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Interaction between the left-handed Z-DNA and polyamine

The crystal structure of the $d(CG)_3$ and *N*-(2-aminoethyl)-1,4-diamino-butane complex

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The DNA fragment $d(CG)_3$ was co-crystallized with *N*-(2-aminoethyl)-1,4-diaminobutane (PA(24)), a chemically synthesized polyamine. The complex crystal contained one polyamine, 3 magnesium cations and one sodium cation per duplex of $d(CG)_3$, and well diffracted the X-ray intensities up to 1.0 Å resolution. The $d(CG)_3$ took a left-handed Z-DNA conformation, and the PA(24) molecule electrostatically interacted with the phosphate groups of the $d(CG)_3$ duplex.

Z-DNA; Polyamine; Crystal structure; Molecular interaction; Molecular conformation

1. INTRODUCTION

Polyamine could be one of the promoting factors in carcinogenesis because it is known that the content of polyamine is ten times higher in the cancerous organ compared to the normal one [1-6]. The causality between the polyamine and carcinogenesis has been investigated by biological and molecular biological techniques.

Recently the genes which are activated in some types of cancers were identified, and it became clear that left-handed DNA played an important role in the mechanism of carcinogenesis [7]. Thus, the study of interaction between DNA and polyamine has been receiving increasing interest. Here we deal with the crystal structure (resolution = 1.0 Å) of $d(CG)_3$ and *N*-(2-aminoethyl)-1,4-diaminobutane complex (PA(24)), where $d(CG)_3$ is a Z-DNA fragment and PA(24) is an artificially synthesized polyamine. The chemical structure of PA(24) is shown in Fig. 3.

2. EXPERIMENTAL

$d(CG)_3$ and PA(24) were chemically synthesized. Since it is well known that both the polyamine and metal cations stabilize the DNA conformation [8], we used magnesium ions for the crystallization of the $d(CG)_3$-PA(24) complex. Single crystals were obtained within 2 weeks from a mixture containing 2 mM ammonium salt of $d(CG)_3$, 10 mM PA(24) trichloride, and 15 mM $MgCl_2$, in 30 mM sodium cacodylate buffer (pH 7.0) using the vapor diffusion method. The crystal size used for the X-ray work had the dimensions $0.5 \times 0.3 \times 0.5$ mm^3. Precession photographs showed that the cell dimensions were: $a = 17.94(1)$ Å, $b = 31.23(1)$ Å, $c = 44.55(1)$ Å, with space group $P2_12_12_1$. From the unit cell volume in an asymmetric unit, the existence of the one duplex containing 12 nucleotides was estimated.

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 devised by North et al. [9], and the X-ray damage was corrected as a function of time.

Since the cell dimensions were almost the same as those of the $d(CG)_3$ crystal [10], the phase problem was solved by the molecular replacement method [11] using atomic coordinates of the $d(CG)_3$ [10]. A PA(24) molecule, 3 magnesium ions and a sodium ion were identified by the difference Fourier synthesis. The existence of approximately 100 water molecules became clear through the successive Fourier syntheses, where the Fourier syntheses were carried out using 8921 reflections with $|F_o| > 3\ \sigma(F_o)$ to 1.0 Å resolution. The model building of the complex structure was performed using the program FRODO [12,13] on an IRIS 2400 turbo workstation and was refined by the stereochemically-restrained least-squares method [14] to a residual value (*R*-value) of 0.161. The averaged r.m.s. deviation for atomic coordinates was 0.014. The radial distribution of the final *R*-factor gives a mean co-ordinate error of 0.10 [15]. All calculations were carried out on a Micro VAX-II at the Computer Center, Osaka University of Pharmaceutical Sciences, and on an Acos-930 system at the Research Center for Protein Engineering of Institute for Protein Research, Osaka University.

3. RESULTS AND DISCUSSION

The molecular conformation of $d(CG)_3$ constituted a Z-type DNA fragment with a left-handed form, as is

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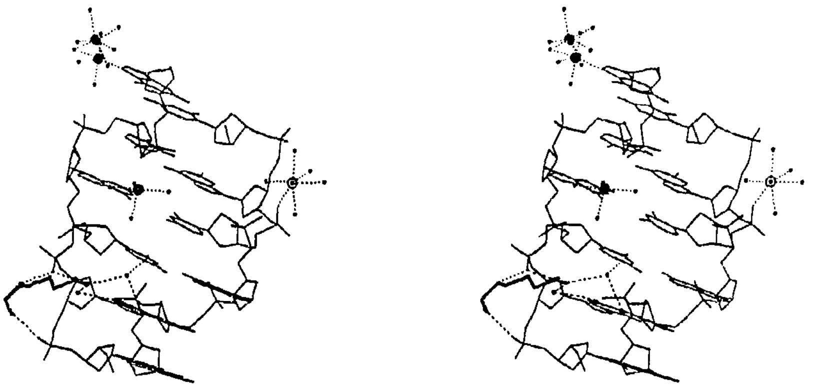


Fig. 1. Wire model diagram of d(CG)$_3$-PA(24) complex structure. The PA(24) molecule is shown with thick lines. The sodium ⊙ and magnesium ● ions are placed on the upper side of the d(CG)$_3$ molecule. The dotted lines show the coordination bonds or hydrogen-bonds.

shown in Fig. 1. Two different conformations were found for the GpC phosphate group (P9), as shown in Fig. 2. One is the frequently observed Z-I type, where the ξ torsion angle of the guanosine group (G8) is in the $-sc$ conformation and the α angle of the cytidine group (C9) is in *ap* conformation. The other is a relatively unique Z-II type, in which both of the preceding ϵ and ξ torsion angles change from $-sc/-sc$ (Z-I type) to $ap/+sc$. The refined structure showed that one PA(24) molecule possessing 3 positive charges per molecule, one sodium ion and 3 magnesium ions exist in an asymmetric unit and neutralize the negative charges of the phosphate groups of the d(CG)$_3$ duplex molecule. The interaction mode between the PA(24) and d(CG)$_3$ is shown in Fig. 3 and the coordination geometries of the sodium and magnesium ions are shown in Fig. 4.

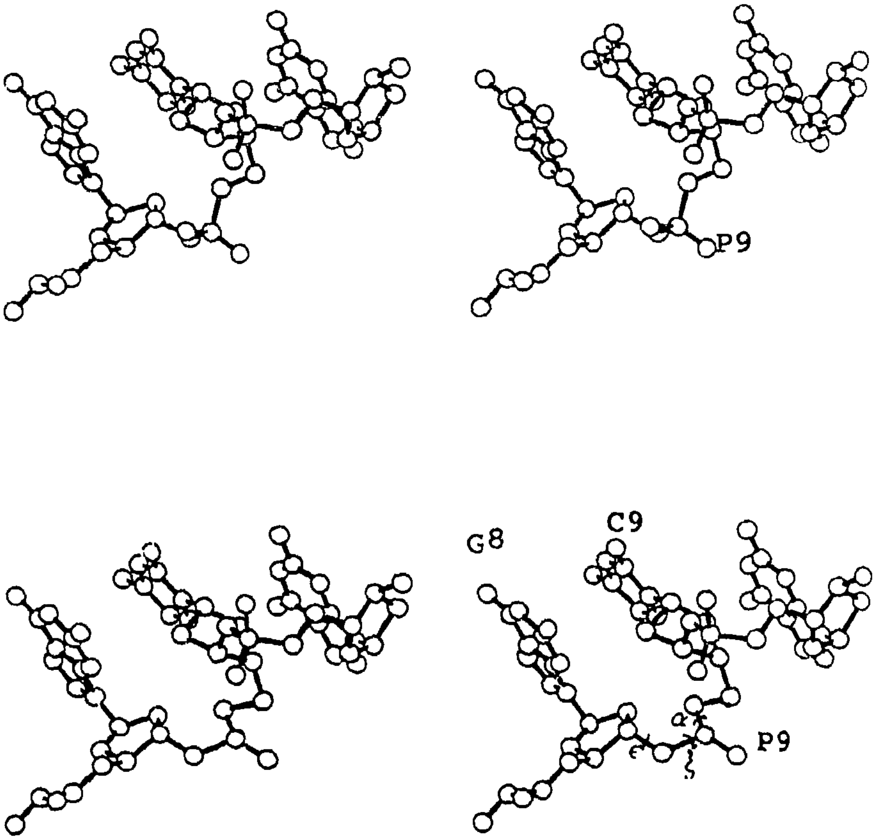


Fig. 2. The Z-I and Z-II conformation around the P9 phosphate group observed in the d(CG)$_3$. The upper and lower stereo diagrams show the Z-I and Z-II conformations, respectively.

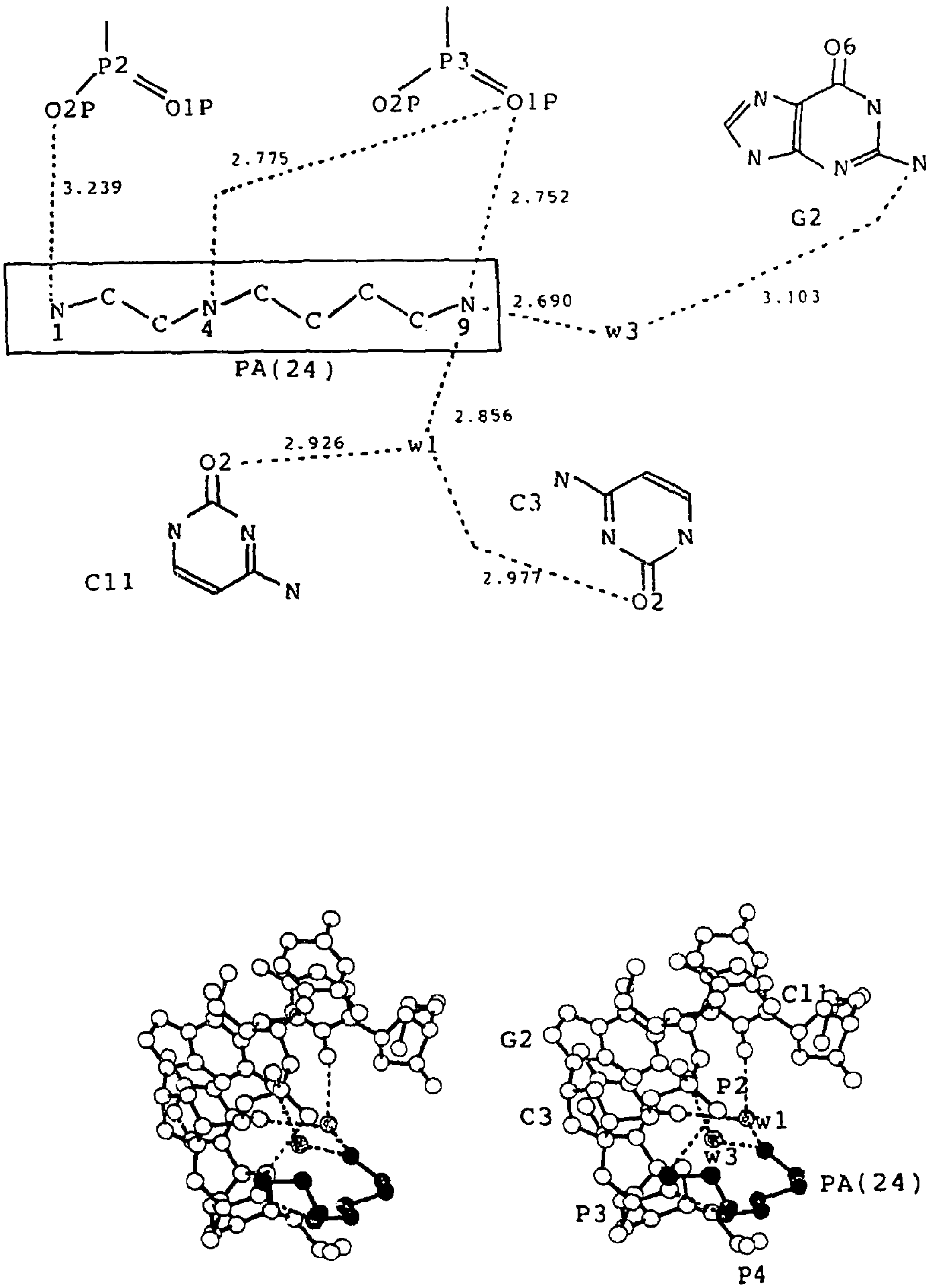


Fig. 3. The interaction between the PA(24) and d(CG)$_3$. The upper part of the figure shows a schematic sketch of the interaction, and the lower part, a stereoscopic drawing. The PA(24) molecules are shown with the black balls and two water molecules are shown with the shaded balls. Hydrogen bonds are shown as dotted lines.

The PA(24) molecule took a warped conformation around the d(CG)$_3$. The nitrogen atoms of two terminal amino (N9) groups and an imino (N4) group participated in the binding to the DNA fragment. The terminal and the imino nitrogen atoms were hydrogen-bonded to the oxygen atoms of the P3 phosphate group. The N9 nitrogen atom of PA(24) was further weakly bound to the N2 of G2, O2 of C3 and O2 of C11 base group through hydrogen-bonds via water molecules. On the other hand, the other terminal N1 atom of

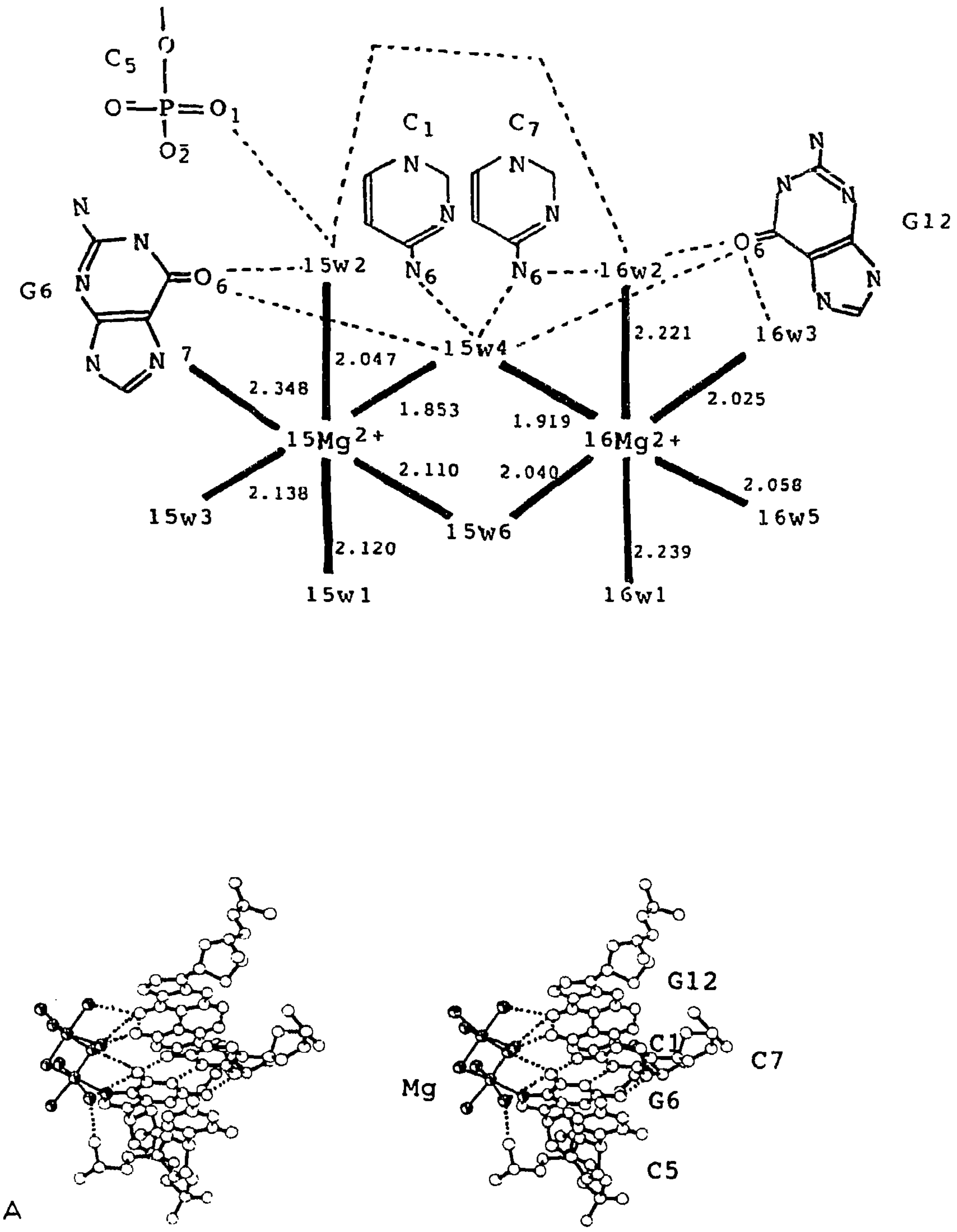
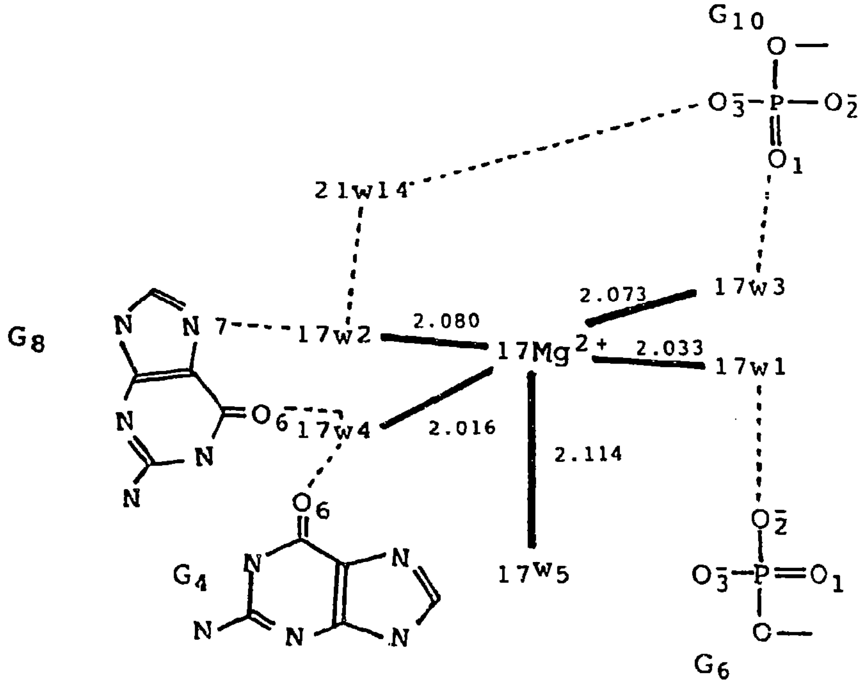
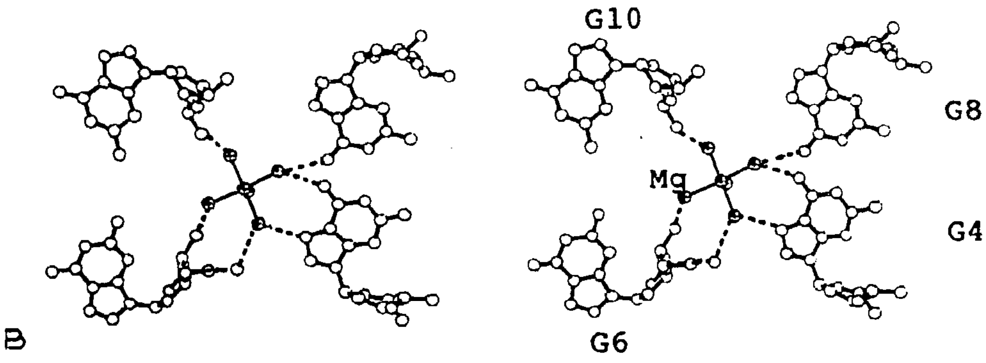


Fig. 4. The coordination geometries of the magnesium ions (A and B) and sodium ion (C). The upper parts of the respective figures show schematic sketches of the coordination, and the lower parts, stereoscopic drawings. Dark lines represent coordination bonds. Hydrogen bonds are shown as dotted lines. (Fig. 4B and C, see p. 242 and 243).

PA(24) was hydrogen-bonded to the oxygen atom of the P2 phosphate group. Thus the PA(24) molecule binds directly to the one side of the duplex with a fashion of twining its molecule around the helix of the duplex molecule. In this complex structure, the weak bridge formations between the interstrand $d(CG)_3$ molecules were formed by the hydrogen bond via the PC(24) and water molecules. Such bridge formations have also been observed in the structures of Z-DNA [16] and B-DNA [17] complexed with spermine.

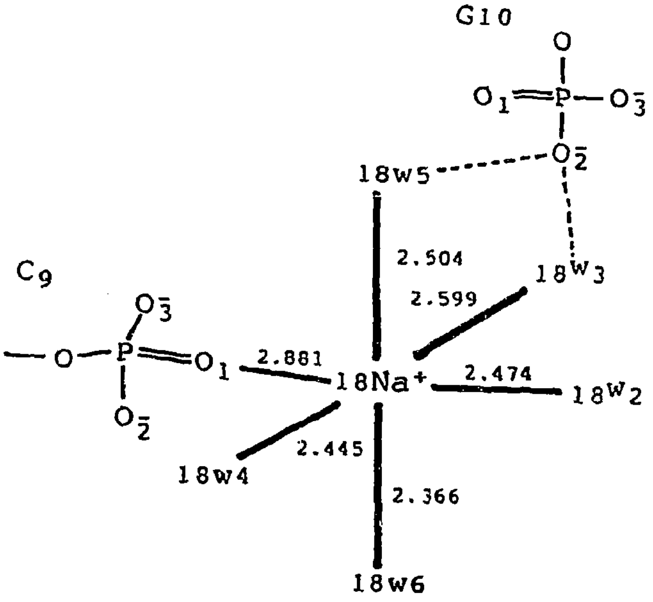


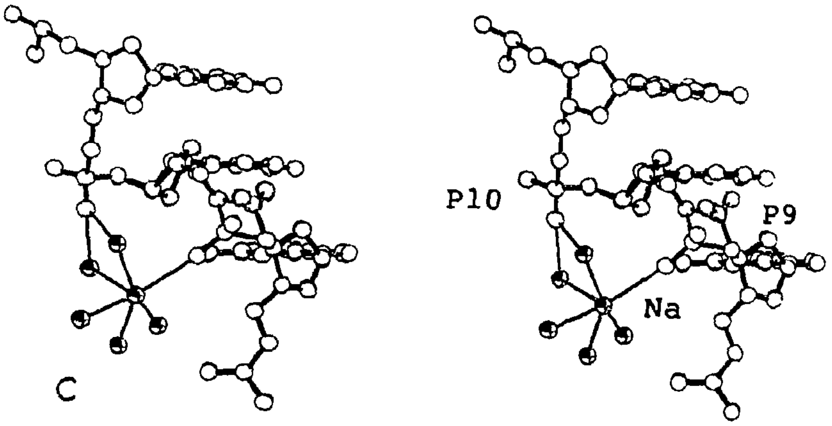


(Fig. 4B).

Three types of coordination polyhedra around the sodium and magnesium cations were observed. As is shown in Fig. 4C, one sodium cation was coordinated with 5 water molecules and an oxygen atom of the phosphate group (C9) of a Z-I conformation. Furthermore 2 of the 5 coordinated water molecules were hydrogen-bonded to the oxygen atom of the phosphate group (G10).

One of 3 magnesium cations was coordinated with 5 water molecules (Fig. 4B) so that the coordination





(Fig. 4C).

mode belongs to a kind of tetrahedron style, not an octahedron one. These water molecules participate in many hydrogen bonds with bases and phosphate groups. On the other hand, the cluster of magnesium cations which were connected to 9 water ligands was observed (Fig. 4A), and interestingly, the N7 atom of the guanine base (G6) participated in the coordination geometry. Water molecules were hydrogen-bonded to the O6 atom of guanine base (G6,G12), O1 atom of the phosphate group (C5) and N6 atoms of cytosine base (C1,C7). Those coordination geometries are similar to those of native Z-DNA [10].

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