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Crystallographic studies of metal ion – DNA interactions: different binding modes of cobalt(II), copper(II) and barium(II) to N^7 of guanines in Z-DNA and a drug – DNA complex

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ABSTRACT

Metal ion coordination to nucleic acids is not only required for charge neutralization, it is also essential for the biological function of nucleic acids. The structural impact of different metal ion coordinations on DNA helices is an open question. We carried out X-ray diffraction analyses of the interactions of the two transition metal ions Co(II) and Cu(II) and an alkaline earth metal ion Ba(II), with DNA of different conformations. In crystals, Co(II) ion binds exclusively at the N^7 position of guanine bases by direct coordination. The coordination geometry around Co(II) is octahedral, although some sites have an incomplete hydration shell. The averaged Co-N^7 bond distance is 2.3 Å. The averaged Co-N^7-C^8 angle is 121°, significantly smaller than the value of 128° if the Co-N^7 vector were to bisect the C^5-N^7-C^8 bond angle. Model building of Co(II) binding to guanine N^7 in B-DNA indicates that the coordinated waters in the axial positions would have a van der Waals clash with the neighboring base on the 5′ side. In contrast, the major groove of A-DNA does not have enough room to accommodate the entire hydration shell. This suggests that Co(II) binding to either B-DNA or A-DNA may induce significant conformational changes. The Z-DNA structure of Cu(II)-soaked CGCGTG crystal revealed that the Cu(II) ion is bis-coordinated to N^7 position of G10 and #G12 (# denotes a symmetry-related position) bases with a trigonal bipyramid geometry, suggesting a possible N^7-Cu-N^7 crosslinking mechanism. A similar bis-coordination to two guanines has also been seen in the interaction of Cu(II) in m^5CGUAm^5CG Z-DNA crystal and of Ba(II) with two other Z-DNA crystals.

INTRODUCTION

DNA is a polyelectrolyte with negative charges associated with its phosphate groups (1). In solution, charge neutralization is mostly attained from the positive charges of metal ions. It is therefore not surprising to find nucleic acids bound with metal ions *in vivo*. The coordinated metal ions are important for the biological action of nucleic acids. It is believed that metal ions serve to screen the negative charges by direct interactions with the phosphate oxygen atoms. The most common intracellular metal ion is the potassium ion. Magnesium is another important metal ion for the function of nucleic acids. For example, magnesium ion plays an integral role in the folding of transfer RNA (2–4). It has also been shown to be essential in the action of ribozyme action (5). To visualize how these metal ions bind to nucleic acids, we used high resolution x–ray diffraction studies of oligonucleotides which offer reliable answers that complement other spectroscopic studies. For example, the structures of several Z-DNA structures (6–8), B-DNA structures (9–12) and drug-DNA complexes (13–18) revealed the locations of magnesium and sodium ions. In crystals, both sodium and magnesium ions have been found to interact with various sites on nucleic acids, including phosphate oxygen and N^7 of guanine. Interestingly, the interactions may be either through direct metal ion coordination or mediated through water molecules of the metal ion's hydration shell.

Transition metal ions such as cobalt(II), copper(II) have different binding characteristics toward DNA (19). They bind almost exclusively by coordinating to the N^7 position of purine, especially of guanine. While there have been extensive structural studies of heavy metal ion interactions with nucleic acid components (e.g., bases, nucleosides) (20, reviewed in 1), very little information is available on how they interact with macromolecular nucleic acid at the molecular level. A limited number of studies on the interaction of Pb(II) with tRNA indicated that it attacked the phosphate group, causing a backbone breakage under neutral pH condition (21).

More recently, the availability of oligonucleotide crystals made it possible to diffuse different metal ions into the crystal lattice. The reasonably open solvent channels afforded the diffusion of metal ions. The first CGCGCG Z-DNA structure (spermine form) was solved by the multiple isomorphous replacement (MIR) method using Ba(II) and soaked-Co(II) as heavy atoms (6). Co(II)

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was found to coordinate to the N^7 of G6 residue. Subsequently, the studies on the interactions of $Ru(NH_3)_6^{+3}$ (22), $Co(NH_3)_6^{+3}$ (23) and Cu(II) (24, 25) with Z-DNA have been carried out. In a study of the cisplatin-CGCGAATTCGCG complex, cisplatin [i.e., cis-diamminodichloroplatinum(II)] was diffused into the B-DNA crystals. The N^7 of guanine was observed as one of the square planar Pt ligands (26).

We extended the study further to Co(II) as well as Ba(II) ions and compared it with that of the Cu(II) ion (24, 25). The goal is to understand the different preferred coordination geometries of Co(II), Ba(II) and Cu(II) to DNA. Our approach takes advantage of the excellent crystal quality of Z-DNA (6, 8) and that of daunorubicin-DNA complex (14). This affords a reliable determination of the metal ion locations and the water molecules coordinated to it. We describe six new DNA crystal structures in which the different modes of interaction of Co(II), Cu(II) and Ba(II) ions to DNA have been carefully analyzed.

MATERIALS AND METHODS

The preparation of the 'native' crystals used here have been described in earlier studies. They are the magnesium and spermine forms of CGCGCG (6, 8), the magnesium form of CGCGTG (27), and the tetragonal form of MAR70-CGT[2-amino-A]CG formaldehyde-crosslinked complex (18). After each crystal appeared and stopped growing, a small volume of metal ion solution (1 mM solution of $CoCl_2$ or $CuCl_2$) was added to each crystallization dip and allowed to equilibrate with the reservoir. This was repeated until metal ion precipitated from the crystallization dip. The crystals were allowed to soak in the solution for one to seven days, depending upon their stability and color change. The Co(II)-soaked crystals had a light pink color, whereas the Cu(II)-soaked crystals were light green in color.

Suitable crystals were chosen for crystallographic studies. Each metal ion soaked crystal was mounted in a thin-walled glass capillary and sealed with a droplet of the crystallization mother liquor for data collection. The metal ion soaked crystals were isomorphous with their respective 'native' crystals. The diffraction data sets were collected by the ω-scan mode, to a resolution of 1.5 Å or higher, at room temperature on a Rigaku AFC-5R rotating-anode diffractometer. The AFC-5R was at a power of 50 KV and 180 mA with graphite monochromated CuK_α radiation (1.5418 Å). Lorentz-polarization, absorption and decay corrections were applied to all data sets.

A total of six new structures has been analyzed. In each case, the atomic coordinates from the 'native' crystal structures were used as the starting model for the refinement by the Konnert-Hendrickson constrained refinement procedure (28, 29). After many cycles of refinement with all available data, the R-factor in general reached ~30% at the limiting resolution. If the metal ion had a reasonable occupancy, it could be easily located from the Fourier $(2|F_o| - |F_c|)$ maps. Inclusion of the metal ion(s) normally improved the R-factor by about 5% to 8%, depending on the number and occupancy of the metal ion sites. Water molecules were then located from subsequent Fourier $(2|F_o| - |F_c|)$ maps and added to the refinement. On the average, the final crystallographic R-factor is ~20%, a typical value seen in many other oligonucleotide crystal structures. The crystallographic data and the refinement results are summarized in Table 1. We noted that the temperature factor of some metal ions were high, likely due to their low occupancies. Refinement

Table 1. Crystal data of metal-DNA complexes

Complex	Unit Cell Dimension (Å) a	b	c	Space Group	R-factor	Resolution (Å)	No.of Fo (>2σ(Fo))	Rmsd (Å)	Reference
Co-CGCGCG	17.78	31.15	44.63	$P2_12_12_1$	0.196	1.5	2,386	0.025	This work
Co-CGCGCG-Spm[a]	17.94	31.14	44.77	$P2_12_12_1$	0.237	1.5	2,777	0.026	This work
Co-CGCGTG	17.18	31.41	45.62	$P2_12_12_1$	0.215	1.5	1,894	0.021	This work
Co-CGCGCG-MAR70[b]	28.00	28.00	52.54	$P4_12_12$	0.202	1.5	2,044	0.014	This work
Cu-CGCGTG	17.16	31.42	45.53	$P2_12_12_1$	0.180	1.5	2,122	0.016	This work
Ba-m^5CGm^5CGTG	17.16	31.42	45.53	$P2_12_12_1$	0.197	1.3	4,201	0.023	This work
Ba-NH_2-CGbr^{5}CGCG	51.13	18.44	34.67 β=120.9°	C2	0.164	1.4	3,727	0.011	(#34)
Cu-CGCGCG	18.01	31.03	44.80	$P2_12_12_1$	0.198	1.2	4,107[c]	---	(#24)
Cu-m^5CGUAm^5CG	17.59	30.58	44.52	$P2_12_12_1$	0.209	1.3	2,587[c]	---	(#25)

[a] Spm is the spermine form of CGCGCG Z-DNA crystals (Wang *et al.*, 1979, reference 6).
[b] MAR70 is a derivative of daunorubicin (Gao *et al.*, 1991a, reference 10, 18).
[c] No. of Fo>2σ(I).

of the metal ions' occupancy were tested, but they did not yeild unequivocally better results. We decided to use only temperature factors as indicators of occupancy. The final atomic coordinates of these structures have been deposited at Brookhaven Protein Databank.

RESULTS

Co(II) and Z-DNA interactions

Co(II) ion has been successfully soaked into three different Z-DNA crystals, i.e., the magnesium forms of CGCGCG (8) and CGCGTG (27) and the spermine form of CGCGCG (6). The solvent channels of this crystal lattice are distributed between the Z-DNA helices stacked end-over-end along the c-axis direction. Figure 1 shows three CGCGCG hexamer Z-DNA helices (Mg-form) along with some of the coordinated Co(II) ions, viewed down the helix axis direction.

In the magnesium form of the CGCGCG and CGCGTG native crystals (8, 27), magnesium and sodium ions were the only counter ions used for the crystallization. The diffusion of Co(II) ions into the crystals presumably displaced some of the originally bound Mg(II) and Na(I) ions. It may be a result of the stronger binding between Co(II) and DNA. This is illustrated in Figure 2, which compares two base pairs of the CGCGTG structure *before* and *after* the Co(II) soaking. In the native crystal, a fully-hydrated magnesium ion is seen to interact with the base pairs on the major groove side. The water molecules coordinated to Mg(II) ion form hydrogen bonds with the guanine bases. After the Co(II) soaking, two Co(II) ions were found to directly coordinate to two separate guanines (G4 and G8). Note that the magnesium ions at these

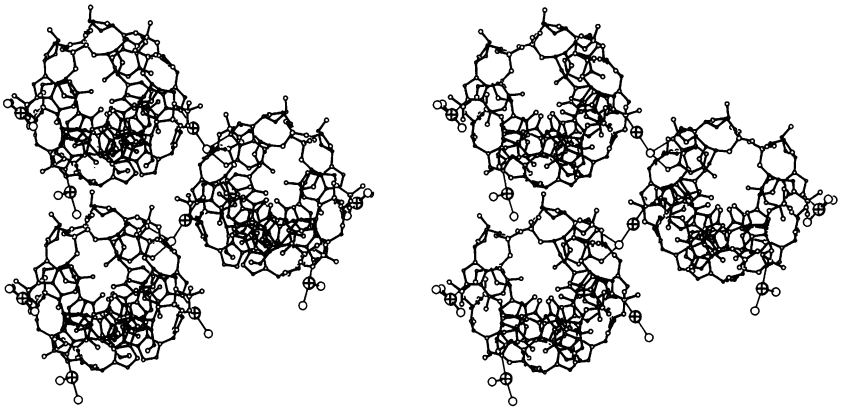

Figure 1. Stereoscopic skeletal drawing of the packing of three Z-DNA helixes viewed along the helix axis (c-axis). The coordinated Co(II) ions are shown as larger crossed circles and the open circles associated with these are the waters. The waters can be seen hydrogen bonded to the symmetry related helices.

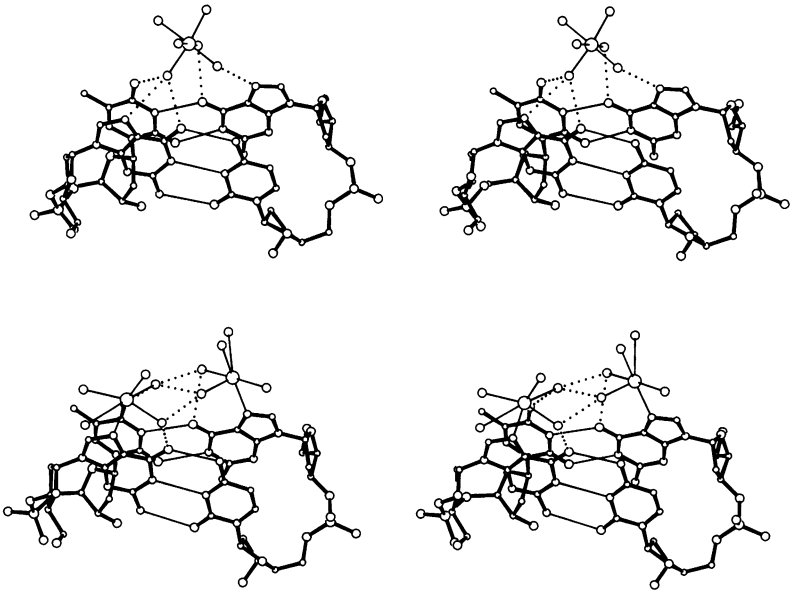

Figure 2. Comparison of the interaction between metal ions and two base pairs (T5-G8 and G4-C9) in the CGCGTG structures. (Top) Magnesium form. (Bottom) Co(II)-soaked form.

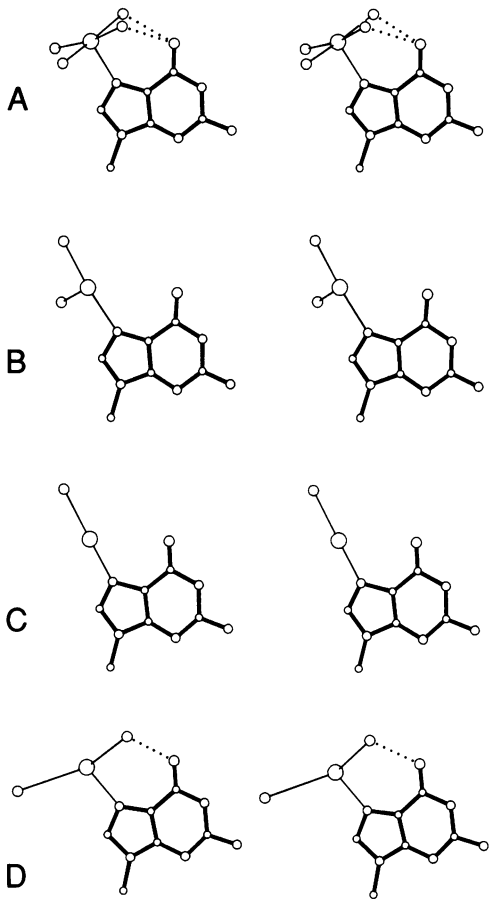

Figure 3. Comparison of the interaction between Co(II) and different guanine bases in Mg-form (A−C) and spermine-form (D) of CGCGCG Z-DNA structures. **(A)** Co2-G6. **(B)** Co1-G4. **(C)** Co3-G10. **(D)** Co-G6. See Table 2 for their coordination geometry.

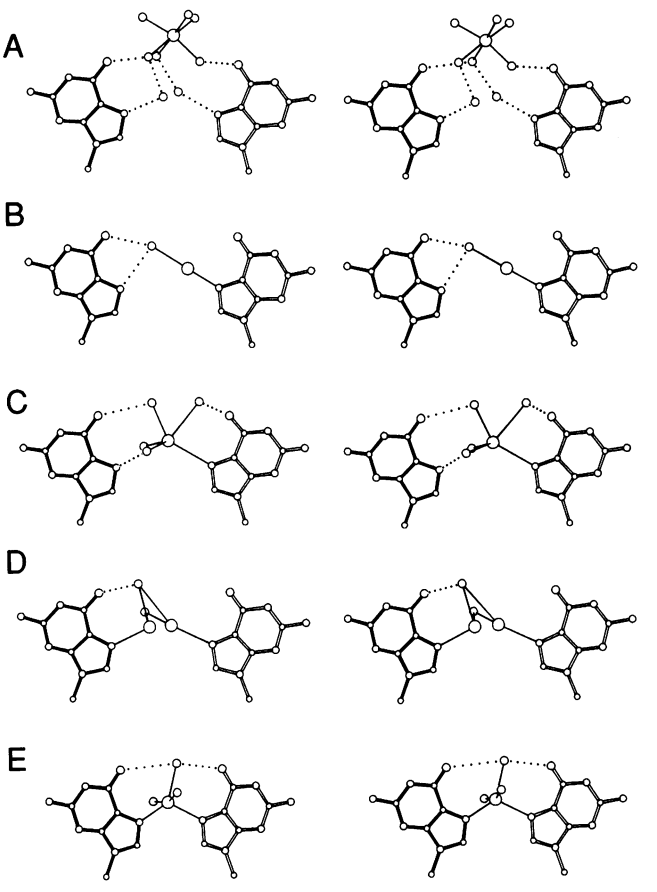

Figure 4. Comparison of the environment around the G10 and #G12 region in five different Z-DNA structures. **(A)** Hexahydrated Mg ion in the Mg-form of CGCGCG. **(B)** Co3 in the Co(II)-soaked Mg-form of CGCGCG. **(C)** Pentahydrated Na(I) ion in the CGCGTG structure. **(D)** Disordered Co3 ions in the Co(II)-soaked CGCGTG structure. **(E)** Cu(II) ion in the Cu(II)-soaked CGCGTG structure.

sites were completely displaced. Both Co(II) ions were coordinated directly to the N^7 of guanines and had a full hydration sphere (5 waters) despite a somewhat distorted octahedral geometry. The two Co(II) ions are 6.1 Å apart and are separated by water molecules. A third Co(II) ion was found to occupy a location between two guanine bases, G10 and #G12, of the CGCGTG structure (#G12 belongs to a symmetry related helix). It turned out that this Co(II) ion was disordered between two possible positions, either coordinating to G10 or to #G12, but not to both simultaneously (*vide infra*).

In the magnesium form of CGCGCG structure, three Co(II) ions were found to coordinate to G4, G6 and G10 bases. While they are well-occupied (temperature B-factors 23−35 $Å^2$), their hydration shells are incomplete, as shown in Figure 3(A−C). In contrast, only one Co(II) ion was located in the spermine form of CGCGCG structure (6). This is probably due to the stronger binding of the two spermine molecules in this crystal lattice. They could not be displaced by the diffusible Co(II) ion. In our previous analysis, we found a Mg(II) ion coordinated to the N^7 of G6 base (7). The Mg(II) ion helped to stabilize the Z_{II} phosphate conformation at the G4pC5 step. This Mg(II) ion was replaced by a Co(II) ion, as shown in Figure 3D.

Table 2 summarizes the interactions of metal ions Co(II), Cu(II) and Ba(II) with DNA in six new structures. Both the transition metal ions Co(II) and Cu(II) coordinate exclusively to the N^7 position of guanine. In some structures we observe an incomplete hydration shell. Ba(II) ions prefer to coordinate with both N^7 and O^6 positions of guanine in DNA. The Ba(II) ion simultaneously coordinates with two guanines on two symmetry related helices.

Bridging metal ion between two guanines

An interesting place in the lattice of the $P2_12_12_1$ crystal form of hexamer Z-DNA, is the space between the G10 and a symmetry-related (by 2_1-screw axis) #G12 base of the neighboring helix. The two guanine bases face each other so that their N^7 and O^6 atoms point to the same area, creating a cavity about 3 Å in diameter. In the Mg-form of CGCGCG Z-DNA crystal, this cavity is occupied by a fully-hydrated Mg ion (Figure 4A). The hydrated Mg ion cluster interacts with the two guanines in a very similar manner. One of the hydration waters is directly hydrogen bonded to the O^6 of guanine, while another hydration water is bonded to the N^7 of the same guanine through a bridging water molecule. The Mg(II) ion is slightly out of the plane of G10/#G12. In contrast, the Z−DNA crystal of CGCGTG (Mg-form) is found to have a Na(I) ion in this cavity. The sodium ion is directly coordinated to the G10 (Figure 4C). In this case, only four hydration waters were found, since the fifth hydration site is excluded by the two H8 hydrogen atoms of guanines.

Table 2. Coordination geometries around metal ion

Complex	Metal ion-Base	Distances (Å)		Angle(°)		Temp. factor
		M^{2+}-N^7	M^{2+}-O^6	M^{2+}-N^7-C^8 (°)	M^{2+}-N^7-C^5 (°)	B(Å^2)
Co-CGCGCG	Co 1 ---- G 4	2.28	3.87	115.6	140.4	27.5
	Co 2 ---- G 6	2.15	3.60	119.5	135.2	23.2
	Co 3 ---- G10	2.10	3.44	125.4	133.1	34.7
Co-CGCGCG-Spm	Co 1 ---- G 6	2.18	3.86	115.0	139.0	47.6
Co^{2+}CGCGTG	Co 1 ---- G 4	2.14	3.70	119.0	134.6	23.4
	Co 2 ---- G 8	2.32	3.81	116.4	136.5	25.0
	Co 3 ---- G10[a]	2.60	3.21	123.0	131.3	54.2
	Co 3 ---- G12[a]	2.67	3.79	127.8	126.2	54.5
Cu-CGCGTG	Cu 1 ---- G10	2.27	3.67	121.5	133.7	20.7
	Cu 1 ---- G12[b]	2.10	3.35	128.6	125.8	20.7
Co-CGCGCG-MAR70	Co 1 ---- G 2	2.18	3.62	124.2	130.9	22.0
	Co 2 ---- G 6	2.64	4.05	117.4	139.4	23.1
Ba-m^5CGm^5CGTG	Ba 1 ---- G 4	2.88	2.85	145.0	107.3	41.7
	Ba 2 ---- G10	2.88	3.06	150.3	105.5	9.9
	Ba 2 ---- G12[b]	2.91	2.88	151.8	104.9	9.9
Ba-NH_2-CGbr^{5}CGCG	Ba 1 ---- G10	3.01	2.77	153.5	102.6	7.8
	Ba 1 ---- G08[c]	3.12	2.80	154.8	101.5	7.8

[a] Co is disordered between two coordinations sites to G10 or G12.
[b] Cu 1 or Ba 2 is coordinated to G10 and G12 simultaneously.
[c] Ba 1 is coordinated to G10 and G8 simultaneously.

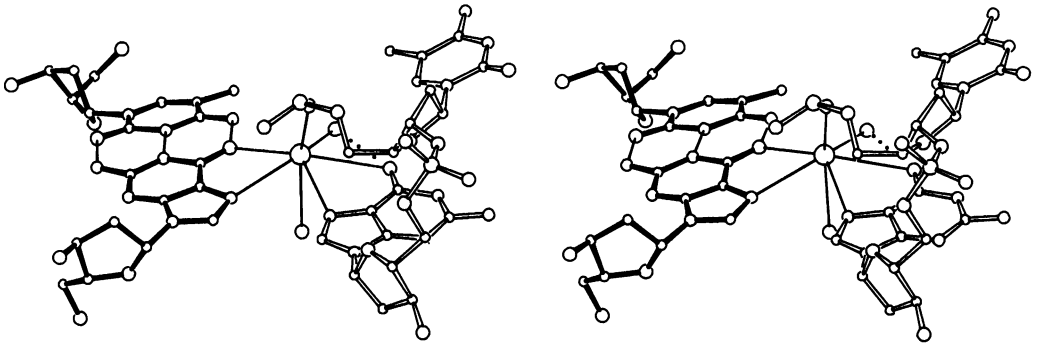

Figure 5. A stereoscopic view of Ba(II) ion bis-coordination to G10 and #G12 in the crystals of m^5CGm^5CGTG grown in the presence of Ba(II). (#G12 belongs to a symmetry related helix and is shown in filled bonds.)

When these two crystals (Mg-form of CGCGCG and CGCGTG) were soaked with Co(II) ion, different results were obtained. In the Co-CGCGCG structure, only one Co(II) ion was found to coordinate with G10 (Figure 4B). On the other hand, two disordered Co(II) ions were found in the Co-CGCGTG structure, one coordinating with G10 and the other coordinating with #G12 (Figure 4D). Because of the disordered arrangement, the Co(II) ions have high temperature B factors, reflecting their low occupancies (Co3 of CGCGTG in Table 2).

A very different situation was observed when the CGCGTG crystal was soaked with Cu(II) ion. In this case, the Cu(II) ion coordinated simultaneously to the N^7 sites of both G10 and #G12 guanines (Figure 4E). The Cu(II) ion had a trigonal bipyramid coordination geometry with three hydration waters.

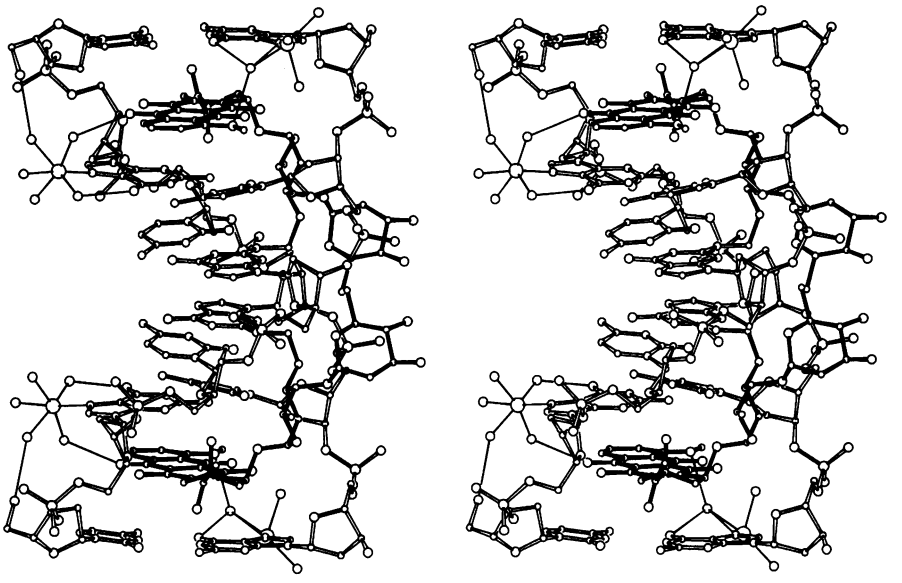

Figure 6. A stereo view of the anthracycline drug MAR70 complexed to CGCGCG. Four Co(II) ions per complex are seen to coordinate to the four outer guanine bases. Note that the central two guanines which are in the near normal B-DNA conformation do not have any Co(II) ion bound to the N^7 position.

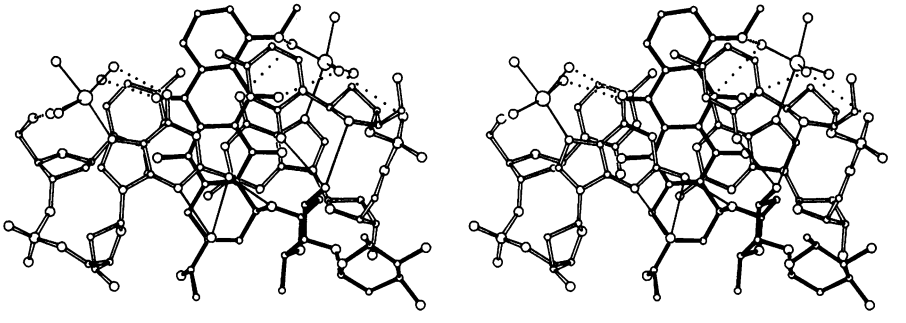

Figure 7. A view of the intercalated anthracycline drug MAR70 and the two adjacent G-C base pairs from a direction perpendicular to the base plane. The long dimension of the aglycone chromophore is nearly perpendicular to the $C^{1'}$-$C^{1'}$ vectors of the base pairs.

Two of the coordination sites in the trigonal plane were occupied by the N^7 of G10 and $^{\#}$G12, while the third site was occupied by a water which bridges the O^6 atoms of both guanines. The Cu-G10N^7 and Cu-$^{\#}$G12N^7 distances were 2.27 Å and 2.10 Å, respectively. This simultaneous coordination brought the two guanines closer together than they were in the Mg-form or the Co-form of CGCGTG (Figure 4C and 4D). The distance between G10N^7 and $^{\#}$G12N^7 in the Cu-form is 3.83 Å, which is significantly shorter than that of the Mg-form (5.35 Å). This indicates that Cu(II) is capable of pulling guanine away from the helix axis in this crystal lattice without destabilizing the crystal. (Note that the unit cell dimensions of the Co-form and Cu-form of CGCGTG crystals in Table 1 are nearly identical.)

Another example of bis-coordination by a metal ion was seen in the Ba-form of the aminohexyl-CGCGCG Z-DNA structure (34). A Ba(II) ion was located between two guanines, simultaneously coordinated to the N^7 and O^6 of both guanines. In a new crystal of m^5CGm^5CGTG grown in the presence of Ba(II), a similar bis-coordination of Ba(II) was seen, but in this case the lattice type remained that of the canonical Z-DNA ($P2_12_12_1$). The Ba(II) bridges two DNA helices related by symmetry. The helices were drawn closer to allow for the simultaneous coordination of Ba(II) ion to the N^7 and O^6 of guanines on both helices. The bis-coordination of Ba(II) ion to G10 & $^{\#}$G12 of m^5CGm^5CGTG is shown in Figure 5.

Co(II) and DNA-MAR70 interactions

Many metal ions, e.g., Fe(III) ion, play an important role in the activities of daunorubicin/doxorubicin drugs (30, 31). The metal ion may form a chelation complex with the free drug. But as far as the metal ion binding is concerned, it is less clear what happens when the drug is already intercalated in the DNA double helix. In the previous daunorubicin-CGTACG structure (14), a sodium ion was found to coordinate simultaneously with N^7 of G6 base and O^4/O^5 of daunorubicin. However, the sodium coordination is expected to be rather weak and should be easily displaced by other metal ions in solution. We are interested in knowing how metal ions such as Co(II) may interact with a daunorubicin-DNA complex.

We have chosen the crystal of MAR70-CGCGCG formaldehyde-crosslinked adduct (18, 32), (MAR70 is a daunorubicin derivative) for the soaking experiment. This crystal

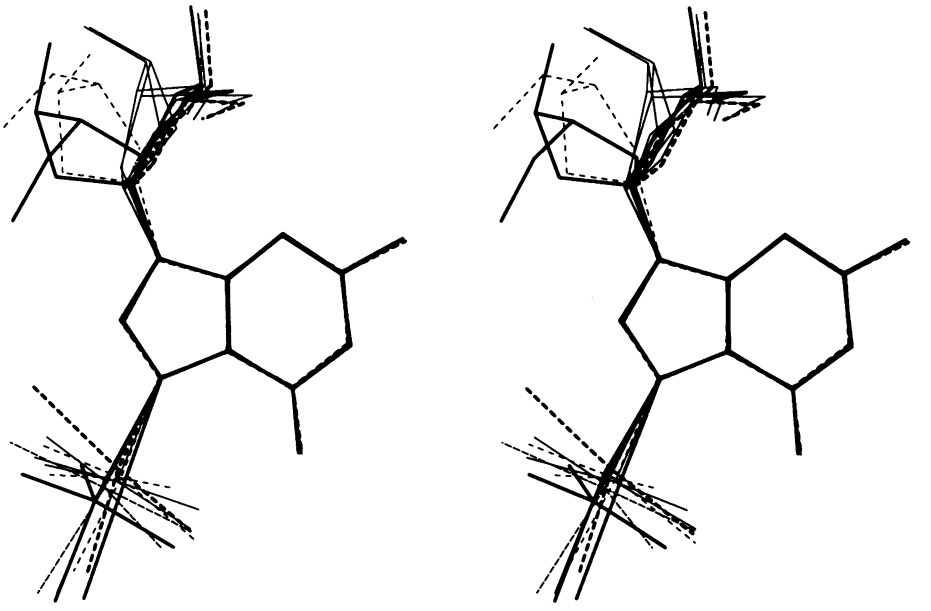

Figure 8. Superposition of ten Co(II)-coordinated guanines (selected from the list in Table 2).

appeared to be very robust and could withstand the diffusion of Co(II) into the crystal lattice without being destroyed. Unfortunately, the formaldehyde crosslinked crystals of daunorubicin-CGCGCG complex (15) were destroyed by the Co(II) soaking and could not be used for further studies. The Co(II)-soaked crystal structure of MAR70-CGCGCG has been refined to an R-factor of 0.208. Two unique Co(II) ions have been located as shown in Figure 6 (the two strands of DNA double helix are related by a 2-fold axis).

Figure 7 shows a part of the Co(II) soaked MAR70-CGCGCG formaldehyde-crosslinked complex in detail. One Co(II) ion is coordinated with the N^7 of G6 (Co-N^7 distance 2.64 Å), displacing the Na(I) ion originally near the same site. However, this Co(II) ion has shifted relative to the old Na(I) position so that it is in plane with the guanine base and is no longer coordinated to the O^4/O^5 of MAR70. The second Co(II) ion is coordinated to N^7 of G2 base (Co-N^7 distance 2.18 Å), which is adjacent to the aglycone but on the other side of G6. This Co(II) ion has a complete hydration shell, with its water molecules hydrogen bonded to nearby oxygen atoms (O^6 of G6, $O^{5'}$ of C1 and O^{10} of MAR70) (see (18) for a numbered schematic of MAR70).

Comparison of Co-DNA coordination geometries

In this study, we analyzed five new crystal structures in which Co(II) and Cu(II) have been incorporated. Ten Co(II) binding sites have been located (Table 2), of which eight were well-ordered (except Co3 of Co-CGCGTG structure). All of them coordinated to the N^7 of different guanines. Figure 8 shows a composite of selected Co(II)-coordinated guanine bases. Most of the Co-N^7 distances range from 2.10 Å to 2.67 Å with an averaged distance of 2.3 Å. The Co-N^7 distance values are a little larger than the value of 2.16 Å in the Co(II)-5'-IMP structure (33). It is interesting to note that the coordination geometry at N^7 has the Co-N^7 vector slightly tilted away from the O^6 atom. The average Co-N^7-C^8 and Co-N^7-C^5 angles are 121° and 134° respectively. The observed average Co-N^7-C^8 angle is smaller than the theoretical value (128°), if the Co-N^7 vector were to symmetrically bisect the C^5-N^7-C^8 angle.

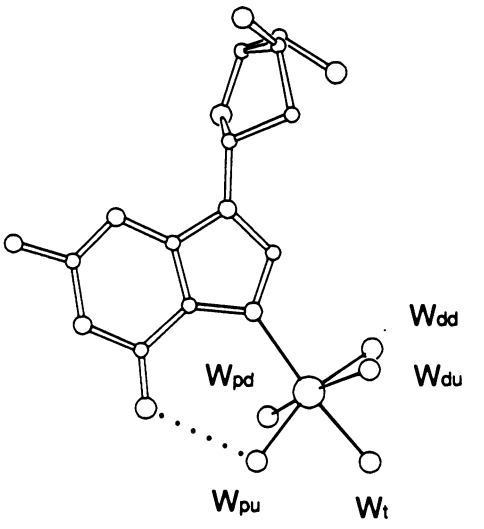

Figure 9. A diagram showing a typical Co(II) coordination geometry at the N^7 position with its full hydration shell. The five hydration water molecules are denoted as W_t (t=trans), W_{pd} (pd=proximal-down), W_{pu} (proximal-up), W_{dd} (dd=distal-down) and W_{du} (distal-up).

The preferred Co(II)-coordination geometry may be understood from Figure 9. Here we label the water molecules of the hydration shell with specific identification W_t, W_{dd}, W_{du}, W_{pd}, and W_{pu} as explained in the figure legend. Most of the Co-W distances range from 1.94 Å to 2.43 Å, with a few exceptions (Table 3). An interesting observation is that the W_{pu} water is hydrogen bonded to the O^6 of the same guanine base. To fulfill this hydrogen bonding geometry, the W_{pu} water is required to be slightly out of the plane of the guanine base. In other words, the vector of W_{pu}-Co-W_{dd} is making an angle of approximately 36° ~ 65° to the plane of the guanine base. In addition, the two

Table 3. Hydration shell geometry around metal ion

Complex	Metal Ion	Guanine (at N^7)	Wt	Wpu	Wpd	Wdu	Wdd
Co-CGCGCG	Co 1	G 4	2.32	---	---	---	2.23
	Co 2	G 6	---	2.22	2.35	2.18	2.06
	Co 3	G10	2.43	---	---	---	---
Co-CGCGCG-Spm	Co 1	G 6	---	2.48	---	3.23	---
Co-CGCGTG	Co 1	G 4	2.34	2.32	2.90	3.05	2.02
	Co 2	G 8	2.37	2.26	2.11	3.30	2.58
Cu-CGCGTG	Cu 1	G10	---	2.01	2.21	---	1.94
Co-CGCGCG-MAR70	Co 1	G 2	2.02	2.01	2.01	2.00	2.04
	Co 2	G Ω6	2.04	---	2.02	2.01	2.06

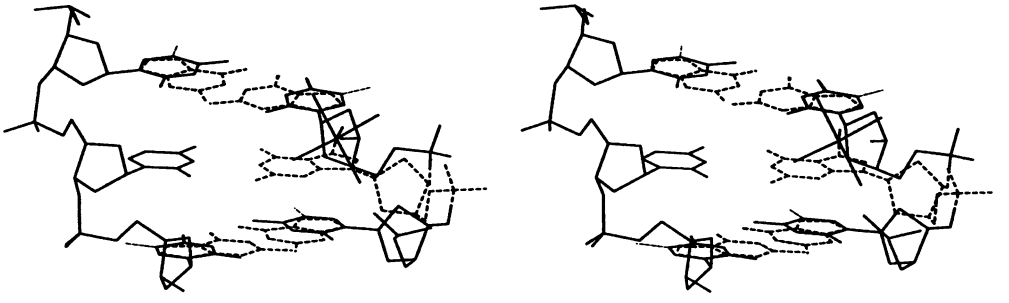

Figure 10. A diagram showing a view of superposed models of Co(II)-coordination at the N^7 position of guanine in B-DNA with its full hydration shell. The preferred coordination geometry was searched by rotating the hydration shell every 15° with respect to the vector of N^7-Co-W_t. All sixteen models of possible triplet sequences of B-DNA with one conformation of the coordinated cobalt ion are superposed. A steric clash of hydration shell waters with the base on the 5′ side of the coordination site was observed for all angles, while the 3′ side was unhindered.

other water molecules (W_{pd} and W_{pu}) are now in somewhat axial positions. If the Co(II) ion has a complete hydration shell when bound to a DNA double helix, one needs to consider the possible consequence on DNA conformation due to the van der Waals interference of axial waters on the neighboring bases.

To address this issue, we carried out an exhaustive model building study by putting a Co(II)-coordinated guanine in the middle of trinucleotide base pairs of B-DNA and A-DNA. All sixteen combinations of nucleotide triplets were tested for both A and B-DNA. We chose to scan the entire conformational space available for the hydrated cobalt ion when coordinated with a given DNA sequence in A or B conformation. The disposition of the hydration shell was varied by rotating the plane of Co-W_{dd}-W_{du}-W_{pu}-W_{pd} every 15° with respect to the axis of N^7-Co-W_t (Figure 9) for each model. For B-DNA, it was found that there is always a short contact between either the W_{du} or W_{pd} waters with the 5′-side neighboring base while the 3′-side is unrestrained (Figure 10). It seems that when Co(II) is bound to a guanine base of B-DNA, it can not maintain a complete octahedral hydration shell unless there is a conformational change in the adjacent base pairs. For A−DNA, the clash between $W_{pu/du}$ and the base on the 5′-side is particularly severe, while the 3′ side is free (not shown). This is because the N^7 of guanine is much less accessible in the deep major groove of A-DNA. A larger conformational change of adjacent base pairs would be expected if a fully hydrated Co(II) binds to A-DNA.

DISCUSSION

The study of two transition metal ions (Co(II) and Cu(II)) binding to DNA using high resolution x-ray diffraction offered us an unique opportunity to visualize their various binding modes at the molecular level. An interesting observation is that they bind exclusively to N^7 of guanines with direct coordination at different sequence locations. Even though the negatively charged phosphates are exposed to the solvent channels, no metal binding to phosphate oxygen atom was seen. Therefore charge-charge interaction with phosphate oxygens is not a dominant force in the transition metal ion binding to DNA.

It should be noted that for Co(II) ion different guanines have different affinities. For example, the Co(II) ion binds to the magnesium form of CGCGCG at G4, G6 and G10 sites, but to the magnesium form of CGCGTG at G4, G8, G10 and #G12 sites (Table 2). The local environment of the guanine base apparently plays some role in affecting the reactivity of N^7 toward the Co(II) ion. In the case of Cu(II) soaking with the magnesium form of CGCGCG, *every* guanine was attacked to a certain degree (24). It is possible that if we had soaked the

Z-DNA crystals with Co(II) ion for a very long time (e.g., several months), other sites may be attacked. Nonetheless our experiments suggested a gradation of reactivities toward Co(II) coordination in the crystal lattice.

Another significant observation was that the Cu(II) ion was capable of pulling two guanines (G10 and #G12 of CGCGTG) closer by about 1.5 Å to form a bis-coordination linkage. This suggests that Cu(II) ion may be very effective in causing a 'crosslink' between two different DNA duplexes, at least in the case of Z-DNA. It should be pointed out that in the above studies of Z-DNA, the guanine bases, the targets of Co(II) and Cu(II) coordination, are in the syn conformation. The N^7 site on Z-DNA is very exposed, thus prone to attack by the metal ions. We have recently shown that Ba(II), a larger metal ion, is also capable of bridging two guanines through the N^7 and O^6 positions (34). In the Ba(II) case, the interaction is reversible, in contrast to the irreversible coordination of Cu(II) ion.

Our model building studies suggested that the binding of Co(II) to DNA guanine N^7 is likely to cause some conformational change of DNA in solution. This was based on the assumption that Co(II) ion tends to maintain a complete octahedral hydration shell. In B-DNA, the base pair in the adjacent (5'-side) position could adjust its stacking pattern (e.g., change helical twist angle) to accommodate the axial water molecule with new hydrogen bonds between water and DNA. This was borne out by the structure of CGCGCG-MAR70 soaked with cobalt (Figures 6 & 7). Here the DNA is a somewhat distorted B-DNA. In this structure, the N^7 of G4 residue is completely exposed in the crystal lattice. However, no Co(II) ion is found to coordinate to the G4N^7 site. Since the base pair can not move too much in the crystal lattice, the potential van der Waals clash (*vide supra*) prevents a Co(II) ion from coordinating to the G4N^7 site.

In the case of A-DNA or RNA, the preferred binding site, i.e., N^7 of guanine, is now buried in the very deep and narrow major groove. It seems unlikely that a hydrated Co(II) ion can be accommodated in the deep major groove of A-DNA or RNA double helix. Whether other sites (e.g., N^3 of purine) in the more exposed minor groove become the preferred binding sites, or a substantial conformation change occurs for the binding at N^7 remains to be determined. We are testing this by soaking Co(II) into several A-DNA crystal structures.

The coordination geometry around the metal ion has a certain degree of variation. For example, the Co-N^7 coordination bond distance may vary between 2.0 Å to 2.6 Å (Table 2 and Figure 8). While the octahedral geometry is preferred, it may be distorted depending on the local environment. It should be noted that we have observed previously that an N^7-coordinated Co(II) ion could be slightly out of the plane of guanine. In the structure of the tetragonal form of cyclic-(pGpG) (35), a Co(II) ion was found to coordinate to two adjacent (intra-strand) guanines, causing a de-stacking of the two guanines (a 36° dihedral angle between them).

In conclusion, the detailed structural information of how transition metal ion such as Co(II) or Cu(II) interact with polymer DNA (using oligonucleotides as model systems) may be valuable in understanding the roles of metal ions in biology. It is possible that bases that are exposed in certain higher ordered structures may be the probable sites for specific interaction with transition metal ions. For example, the bases in a loop region of a hairpin may be a better target for metal ion interaction. This has been observed in the tRNA structure (21). Alternatively, metal ion may bind to guanine base and force the helix to open up (19, 36). These speculations may be tested by further experiments.

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