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The rare crystallographic structure of d(CGCGCG)$_2$: The natural spermidine molecule bound to the minor groove of left-handed Z-DNA d(CGCGCG)$_2$ at 10 °C

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Abstract

Several crystal structure analyses of complexes of synthetic polyamine compounds, including N^1-(2-(2-aminoethylamino))ethyl)ethane-1,2-diamine PA(222) and N^1-(2-(2-(2-aminoethylamino)ethylamino)ethyl)ethane-1,2-diamine PA(2222), and left-handed Z-DNA d(CGCGCG)$_2$ have been reported. However, until now, there have been no examples of naturally occurring polyamines bound to the minor groove of the left-handed Z-DNA of d(CGCGCG)$_2$ molecule. We have found that spermidine, a natural polyamine, is connected to the minor groove of left-handed Z-DNA of d(CGCGCG)$_2$ molecule in a crystalline complex grown at 10 °C. The electron density of the DNA molecule was clear enough to determine that the spermidine was connected in the minor groove of two symmetry related molecules of left-handed Z-DNA d(CGCGCG)$_2$. This is the first example that a spermidine molecule can form a bridge conformation between two symmetry related molecules of left-handed Z-DNA d(CGCGCG)$_2$ in the minor groove.

Keywords: d(CGCGCG)$_2$; Left-handed Z-DNA; Spermidine; X-ray crystallography; Polyamine

From X-ray crystallographic studies, Wang and Rich discovered that the structure of d(CGCGCG)$_2$ as a totally unexpected left-handed double helix [1]. This observation occurred in 1979, fully 23 years after the initial report of the structure of DNA (B-DNA) by Watson and Crick [2]. The novel structure was named left-handed Z-DNA [3]. According to the fiber diffraction photograph and the NMR spectrum of DNA, the polynucleotide consists of purine bases alternating in turn with pyrimidine bases. The base pairs of poly d(CG) [4], poly d(GC) [5], poly d(AC) [6] easily take the left-handed Z-type conformation if the salt concentration is high enough. In the first example of the crystal structure of the complex of left-handed d(CGCGCG)$_2$ and $H_3N^+(CH_2)_2NH_2^+(CH_2)_2NH_2^+(CH_2)_2NH_3^+$(PA222), the synthetic polyamine molecule was connected to a minor groove of left-handed Z-DNA at room temperature [7]. When d(CGCGCG)$_2$ adopted the left-handed Z-DNA structure, the distances between the bases and the neighboring phosphoric acids within the same strand were almost the same as the distances between adjacent nitrogen atoms in the PA222 molecules. Therefore, PA222 molecules easily connect with d(CGCGCG)$_2$ molecules and stabilize d(CGCGCG)$_2$ molecules. In addition, Mg^{2+} atoms unite coordination with the oxygen atoms of the phosphoric acid of d(CGCGCG)$_2$ molecule and Mg^{2+} atoms form complexes of octahedron coordination and stabilize d(CGCGCG)$_2$ conformations. Spermine ($H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$), spermidine ($H_2N(CH_2)_3NH(CH_2)_4NH_2$), putrescine ($H_2N(CH_2)_4NH_2$) and cadaverine ($H_2N(CH_2)_5NH_2$) are

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well known naturally occurring polyamines [8]. These polyamines have the ability to change right-handed B-DNA to left-handed Z-DNA [9]. Therefore, it is important to investigate the interaction between polyamines and the left-handed Z-DNA in order to understand the mechanism of interconversion of conformation. In examining the interaction modes between polyamines and the left-handed Z-DNA [10], we determined the crystal structure of the $d(CGCGCG)_2$ + spermidine ($H_2N(CH_2)_3NH(CH_2)_4NH_2$) complex from the X-ray diffraction data obtained from crystals grown at 10 °C. The positions of the metal cations and the water molecules around $d(CGCGCG)_2$ molecules were precisely determined.

Materials and methods

DNA $d(CGCGCG)_2$ was synthesized by Takara Bio Inc. Spermidine was purchased from Sigma Aldrich Industries Ltd. 4 mg/ml DNA $d(CGCGCG)_2$, 100 mM $MgCl_2$ and 30 mM spermidine were adjusted in 10 mM cacodylic acid buffer (pH 7.0) and the MPD of the liquid was adjusted at 8% while the MPD of the outside liquid was adjusted by 10–15%. Crystallization was accomplished by the vapor diffusion equilibrium method by leaving the crystallization solution for 2 weeks in the incubator at 10 °C. A hexagonal pillar-shaped colorless crystal, $0.3 \times 0.4 \times 0.1$ mm^3, was obtained after about 2 weeks. Diffraction data were measured by synchrotron radiation by using the beam line BL-6A at The High Energy Accelerator Research Organization. The data measured at the wavelength of the synchrotron radiation of 1.0 Å with the ADSC Quantum-210 CCD camera for data up to 1.0 Å resolution under the Crayo conditions of 95 K. The 120 frame image data (Table 1) were measured at φ angle from −90° to 90° by 1.5° and data exposure time 2 s. Image data processing was obtained with scaling and truncation functions of the CCP4 software package. Completeness at this time was 100%, with R-merge equal to 0.047. Rotation and translation functions [11] was calculated with program MOLREP [12] by using these data, and the phase was determined. Previously published coordinates for the DNA of PA(24) $d(CGCGCG)_2$ complex structure were also used for the phase decision [13]. The positions of Mg^{2+} and Na^+, that of spermidine, and those of water molecules were decided with the program Coot [14] and the program Turbo Frodo [15] by using difference Fourier synthesis map of $2F_o - F_c$. The structure was refined using the anisotropic temperature factor for the DNA, and with isotropic temperature factors for the Mg^{2+}, Na^+, spermidine, and water molecules using the programs X-PLOR [16] and SHELXL [17]; the final R factor value was 0.232. Data collecting statistics for $d(CGCG\ CG)_2$ + spermidine are given in Table 1. The atomic coordinates have been deposited in the Brookhaven Protein Databank (PDB entry number 2ELG).

Table 1
Crystal data of the $d(CG)_3$ + spermidine complex

Cell dimensions	Spermidine complex
a (Å)	17.85
b (Å)	30.99
c (Å)	44.02
α (°)	90
β (°)	90
γ (°)	90
V (Å^3)	24,351
Space group	$P2_12_12_1$
Crystal system	Orthorhombic
No. of reflections measured	79,230
No. of reflections independent	11,287
No. of reflections used 2σ	11,232
Completeness	100
Used reflections completeness	99.5
Resolution (Å)	1.0
Z	4
R-value	0.232

Results and discussion

The crystal lattice of the spermidine + $d(CGCGCG)_2$ complex crystal was approximately the same as the crystal lattice of other left-handed $d(CGCGCG)_2$ crystals. The space group, $P2_12_12_1$, was consistent with an orthorhombic crystal system (Table 1). In a single asymmetric unit of a crystal, a spermidine molecule, one sodium ion, and three magnesium ions were connected to the left-handed Z-DNA of the $d(CGCGCG)_2$ molecule shown in Fig. 1. We recently reported the position of magnesium ions and sodium ion at site 1, site 2 and site 3 of a methylamine and $d(CGCGCG)_2$ complex [18]. In the present crystalline complex with a natural polyamine, the positions of the three magnesium ions and one sodium ion were completely in agreement with the positions of site 1, site 2 and site 3 of the magnesium ions in the methylamine + $d(CGCGCG)_2$ complex crystal. The modes of coordination of the water molecules to the magnesium ions and a sodium ion were also the same as those between the water molecules and the magnesium ions in the methylamine + hexamer complex. In both crystalline complexes, similar positions were observed for the atoms in the bases and the oxygen atoms in the phosphate groups, which were hydrogen-bonded to the water molecules coordinated to the metal cations [18].

Two magnesium ions at site 1 and site 1′ formed a cluster and these magnesium ions coordinated with nine water molecules and the N_7 atom of the G6 base. All coordinate

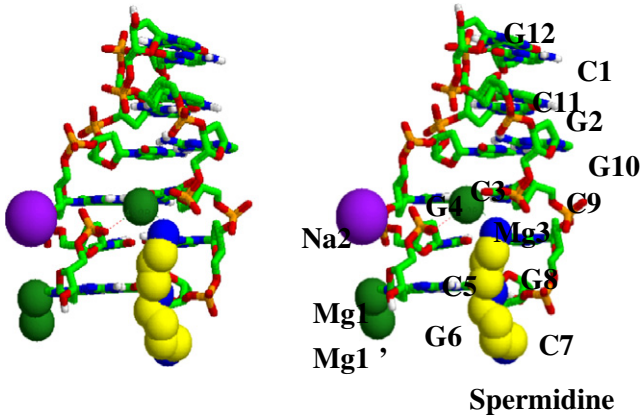

Fig. 1. The stereoscopic diagram of $d(CGCGCG)_2$ + spermidine complex crystal structure. The stick model shows left-handed Z-DNA $d(CGCGCG)_2$. The green sticks show carbon atoms, the blue sticks nitrogen atoms, the red sticks oxygen atoms, the white sticks hydrogen atoms and the orange color sticks show phosphorus atoms. The space filling models show the magnesium ions, a sodium ion and a spermidine molecule. The dark green balls indicate the magnesium ions. The purple ball indicate a sodium ion, the yellow balls indicate the carbon atoms and the blue balls indicate the nitrogen atoms.

bond distances included in this cluster molecule were 1.61–2.48 Å and these distances were ideal. The water molecule is hydrogen-bonded to the O_6 and N_3 atoms of the G6 base, the O_1P atom of phosphate group in C5, and to the O_6 atoms of symmetry-related G10 and G12 bases with symmetry operation $-x+0.5$, $-y$, $z+0.5$.

The sodium ion of site 2 is hydrated with six water molecules and the distances of the coordinate bonds of the sodium ion varied from 2.25 to 2.48 Å, slightly longer than the distances of coordinate bonds of magnesium ion [19]. Therefore, these coordinate bonds were ideal for sodium ions. Furthermore, these waters of coordination are hydrogen bonded to the O_1P atom of the phosphate group and to the $O_{4'}$ atom of the ribose in the G6, as well as to the O_2P atom of the phosphate group of the adjacent C5. These coordination waters are hydrogen-bonded to the O_2P atom of the phosphate group of C9 and to the O_1P atom of phosphate group of G10. C9 and G10 are moved by symmetry operation of $-x+1$, $y-0.5$, $-z+0.5$, respectively.

Magnesium ion of site 3 is coordinated to six water molecules. The distances between the coordinated waters and magnesium ion ranged from 1.84 to 2.24 Å, ideal coordination bonds lengths for magnesium ion. The water molecules coordinated with this magnesium ion were hydrogen-bonded with the O_2P atom of the phosphate group of G6. These coordination waters were also hydrogen-bonded to the O_6 atom of the G4 base, the O_6 atom and the N_7 atom of the G8 base, and to the O_2P atom of phosphate group of G10. G4, G8, and G10 were moved by the symmetry operation of $-x+1$, $y-0.5$, $-z+0.5$.

Spermidine was located near site 4 and was inserted completely in the minor groove of $d(CGCGCG)_2$ (Fig. 2A). Until now, the only examples of polyamines completely in the minor groove of DNA of $d(CGCGCG)_2$ were the synthetic polyamines PA(222) and PA(2222) [7,20]. Here, for the first time, we present the X-ray crystal structure analysis of a natural polyamine (spermidine) that completely enters the minor groove of DNA d(CGCG CG)$_2$. Previously, we crystallized the $d(CGCGCG)_2$ + spermidine complex at the temperature of 4 °C [21] and reported its crystal structure. In the present study, the positions of the sodium ion and the magnesium ions were the same as the positions of site 1, site 2 and site 3 of the earlier crystal structure [21], as illustrated by the stereodiagram (Fig. 2A) even though, in this case, we crystallized this $d(CGCGCG)_2$ + spermidine complex at 10 °C. Spermidine was located near site 4 whether the $d(CGCGCG)_2$ + spermidine complex was crystallized at low temperature or at 10 °C. Under the original crystallization conditions, the spermidine molecule formed a U-shape conformation, with the spermidine molecule bridging neighboring DNA molecules, without being connected in the minor groove of DNA $d(CGCGCG)_2$. In contrast, when the d(CGC GCG)$_2$ + spermidine complex was crystallized at 10 °C, the spermidine molecule assumed an elongated form and penetrated the minor grooves of two molecules of $d(CGCGCG)_2$ in the $d(CGCGCG)_2$ spermidine complex crystal. This is the first observation of either a synthesized polyamine or a natural polyamine penetrating between the minor grooves of two adjacent molecules of DNA.

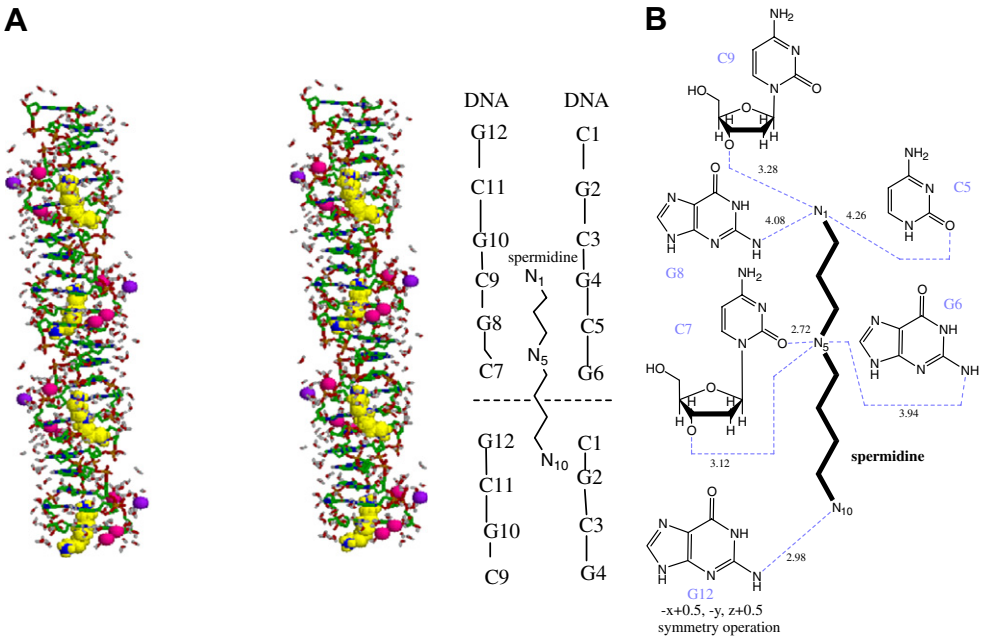

Fig. 2. (A) Four connected DNA molecules which moved with four continuous symmetry operations. The colors of DNA were the same as Fig. 1 except the magnesium atoms were indicated by magenta. Spermidine forms bridges between the minor groove of two molecules of double helical $d(CGCGCG)_2$ molecules. (B) The position of spermidine molecule indicating the mode of hydrogen-bonding between the spermidine molecule and the bases of Z-DNA and the phosphate group of Z-DNA $d(CGCGCG)_2$. G12 is moved by symmetry operation $-x+0.5$, $-y$, $z+0.5$.

The spermidine molecule was tightly connected in the minor groove of the d(CGCGCG)$_2$ molecule. The N_1 atom of spermidine molecule was hydrogen-bond to the O_2 atom of the C5 base, the N_2 atom of the G8 base and the $O_{3'}$ atom of the ribose of C9 (Fig. 2B). The N_5 atom of the spermidine molecule was hydrogen-bonded to the N_2 atom of the G6 base, to the O_2 atom of the C7 base, and to the $O_{3'}$ atom of the ribose of C7. Furthermore, the N_{10} atom of the spermidine molecule hydrogen-bonded to the N_2 atom of the G12 base of the DNA, which moved with symmetry operation of $-x+0.5$, $-y$, $z+0.5$. The positions of the magnesium cluster of site 1, the sodium ion of site 2, and the magnesium ion of site 3 were the same as those in the crystal structures of both the d(CGCGCG)$_2$ + spermidine formed at low temperature [21] and of d(CGCGCG) + PA(24) grown at room temperature [22]. These positions also agree with the position of the magnesium ion of d(CGCGCG)$_2$ + methylamine complex crystal structure[18]. In the electron density map, all atoms of spermidine displayed tight electron density. The DNA molecule was easily traced in this map because its electron density was very clear. The binding states of the coordination waters made it possible to identify the sodium ion and the magnesium ions. The sodium and magnesium ions were clearly distinguished because the lengths of the coordinate bond between coordination waters were longer for sodium ions than for magnesium ion. As shown in (Fig. 2A) four asymmetric units were moved with symmetry operations and aligned lengthwise. The spermidine molecule hydrogen-bonded in the minor groove of C9–C7, G4–G6 of the original d(CGCGCG)$_2$ molecule and also hydrogen-bonded in the minor groove of G12 and C1 of a second d(CGCGCG)$_2$ molecule which moved with symmetry operation of $-x+0.5$, $-y$, $z+0.5$. In this way, we observe the very unusual occurrence of a spermidine molecule connecting to the minor grooves of two consecutive DNA molecules, forming a bridge conformation. Viewed from the top, the spermidine molecule is connected in a ditch of the minor groove of d(CGCGCG)$_2$, with metal ions connected to the bases of the other side of the d(CGCGCG)$_2$ molecule. The water molecules were clustered around the spermidine molecule, the metal ions, and the phosphate group of DNA, thus stabilizing the entire structure of the DNA molecule. These positions of the magnesium ions and the sodium ion were in complete accord with earlier crystals structures of d(CGCGCG)$_2$ in complex with polyamines [21,22] or with methylamine [18].

In the present crystal structure of d(CGCGCG)$_2$ + spermidine complex grown at 10 °C, the spermidine molecule formed an extended structure, but in the crystal structure of d(CGCGCG)$_2$ + spermidine complex at low temperature [21], the spermidine molecule formed a U-shape conformation. These structural changes of spermidine molecules may be brought about by the increase and decrease of entropy. When the entropy decreases, that is to say, at low temperature, a normally linear structure, such as a spermidine molecule, bends and forms a U-shape structure. When the entropy increases, i.e., the temperature rises, the molecule forms an extended structure.

The torsion angles and bond angles of the back bone of the DNA molecules were approximately the same for the crystal structures formed at either low temperature or at 10 °C. Accordingly, the left-handed d(CGCGCG)$_2$ molecule connects to the spermidine molecule in the minor groove stably and the left-handed d(CGCGCG)$_2$ molecule can accommodate the spermidine molecule selectively in the minor groove. These phenomena show that left-handed d(CGCGCG)$_2$ can become an amplexus molecule for the spermidine molecule and may find future application as a separation agent for polyamines.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2007.04.026.

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