Interaction between left-handed Z-DNA and polyamine – 3

The crystal structure of the d(CG)$_3$ and thermospermine complex

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Abstract The DNA fragment, d(CG)$_3$, was co-crystallized with *N*-(3-amino-propyl)-*N*-(5-aminopropyl)-1,4-diaminobutane (thermospermine; PA(334)), a polyamine metabolized from the nucleic acid. By using a good crystal with dimensions of 0.5×0.5×0.5 mm^3, X-ray intensity data were collected up to 1.0 Å resolution. Two thermospermine molecules and a magnesium cation were bound to the left-handed double-helical d(CG)$_3$ molecule. The d(CG)$_3$ molecule adopted the left-handed Z-conformation and two thermospermine molecules and a magnesium cation neutralized the negative charges of the phosphate groups of the d(CG)$_3$ molecule. Furthermore, the binding modes between d(CG)$_3$ and thermospermine were different from those of d(CG)$_3$ complexes with PA(24), spermidine and spermine. This is the first case in which it was determined by X-ray crystallographic analysis that one of two thermospermine molecules bound three d(CG)$_3$ duplexes which were symmetrically related to each other, and the other formed two hydrogen bonds at the N(5) and N(9) atoms with two adjacent nucleotide phosphate groups of a single d(CG)$_3$ strand at the minor groove. Furthermore, no direct coordination bond was found between the d(CG)$_3$ molecule and the magnesium cation.

Key words: Z-DNA; Polyamine; Crystal structure; d(CG)$_3$-thermospermine complex; Molecular interaction; Molecular conformation

1. Introduction

Recently, we reported the crystal structures of the left-handed Z-DNA fragment, d(CG)$_3$, in complexes with *N*-(2-aminoethyl)-1,4-diaminobutane (PA(24)) [1], and with *N*-(2-aminopropyl)-1,4-diaminobutane (spermidine, PA(34)) [2], and discussed the mode of interaction of these complexes [3]. On the other hand, the structure of the d(CG)$_3$+spermine complex was determined by Wang et al. [4]. It was clearly shown that these polyamines interact with the DNA molecule according to a wide variety of modes, and protect against cleavage of the double-stranded DNA. Furthermore, these polyamines are involved with cell proliferation and the activation of DNA, RNA, and proteins [5]. It was considered that polyamines sometimes act as promoting agents for carcinogenesis [6]. Here, we used thermospermine as a polyamine complexed with Z-DNA, and report the crystal structure of a complex between d(CG)$_3$ and thermospermine (PA(334)) at resolutions up to 1.0 Å. The chemical structures of thermospermine, spermine and the d(CG)$_3$ molecule are illustrated in the scheme below. Thermospermine is a rare polyamine extracted from *Thermus thermophilus* [7] and is a so-called lengthy polyamine, as shown in the scheme, of which the chain length is similar to that of spermine but in which one imino nitrogen position differs from that of spermine, and this may cause the variations in binding mode between d(CG)$_3$ and polyamines. The results indicate that the modes of binding are somewhat different among d(CG)$_3$ complexes with polyamines, and therefore, that the chain length of polyamines, and the position and number of the nitrogen atoms of the polyamines are essential to determine the mode of interaction of the d(CG)$_3$ molecule with the polyamine molecule.

thermospermine : N(1)H$_2$CH$_2$CH$_2$CH$_2$N(5)H- CH$_2$CH$_2$CH$_2$N(9)H- CH$_2$CH$_2$CH$_2$CH$_2$N(14)H$_2$
spermine : N(1)H$_2$CH$_2$CH$_2$CH$_2$N(5)H- CH$_2$CH$_2$CH$_2$CH$_2$N(10)H- CH$_2$CH$_2$CH$_2$N(14)H$_2$

d(CG)$_3$ duplex: 5'-OH- C1- p2 - G2- p3 -C3 -p4 -G4-p5-C5-p6-G6-OH-3'
3'-OH- G12 -p12-C11-p11-G10-p10-C9-p9-G8-p8-C7-OH-5'

Scheme

2. Material and methods

The DNA hexamer, d(CG)$_3$ was prepared by the phosphotriester method. Thermospermine was obtained from a natural source and purified by column chromatography. Since it is well known that both polyamines and metal cations may stabilize the DNA conformation [8], we used the magnesium cation for crystallization of the d(CG)$_3$ complex with thermospermine. Single crystals were grown within 2 weeks from a mixture containing 2 mM ammonium salt of d(CG)$_3$, 10 mM thermospermine tetrachloride and 15 mM $MgCl_2$ in 30 mM sodium cacodylate buffer solution (pH 7.0) using the vapor diffusion method. The crystal used for X-ray studies had the dimensions of 0.5×0.5×0.5 mm^3. The cell dimensions refined by precession photographs, $a = 17.98(1)$ Å, $b = 31.51(1)$ Å, $c = 44.38(1)$ Å with orthorhombic space group $P2_12_12_1$, are isomorphous to those of the d(CG)$_3$ crystal [9] and the d(CG)$_3$ complex with spermine [4], and there is one duplex containing 12 nucleotides in an asymmetric unit. The crystals were sealed in thin-walled glass capillaries and the integrated intensities were measured by the continuous ω-scan method. X-ray diffraction data were collected up to 1.0 Å resolution. We applied a semi-empirical absorption correction derived by North et al. [10], and X-ray damage was corrected as a function of time. The phase problem was solved by a molecular replacement method [11] with the

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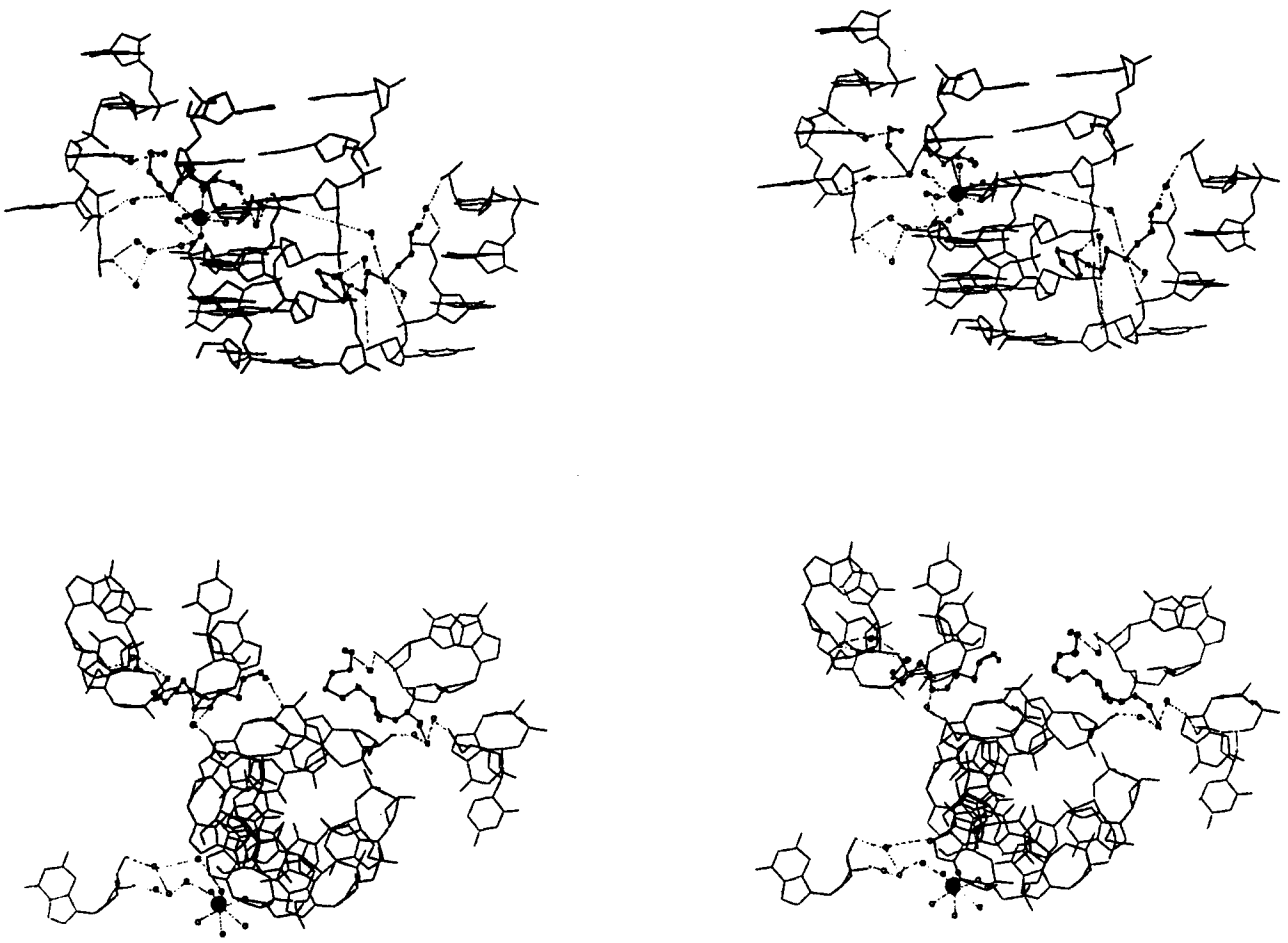

Fig. 1. Wire model of $d(CG)_3$-thermospermine complex structure. Thermospermine molecules are represented with ball and thick stick, and magnesium is shown by a black circle (●). Dotted lines indicate the coordination bonds and hydrogen bonds.

program AMORE in the CCP4 package using the atomic coordinates of the $d(CG)_3$ crystal structure [9], the structure being refined by the program X-PLOR [12] with a rigid body refinement method. Two thermospermine molecules and one magnesium ion were identified by a different Fourier synthesis. 72 water molecules were clearly located through a successive Fourier synthesis using the reflections with $|F_0| > 3\sigma(F_0)$ to 1.0 Å resolution. Model building of the structure of the complex was performed using the program O [13] on an IRIS Indy workstation, and refined using a stereochemically restrained least-squares method [14] with the program NUCLS and a molecular simulation with the program X-PLOR to a residual R value of 0.19. The averaged r.m.s. deviation for atomic coordinate error was 0.20. The radial distribution of the final R factor using the Luzzati plot [15] gives a mean coordinate error of 0.15. All calculations were carried out on the IRIS Indy workstation, Osaka University of Pharmaceutical Sciences, and on an ACOS-3700 System at the Research Center for Protein Engineering, Institute for Protein Research, Osaka University.

3. Results and discussion

The primary structures of $d(CG)_3$ and thermospermine are shown in Section 1. As shown in Fig. 1, the $d(CG)_3$ molecule adopts the left-handed Z-DNA conformation, two thermospermines and one magnesium cation binding to one $d(CG)_3$ molecule of which the binding mode is rather different from those found in the crystal structures of the $d(CG)_3$+*N*-(2-aminoethyl)-1,4-diaminobutane (PA(24)) [1], $d(CG)_3$+spermidine [2], and $d(CG)_3$+spermine complexes [4]. One helical turn of the left-handed Z-DNA self-complementary duplex comprises 24 nucleotides. 10 positive charges in which eight positive charges are supplied by two thermospermines (four positive charges per thermospermine) and two positive charges from one magnesium cation may neutralize 10 negative charges furnished by the phosphate groups of $d(CG)_3$ molecule. As clearly shown in Figs. 2 and 3, two thermospermine molecules interact with the $d(CG)_3$ duplex in different manners. The first thermospermine molecule-1 is connected between the three symmetrically related $d(CG)_3$ duplexes instead of the two $d(CG)_3$ duplexes in the $d(CG)_3$+spermine complex [4], and the second one is bound to the original $d(CG)_3$ molecule at the minor groove. One magnesium cation was tightly bound to the original $d(CG)_3$ molecule with indirect coordination bonds through water molecules. These binding modes may exert an important influence in protecting from physical cutting or hydrolysis of the oligonucleotide and also preventing separation of the double-stranded oligonucleotide. Furthermore, we now know that polyamines and metal cations can promote the so-called B-Z transition. Although a considerable chain length and number of imino nitrogen atoms in the polyamine molecule are essential for its biological activities, it is still unknown whether these phenomena of binding between gene and polyamine are concerned with cell proliferation. The pseudo rotation angles around all the glycosidic bonds in the $d(CG)_3$ molecule in this case were normal and the ε and ζ

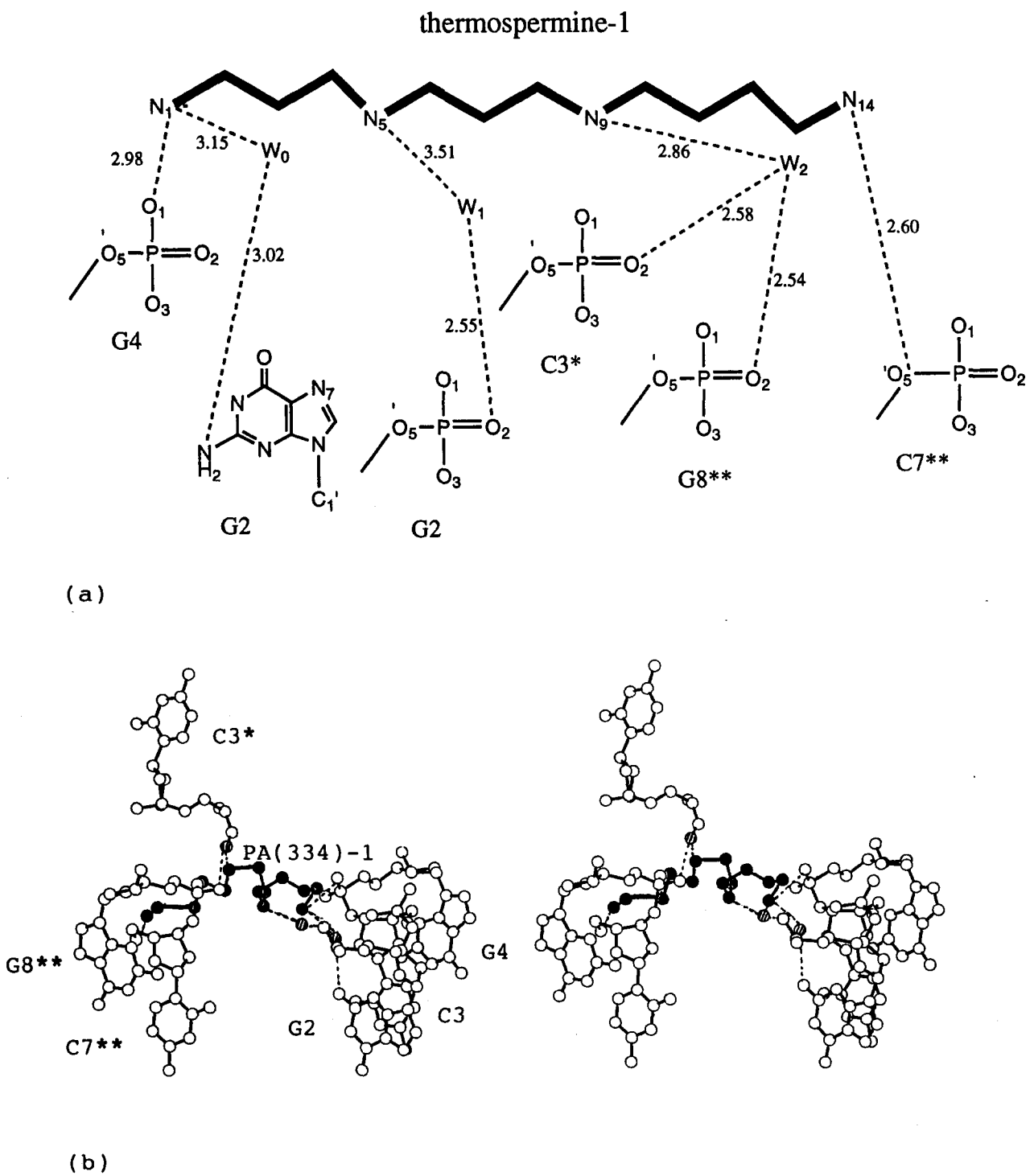

Fig. 2. Interaction between thermospermine-1 and $d(CG)_3$. (a) Schematic sketch of interaction. W denotes water molecule. (b) Stereoscopic drawing around thermospermine-1. The thermospermine-1 molecule is represented by a black ball and stick, and water molecules by shaded balls. Hydrogen bonds are shown by dotted lines.

torsion angles were −sc/−sc (Z-I type). The binding modes around the first thermospermine molecule-1 are shown in Fig. 2a. The terminal amino N(1) atom bound directly to the G4 phosphate oxygen atom and through a water molecule to the amino N2 atom of the G2 base in the original $d(CG)_3$ duplexes. The imino N(5) atom bound to the phosphate oxygen atom of G2 in the original $d(CG)_3$ duplex via a water molecule. On the other hand, the imino N(9) and terminal amino N(14) atoms are connected to the phosphate oxygen atoms in the symmetrically related neighboring $d(CG)_3$ duplexes directly or through water molecules. As indicated in Fig. 2b, the thermospermine molecule-1 linked and formed a bridged conformation binding to three neighboring $d(CG)_3$ molecules. The binding modes of another thermospermine molecule-2 are shown in Fig. 3. Thermospermine molecule-2 bound tightly to the original $d(CG)_3$ duplex at the minor groove, and wrapped

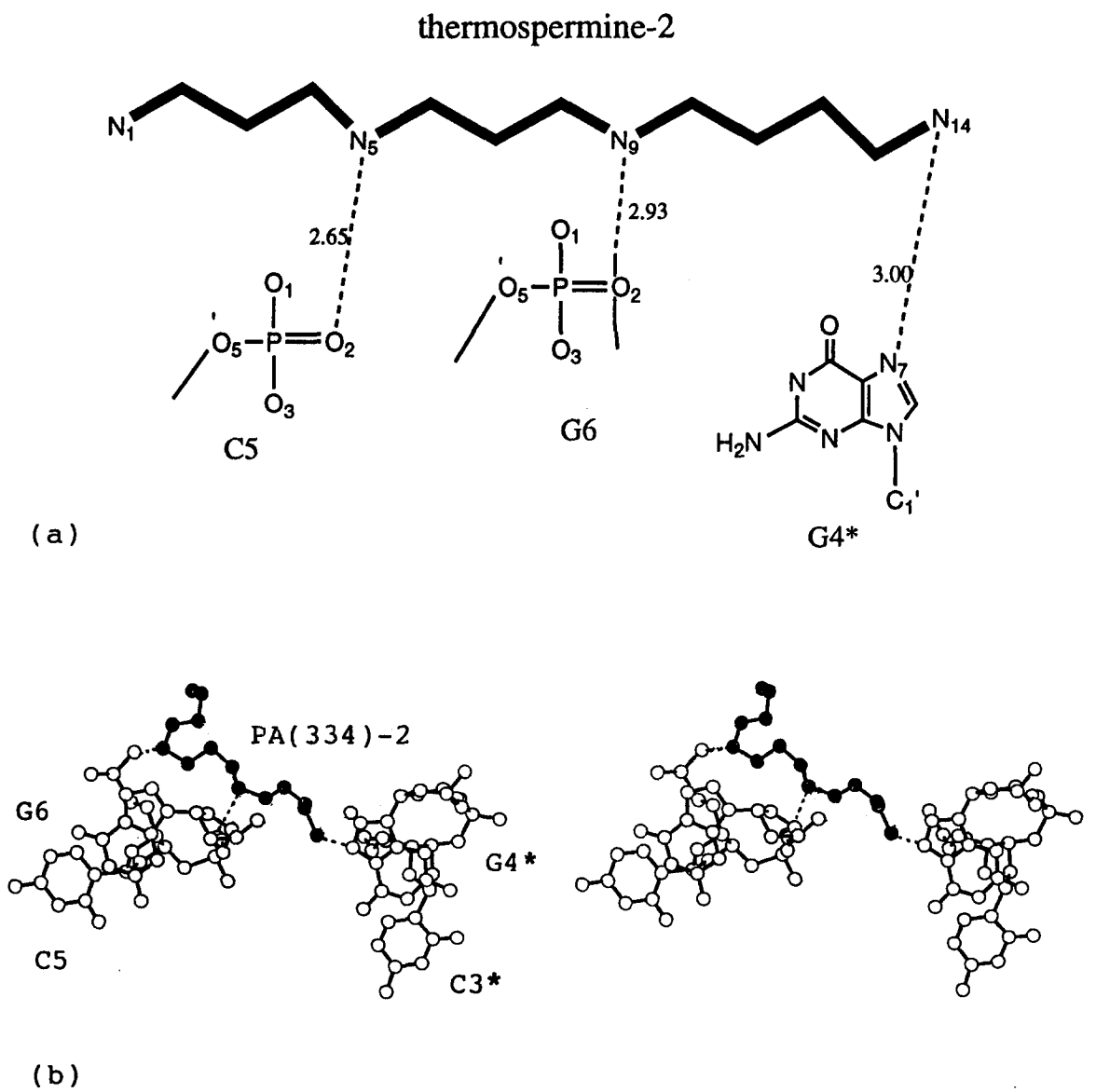

Fig. 3. Interaction between thermospermine-2 and $d(CG)_3$. (a) Schematic sketch of interaction. (b) Stereoscopic drawing around thermospermine-2. The thermospermine-2 molecule is shown by black ball and stick and water molecule is shown by shaded ball. Hydrogen bonds are shown by dotted lines.

up in the minor groove with the hydrogen bonds between the imino N(5) and N(9) atoms and the phosphate oxygen atoms of C5 and G6 of the original $d(CG)_3$ duplex, respectively. Meanwhile, the first spermine molecule in the case of the $d(CG)_3$+spermine complex crystal [4] joined together with the $d(CG)_3$ molecule at the minor groove with the hydrogen bonds between the N(1) and N(5) atoms of the spermine and the phosphate oxygen atoms of C9 and G10 of the original $d(CG)_3$ duplex, respectively. It is interesting to note that the distance between the N(5) and N(9) atoms of thermospermine is the same as that of the N(1) and N(5) atoms of spermine, and that these distances might be suitable for hydrogen bond formation with two adjacent nucleotide phosphate groups in the single $d(CG)_3$ strand at the minor groove of the $d(CG)_3$ duplex. The terminal amino N(14) atom connected directly to the N7 of the G4 base in the symmetrically related neighboring $d(CG)_3$ molecule. This is the first case where one polyamine molecule bound three surrounding left-handed $d(CG)_3$ molecules. As shown in Fig. 4, six water molecules grouped around a magnesium cation (ligancy: 6) and formed a distorted octahedron. Three out of six water molecules bound further to the bases or phosphate groups of the original and neighboring $d(CG)_3$ duplexes directly or through water molecules via several hydrogen bonds. However, a direct coordination bond was not found between the magnesium cation and the bases of the $d(CG)_3$ molecule. In the case of the $d(CG)_3$+Mg [4] and $d(CG)_3$+PA(24) complexes, a direct coordination bond was found between the magnesium cation and the base of the $d(CG)_3$ molecule. However, this was not found in the $d(CG)_3$+PA(34), $d(CG)_3$+PA(334) and $d(CG)_3$+spermine complexes. Therefore, with increasing number of amino groups in the polyamine molecule and greater length of the polyamine molecule, direct binding of the magnesium cation to the base of the $d(CG)_3$ molecule via a coordination bond becomes more difficult. Even though the binding modes between the magnesium cation and the left-

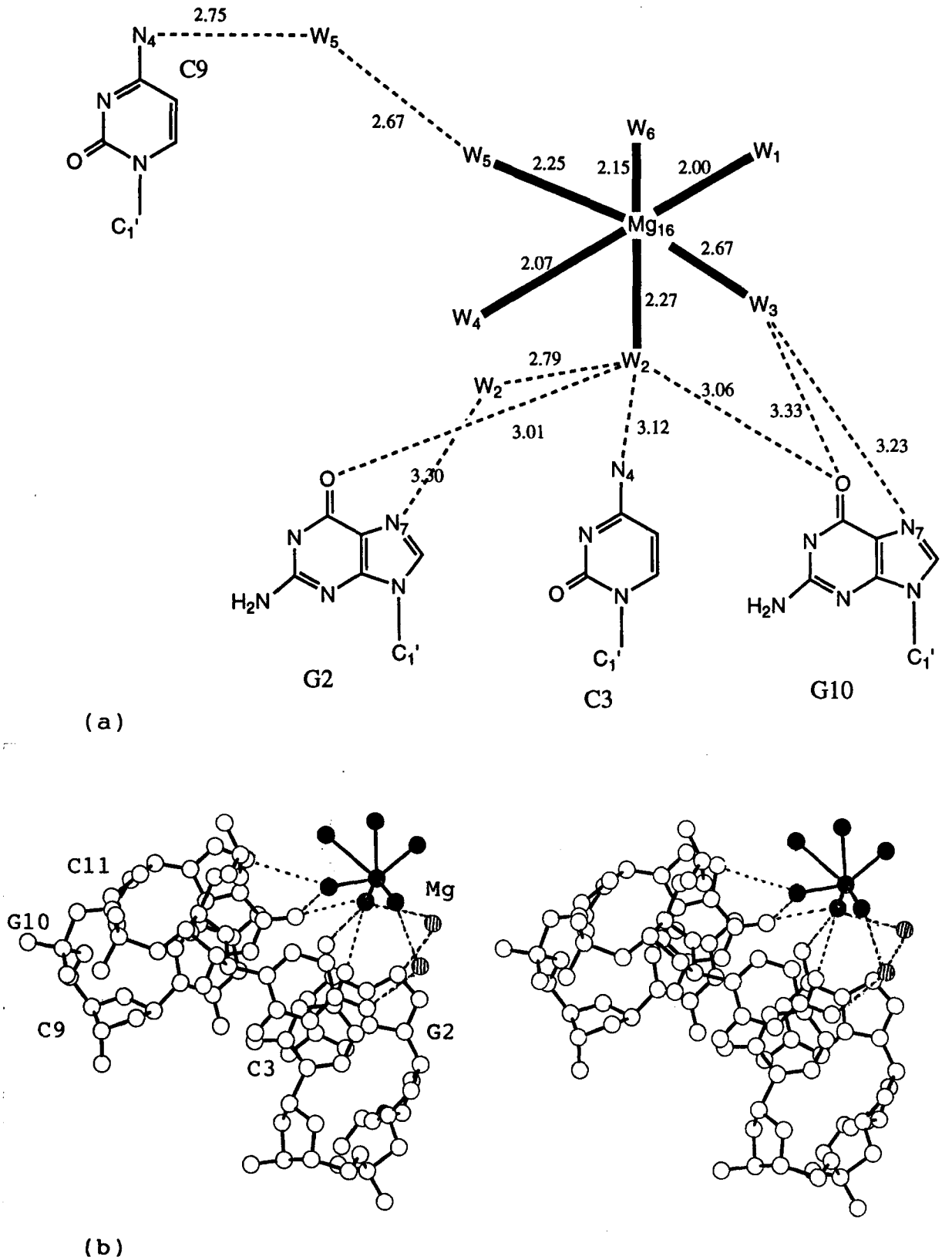

Fig. 4. Coordination geometry around magnesium cation. The upper part shows a schematic sketch of the coordination and hydrogen bonds, and the lower part, a stereoscopic drawing. The heavy line represents coordination bonds around the magnesium cation. Hydrogen bonds are shown by dotted lines.

handed $d(CG)_3$ duplex are rather different from those found in the $d(CG)_3$ complexes with PA(24), spermine and spermidine, it is obvious that metal cations may assist in the stabilization of the left-handed double-helical $d(CG)_3$ molecule;

also, the chain length and number of nitrogen atoms in the polyamine molecule are essential for tight binding and stabilization of the $d(CG)_3$ molecule. From these results, it is suggested that the polyamine plays important roles in the regulation and activation of DNA, RNA and protein and in cell proliferation.

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