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Accelerated Publications

Structural Basis for Stabilization of Z-DNA by Cobalt Hexaammine and Magnesium Cations[†]

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ABSTRACT: In the equilibrium between B-DNA and Z-DNA in poly(dC-dG), the $[Co(NH_3)_6]^{3+}$ ion stabilizes the Z form 4 orders of magnitude more effectively than the Mg^{2+} ion. The structural basis of this difference is revealed in Z-DNA crystal structures of d(CpGpCpGpCpG) stabilized by either Na^+/Mg^{2+} or Na^+/Mg^{2+} plus $[Co(NH_3)_6]^{3+}$. The crystals diffract X-rays to high resolution, and the structures were refined at 1.25 Å. The $[Co(NH_3)_6]^{3+}$ ion forms five hydrogen bonds onto the surface of Z-DNA, bonding to a guanine O6 and N7 as well as to a phosphate group in the Z_{II} conformation. The Mg^{2+} ion binds through its hydration shell with up to three hydrogen bonds to guanine N7 and O6. Higher charge, specific fitting of more hydrogen bonds, and a more stable complex all contribute to the great effectiveness of $[Co(NH_3)_6]^{3+}$ in stabilizing Z-DNA.

The nucleic acids are negatively charged polyelectrolytes, and positively charged cations are known to be important in determining their conformation. Information that we have concerning the role of cations is usually limited to their effects in solution, since structural data based on X-ray diffraction patterns of nucleic acids usually do not have sufficient resolution to determine the details of their interactions. However, single crystals of oligonucleotides sometimes diffract X-rays to atomic or near-atomic resolution.

Right-handed B-DNA and left-handed Z-DNA are two different conformers of the double helix in equilibrium with each other, and ions play an important role in defining the equilibrium [reviewed in Rich et al. (1984)]. High concentrations of sodium chloride or magnesium chloride convert the right-handed B-DNA form of poly(dG-dC) into left-handed Z-DNA, as shown by circular dichroism and Raman spectroscopy (Pohl & Jovin, 1972; Thamann et al., 1981). Behe & Felsenfeld (1981) have shown that the cobalt hexaammine ion is very effective in specifically stabilizing Z-DNA; for example, $[Co(NH_3)_6]^{3+}$ is 5 orders of magnitude more effective than Na^+ and 4 orders of magnitude more effective than Mg^{2+}. It is of interest to inquire about the structural basis of this high level of specificity. Here we report on the crystal-structure analysis of d(CpGpCpGpCpG) [$(dC\text{-}dG)_3$] stabilized either by sodium/magnesium ions or by sodium/magnesium plus cobalt hexaammine ions. The two crystals both diffract to high resolution, and they have been refined to a residual factor (*R* factor) of 18% at 1.25-Å resolution. These two structures reveal the position of the sodium/magnesium plus cobalt hexaammine cations. The cobalt hexaammine cation interacts specifically with the outer convex surface of Z-DNA through a series of five hydrogen bonds to both guanine and phosphate groups. In contrast, the magnesium cation forms three hydrogen bonds through water molecules in its hydration shell. The detailed fitting of cobalt hexaammine to the surface of Z-DNA is partly responsible for its great effectiveness in stabilizing Z-DNA.

EXPERIMENTAL PROCEDURES

The oligonucleotides were synthesized by the phosphotriester method (van der Marel et al., 1981). In the first Z-DNA structure solved (Wang et al., 1979), the oligonucleotide had

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both magnesium and spermine cations in the complex. In the present investigation, the same oligonucleotide d(CpGpCpGpCpG) was crystallized, except that no spermine was used in the crystallization mixture. In the structure stabilized by magnesium cations, the crystallizing mixture contained 1.4 mM oligonucleotide, 20 mM sodium cacodylate (pH 7.0), and 120 mM magnesium chloride. The crystals were formed by vapor-phase equilibration with 10% 2-methyl-2,4-pentanediol (2-MPD). Crystals grew as quasi-hexagonal rods in a 3-day period and had grown to a sufficient size by 2 weeks for single-crystal X-ray diffraction analysis. The crystal was mounted in a sealed glass capillary tube with a droplet of mother liquor and was found to have crystallized in space group $P2_12_12_1$ with dimensions a = 18.01 Å, b = 31.03 Å, and c = 44.80 Å. The crystals diffracted to better than 1.25-Å resolution.

The cobalt hexaammine Z-DNA crystals were formed in a similar manner, except that equimolar amounts of cobalt hexaammine trichloride (Aldrich) were also placed in the solution. After the crystals were grown, additional amounts of cobalt hexaammine trichloride were added up to a final concentration of 23 mM cobalt hexaammine trichloride. The crystals were allowed to stand, and after nearly 3 months of soaking they became deep yellow. Crystallographic analysis of these crystals showed that they had the same space group with unit cell dimensions a = 18.00 Å, b = 30.96 Å, and c = 44.83 Å. These crystals also diffracted to a resolution beyond 1.25 Å. In view of the similarities of the cell dimensions with the spermine–magnesium complex of $(dC\text{-}dG)_3$ (Wang et al., 1979), we assumed that a similar structure was formed, and the earlier coordinates were used as a trial structure. These coordinates were quickly refined by the Konnert–Hendrickson restrained refinement program (Hendrickson & Konnert, 1979). In the course of refining the sodium/magnesium form of the $(dC\text{-}dG)_3$ crystal, approximately 100 water molecules and ions appeared in the successive Fourier maps. Magnesium ions could be identified through the formation of regular octahedral coordination complexes with water molecules with a distance of about 2.0 Å between the magnesium and oxygen atoms of water. Four magnesium complexes were identified per DNA duplex. The final residual R value was 18.0%.

The coordinates of the refined magnesium structure were then used to refine the cobalt hexaammine structure. The cobalt hexaammine ion was identified by difference Fourier analyses, and the structure was then refined to a final R value of 18.5%. A similar number of water molecules were identified. The cobalt hexaammine ion forms a regular octahedron with a cobalt–nitrogen distance of 2.00 Å. One cobalt hexaammine complex was identified per DNA duplex molecule, and it was judged to have an occupancy in excess of 80%. Two magnesium complexes were also found in this structure. The full details of the two crystal structures will be published elsewhere.

RESULTS

The magnesium structure contains four magnesium complexes per DNA duplex, and two of those were also found in the cobalt hexaammine structure. Figure 1 is a van der Waals diagram of three of the hexamer duplexes as they are found in the crystal lattice aligned along the c axis. A heavy line is drawn from phosphate to phosphate, showing the zigzag arrangement of the backbone. The shaded area between the phosphates shows the position of the deep helical groove. The positions of the magnesium and cobalt hexaammine complexes on the surface of Z-DNA are shown in Figure 2. Three hydrated magnesium complexes attached to the surface of the molecule are labeled 1–3. Complexes 1 and 2 are intermolecular, bound to two different molecules. They are, in fact, two views of the same magnesium complex related to each other by a translation along the a axis attached in two different ways. Magnesium complex 3 coordinates directly to the Z-DNA molecule and does not have intermolecular binding. Thus there are two independent magnesium complexes per hexamer duplex.

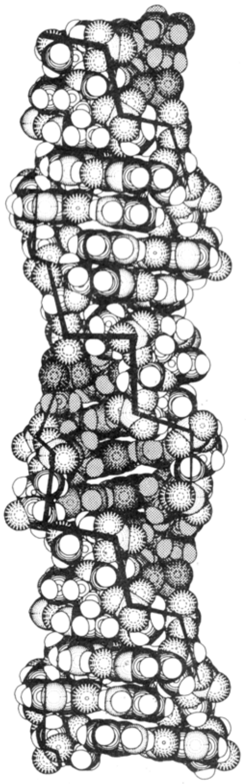

FIGURE 1: van der Waals diagram of Z-DNA formed by d(CpGpCpGpCpG). Three repeats of the DNA hexamer are shown as they are organized along the c axis of the molecule in the crystal. The solid zigzag line goes from phosphate to phosphate along the molecule, including the position between molecules where the phosphate is missing because the crystallized material was a hexanucleoside pentaphosphate. The shaded area is the deep helical groove in Z-DNA. Phosphorus atoms have large stippled circles, oxygen has dashed circles, carbon has solid circles, nitrogen has stippled circles, and hydrogen atoms are empty.

The cobalt hexaammine complex is intermolecular, and the two complexes labeled A and B in Figure 2 are two views of the same complex that binds to the molecule in two different manners. Its presence in the lattice results in the exclusion of two of the magnesium complexes found in the crystal with magnesium alone. It can be seen (Figure 2) that cobalt hexaammine and magnesium complexes 1 and 3 bind predominantly to the outer convex surface of the molecule. Only magnesium complex 2 binds very close to the helical groove of Z-DNA (shown as the shaded portion of the DNA).

Details of the interaction of the cobalt hexaammine complex to Z-DNA in position A are shown in Figure 3. Five hydrogen bonds are formed between the cobalt hexaammine complex and the outer surface of the Z-DNA molecule. Three coordinated ammonia molecules are the hydrogen-bond donors. One donates to phosphate P9 and N7 of guanine 10 below it, and another donates to phosphate P9 and O6 of guanine 10. A third ammonia donates one hydrogen atom to O6 of guanine 10. The three ammonia groups that are involved in hydrogen bonds all come from one face of the octahedron formed around the cobalt ion. The hydrogen-bond lengths for these five

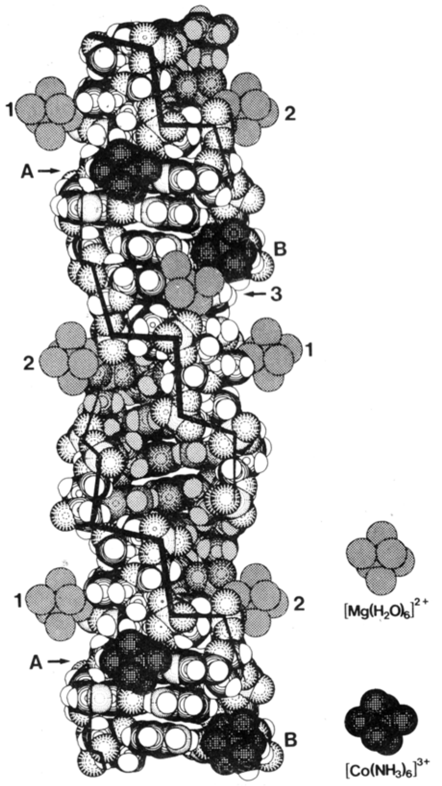

FIGURE 2: Z-DNA with its attached ions in the magnesium/cobalt hexaammine crystal. Two types of octahedral complexes are shown on the surface of the molecule. Magnesium octahedra (1–3) are illustrated with lighter shading and cobalt hexaammine octahedra (A and B) with darker shading. The ion complexes are largely bound between two different DNA duplexes in the lattice. Thus magnesium water octahedra 1 and 2 are two views of the same complex showing two different modes of binding. Magnesium complex 3 is not intermolecular and binds only to this molecule. Cobalt hexaammine complexes A and B are the same complex, and they show the two different ways in which they interact with DNA duplex molecules in the lattice.

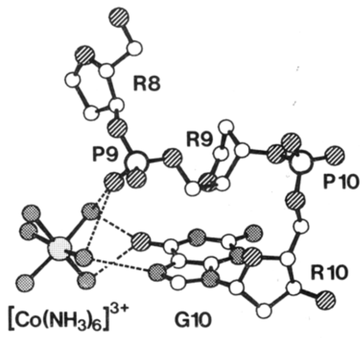

FIGURE 3: Coordination of cobalt hexaammine to Z-DNA. The ammonia molecules of the cobalt hexaammine complex are found hydrogen bonded to the phosphate group P9 and to N7 and O6 of guanine 10 (G10). The phosphate group P9 has rotated down in the cobalt hexaammine crystal to form a $Z_{\rm II}$ conformation that allows it to add the two additional hydrogen bonds. This shows the coordination of position A, Figure 2.

hydrogen bonds are as follows: ammonia nitrogen to N7, 3.15 Å; ammonia nitrogen to O6, 2.83 and 2.73 Å; ammonia nitrogen to OP9, 2.99 and 3.31 Å. The three ammonia molecules that are not shown forming hydrogen bonds in Figure 3 are actually bonding to another adjoining DNA duplex (ion B, Figure 2) in a manner that has some similarities to that shown in Figure 3. The major difference is that ion B binds at the end of the duplex so that it bridges the gap between one duplex and the other duplex stacking underneath it. That gap does not have a phosphate group, and this makes for a slight modification in the hydrogen bonding. However, the bonding shown in Figure 3 is that which occurs in the middle of a continuous duplex, and it is devoid of any end effects due to the finite size of the oligomer.

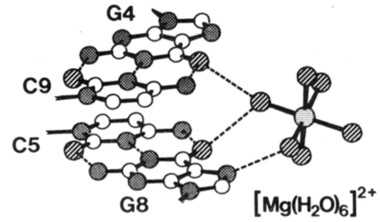

FIGURE 4: Coordination of a magnesium water octahedral complex with the outside of Z-DNA (site 2 of Figure 2). One water molecule binds to O6 of guanine 4 and O6 of guanine 8 while another binds to N7 of guanine 8.

The coordination of a hydrated magnesium ion (site 1, Figure 2) to the outer surface of Z-DNA is shown in Figure 4. This has three hydrogen bonds instead of the five shown in the cobalt hexaammine complex. The hydrogen bonds are formed between base pairs and the octahedrally coordinating water molecules surrounding the magnesium ion. One water molecule donates a hydrogen to O6 of guanine 4 (3.0 Å) and to O6 of guanine 8 (3.1 Å). Another water molecule donates a hydrogen to N7 of guanine 8 (2.8 Å). The bonding to the right side of the hydrated magnesium complex is not shown in Figure 4. That coordination corresponds to site 2 of Figure 2, and it forms a solitary hydrogen bond between one coordinating water molecule and the oxygen of P10 (2.7 Å).

Previously, it was shown that Z-DNA exists in two different forms called $Z_{\rm I}$ and $Z_{\rm II}$ (Wang et al., 1981). They differ principally in the rotation of a phosphate group in a GpC sequence. Form $Z_{\rm II}$ was found to occur when a magnesium ion coordinated directly with the N7 position of a guanine at the other surface of Z-DNA. One of its five octahedral water molecules is then in a position so that it can form a hydrogen bond with a neighboring phosphate group when it moves into the $Z_{\rm II}$ conformation. What is interesting in the present study is that phosphate P9 in the magnesium structure is in the $Z_{\rm I}$ conformation. However, formation of the cobalt hexaammine complex resulted in a local conformational change so that phosphate P9 has now rotated into the $Z_{\rm II}$ conformation where it can receive the two hydrogen bonds shown in Figure 3. Thus, part of the detailed fitting of the cobalt hexaammine ion to the outer surface of Z-DNA is associated with a local conformational change from a $Z_{\rm I}$ to a $Z_{\rm II}$ configuration with additional hydrogen-bonding interactions.

Magnesium complex 3 (Figure 2) is seen to have only four water molecules rather than the six found in complexes 1 and 2. This is because that magnesium ion coordinates directly with N7 of guanine 6 and results in a local $Z_{\rm II}$ conformation in a manner analogous to that which has been seen previously in the spermine complex of Z-DNA (Wang et al., 1981). However, the presence of the nearby cobalt hexaammine complex has resulted in the loss of a second water molecule of its octahedral complex, reducing the total to four remaining coordinating waters.

It is interesting that a cobalt hexaammine complex and a magnesium complex are crowded close together in this structure (B and 3 in Figure 2). This situation is somewhat similar to the clustering of cations that was seen in the crystal structure of d(m^{5}CGTAm^{5}CG) in which a series of four positively charged cations are close together on the surface of the Z-DNA crystal (Wang et al., 1984). In the present case, the two ion complexes have a total charge of 5+. In the crystal structure stabilized with only magnesium ions, two magnesium octahedral complexes share an edge when bound in this position on the outer surface of the nucleic acid.

DISCUSSION

One of the questions this study can address is why the cobalt hexaammine ion is so effective in stabilizing Z-DNA relative to B-DNA. Part of the reason is related to the fact that the surface of Z-DNA provides an environment where five hydrogen-bonding interactions can be formed. This is in contrast to B-DNA, which does not have a comparable surface that can form such a detailed fitting. As mentioned above, cobalt hexaammine is 5 orders of magnitude more effective in stabilizing Z-DNA than is the sodium ion and 4 orders of magnitude more effective than magnesium in stabilizing Z-DNA (Behe & Felsenfeld, 1981). This structural study suggests that an important component in the stabilization of a nucleic acid conformation by cations may be the detailed fitting of the cation to the macromolecule. In the present case, the formation of five hydrogen bonds with a central charge of 3+ is much more effective than the formation of three hydrogen bonds in the magnesium complex with a central charge of 2+. This may be partly related to the fact that the cobalt hexaammine ion has a total of 18 hydrogen atoms that are capable of forming hydrogen bonds outside the complex while the magnesium water octahedron has as many as 12, assuming no intracomplex hydrogen bonding. Because the hydrogen bonds from the cobalt hexaammine are all donated from one face of the octahedron, the other three coordinate sites on the opposite octahedral face could be occupied by substances other than ammonia molecules. This might represent a way of using such an ion as a carrier for bringing other molecules close to the vicinity of Z-DNA.

Another factor is related to the relative stability of the cobalt hexaammine complex compared to the magnesium water complex. The former is very stable, while the latter is relatively unstable with a constant interchange of water molecules between those coordinating to the magnesium ion and those in the bulk of the solution. This would represent a relative destabilizing factor compared to the more fixed position of the ammonia molecules clustered about cobalt.

Finally, it is likely that the coordination shown in Figure 3 is responsible for the remarkable stabilization of poly(dC-dG) as Z-DNA in solution. This interaction is found at the middle of the $(dC\text{-}dG)_3$ hexamer away from the end and could be repeated many times along the DNA molecule.

Registry No. d(CpGpCpGpCpG), 58927-26-7; Mg, 7439-95-4; $[Co(NH_3)_6]^{3+}$, 28219-81-0.

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