Polyamine interaction with Z-DNA

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In order to elucidate the detailed Z-DNA interaction with polyamines and also to clarify the mutual molecular recognition between the left-handed helix and the biologically important polyamine molecule, several polyamine-Z-DNA hexamer complexes were crystallized and their crystal structures were determined by X-ray diffraction. The general interaction modes found in these crystal structures were discussed in comparison with those in the complexes between polyamine and the right-handed DNA or RNA.

Keywords: polyamine-Z-DNA complexes, X-ray structure analysis

INTRODUCTION

Polyamines are widely distributed aliphatic polycationic compounds that are found in all cells and play a significant role in the regulation of normal and malignant cell proliferation.[1] Polyamines affect cell proliferation, differentiation, DNA replication, protein synthesis and the activity of several enzymes, including kinases. Despite the importance of polyamines in various processes involved in cell growth, their primary role in cell biology is not known.

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Polyamines are positively charged under physiological ionic conditions. The negatively charged phosphate groups in DNA are therefore prime targets for the interaction of polyamines by electrostatic forces. In agreement with this hypothesis, polyamines stabilize DNA against thermal denaturation[2] and radiation damage.[3] Furthermore, polyamines are capable of provoking a conformational transition in poly(dG-dC) from its usual right-handed B-DNA form to a left-handed Z-DNA form at physiologically relevant cationic concentrations.[4] The enzyme immunoassay, using monoclonal anti-Z-DNA antibody, indicated a corresponding increase in polyamine concentrations with the completion of the B-DNA to Z-DNA transition.[5]

The polyamine interaction with nucleic acids was theoretically simulated using semiempirical energy formulae and molecular mechanics calculations. For instance, spermine docked into the major groove of B-DNA stabilized the complex by maximizing interactions between the proton acceptors on the DNA oligomer and the proton donors on spermine.[6]

On the other hand, the experimental results on spermine interaction with B-DNA were obtained by X-rays, one by the X-ray diffraction technique[7] and the other by X-ray single crystal analysis of B-DNA dodecamer.[8]

In the present study, we describe the detailed crystal structures of the complexes of Z-DNA hexamer, $d(CG)_3$, with four different polyamines and compare the interactions between the Z-DNA and the polyamines at the molecular level. The chemical structures of the polyamines used are shown in Figure 1.

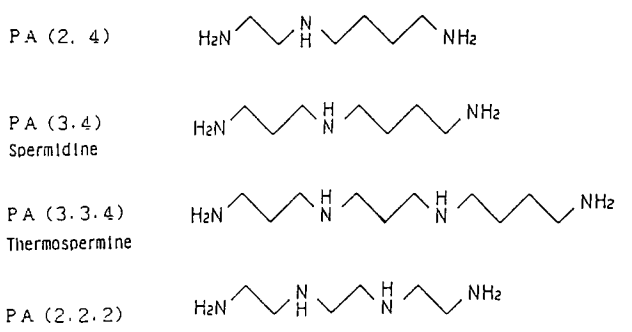

Figure 1. Chemical structures of polyamines

METHODS

The DNA hexamer, d(CGCGCG),* was prepared by a phosphotriester method.[9] By using the vapor diffusion method with 3.0% polyethyleneglycol (PEG) as the precipitating agent, crystals were obtained from solutions containing sodium cacodylate buffer (pH 7.0), magnesium chloride and DNA hexamer with or without polyamine. Table 1 shows the crystallization condition for the Z-DNA-PA(2,4) complex (complex I) crystal. In the first $d(CG)_3$ Z-DNA structure,[10] the DNA hexamer had spermine cations in the complex, but later it was shown that the isomorphous Z-DNA crystals can be obtained without spermine in the crystallization mixture.[11] Therefore, four polyamine complexes with the DNA hexamer were crystallized as isomorphous to the Z-DNA hexamer crystal[11] without polyamine. Crystallographic data for the polyamine complexes and polyamine free crystal form are given in Table 2. The individual polyamine complex crystal was highly ordered and the complete three-dimensional (3D) data were collected to a resolution of 1.0–1.1 Å using a Picker diffractometer.

The structure of each polyamine complex except complex IV was determined by the isomorphous replacement method; that is, the structure was solved by placing the polyamine free hexamer coordinates in the polyamine hexamer complex lattice and calculating the phases of each reflection. The polyamine binding region was located easily in the difference electron density map. Structures were refined by the Hendrickson-Konnert restraint least-squares refinement procedure.[12] The difference electron density map is quite clear, and all electron density peaks of height greater than 3σ have been interpreted, and assigned, depending on their shapes—elongated density peaks to polyamines; large spheres of high density to hydrated magnesium ions; and small, isolated spheres to solvent water molecules. Octahedral magnesium hydrates and sodium hydrates were distinguishable. The X-ray structure determination of the complex IV is now underway.

All the numerical calculations for determining the structures were carried out on an ACOS 850 computer at the Crystallographic Research Center, Institute for Protein Research, Osaka University. The IRIS display was used for the molecular graphics with the program VENUS.[13]

*The numbering system of DNA hexamer is:
C1-p2- G2-p3- C3-p4- G4-p5- C5-p6- G6
. . . . . .
. . . . . .
. . . . . .
G12p12C11p11G10p10C9-p9- G8-p8- C7

Table 1. Crystallization condition for complex I

d(CGCGCG)	1.5 mM
Sodium cacodylate	12.5 mM
$MgCl_2$	7.5 mM
Polyamine PA(2,4)	2.5 mM
Precipitant	1.9 v/v% PEG 600
Reservoir precipitant	3.0 v/v% PEG 600

RESULTS AND DISCUSSION

Molecular structure of DNA hexamer in polyamine complexes

As already mentioned, the DNA hexamer, $d(CG)_3$, forms a left-handed helix called Z-DNA even with no polyamine in the crystallization solution. The refinement procedure indicates that the overall molecular conformation of the Z-DNA hexamer, $d(CG)_3$, in the polyamine free crystal is roughly similar to that in the polyamine complex crystal: 12 residues per turn with a helix pitch of ca. 45 Å, the glycosidic torsion angle

Table 2. Crystals of Z-DNA with and without polyamine

Name	Z-DNA	Complex I	Complex II	Complex III	Complex IV
Oligomer	$d(CG)_3$	$d(CG)_3$	$d(CG)_3$	$d(CG)_3$	$d(CG)_3$
Polyamine	—	PA(24)	PA(34)	PA(334)	PA(222)
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
a(Å)	17.98	17.94	17.93	17.98	17.93
b(Å)	30.94	31.23	31.23	31.51	31.36
c(Å)	44.81	44.55	44.64	44.38	44.62
Z	4	4	4	4	4
Volume /base pair (Å^3)	1039	1040	1041	1048	1045
Resolution (Å)	1.8	1.1	1.0	1.0	1.0
R-value	0.13	0.17	0.19	0.19	—

of deoxyguanosine is *syn*, but that of deoxycytidine is *anti*, as commonlly found in normal right-handed B-DNA. In yeast phenylalanine tRNA structure, the spermine produces a bend in the double helix of RNA,[14] and in this DNA hexamer, especially in the complex II (Z-DNA hexamer with PA(3,4)) as shown in Figure 2, the electrostatic interaction between the terminal positively charged amino nitrogen atom, N(1), and the five negatively charged phosphates, p4, p6, p10, p11 and p12, causes an opening in the minor groove of Z-DNA double-helix. The PA(3,4) molecule penetrates the minor groove of Z-DNA and the helical axis shows a bend toward the major groove.

The structural stabilization with charge neutralization

By carefully inspecting the difference electron density map, we could distinguish the metal ions, magnesium and sodium ions, from the solvent water molecules. For instance, in the complex I crystal one sodium ion and three magnesium ions—of which two magnesium ions form a dimeric cluster—were found per one double-helical Z-DNA hexamer in addition to one polyamine molecule, PA(2,4). The coordination polyhedra around these ions are shown in Figure 3. One sodium ion was coordinated with five water molecules and one oxygen atom of the phosphate (p9) near the minor groove to form a distorted octahedron. Furthermore, two of five water molecules strongly hydrogen-bonded with two oxygen atoms of the neighboring phosphate group (p10). One magnesium ion with five water ligands was located in the major groove, and one coordinated water molecule formed two hydrogen bonds with each O(6) oxygen atom of the guanine bases of residues G4 and G8. These two hydrogen bonds with distances of 3.06 Å and 3.01 Å, respectively, may stabilize the two adjacent G–C base-pairings (G4–C9 and C5–G8) by reducing fluctuation in the stacking interactions. Another coordinated water molecule participates in a hydrogen bond with N(7) atom of the G8 residue with a distance of 2.75 Å. The rest

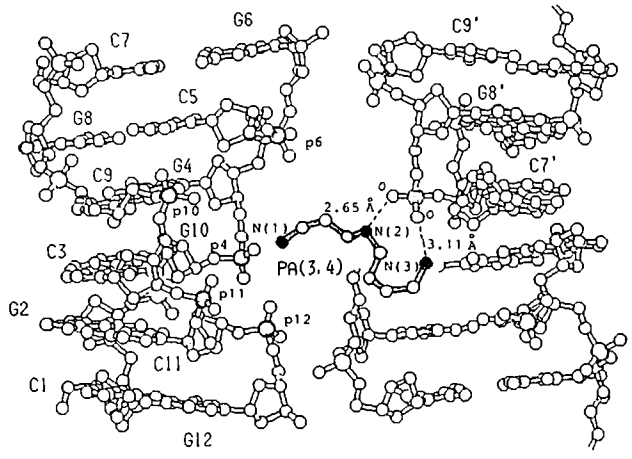

Figure 2. Polyamine interaction with Z-DNA in complex II crystal

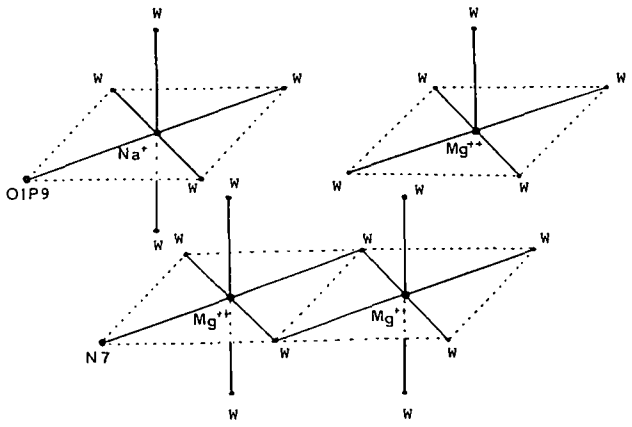

Figure 3. Coordination geometry of ions in complex I

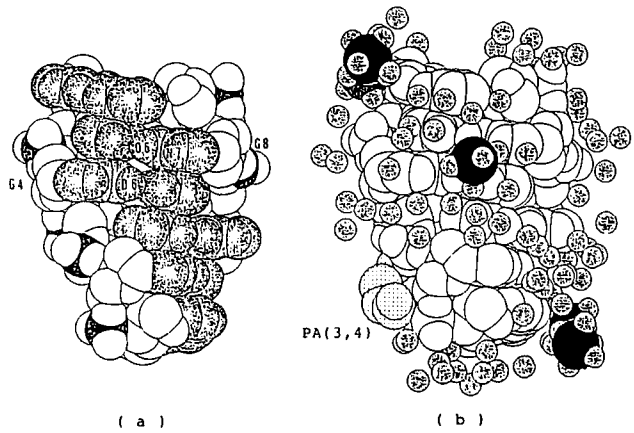

Figure 4. CPK model of Z-DNA hexamer (a) and complex II (b)

of the magnesium cluster, which forms a dimeric coordination geometry as shown in Figure 3, was also located in the major groove of the Z-DNA double helix.

A particularly interesting feature of the interaction is that one magnesium ion in the cluster is directly coordinated to the N(7) atom of the guanine base in residue G6 with a distance of 2.39 Å. Two coordinated water molecules participate in the hydrogen bonding with N(4) of the cytosine base in the C7 residue and also with O(6) atom of the guanine base in resiude G6, stabilizing the terminal G–C base pairing region. Figure 4 represents the computer-drawing CPK model of the Z-DNA double helix (Figure 4a) with the polyamine PA(2,4) (the dotted circle in this figure), the magnesium ions (the large black circle), and the solvent water molecules (the small gray circle) (Figure 4b). The sodium ion cannot be seen because it is located on the back side of the complex in this projection.

In the case of the complex II crystal, three magnesium ions and one sodium ion were found in the difference electron density map beside one PA(3,4) molecule. The location and coordination geometry around the magnesium ions, were quite similar to that of the complex I crystal. The sodium ion was located near the C4 and C5 residues instead of the G8 and G9 residues in the

Table 3. Different polyamines in Z-DNA crystals of d(CG)$_3$

Cation	PA(24)	PA(34)	PA(334)	PA(222)
Polyamine	1 (+3)	1 (+3)	2 (+8)	—
Magnesium	3 (+6)	3 (+6)	1 (+2)	—
Sodium	1 (+1)	1 (+1)	0	—
Water	85	97	80	—
R value	0.17	0.19	0.19	—
Negative charges in Z-DNA	−10	−10	−10	−10

Total number of positive charges is shown in parentheses

complex I crystal; that is, the sodium ions in both complex I and II are located in the G–C base-pair region of the minor groove in the Z-DNA double helix and interact with the phosphate group through the coordinated water molecules. Table 3 is a list of the charge balance in three polyamine-Z-DNA complex crystals. As already mentioned, in the complex I crystal, the total number of the positive charges is 10; three positively charged nitrogen atoms in the PA(2,4) molecule, six positive charges of three magnesium ions, and one positive charge of one sodium ion, whereas the number of the negatively charged phosphates in Z-DNA double helix is also 10. Hence, the positive and negative charges in the asymmetric unit of the complex I crystal neutralize each other. As shown in Table 3, in complex II and the complex III, the charge neutralization is also strictly conserved.

The polyamine interaction mode found in the polyamine-Z-DNA complex crystals

The polyamine-Z-DNA interaction mode should be different in each of the three polyamine-Z-DNA complex crystals because, as indicated in Table 3, the complex III crystal contains two polyamine molecules per an asymmetric unit, whereas only one polyamine molecule was found in the cases of the complex I and the complex II. Furthermore, the positively charged primary and

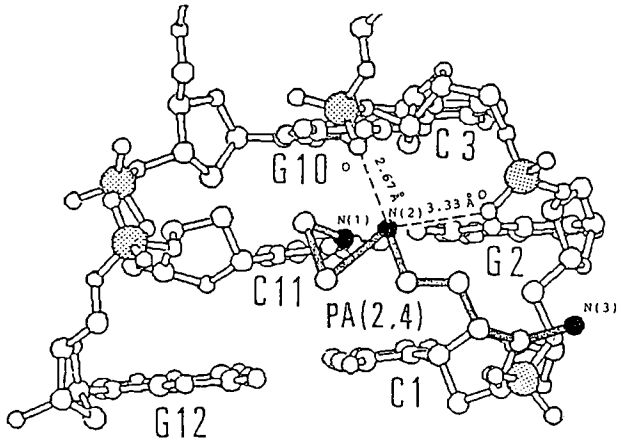

Figure 5. Polyamine interaction with Z-DNA in complex I crystal

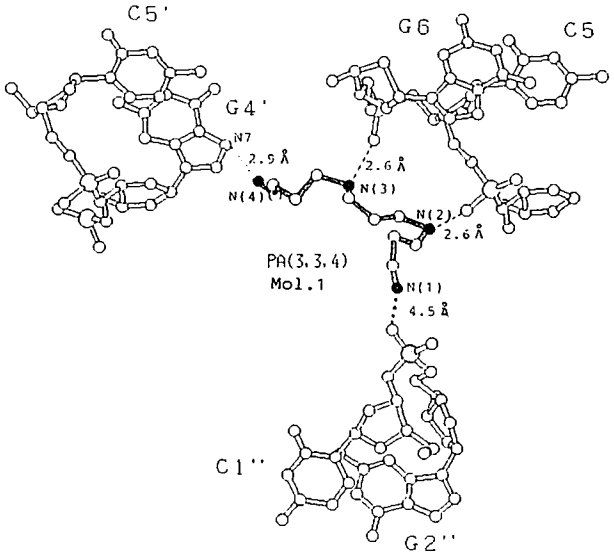

Figure 6. Polyamine interaction with Z-DNA in complex III crystal

secondary amine can electrostatically interact as well as form hydrogen bonds to the DNA molecule or the solvent water molecule. Figure 5 shows the hydrogen bonding interactions between the PA(2,4) molecule and the terminal three base-pair portion of the Z-DNA double helix. The nitrogen atoms at both ends, N(1) and N(3), form several hydrogen bonds to the solvent water molecules but none with the Z-DNA double helix. The N(1) atom penetrates the minor groove of the Z-DNA helix, while the other terminal nitrogen atom, N(3), interacts with the adjacent Z-DNA helix by Coulomb force. The central imino nitrogen atom of the PA(2,4) molecule, N(2), forms two hydrogen bonds to the oxygen atoms of the phosphate groups p3 and p4, with bond distances of 3.3 Å and 2.67 Å, respectively.

In the complex II crystal, the polyamine, P(3,4), forms two hydrogen bonds between the central nitrogen atom, N(2), and one oxygen atom of the phosphate p8 with an interatomic distance of 2.65 Å, and between the terminal nitrogen atom, N(3), and one oxygen atom of the phosphate p8 with an interactomic distance of 3.11 Å, as shown in Figure 2. The remaining terminal nitrogen atom, N(1), penetrates the minor groove of the adjacent Z-DNA helix roughly in the center of the minor groove of the adjacent Z-DNA molecule. As already mentioned, the positively charged terminal nitrogen atom interacts electrostatically with five negatively charged phosphates, causing an expansion of the minor groove of the Z-DNA helix. Figure 6 indicates the interaction mode of the polyamine, the PA(3,3,4) Mol.I, with the three surrounding Z-DNA helices. In this case, PA(3,3,4) molecule contains two central imino nitrogen atoms, N(2) and N(3), which form two hydrogen bonds with the phosphate oxygen atoms with interatomic distances of 2.6 Å and 2.6 Å, respectively. The two terminal amino nitrogen atoms, N(1) and N(4), interact with the neighboring DNA molecules mainly by electrostatic force. The N(1) atom forms a so-called salt bridge with the negatively

charged phosphate (p2) oxygen atom with a distance of 4.5 Å. The distance between the N(4) atom and the N(7) atom of the neighboring guanine base of residue G4 is 2.9 Å. The N(4) atom is 2.4 Å out of the guanine base plane, hence the N(4) atom probably forms a very weak hydrogen bond.

The general interaction modes and the molecule recognition found in the Z-DNA-polyamine complex crystals

It is worthwhile to compare the three polyamine-Z-DNA interaction modes in order to establish a general description of the interaction should be found in any polyamine-Z-DNA complex crystal. We now have three polyamine-Z-DNA complex crystals as examples for comparison with other complexes. Summarized below are the general rules describing their interaction modes:

(1) The terminal amino nitrogen atom of polyamine is located near the minor groove of the Z-DNA double helix or penetrates it, and interacts electrostatically with the phosphate group.
(2) The central imino nitrogen atoms—irrespective of their number—form strong hydrogen bonds to the phosphate oxygen atoms in the minor groove.
(3) The molecular conformation of the polyamine is not always extended (a *trans*-zigzag form). Sometimes the polyamine molecule is shaped rather like a fish-hook, which is the shape of the spermine molecule in the yeast phenylalanine transfer RNA crystal.[14]
(4) The entire polyamine molecule does not always adhere to the concave surface of the minor groove of the Z-DNA double helical molecule. A polyamine molecule with a relatively long chain has a tendency to lie across or between two or three neighboring Z-DNA double helices.

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