abstract

In crystals of complexes of thermine and d(CGCGCG)₂ molecules grown at 4, 10, and 20 °C, the numbers of thermine molecules connected to the DNA molecule were dependent on the temperature of the crystallization. Two molecules of thermine and one Mg²⁺ ion were connected to DNA molecule when thermine and d(CGCGCG)₂ were co-crystallized at 4 and at 20 °C. When an increased concentration of magnesium and thermine molecules were co-crystallized with d(CGCGCG)₂ molecules at 10 °C, three Mg²⁺ ions and only one thermine molecule were bound with a d(CGCGCG)₂ molecule. The number of polyamines and of Mg²⁺ ions connected to DNA was dependent on the atomic values of the polyamine and of the metal ion. The binding of more Mg²⁺ ions occurred when the atomic value of Mg²⁺ exceeded that of the corresponding mono- or polyamine, and when the Mg²⁺ ion concentration was elevated. Furthermore, this study is the first documentation of a naturally occurring polyamine bound to the minor groove of DNA in a crystal structure.

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Biogenic polyamines have been extensively studied, especially with respect to their possible relationship to gene expression and to carcinogenesis. These investigations include studies of polyamines and the possible relationship between their metabolites and neoplastic growth, their possible roles as markers of malignancy [1,2], and their use as indicators of disease activity and response to therapy in cancer patients. α -Difluoromethylornithine, an inhibitor of polyamine biosynthesis, in combination with α -interferon, suppressed tumor growth in mice. Changes in poly-

amine metabolism in tumor-bearing hosts with total parenteral nutrition and intravenous infusion of α -difluoromethylornithine were also observed [3].

Thermine is a tetraamine polyamine, with a hydrocarbon chain structure of NH₂(CH₂)₃NH(CH₂)₃NH(CH₂)₃NH₂ PA(333). Thermine was first found in the extremely thermophilic bacterium *Thermus thermophilus* and is also reported to exist in plants, but it is not found in animal tissues. Polyamines can modulate the activity of several enzymes, including kinases and endonucleases. Of the polyamines, thermine shows activity not only at low temperature, but also at high temperatures where other polyamines are not active.

These tetraamine polyamines have been isolated and purified from the thermophile cell extracts and their chemical structures have been confirmed by comparing the physicochemical spectra of the isolated polyamines

Abbreviations: PA(2222), N¹-[2-(2-(2-aminoethylamino)ethylamino)-ethyl]-ethane-1,2-diamine; PA(222), N¹-[2-(2-aminoethylamino)-ethyl]-ethane-1,2-diamine.

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with those of chemically synthesized authentic amines. Trace amounts of 1,3-diaminopropane were occasionally detected in the cell extracts. However, the presence of 1,3-diaminopropane as a precursor of norspermidine, thermine and other longer polyamines was suggested by enzymatic studies since this compound served as a substrate for the aminopropyltransferase extracted from this thermophile.

The major polyamine components in *T. thermophilus* grown at 75 °C are two tetraamines thermine [4] and thermospermine [5]. The polyamine composition varied markedly depending on culture medium, culture temperature, growth stage, and other conditions. The novel polyamines found in *T. thermophilus* were also present in some mesophilic microorganisms and higher organisms. Norspermidine and/or thermine have also been found in mesophilic bacteria [6], algae [7], euglena [8], plant viruses [9], sea animals [10], shrimps [11], and insects [12]. When radioactive putrescine was added to the cell-free extract of *T. thermophilus* in the presence of unlabeled decarboxylated *S*-adenosylmethionine, labeled spermidine and thermospermine and/or spermine were found in the cells. Likewise, the *in vitro* production of norspermidine, thermine, and caldopentamine from 1,3-diaminopropane were observed. These findings suggest that polyamines are synthesized from the corresponding shorter polyamines by transaminopropylation in the thermophile cells.

Thermine is a repeat polyamine of propylamine. We have already analyzed by X-ray crystallography the repeat synthetic polyamines PA(222) [13] and PA(2222) [14] in complex with d(CGCGCG)₂. Both polyamines PA(222) and PA(2222) were bound to the minor groove of d(CGCGCG)₂. However, no natural polyamines have been found connected to the minor groove of d(CGCGCG)₂, and only in crystals grown at 4 °C has spermidine was reported to be bound to the minor groove of d(CGCGCG)₂ [15,17]. In the present study, two thermine molecules were found bound to the minor groove of the d(CGCGCG)₂ molecule. This result is the first discovery of a natural polyamine perfectly bound to the minor groove of d(CGCGCG)₂ molecule. These findings suggest that DNA acts as a subsumption compound, and this discovery assumes that DNA has a possible role in polyamine classification.

Results and discussion

When thermine was crystallized with d(CGCGCG)₂ at three different temperatures, at 4, 10, and 20 °C, and with high concentrations of MgCl₂, the d(CGCGCG)₂ adopts a left-handed Z-DNA conformation and the thermine molecule is clearly connected to the minor groove of the left-handed Z-DNA (Fig. 1). The cell constants of the crystals containing thermine grown at three different temperatures were almost the same and were comparable to other crystal lattices which were crystallized under high concentrations of salt. The values of cell constants, $a = 18$ Å, $b = 31$ Å,

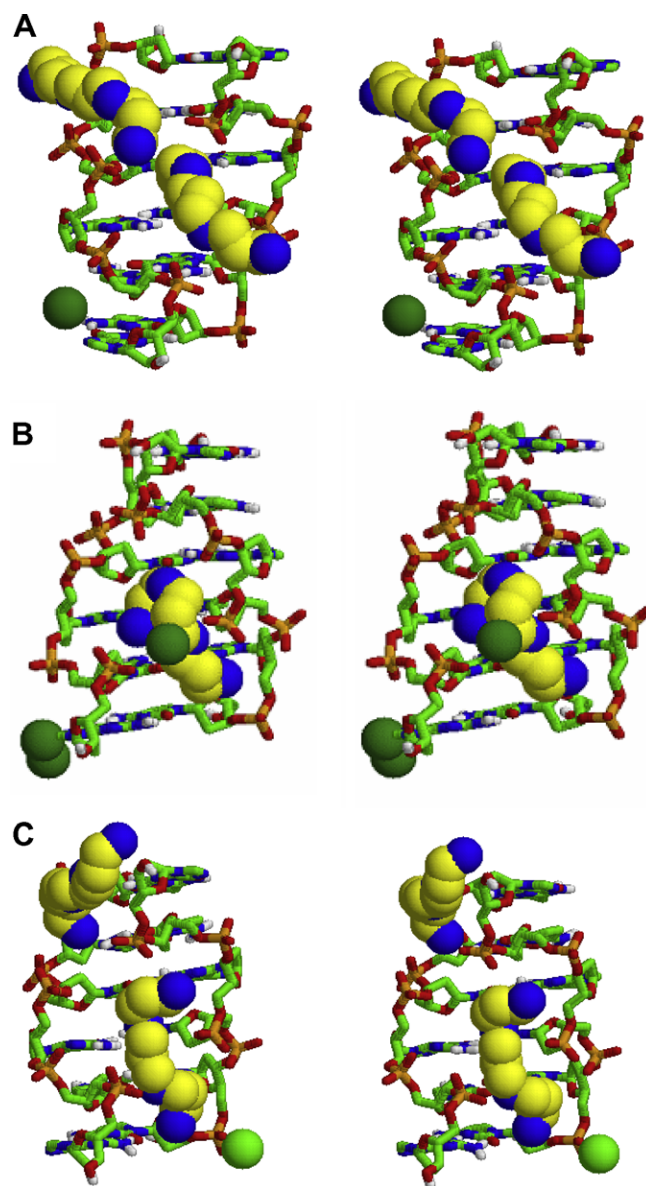


Fig. 1. (A) Drawing of the structure of one double strand helix of the d(CGCGCG)₂–thermine complex crystallized at room temperature. Two thermine molecules (blue and yellow spheres) are positioned in the minor groove of the d(CGCGCG)₂ molecule. (B) Drawing of the structure of one double strand helix of the d(CGCGCG)₂–thermine complex crystallized high salt conditions at 10 °C. One thermine molecule (blue and yellow spheres) is positioned in the minor groove of d(CGCGCG)₂ molecule. (C) Drawing of the structure of one double strand helix of the d(CGCGCG)₂–thermine complex crystallized at low temperature. Two thermine molecules (blue and yellow spheres) are positioned in the minor groove of the d(CGCGCG)₂ molecule.

$c = 44$ Å before and after. The crystal system was orthorhombic and the space group was $P2_12_12_1$ at all three temperatures.

The crystal structures of the thermine and d(CGCGCG)₂ complexes are shown in Fig. 1. Magnesium ion directly coordinated to the phosphate of the G2 base, and notably, this is the first example of an X-ray crystal structure with two natural polyamines connected to the

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