Interaction between the left-handed Z-DNA and polyamine-2

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

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Abstract **This paper deals with the crystal structure of d(CG)$_3$-spermidine complex. The DNA fragment, d(CG)$_3$, was crystallized with *N*-(2-amino-propyl)-1,4-diamino-butane, PA(34), spermidine. The results of its X-ray crystallographic analysis showed many intermolecular contacts between d(CG)$_3$ and spermidine, but the binding mode of spermidine to the d(CG)$_3$ molecule is different from that of the d(CG)$_3$ and *N*-(2-amino-ethyl)-1,4-diamino-butane [PA(24)] complex: a spermidine molecule bound to the d(CG)$_3$ and its symmetrically related neighboring d(CG)$_3$ molecules through the water molecules with hydrogen bonds, while one PA(24) molecule connected directly to one d(CG)$_3$ molecule, but not to its neighboring d(CG)$_3$ molecule. In the crystal, the d(CG)$_3$ molecule was the left-handed Z-form, and three magnesium cations and a sodium cation were observed around the d(CG)$_3$ moiety with different binding modes from the case of the d(CG)$_3$-PA(24) complex.**

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

1. Introduction

We recently reported the crystal structure of the left-handed Z-DNA, d(CG)$_3$, and *N*-(2-aminoethyl)-1,4-diamino-butane [PA(24)] complex [1], in which the PA(24) molecule hydrogen-bonded tightly to the minor groove of d(CG)$_3$ and the sodium and the magnesium cations played an important role in the complex stabilization. It is well known that polyamine derivatives are essential for promoting cell growth, for inducing the biosynthesis of DNA, RNA and proteins [2], and for regulating the various enzyme activities in vitro [3]. We thought that the polyamine derivatives contributed to the inter-molecular interaction of the Z-DNA fragment, d(CG)$_3$, and the chain length of polyamine might be essential to control the strength of these interactions. Therefore, we cocrystallized d(CG)$_3$ and *N*-(2-amino-propyl)-1,4-diaminobutane named spermidine [PA(34)], which is longer than the PA(24) molecule by one ethyl group, and solved the complex crystal structure by X-ray diffraction method.

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spermidine $NH_2CH_2CH_2CH_2NHCH_2CH_2CH_2CH_2NH_2$

double helical 5'-OH-C1 -p2 -G2 -p3 -C3 -p4 -G4-p5-C5-p6-G6-OH-3'

d(CG)$_3$ 3' -OH-G12-pl2-C11-p11-G10-pl0-C9-p9-G8-p8-C7-OH-5'

2. Materials and method

The DNA hexamer d(CG)$_3$ was chemically synthesized and spermidine was purified from the organs of rat fetuses. As is well known, polyamine and metal cations are important for the conformational stability of DNA oligomers [4]. Magnesium cations are usually adequate for the crystallization of DNA oligomer and along with increases in the concentrations of polyamine, salt, and metal ion, the DNA oligomer, d(CG)$_n$, is converted from the right-handed B-form DNA to the left-handed Z-form DNA [5], and therefore, in this case, magnesium cation was added for the crystallization of the Z-form d(CG)$_3$ and spermidine complex. Single crystals were obtained within 2 weeks from a mixture containing 2 mM ammonium salt of d(CG)$_3$, 10 mM spermidine trichloride, and 15 mM $MgCl_2$ in 30 mM sodium cacodylate buffer (pH 7.0) using the vapor diffusion method. The crystal used for the X-ray study had dimensions of $0.5\times0.3\times0.3$ mm^3. The refined cell constants are: $a=17.93(1)$ Å, $b=31.23(2)$ Å, $c=44.64(1)$ Å with space group $P2_12_12_1$. These values were similar and isomorphous to those of the d(CG)$_3$ crystal and the d(CG)$_3$-PA(24) complex crystal. The unit cell volume indicates that one duplex DNA oligomer containing 12 nucleotides was involved in an asymmetric unit. The crystal was sealed into thin-walled glass capillary and the integrated intensities were measured by the continuous ω-scan method with monochromated Cu-K α radiation ($\lambda=1.5418$ Å) using the Nicolet P3 automated four-circle diffractometer at the temperature of 10°C. The intensity data were collected up to 1.0 Å resolution with the scan speed 4°/min. We applied a semi-empirical absorption correction derived by North et al. [6], to observed intensities, and the X-ray damage was corrected as a function of time. Since the cell dimensions were isomorphous with those of the d(CG)$_3$ crystal, the phases of each structure's factors were solved by the molecular replacement method with atomic coordinates of the d(CG)$_3$ [7]. Using these phases, the atomic coordinates were refined by the stereochemically restrained least squares method (program NUCLS) [8] (the initial *R*-value was 0.31). The spermidine molecule, three magnesium cations and one sodium cation were clearly located on the 2[Fo]-[Fc] map calculated by successive Fourier synthesis (program PROTEIN) [9]. As the result of 120 cycles refinement and Fourier synthesis using 6301 reflections with $[Fo]>3\sigma(Fo)$ to 1.0 Å, 117 water molecules were identified. The model building of the complex structure was performed using the program FRODO [10] on IRIS 2400 TURBO workstation and the structure was refined up to the residual (the *R*-value) of 0.191. The averaged r.m.s. deviation for atomic coordinates was 0.016 and the radial distribution of the final *R*-value gives a mean coordinate error of 0.10 from the Luzzati plot [11]. All calculations were carried out on a Micro VAX-II at the Computer Center, Osaka

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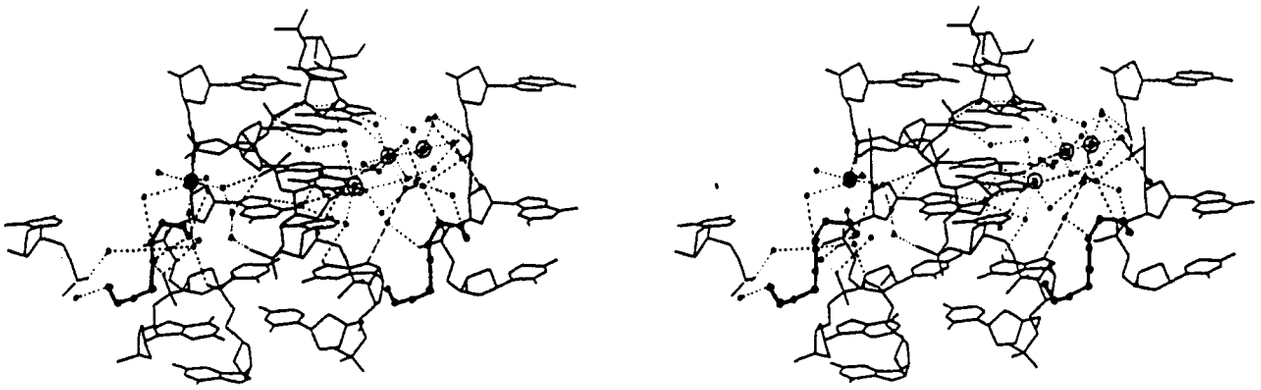

Fig. 1. Wire model stereodiagram of the molecular structure of the $d(CG)_3$-spermidine complex structure. The spermidine molecule is shown with thick lines. The dotted lines indicate the coordination bonds or hydrogen bonds around the magnesium (⊙) ion, sodium (●) ion and water molecule (•).

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 discussions

The chemical structure of double helix $d(CG)_3$ and spermidine is shown in the scheme in Section 1. Fig. 1 gives the stereoview of the molecular structure of the $d(CG)_3$ and spermidine complex determined by X-ray crystallography. As clearly shown in this figure, the $d(CG)_3$ molecule took the double helical left-handed Z-DNA conformation which contains 12 nucleotide residues having cytosine and guanine bases within one helical turn, and the $d(CG)_3$ molecules lined parallel to the *c*-axis were piled up continuously by the head-to-tail type stacking interaction between bases. A spermidine molecule, three magnesium cations and a sodium cation bound to the double helical $d(CG)_3$ molecule by several hydrogen bonds or coordination bonds via water molecules, and 10 positive charges summed up with a spermidine molecule, three magnesium cations and a sodium cation were neutralized by 10 negative charges counted in the phosphate groups of the double helical $d(CG)_3$ molecule. As shown in Fig. 2, the N1 atom of spermidine was involved in binding to the phosphate groups of G6 and G12 through water molecule with hydrogen bond, and both the imino nitrogen atom of N5 and the terminal amino nitrogen atom of N10 of the spermidine molecule participated in the hydrogen bonds to an oxygen atom of the P3 phosphate group of the symmetrically related neighboring $d(CG)_3$ molecule via water molecules, while the N1, the imino N4 nitrogen atom and the terminal N9 atom of the PA(24) molecule in the complex molecule directly bound to the phosphate groups of the $d(CG)_3$ in the minor groove, and further the terminal N9 atom hydrogen-bonded to the base groups of the neighboring $d(CG)_3$ molecule through water molecules. In the $d(CG)_3$-spermidine complex, the bridge conformation was formed between the

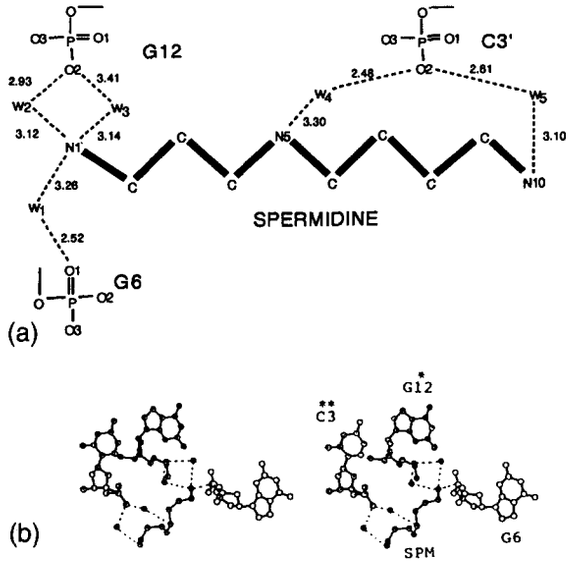

Fig. 2. The interaction between spermidine and $d(CG)_3$ molecules. (a) Schematic representation. The dotted lines show hydrogen bonds through water molecules (w). (b) Stereoscopic drawing. The base with one asterisk indicates the base of the opposite strand in the original $d(CG)_3$ duplex, and the base with two asterisks shows the base in the symmetrically related neighboring $d(CG)_3$ molecule. The numerical values are hydrogen bond distances.

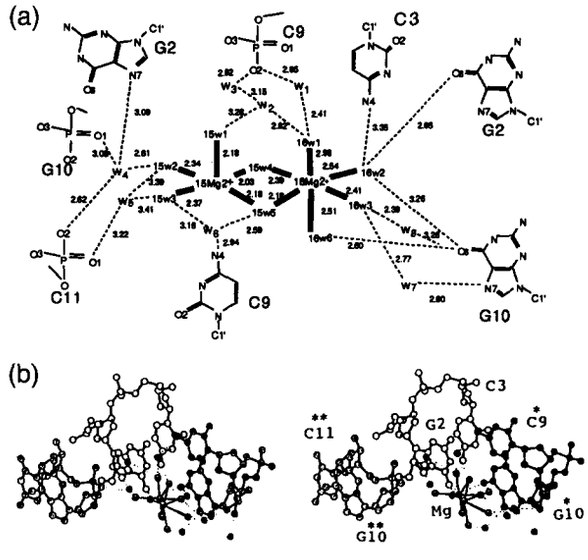

Fig. 3. The coordination geometry of the ion cluster formed by two magnesium cations (15Mg and 16Mg). (a) Schematic representation. The bold lines show the coordination bond, and the dotted lines indicate the hydrogen bonds. (b) Stereoscopic representation.

interstrand $d(GC)_3$ molecules by the hydrogen bonds which may protect a strand separation, a physical cutting or a hydrolysis of the duplex $d(CG)_3$ molecules [12]. It was indicated that all kinds of polyamine molecules may not necessarily bind in the minor groove of the DNA oligomer. However, in any case, it seems likely that the polyamine molecule is located near the minor groove of the DNA oligomer and contributes to the stabilization of the DNA oligomer [5], and furthermore, with the chain length of polyamine longer, the number of short contacts between the symmetrically related neighboring DNA oligomers tends to increase. The amount of spermidine in the tissue of the fetal rat is many times larger than that in the tissue of the mature rat. This effect shows that the spermidine molecule plays a very important role in the increasing of the cells [13]. Furthermore the $d(CG)_3$-spermine complex and $d(CG)_3$-thermospermine complex have been analyzed by X-ray crystallography ([14]; Ohishi, unpublished data), however the spermine and thermospermine molecules are too long so stabilize the double strand of the helix easily and protect against the physical cutting of the $d(CG)_3$ molecule, the spermidine molecule is too short, so far as we think that the spermidine molecule is not stabilize the double strand of the helix in the case of the $d(CG)_3$-spermine complex and the $d(CG)_3$-thermospermine complex. But the result of X-ray crystallography of the $d(CG)_3$-spermidine complex shows that the spermidine bound to the symmetrically related $d(CG)_3$ molecules, stabilized the complex crystal and protected against the physical cutting of $d(CG)_3$. It is surprising that the short chain natural polyamine played an important role in the stabilization and protection of the $d(CG)_3$ molecule. In the $d(CG)_3$-spermidine complex, three magnesium cations (15Mg, 16Mg and 17Mg) and a sodium cation (18Na) were observed. As shown in Fig. 3, 15Mg and 16Mg formed an ion cluster where 15Mg and 16Mg were coordinated with five and six water molecules to form a distorted tetrahedron and octahedron, respectively, and the cluster connected one $d(CG)_3$ duplex and its symmetrically related neighboring $d(CG)_3$ molecule by many hydrogen bonds via

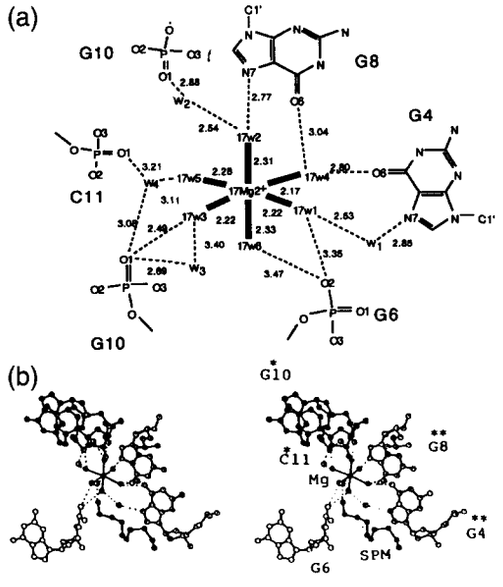

Fig. 4. The coordination geometry around the magnesium cation (17Mg). (a) Schematic drawing. (b) Stereoscopic drawing.

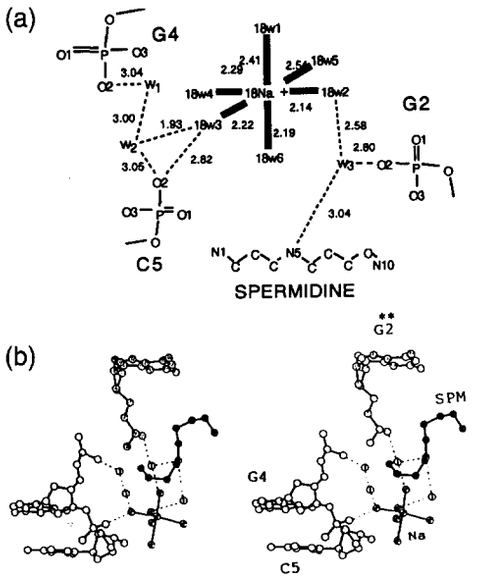

Fig. 5. The interaction of the sodium cation (18Na) with the spermidine and the $d(CG)_3$ molecules. (a) Schematic representation. (b) Stereoscopic representation.

water molecules. As shown in Figs. 4 and 5, both 17Mg and 18Na were coordinated with six water molecules to form an octahedron and the weak bridge formations between the interstrand $d(CG)_3$ molecules and also between one $d(CG)_3$ duplex and its symmetrically related neighboring $d(CG)_3$ molecules, but the magnesium or sodium cations did not directly bind to the phosphate groups or bases. In the $d(CG)_3$-PA(24) complex, on the other hand, a similar kind of ion cluster was formed by two magnesium ions connected to the interstrand $d(CG)_3$ molecules via water molecules. These results indicate that the $d(CG)_3$ molecules were stabilized by base stacking interactions, hydrogen bonds and Coulomb interactions among spermidine, metal cations, the phosphate groups of the Z-DNA fragment and the surrounding water molecules in the crystal. The bond lengths, bond angles and the G-C base-pair interaction mode of the $d(CG)_3$ duplex exhibited no difference from those of the $d(CG)_3$-spermine complex [4]. The rotation angle of the glycosyl bond of the cytidine residues took the *anti* conformation and that of the guanosine residues was *syn*. The rotation angle around the P9 phosphate was the standard Z-I type (−sc/−sc), while in the case of the $d(CG)_3$-PA(24) complex, that was the unusual Z-II type (ap/+sc) [1].

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References

[1] Ohishi, H., Kunisawa, S., van der Marel, G., van Boom, J.H., Rich, A., Wang, A. H. J., Tomita, K. and Hakoshima, T. (1991) FEBS Lett. 284, 238–244.
[2] Elizabeth, R. and Williams, R.J.P. (1987) Biochem. J. 245, 641–647.
[3] Alexandre, H. and Geuskens, M. (1984) Arch. Biol. 95, 55–70.
[4] Gessner, R.V., Frederick, C.A., Quigley, G.J. and Rich, A.H.J. (1989) J. Biochem. 264, 7921–7935.
[5] Crawford, J.H., Kolpak, F.J., Wang, A.H.J., Quigley, G.J., van

Boom, J.H., van der Marel, G. and Rich, A. (1980) Proc. Natl. Acad. Sci. USA 77, 4016–4020.
[6] North, A.C.T., Phillips, D.C. and Matthews, F.S. (1968) Acta Crystallogr. A24, 351–359.
[7] Wang, A.H.J., Quigley, G.J., Kolpk, F.J., Crawford, J.L., van Boom, J.H., van der Marel, G. and Rich, A., (1979) Nature 282, 680–686.
[8] Hendrickson, W.A. and Konnert, J.H. (1980) in: Biomolecular Structure, Function, Conformation and Evolution (Srinivasan, R., Ed.) Vol. 1, pp. 43–57, Pergamon Press, Oxford.
[9] Steigeman, W. (1974) Dissertation, TU, Munich.
[10] Jones, T.A. (1978) J. Appl. Crystallogr. 11, 268–272.
[11] Luzzati, P.V. (1952) Acta Crystallogr. 5, 802–810.
[12] Morgan, J.E. and Blankenship, S.W. (1986) Arch. Biochem. Biophys. 24, 225–232.
[13] Alexandre, H. and Geuskens, M. (1984) Arch. Biol. 95, 55–70
[14] Gessner R.V., Frederick C.A., Quigley G.J., Rich A. and Wang, A.H.-J. (1989) J. Biol. Chem. 264, 7921–7935.