The crystal structure of N^1-[2-(2-amino-ethylamino)-ethyl]-ethane-1,2-diamine (polyamines) binding to the minor groove of d(CGCGCG)$_2$, hexamer at room temperature

Hirofumi Ohishi[a,*], Kenji Suzuki[b], Masahiro Ohtsuchi[c], Toshio Hakoshima[d], Alexander Rich[e]

[a] *Osaka University of Pharmaceutical Sciences, Nasahara, Takatsuki, Osaka 569-l094, Japan*
[b] *Chemistry Research Laboratories, Dainippon Pharmaceutical Co., Ltd., Enoki, Suita, Osaka 564-0053, Japan*
[c] *Research Division, Taiyo Yakuhin Kogyo Co., Akizawa, Gifu, Gifu 501-1182, Japan*
[d] *Department of Molecular Biology, Nara Institute of Science and Technology, Takayama, Ikoma, Nara 630-0101, Japan*
[e] *Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA*

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Abstract The crystal structure of a left-handed Z-DNA hexamer, d(CG)$_3$ in complex with a synthetic polyamine, N^1-[2-(2-amino-ethylamino)-ethyl]-ethane-1,2-diamine, NH_3^+-$(CH_2)_2$-NH_2^+-$(CH_2)_2$-NH_2^+-$(CH_2)_2$-NH_3^+ [PA(222)], has been determined by the X-ray diffraction method at 1.0 Å resolution. In an orthorhombic crystal, the d(CG)$_3$ duplex binds two PA(222) molecules, and this synthetic polyamine exhibits dual conformational properties. One of the two PA(222) molecules resides on the floor of the minor groove of a Z-DNA duplex and imino groups bridge the two phosphate chains across a double helix, while the terminal amino groups link the oxygen atoms O2 of four cytosine bases. This PA(222) molecule makes a U-turn like a fishhook at one of its ends to provide a micro-environmental network previously unseen in complexes of DNA with polyamines. The width of the minor groove does not become considerably greater with the looped end of the polyamine, indicating conformational rigidity of the Z-DNA backbone imposed by the high stacking energy of the GC base pairs. While polyamine binding to the minor groove has been postulated by theoretical studies for stabilizing the Z-DNA double helical conformation, the finding in the crystal of the looped polyamine chain binding the minor groove of Z-DNA is observed for the first time from the data collected at 10°C (so-called room temperature data). Another PA(222) molecule binds on the convex outer surface of the major groove of the Z-DNA duplex and links three d(CG)$_3$ duplexes which are symmetrically related to each other. The structure of this PA(222) presents the previously reported zigzag type conformation [Egli et al., Biochemistry 30 (1991) 11388–11402]. Comparison of this structure with other polyamine–DNA cocrystals reveals structural themes and differences that may relate to the length of the polyamine.

*Corresponding author. Fax: (81)-726-90 1090.
E-mail address: ohishi@gly.oups.ac.jp (H. Ohishi).

Abbreviations: d(CG)$_3$, d(CGCGCG)$_2$; d(GC)$_3$, d(GCGCGC)$_2$; d(CGCGAATTCGCG), d(CGCGAATTCGCG)$_2$; d(GTGTACAC), d(GTGTACAC)$_2$; PA(222), N^1-[2-(2-amino-ethylamino)-ethyl]-ethane-1,2-diamine; PA(343), NH_3^+-$(CH_2)_2$-NH_2^+-$(CH_2)_2$-NH_2^+-$(CH_2)_2$-NH_3^+; spermine, NH_3^+-$(CH_2)_3$-NH_2^+-$(CH_2)_4$-NH_2^+-$(CH_2)_3$-NH_3^+

Key words: Z-DNA:polyamine complex; Binding in minor groove; X-ray crystal structure

1. Introduction

The importance of polyamines in biological systems and their function in mediating the interactions of biological anions such as nucleic acids are well known [2]. There are numerous reports on the interaction of natural and synthetic polyamines with nucleic acids, and on the phenomena of the B-DNA to Z-DNA transition of poly[d(G-m^{5}C)] and poly-[d(G-C)] [3,4]. In the A-DNA octamer, d(GTGTACAC), the spermine binds to the floor of the major groove and takes an S-shaped configuration by adopting the gauche conformation around the near central C–N and C–C bonds [5]. In the crystal structure of a B-DNA dodecamer d(CGCGAATTCGCG) [6], only one spermine molecule was found to be spanning the upper ends of the major groove. Most polyamines in complex with Z-DNA oligomers [1,7–15] were found to be linkers of two self-complementary strands or to bridge neighboring double-stranded helices, with the exception of the d(CG)$_3$–spermine complex, whose structure indicated the binding of spermine in the minor groove of a Z-DNA hexamer [11]

In theoretical studies on polyamine–nucleic acid interactions, the best known molecular models for the B-DNA–spermine complex are those by Subirana and by Liquori [16–18]. In these models, the spermine molecule is expected to bridge two backbone strands across a B-DNA minor groove and two terminal amino groups of spermine would neutralize two phosphates of opposite chains. A model for the interaction of polyamines with B-DNA simulated by theoretical calculations [19,20] indicated the best fitting of spermine in the minor groove of the center of d(CGCGAATTCGCG). In contrast, using conformational energy and molecular mechanics calculations Feuerstein et al. [21] obtained the best model when an extended spermine molecule bridged two complementary strands across the major groove of the d(GC)$_3$ duplex.

To compare the theoretical calculations with the experimen-

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Table 1
The crystal data of $d(CG)_3$–PA(222) complex

Cell dimensions	PA(222)
a (Å)	17.93
b (Å)	31.36
c (Å)	44.62
α (°)	90
β (°)	90
γ (°)	90
Space group	$P2_12_12_1$
Crystal system	Orthorhombic
No. of reflections measured	30 955
No. of reflections independent	10 897
No. of reflections used 3σ	9 119
Completeness	100
Used reflections completeness	83.7
Resolution (Å)	1.0
Z	4
R-value	0.138

tal results and to investigate the binding properties of other than spermine polyamines, we crystallized the $d(CG)_3$ hexamer in the presence of the synthetic polyamine PA(222). The crystal structure of the $d(CG)_3$ duplex in complex with PA(222) has revealed that one polyamine molecule binds to the floor of the minor groove of the Z-DNA hexamer while the other serves as a bridge to link the double-stranded helices in the crystal lattice according to the motif previously observed by Egli et al. [1].

2. Materials and methods

The DNA hexamer $d(CG)_3$ was purchased from Bex and the polyamine, PA(222), $NH_2CH_2CH_2NHCH_2CH_2NHCH_2CH_2NH_2$ N^1-[2-(2-amino-ethylamino)-ethyl]-ethane-1,2-diamine were purchased from Sigma-Aldrich. In the abbreviations used for polyamines the numerals after PA given in parentheses indicate the number of methylene groups separating amino and imino functions in polyamine. Single crystals were obtained within 2 weeks at +15°C from a solution containing 2 mM ammonium salt of $d(CG)_3$, 10 mM appropriate polyamine tetrachloride salt, 15 mM $MgCl_2$ in 30 mM sodium cacodylate buffer (pH 7.0), and 20% MPD using the vapor diffusion method. X-ray diffraction data were collected at KEK (BL18B) synchrotron and SPring-8 (BL41XU) facilities. The 120-frame image data were collected by 1.5° up to 1.0 Å resolution. The image data were processed and integrated by the program MOSFLM, and scaling was performed by the program SCALA in the CCP4 package [22], truncate and agrovata were carried out by the CCP4 package.

Structures were solved using the Amore CCP4 package by rotation and translation function and the program Turbo Frodo [23] for display.

Refinement initially used the simulated annealing and molecular dynamics method with the program X-PLOR [24]. Following this, the residual-block diagonal least-squares method with anisotropic temperature factors for the nucleic acid oligomer $d(CG)_3$ and with isotropic temperature factors for the appropriate polyamine was applied using the program SHELXL-97 [25]. All calculations were carried out on a Compaq Alpha DS10 (Compaq, Maynard, MA, USA) and O2 workstation (SGI, Mountain View, CA, USA) at the Information Science Center, Osaka University of Pharmaceutical Sciences. Data collecting statistics for $d(CG)_3$–PA(222) are given in Table 1. The atomic coordinates have been deposited in the Brookhaven Protein Databank (entry number 1DJ6).

3. Results and discussion

3.1. Packing of $d(CG)_3$–PA(222) complex and overall structure

Three crystal structures of Z-DNA–polyamine complexes determined in this study, $d(CG)_3$–PA(222), are isomorphous to those of other $d(CG)_3$ hexamer crystals presented earlier [8,9]. However, in the present structures each polyamine binds to double-stranded helices according to its own hydrophobic properties and in a quite different manner. Since we only discuss briefly the packing of double-stranded helices for $d(CG)_3$–PA(222), and we will focus on the structural features of polyamines bound to the minor groove of $d(CG)_3$, the helical axis of the Z-DNA hexamer duplex coincides with the two-fold screw axis, and the Z-DNA hexamer duplexes stack in an end-to-end fashion to form an infinite helix. The length of the c-axis, 44.62 Å, is just enough to accommodate one helical turn (12 base pairs per turn) with two piled Z-DNA hexamer duplexes. In this paper Fig. 1 presents the projection along the c-axis of $d(CG)_3$–PA(222) and shows two PA(222) molecules, the one of an intra-helix mode occupies the minor

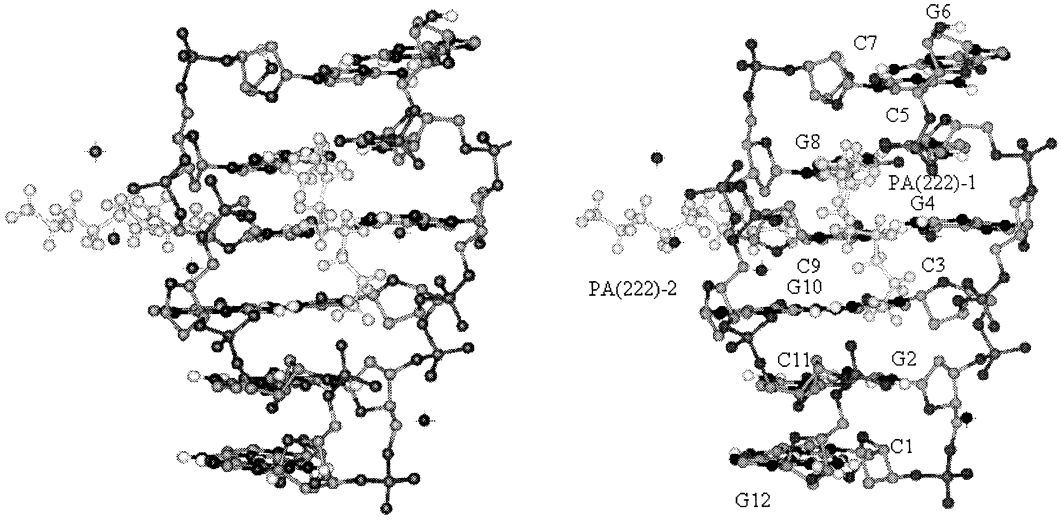

Fig. 1. Stereodrawings of a view into the minor groove in structures of the $d(CG)_3$–PA(222) complex. Intra-bound PA(222)-1 sits in the minor groove at the center of the double-stranded helix between G2–C11 and C5–G8 base pairs, and inter-bound PA(222)-2 is positioned perpendicularly to the helix axis and connects surrounding helices.

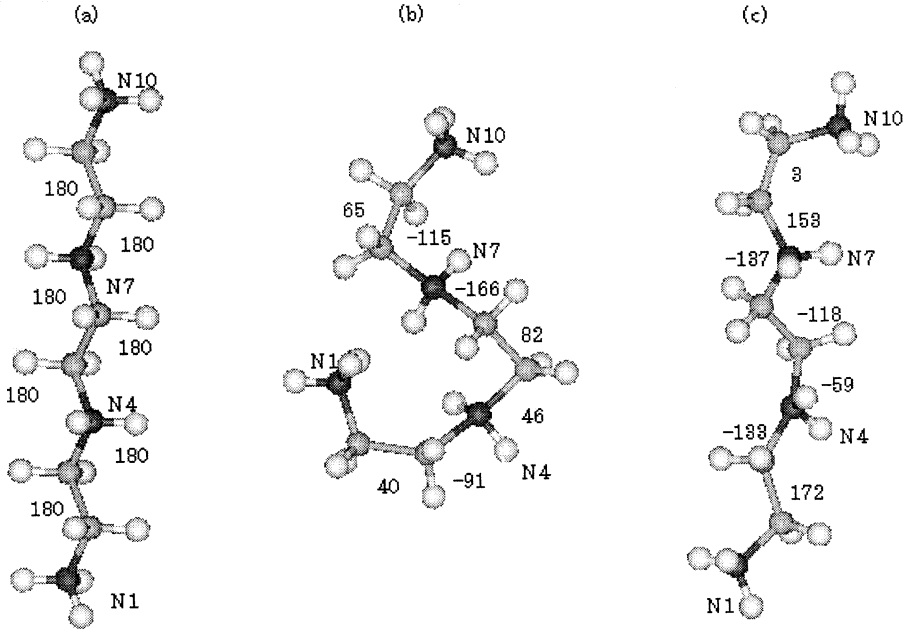

Fig. 2. Presentation of the conformations of polyamines from theoretical calculations and from the results of crystal structure determination of $d(CG)_3$–polyamine complexes. The bond lengths and torsion angles are given next to each bond of the polyamine molecule. The N1 amino group is at the lowest end of each polyamine chain. a: Calculated by MOPAC the most stable zig-zag conformation of PA(222). b: The loop-type conformation of PA(222) found in the crystal of the $d(CG)_3$–PA(222) complex. This PA(222)-1 molecule resides in the minor groove of the $d(CG)_3$ duplex. c: Disordered zig-zag conformation of PA(222)-2 molecule which is found to be a spacer bridging the parallel helices in the crystal lattice.

groove, and the other molecule of an inter-helix mode binds to the surface of the $d(CG)_3$ duplex.

In the $d(CG)_3$ duplexes the phosphorus atoms of one strand are numbered as P2 following the first base up to P6, and those of the complementary strand are numbered P8–P12, respectively. In Fig. 1 the cytosine C1 is at the lowest part of the helix on its right side.

3.2. Conformations of PA(222) molecules in the $d(CG)_3$–PA(222) complex

Two PA(222) molecules are found in this crystal of the $d(CG)_3$–PA(222) complex. One molecule is bound to the floor of the minor groove of the left-handed $d(CG)_3$ hexamer duplex and is referred to as 'intra-helix PA(222)' or PA(222)-1 (Fig. 1). The other PA(222) molecule is present on the convex surface of the Z-DNA duplex and mediates the contact between neighboring duplexes using several hydrogen bonds that stabilize the crystal lattice. This is called the 'inter-helix PA(222)' or PA(222)-2. The intra-helix PA(222)-1 molecule does not preserve an energetically preferred extended zig-zag conformation (Fig. 2a) but instead, as is shown in Fig. 2b, the overall conformation of PA(222)-1 changes because of a kink produced at the N1 amino group. This results in U-turning of PA(222)-1 apparently due to the bend occurring between the C3–N4–C5–C6 bonds. In contrast, in the inter-helix PA(222)-2 molecule, the torsion angles around several bonds correspond to the *trans* forms and the overall PA(222)-2 conformation is close to the extended zig-zag form shown in Fig. 2c.

3.3. Intra-helix PA(222)-1:Z-DNA interaction

The Fourier electron density map in Fig. 3a shows the PA(222)-1 molecule located close to the floor of the minor groove of the left-handed $d(CG)_3$ duplex.

The previously reported structure of the $d(CG)_3$–PA(343) complex, the so-called pure-spermine $d(CG)_3$, already has revealed an affinity of PA(343) to bind in the minor groove of the $d(CG)_3$ hexamer [11]. Comparison of the binding patterns seen in our structure of the $d(CG)_3$–PA(222) complex with the structure of the $d(CG)_3$–PA(343) crystal shows many structural differences:

1. The binding site of PA(222)-1 is found to be in the central region of the $d(CG)_3$ duplex between base pairs G2–C11 and C5–G8 (Fig. 1) while PA(343) begins its N1 end at the inter-helix junction of two stacked $d(CG)_3$ duplexes and extends its N14 end up to phosphate P10 (see fig. 6 in the paper by Bancroft [11]).
2. PA(222)-1 resembles the conformation of a particular shape, like that of a fishhook, while PA(343) assumes the shape of a twisted U because of the sharp bend of both its ends.
3. The most noticeable differences in the structure of the double helix between the set of PA(222) and PA(343) are the torsion angles around phosphates P5 and P9. Phosphate P5 in PA(222) and also phosphate P9 in PA(343) show the conformational rearrangement from the Z-I form to the Z-II form (α and β change to positive values) apparently due to the presence of the metal ions (magnesium ion of PA(222) and sodium ion of PA(343)) coordinated to the oxygen atoms of the respective phosphate [11].
4. The N1 and N10 atoms of the amino groups of PA(222)-1 recognize cytosine bases O2 of both strands directly or through water molecules, and the N4 and N7 imino nitrogens are bound to phosphate oxygens of both strands directly or also through water molecules. In the structure of PA(343) the recognition patterns are reversed. The imino nitrogens N5 and N10 recognize cytosine O2 of both strands, and the N1 and N14 amino nitrogens recognize the oxygen atoms of phosphates of G residues of the op-

posite strand, respectively. These minor differences may be due to the different number of methylene groups engaged as spacers between the nitrogen atoms in the PA(222) and PA(343) molecules [11].

Binding of PA(222)-1 in the minor groove of $d(CG)_3$ duplex neither expands nor elongates the double helix, and the minor groove width remains virtually the same compared with the standard Z-DNA structure with no polyamine in the minor groove [8,9].

As in a typical Z-form the phosphate groups flanking the minor groove are connected in two zig-zag lines, and two kinds of P–P distances between the opposite strands are found. There is a long distance between P5 and P11 (10.62 Å) and a shorter distance between P4 and P12 (2.80 Å) as well as between P6 and P10 (3.04 Å) (distances are less 5.8 Å, the radius of the phosphorus atom).

PA(222) liganded to $d(CG)_3$ widens an inherently minor groove only between phosphates P6 and P8 by as little as 1.42 Å compared to $d(CG)_3$ alone. P5–P11 distances in the $d(CG)_3$–PA(222)-1 structure are even shorter by 1.68 Å. Thus binding of PA(222)-1 in the minor groove does not cause a serious extension of the P–P distances compared to standard Z-DNA. Calculated local helix parameters using the FREEHELIX program [26] indicate only very small deviations from the standard Z-DNA structure. The largest differences in the helical twists between both structures, $d(CG)_3$–PA(222)-1 and the standard $d(CG)_3$, do not exceed 3.88°. However, the roll deformation of the C5–G6 base steps is more pronounced in the structure of $d(CG)_3$ (6.5°) than in the structure of $d(CG)_3$–PA(222)-1 where the roll is only 0.14°. For the $d(CG)_3$–PA(222)-1 structure, the only rise of the C3–G4 base step is 3.4 Å rather corresponding to the B-form; however, this single base step is counterbalanced by an increased rise of the C5–G6 base step (4.0 Å), and the overall complex remains Z-form. Thus, the P5–P11 distance in this structure is even shorter by about 2 Å than that of the standard $d(CG)_3$ structure. The conformational transition around the P4 phosphorus atom from the Z-I form (in the standard Z-DNA structure) to the Z-II form in this crystal is another contributing factor compressing the minor groove.

As noted in Section 1, Liquori et al. [17] pointed out that the most stable conformation of the spermine molecule takes a fully extended *trans* zig-zag form. It would be reasonable to expect that the smooth elongation of terminal amino groups of polyamine along the minor groove would reach the target, the most distant phosphates of the opposite ends of DNA. However, the crystal structure of $d(CG)_3$–PA(222)-1 shows that PA(222), instead of being of a *trans* zig-zag conformation, makes a loop within a minor groove, and the terminal amino groups participate in a hydrogen bond network directly with the phosphates P6 and P10 and with oxygen O2 of C3 and C11. The Z-DNA conformation is considerably more rigid than the A or B forms, and these specific features of a Z-form of the $d(CG)_3$ hexamer lead to inducing a U-turn of the N1 amino-ethylene terminal. The kink particularly involves bonds C3–N4–C5–C6 followed by a change in the zig-zag conformation around these atoms, and its N10 terminal makes connections with the oxygen atoms O2 of cytosine C5 and C9 (Figs. 2b and 4a).

It is worth mentioning that a fishhook-like-shaped spermine molecule was also found in the major groove of the tRNA double helix near the junction of the D stem and the anticodon stem in the phenylalanine tRNA crystal [27]. Our structure is a second example that shows the looped polyamine.

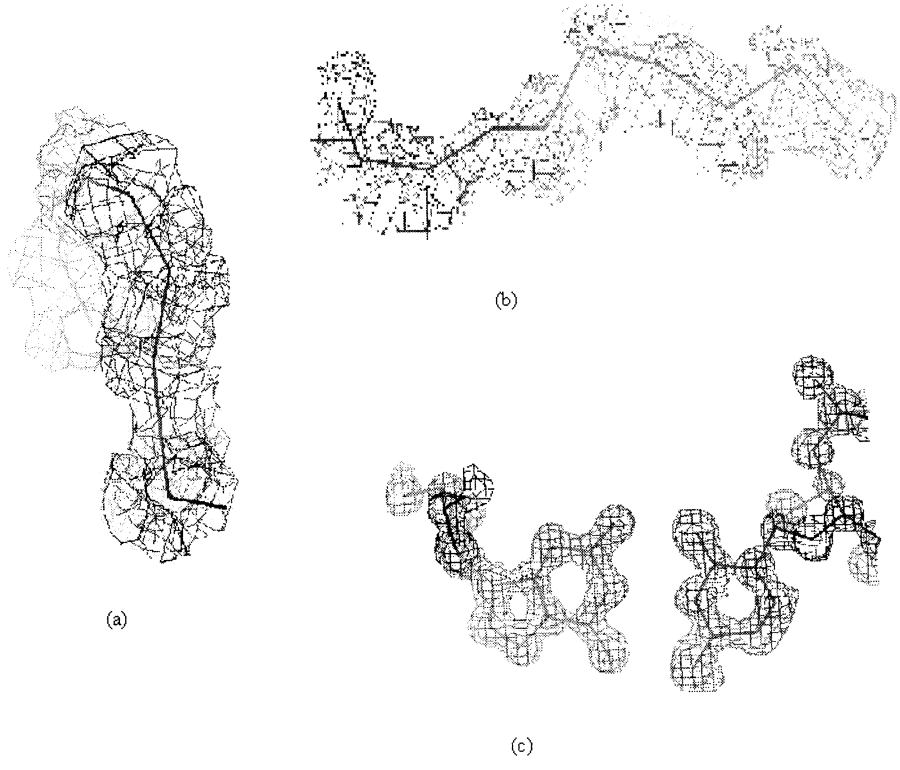

Fig. 3. Fourier electron density map of polyamines located in the minor groove of the $d(CG)_3$ duplex. a: PA(222)-1; b: PA(222) in the outer side of minor groove; c: Base pair of G12–C1.

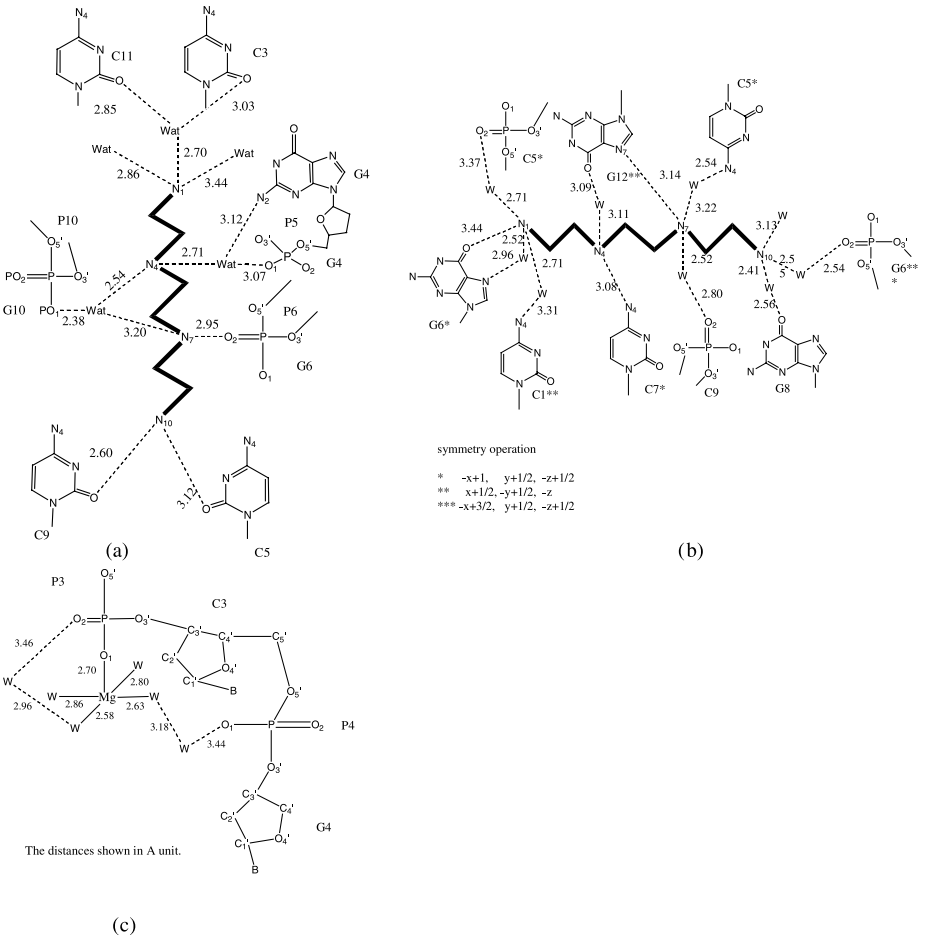

Fig. 4. Schematization of binding of polyamines to the double-stranded $d(CG)_3$ hexamer. Unrolled helix backbone diagram shows the polyamines closest contact to the floor of the minor groove. Except PA(222)-2 all other polyamines reside in the minor groove of helix. a: Minor groove bound PA(222)-1; b: inter-helix bound PA(222)-2; c: geometry of magnesium ion located on the edge of the double helix, found only in the $d(CG)_3$–PA(222) complex. The drawing shows the hydrated magnesium ion connected to the wall of the helix via oxygen of phosphates P3 and P4.

The reasons for formation of a U-turn-like structure by certain polyamines and its implications in realistic biological systems are still not elucidated.

3.4. Inter-helix PA(222)-2:Z-DNA interaction

It is only mentioned briefly that the inter-helix PA(222)-2 molecule is located on the convex outer surface of the major groove of the Z-DNA duplex. It is surrounded by three symmetry-related Z-DNA duplexes. As described above, the PA(222)-2 molecule takes roughly an extended form (Figs. 1 and 2c), and interacts simultaneously with three neighboring $d(CG)_3$ duplexes directly or through water molecules (Fig. 4b). The nitrogen atoms, N1 and N4, that contact the neighboring $d(CG)_3$ duplex residues are marked in Fig. 4b with single and double asterisks. The N7 imino nitrogen atom of PA(222) participates in a hydrogen bonding directory to the N7 of the guanine base of G12 (residue with double asterisks), and to the N4 of cytosine C5 (residue with a single asterisk) via a water molecule.

The nitrogen atom of the amino group, N10, is hydrated with three water molecules, and one of these three waters additionally makes a hydrogen bond with the oxygen of phosphate P6 (G with triple asterisks). Thus, the PA(222)-2 molecule participates in strong interactions with three surrounding Z-DNA duplexes, by involving its inherent positive charges to neutralize the phosphate negative charges.

3.5. Magnesium ion

Besides two PA(222) molecules, one magnesium ion was found in the asymmetric unit of this crystal. Eight positive charges of two PA(222) molecules and one magnesium ion completely neutralize the 10 negative charges of the phosphate groups of the $d(CG)_3$ hexamer duplex. A distorted tetrahedral pyramidal coordination geometry around the magnesium ion with the five surrounding ligands (four water molecules and one phosphate oxygen atom of P3) is shown in Fig. 4c. In this magnesium cluster, two of four coordinated water molecules extend the hydrogen bonds through other shell water molecules to connect the oxygen atoms of phosphates P3 and P4, respectively. As noted above, these hydrogen bonds may be responsible for the Z-I to Z-II conformational transition around P4, and also for the stabilization of a Z-form DNA hexamer duplex. Such pyramidal geometry of water molecules coordinated to the magnesium ion was also found in the two structures of the $d(CG)_3$–PA(24) complex and the $d(CG)_3$–PA(34) complex [13,14].

3.6. Conclusion

Analysis of crystal structures of different polyamines in complex with $d(CG)_3$ indicates a variety of affinities of the polyamines toward the CGCGCG sequence [28]. This accounts for the short polyamines bridging two complementary strands across the major groove of the $d(CG)_3$ duplex, and those which structures are rather read out by the minor groove. Each polyamine bound to the $d(CG)_3$ helix demonstrates a different effect on stabilization of the Z-DNA. Short polyamines containing two or three amino/imino groups tend to bind to the phosphate counter-anions while the longer polyamines are found to be more specific in recognition of the minor groove. Minor groove binding polyamines afford a small molecule recognition code that identifies the minor groove of GC base pairs by reading out DNA bases, perhaps as a sequence or by distinguishing the structure and the polarized CG and GC base pair. The designed match is achieved by a factor of good correlation of the distances between the NH groups of polyamines with a phosphodiester backbone.

Recognition of the $d(CG)_3$ hexamer by PA(222) and previously reported smaller polyamines provides us impetus to further explore the extent of CG base pair recognition [29], which is driven not only by a sequence but also may be directed by the structure and hydrophobic properties of the minor groove. Thus, the details of polyamine–DNA interactions should contribute to gain insight into the molecular level to develop biological research for designing drugs specifically binding selected DNA sequences.

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References

[1] Egli, M., Williams, L.D., Gao, Q. and Rich, A. (1991) Biochemistry 30, 11388–11402.
[2] Tabor, C.W. and Tabor, H. (1984) Annu. Rev. Biochem. 53, 749–790.
[3] Behe, M.J. and Felsenfeld, G. (1981) Proc. Natl. Acad. Sci. USA 78, 1619–1623.
[4] Chen, H.H., Behe, M.J. and Rau, D.C. (1984) Nucleic Acids Res. 12, 2381–2389.
[5] Jain, S., Zon, G. and Sundaralingan, M. (1989) Biochemistry 28, 2360–2364.
[6] Chiu, T.K., Kaczor-Grzeskowiak, M. and Dickerson, R.E. (1999) J. Mol. Biol. 292, 589–608.
[7] Crawford, J.L., 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.
[8] Wang, A.H.J., Quigley, G.J., Kolpak, F.J., Crawford, J.L., van Boom, J.H. and Rich, A. (1979) Nature 282, 680–686.
[9] Wang, A.H.J., Quigley, G.J., Kolpak, F.J., van der Marel, G.A., van Boom, J.H. and Rich, A. (1981) Science 211, 171–176.
[10] Fujii, S., Wang, A.H.J., van der Marel, G.A., van Boom, J.H. and Rich, A. (1982) Nucleic Acids Res. 10, 7879–7892.
[11] Bancroft, D., Williams, L.D., Rich, A. and Egli, M. (1994) Biochemistry 33, 1073–1086.
[12] Tomita, K., Hakoshima, T., Inubushi, K., Kunisawa, S., Ohishi, H., van der Marel, G.A., van Boom, J.H., Wang, A.H.J. and Rich, A. (1989) J. Mol. Graph. 7, 71–75.
[13] Ohishi, H., Kunisawa, S., Van der Marel, G.A., van Boom, J.H., Rich, A., Wang, A.H.J., Tomita, K. and Hakoshima, T. (1991) FEBS Lett. 284, 238–244.
[14] Ohishi, H., Nakanishi, I., Inubushi, K., van der Marel, G.A., van Boom, J.H., Rich, A., Wang, A.H.J., Hakoshima, T. and Tomita, K. (1996) FEBS Lett. 391, 153–156.
[15] Ohishi, H., Terasoma, N., Nakanishi, I., van der Marel, G.A., van Boom, J.H., Rich, A., Wang, A.H.J., Hakoshima, T. and Tomita, K. (1996) FEBS Lett. 398, 291–296.
[16] Shui, X., McFail-Isom, L., Hu, G.G. and Williams, L.D. (1998) Biochemistry 37, 8341–8355.
[17] Liquori, A.M., Constantino, L., Crescenzi, V., Ella, V., Giglio, E., Puliti, R., De Santis Savine, M. and Vitaglino, V. (1967) J. Mol. Biol. 24, 113–122.
[18] Suwalsky, M., Traub, W., Shlnueli, U. and Subirana, J.A. (1969) J. Mol. Biol. 42, 363–373.
[19] Zakrzewska, K. and Pullman, B. (1986) Biopolymers 25, 375–392.
[20] Sy, D., Hugot, S., Savoye, C., Ruiz, S., Charlier, M. and Spotheim-Maurizot, M. (1999) Int. J. Radiat. Biol. 75, 953–961.
[21] Feuerstein, B.G., Pattabiraman, N. and Marton, L.J. (1986) Proc. Natl. Acad. Sci. USA 83, 5948–5952.
[22] Collaborative computational project, number 4 (1994) Acta Crystallogr. D50, 760–763.
[23] Jones, T.A., Zou, J.Y., Cowan, S.W. and Kjeldgaard, M. (1991) Acta Crystallogr. A47, 110–119.
[24] Brunger, A.T. (1992) X-PLOR Version 3.l: A System for X-ray Crystallography and NMR. Yale University Press, New Haven, CT.
[25] Sheldrick, G.M. (1997) SHELXL97. Program for Refinement of Crystal Structure, University of Göttingen, Göttingen.
[26] Dickerson, R.E. (1998) Nucleic Acids Res. 26, 1906–1926.
[27] Quigley, G.J., Teeter, M.M. and Rich, A. (1978) Proc. Natl. Acad. Sci. USA 75, 64–68.
[28] Korolev, N., Lyubartsev, A.P., Nordenskiold, L. and Laaksonen, A. (2001) J. Mol. Biol. 308, 907–917.
[29] Swalley, S.E., Baird, E.E. and Dervan, P.B. (1997) J. Am. Chem. Soc. 119, 6953–6961.