Neomycin, spermine and hexaamminecobalt(III) share common structural motifs in converting B- to A-DNA

Howard Robinson and Andrew H.-J. Wang

Biophysics Division and Department of Cell and Structural Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

Received October 18, 1995; Revised and Accepted December 11, 1995

ABSTRACT

The (dG)n·(dC)n-containing 34mer DNA duplex [d(A2G15C15T2)]2 can be effectively converted from the B-DNA to the A-DNA conformation by neomycin, spermine and Co(NH3)63+. Conversion is demonstrated by a characteristic red shift in the circular dichroism spectra and dramatic NMR spectral changes in chemical shifts. Additional support comes from the substantially stronger CH6/GH8–H3′ NOE intensities of the ligand–DNA complexes than those from the native DNA duplex. Such changes are consistent with a deoxyribose pucker transition from the predominate C2′-endo (S-type) to the C3′-endo (N-type). The changes for all three ligand–DNA complexes are identical, suggesting that those three complex cations share common structural motifs for the B- to A-DNA conversion. The A-DNA structure of the 4:1 complex of Co(NH3)63+/d(ACCCGCGGGT) has been analyzed by NOE-restrained refinement. The structural basis of the transition may be related to the closeness of the two negatively charged sugar–phosphate backbones along the major groove in A-DNA, which can be effectively neutralized by the multivalent positively charged amine functions of these ligands. In addition, ligands like spermine or Co(NH3)63+ can adhere to guanine bases in the deep major groove of the double helix, as is evident from the significant direct NOE cross-peaks from the protons of Co(NH3)63+ to GH8, GH1 (imino) and CH4 (amino) protons. Our results point to future directions in preparing more potent derivatives of Co(NH3)63+ for RNA binding or the induction of A-DNA.

INTRODUCTION

A number of aminoglycoside antibiotics are potent inhibitors of the interactions of proteins and RNA. For example, neomycin (Fig. 1) at 1 µM concentration inhibits the binding of HIV Rev protein to the Rev-responsive element (RRE) RNA hairpin–bulge sequence (1). Similar inhibitions are found in the splicing of group I introns (2), ribozymes (3) and the RNA ligand of the 30S ribosome subunit (4). In contrast, streptomycin is not effective. The molecular basis for such binding specificity is not yet known.

Neomycin has six primary amine groups distributed over three of its four sugars, creating a network of positively charged amino hydrogen bond donors under physiological conditions. Such a molecular architecture is reminiscent of the hexaamminecobalt(III) ion [Co(NH3)63+], with six ammonia molecules surrounding the Co(III) ion. Co(NH3)63+ is extremely potent in promoting, at sub-micromolar concentrations, the transition of poly[d(m5C-G)] from right-handed B-DNA to left-handed Z-DNA (5–7). It is equally effective in promoting formation of DNA four-way junctions (Holliday junctions) (6). In many ways Co(NH3)63+ behaves similarly to the biologically relevant polyamines spermine and spermidine, which are also capable of promoting the two above-mentioned DNA conformational transitions (5–9). Thus these three amine-containing ligands, neomycin, spermine and hexaamminecobalt(III) ion, may share certain similar DNA/RNA interaction properties.

Recently it has been suggested that Co(NH3)63+ induces A-DNA structure in DNA with stretches of GG sequences, as evidenced, among other data, by characteristic changes in their circular dichroism spectra (10). Although (dG)n·(dC)n sequences are known to have a propensity to form A-DNA (11), the structural study of A-DNA in solution has not been straightforward. Therefore, that Co(NH3)63+ has strong effects on DNA conformation in general (12–15) and that it can facilitate the formation of A-DNA in particular are significant, since it has been shown that while B-DNA is considered to be the predominant conformation in vivo, A-DNA may play important biological roles. For example, the recent crystal structure of TATA box binding protein complexed to the TATA-containing sequence revealed that the DNA conformation is closer to A-DNA in several parameters (16,17). Moreover, A-DNA is conformationally very similar to RNA. Thus knowledge of how Co(NH3)63+ binds to A-DNA may provide information about its interaction with RNA. In this paper we study the influence of several cationic ligands, including neomycin, spermine and Co(NH3)63+, on the conformation of a 34mer DNA duplex [d(A2G15C15T2)]2, which is more than three full turns of double helix. The binding sites on DNA for Co(NH3)63+ have also been identified.

* To whom correspondence should be addressed
MATERIALS AND METHODS

The 34mer DNA d(A2G15C15T2) was chosen to contain a long stretch of consecutive (dG)ₙ,(dC)ₙ sequence which is capped with two AT base pairs to prevent concatenation of duplexes. In addition, the DNA decanucleotide d(ACCCGGCGGT) was prepared for high definition structural analysis. DNA oligonucleotides were synthesized at the Genetic Facility of UIUC. They were purified and desalted on a Sepharose S-100 column. Solutions of the DNA oligomers for NMR studies were prepared as described earlier (18,19). For the Na-form of the 34mer DNA sample 3.25 mg 34mer lyophilized powder was dissolved in 0.55 ml H₂O containing 20 mM phosphate buffer, pH 7.0, resulting in a 0.26 mM duplex solution. For the ligand–DNA sample an ∼0.4 mM DNA duplex solution was similarly prepared. Aliquots of the stock solution of ligands [neomycin, spermine, Co(NH₃)₆³⁺, streptomycin and Pt(NH₃)₄²⁺] were purchased from Sigma Chemical Co., St Louis, MO] were individually added to the DNA solution to produce different ratios of ligand to duplex.

NMR spectra were collected on a Varian VXR500 500 MHz spectrometer and the data were processed with FELIX version 1.1 (Hare Research, Woodinville, WA). The non-exchangeable two-dimensional NOE spectra for the 34mer were collected at 25°C in 90% H₂O/10% D₂O with the 1-1 pulse sequence as the read pulse of the NOEY. Twenty four transients were averaged with a recycle delay of 2.9 s and a mixing time of 100 ms. The excitation offset was set to one quarter of the spectral bandwidth, which was set to 12 000 Hz.

Refinements of both the Na-form and Co-form of the decamer (dACCCGGCGGT) have been carried out. Starting models for the decamer in the B- and A-DNA conformations were built using QUANTA, version 4.0 (MSI, Massachusetts, MA). Structural refinement was carried out by the procedure SPEDREF (21). The mixing time used in the refinement was 100 ms. The inversion recovery experiment determined the T₁ relaxation time for every spin. The recycle time was 4.41 s. The correlation time τ_c was determined to be 7 ns using the SPEDREF procedure described before (21). The starting models were refined within the program X-PLOR (22) in conjunction with the SPEDREF procedure. During the first 40 refinement cycles the molecules were given a random set of velocities equivalent to 300 K. NOE-restrained molecular dynamics were then run for 0.1 ps, the temperature coupling bath lowered by 25 K and the process repeated until the molecules had been cooled to 50 K. After each annealing the molecular dyad symmetry was slowly imposed while running an additional 100 cycles of NOE-restrained conjugate gradient minimization. During the last 20 cycles the molecules were refined with only NOE-restrained conjugate gradient minimization. The simulated NOE relaxation rates and NOE intensities for the refined models were calculated by the program MORASS (23). Simulated two-dimensional NOESY spectra were produced by the program CSL (in the SPEDREF package) using the NOE intensities from the simulation and the line shapes and chemical shifts previously obtained from MYLOR. The final structure of the Co-form was derived from NOE-restrained conjugate gradient minimization only, since the starting model was very close to the observed data and simulated annealing could not further improve on the refinement. The NMR R factor was defined as R = \sum |N_o - N_r|/\sum N_o, where N_o and N_r are the experimental and calculated NOE integrals respectively.

Circular dichroism (CD) spectra were recorded on a Jasco J720 spectrometer. The concentration of the 34mer DNA solutions was kept at 10 μM duplex in 20 mM phosphate buffer, pH 7.0.

RESULTS AND DISCUSSION

Evidence of B- to A-DNA transition

Circular dichroism and one-dimensional NMR spectra were taken to monitor titration of the 34mer DNA. Figure 2A shows the CD spectra of the titration of neomycin. The characteristic spectral shift toward the longer wavelength associated with a B- to A-DNA transition is evident. It appeared that the conformational transition was nearly complete at a 4:1 ratio of neomycin.

Two-dimensional NOESY spectra at 25°C in 90% H₂O/10% D₂O were collected with the 1-not-1 pulse sequence as the read pulse of the NOESY. Twenty four transients were averaged with a recycle delay of 2.9 s and a mixing time of 100 ms. The excitation offset was set to one quarter of the spectral bandwidth, which was set to 12 000 Hz.

Refinements of both the Na-form and Co-form of the decamer (dACCCGGCGGT) have been carried out. Starting models for the decamer in the B- and A-DNA conformations were built using QUANTA, version 4.0 (MSI, Massachusetts, MA). Structural refinement was carried out by the procedure SPEDREF (21). The mixing time used in the refinement was 100 ms. The inversion recovery experiment determined the T₁ relaxation time for every spin. The recycle time was 4.41 s. The correlation time τ_c was determined to be 7 ns using the SPEDREF procedure described before (21). The starting models were refined within the program X-PLOR (22) in conjunction with the SPEDREF procedure. During the first 40 refinement cycles the molecules were given a random set of velocities equivalent to 300 K. NOE-restrained molecular dynamics were then run for 0.1 ps, the temperature coupling bath lowered by 25 K and the process repeated until the molecules had been cooled to 50 K. After each annealing the molecular dyad symmetry was slowly imposed while running an additional 100 cycles of NOE-restrained conjugate gradient minimization. During the last 20 cycles the molecules were refined with only NOE-restrained conjugate gradient minimization. The simulated NOE relaxation rates and NOE intensities for the refined models were calculated by the program MORASS (23). Simulated two-dimensional NOESY spectra were produced by the program CSL (in the SPEDREF package) using the NOE intensities from the simulation and the line shapes and chemical shifts previously obtained from MYLOR. The final structure of the Co-form was derived from NOE-restrained conjugate gradient minimization only, since the starting model was very close to the observed data and simulated annealing could not further improve on the refinement. The NMR R factor was defined as R = \sum |N_o - N_r|/\sum N_o, where N_o and N_r are the experimental and calculated NOE integrals respectively.

Circular dichroism (CD) spectra were recorded on a Jasco J720 spectrometer. The concentration of the 34mer DNA solutions was kept at 10 μM duplex in 20 mM phosphate buffer, pH 7.0.

RESULTS AND DISCUSSION

Evidence of B- to A-DNA transition

Circular dichroism and one-dimensional NMR spectra were taken to monitor titration of the 34mer DNA. Figure 2A shows the CD spectra of the titration of neomycin. The characteristic spectral shift toward the longer wavelength associated with a B- to A-DNA transition is evident. It appeared that the conformational transition was nearly complete at a 4:1 ratio of neomycin. Comparisons of the difference spectra from the titration of five multivalent cations [neomycin, spermine, Co(NH₃)₆³⁺, streptomycin and Pt(NH₃)₄²⁺] were made to more easily see the changes (Fig. 2B). Neomycin, spermine and Co(NH₃)₆³⁺ all caused a transition at 8:1, but streptomycin induces only a partial transition and no change was detected for Pt(NH₃)₄²⁺ at 8:1.

We further used one-dimensional NMR to monitor the transition. In Figure 3 the transition is shown to be complete on the addition of eight equivalents of Co(NH₃)₆³⁺. This is evident
Figure 2. Titration of ligands with the solution of A₂G₁₅C₁₅T₂ duplex as monitored by their CD spectra. (A) CD spectra of free 34mer duplex (10 µM DNA in 20 mM phosphate pH 7.0) and the 4:1 and 8:1 neomycin/duplex complexes. The maximum of the positive band shifts from 261 to 270 nm, consistent with a B- to A-DNA transition. (B) Difference CD spectra of various ligands [neomycin, spermine, Co(NH₃)₆³⁺, streptomycin and Pt(NH₃)₄²⁺] in complex with 34mer DNA at an 8:1 ratio and free DNA. Changes are seen for all complexes, except for the Pt(NH₃)₄²⁺ complex.

Figure 3. Spectra of the A₂G₁₅C₁₅T₂ duplex with Co(NH₃)₆³⁺. (A) NOEs from Co(NH₃)₆³⁺ to the A₂G₁₅C₁₅T₂ duplex. (B) Titration of Co(NH₃)₆³⁺ with the solution of A₂G₁₅C₁₅T₂ duplex. The movements of several major resonances associated with the uniform (dG)ₙ·(dC)ₙ·(dG)ₙ·(dC)ₙ sequence, including GH₁, GH₈, CH₆ and CH₄, are indicated by vertical lines. Other unassigned resonances are from the AT base pairs, GC base pairs near the ends and the central GpC step of the duplex. The spectra were collected at 25°C.

Figure 4. Result of titrations of ligands [Co(NH₃)₆³⁺, neomycin, spermine, streptomycin and Pt(NH₃)₄²⁺] at an 8:1 ratio with the solution of A₂G₁₅C₁₅T₂ duplex as monitored by their one-dimensional NMR spectra. All spectra were at 2°C except Co(NH₃)₆³⁺, which was at 25°C.

from the chemical shift changes of both the imino and the aromatic resonances of the DNA. It should be emphasized that the linewidths of the resonances are relatively sharp (~20 Hz), considering that the molecular weight of the duplex is ~22 000. Consistent with the CD data, Figure 4 shows that for the titrations with Co(NH₃)₆³⁺, neomycin and spermine the transitions are fully effected at 8:1 ratio. The remarkable similarity between the three liganded forms is evident, suggesting that the effect of the three ligands on the conformational change is identical. For streptomycin the induction is intermediate, whereas Pt(NH₃)₄²⁺ is completely ineffective.

The resonances of free DNA, neomycin-bound DNA and Co(NH₃)₆³⁺-bound DNA have been assigned for the 34mer DNA by standard sequential assignment procedures using two-dimensional NOESY data (see Fig. 5A–C). Inspection of their chemical shifts revealed characteristic changes associated with a B- to A-DNA transition (10). The GH₈ resonance moved significantly upfield (from 7.51 to 7.29 p.p.m.; see Table 1), whereas the CH₆ resonance moved downfield and became spread out (from 7.55 to 7.61–7.89 p.p.m.). For the Co-form the guanine GH₁ imino proton resonance moved upfield from 12.95 to 12.54 p.p.m. (see Fig. 3). The B to A transition was judged to be nearly complete at an 8:1 ratio based on the chemical shift (δ) difference of various DNA resonances (see Figs 3 and 4). For example, the GH₈ resonance at 7.29 p.p.m. in the Co-form of the 34mer agrees with the limiting δ values, 7.28 and 7.32 p.p.m. respectively, of the G6 and G7 bases of d(CCCCGGGG) deduced from the results of the CD/NMR studies of Xu et al. (10). Similarly, the CH₆ resonance at 7.89 p.p.m. agrees well with the average limiting δ values of the C₃ (7.78) and C₄ (7.68) bases of d(CCCCGGGG).

An interesting observation here is that at low ligand to DNA ratios (e.g. 1:1) the resonances associated with two DNA conformations can barely be discerned. This suggests that the equilibrium between the two conformers is intermediate on the NMR time scale.

Informative clues regarding the DNA conformations are found in a number of key two-dimensional NOESY cross-peaks. Figure 5A–C compares the aromatic–H₁′/H₃′ cross-peak regions of the two-dimensional NOESY spectra of the three DNA forms. The NOE intensity of the H₆/H₈ protons compared with the H₁′ protons shows that all nucleotides remain in the anti conformation.
Table 1. Chemical shifts (p.p.m.) at 25°C of the Na-form, Co-form and neomycin-form of d(A2G15C15T2)2

<table>
<thead>
<tr>
<th></th>
<th>Na-form</th>
<th>Co-form</th>
<th>Neomycin-form</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH1</td>
<td>12.95</td>
<td>12.54</td>
<td>12.59</td>
</tr>
<tr>
<td>GH8</td>
<td>7.51</td>
<td>7.29</td>
<td>7.27</td>
</tr>
<tr>
<td>GH1'</td>
<td>5.72</td>
<td>6.01</td>
<td>5.98</td>
</tr>
<tr>
<td>GH3'</td>
<td>4.88</td>
<td>4.52</td>
<td>4.51</td>
</tr>
<tr>
<td>GH4'</td>
<td>4.32</td>
<td>4.18</td>
<td>4.18</td>
</tr>
<tr>
<td>GH5'</td>
<td>4.15</td>
<td>4.24</td>
<td>4.27</td>
</tr>
<tr>
<td>GH5''</td>
<td>4.11</td>
<td>4.06</td>
<td>4.04</td>
</tr>
<tr>
<td>GH2''</td>
<td>2.67</td>
<td>2.70</td>
<td>2.69</td>
</tr>
<tr>
<td>GH2'</td>
<td>2.51</td>
<td>2.58</td>
<td>2.58</td>
</tr>
<tr>
<td>CH4</td>
<td>8.42</td>
<td>8.43</td>
<td>8.45</td>
</tr>
<tr>
<td>CH4</td>
<td>6.73</td>
<td>6.80</td>
<td>6.73</td>
</tr>
<tr>
<td>CH6</td>
<td>7.55</td>
<td>7.89</td>
<td>7.88</td>
</tr>
<tr>
<td>CH1'</td>
<td>5.87</td>
<td>5.74</td>
<td>5.72</td>
</tr>
<tr>
<td>CH5</td>
<td>5.56</td>
<td>5.48</td>
<td>5.50</td>
</tr>
<tr>
<td>CH3'</td>
<td>4.77</td>
<td>4.76</td>
<td>4.65</td>
</tr>
<tr>
<td>CH4'</td>
<td>4.17</td>
<td>4.16</td>
<td>4.14</td>
</tr>
<tr>
<td>CH5'</td>
<td>4.12</td>
<td>4.26</td>
<td>4.26</td>
</tr>
<tr>
<td>CH5''</td>
<td>4.07</td>
<td>4.10</td>
<td>4.09</td>
</tr>
<tr>
<td>CH2''</td>
<td>2.46</td>
<td>2.56</td>
<td>2.55</td>
</tr>
<tr>
<td>CH2'</td>
<td>2.23</td>
<td>2.51</td>
<td>2.49</td>
</tr>
</tbody>
</table>

When we compare the NOE cross-peak intensities of the H6/H8 protons with the H3' protons significant differences are found. (All cross-peaks have been normalized to the CH5–CH6 cross-peaks.) The CH6/GH8–H3' intensities of the neomycin-form and the Co-form are substantially stronger than the corresponding cross-peaks of the Na-form. This is consistent with a deoxyribose pucker transition from C2'-endo (S-type) to C3'-endo (N-type). The distances between the CH6/GH8 and H3' protons of the same sugar are 2.60/2.83 and 3.84/4.20 Å respectively for A- and B-DNA. In conjunction with earlier data from Xu et al. (10) our data firmly establish that the A-DNA conformation for A2G15C15T2 is fully attained upon the addition of neomycin, spermine or Co(NH3)63+ ions. It is worth pointing out that the chemical shift of CH6 resonances is more dispersive in the Co-form, suggesting that the structure associated with the (dC)n backbone is more variable than the (dG)n backbone.

Structural refinement of A-DNA

To further firmly establish the binding mode we studied the solution structure of the 4:1 complex of Co(NH3)63+ and d(ACCCCGGGGT). Examination of the one-dimensional NMR spectra of the free DNA and the 4:1 complex showed significant changes (see Fig. 6). All resonances from both forms have been assigned using two-dimensional NOESY and TOCSY data with sequential assignment tracing through the aromatic–H1'/H3' regions (see the top sections of Fig. 7). The NMR spectral changes associated with Co(NH3)63+ complexation to this decamer are consistent with the B- to A-DNA transition seen in the 34mer (Fig. 5B and C). Note that the H8 protons of G7, G8 and G9 are

Figure 5. The expanded aromatic–H1'/H3' region of the non-exchangeable proton two-dimensional NOESY spectra of A2G15C15T2 collected at 25°C shows the nucleotide conformations of the three forms. (A) The neomycin-form. (B) The Co-form. (C) The Na-form.

Figure 6. NMR spectra of the 4:1 complex of Co(NH3)63+ ion and the d(ACCCCGGGGT) duplex. The top trace is a slice of the exchangeable proton two-dimensional NOESY through the proton resonance of the Co(NH3)63+ ion at 3.65 p.p.m. Strong cross-peaks are seen from the Co(NH3)63+ protons to GH8, GH1 and CH4 protons. They provide evidence that the Co(NH3)63+ ions are bound in the major groove at the G-N7/O6 sites of the Watson-Crick base pairs as observed in the crystal structure (25). The middle and the bottom traces are the one-dimensional NMR spectra in H2O.
shifted significantly upfield (see Table 2). Moreover, the CH6/GH8–H3′ cross-peaks (located within the dashed boxes in Fig. 7) in the Co-form are stronger than in the Na-form, further evidence of the N-type sugar pucker for the Co-form DNA.

NOE-constrained refinements of the structure of both the native d(ACCCCGGGGT) and of the Co(NH3)63+ complexed form of d(ACCCCGGGGT) (4:1 ratio at 20 mM salt) have been carried out using the crystal structure (24) and canonical A- and B-DNAs as the starting models. Figure 7 shows the spectra simulated from the refined structures. For native d(ACCCCGGGGT) the refined structure conforms to the B-DNA family, while the structure with Co(NH3)63+ is in the A-DNA family (Fig. 8). The refined Co(NH3)63+-induced structure has strong A-DNA characteristics, with the following averaged conformational parameters: x displacement of the base pairs, 4.0 Å; base pair tilt angle, 17°; N-type pseudorotation angle, 25°; rise per residue, 2.65 Å.

Molecular basis of the ligand interactions

How do the ligands bind to DNA to promote the B to A transition? This information was obtained through the detection of direct NOE cross-peaks between the protons of Co(NH3)63+ and the DNA protons. Figure 6 (top) shows a slice of the exchangeable two-dimensional NOESY in H2O through the proton resonance of Co(NH3)63+. The significant NOE cross-peaks are from the Co(NH3)63+ protons to GH8, GH1 (imino) and CH4 (amino), but not to CH6, protons. These peaks are direct NOE peaks, i.e. they are not exchange peaks. The basicity of the NH3 proton of Co(NH3)63+ does not produce significant exchange with H2O protons, since only a very small peak is seen between the NH3 protons of Co(NH3)63+ and the H2O resonance. This clearly suggests that Co(NH3)63+ adheres to guanine bases in the deep major groove of the double helix. The same type of NOEs are observed in the case of the 34mer (Fig. 3A).

A major binding mode is likely analogous to that seen in the crystal structure of the Co(NH3)63+-d(ACCGGCCGGT) complex, in which the Co(NH3)63+ ions are located in the major groove of the decamer A-DNA duplex at the G4pG5 and the G8pG9 steps (24). It is worthwhile noting that the hydrogen bonding interactions between the Co(NH3)63+ ammonia molecules and the GpG step involve three NH3 molecules from one face of the octahedral coordination sphere surrounding the Co(III) ion. Thus the spatial arrangement of the hydrogen bond donors is important in dictating the effectiveness of ligand binding. The triangular arrangement of the three NH3 molecules permits effective binding to the N7/O6 sites from two neighboring intrastrand guanines. Such a binding mode places the NH3 molecules of Co(NH3)63+ close to the C-NH4 amino group and G-H8 protons and accounts for the observed NOE cross-peaks. Interestingly, the interactions of Co(NH3)63+ with Z-DNA have also been studied by X-ray diffraction and the structure showed that the same triangular arrangement of the three NH3 molecules from Co(NH3)63+ was employed. Such a spatial requirement may
explain the observation that square-planar Pt(NH$_3$)$_6^{2+}$ is not effective in B- to A-DNA conversion. However, spermine can penetrate into the deep groove of both A-DNA (major groove) (25) and Z-DNA (minor groove) (26), where hydrogen bonds between the amines of spermine and the DNA acceptors (O6/N7 of G and O2 of C for A- and Z-DNA respectively) are found.

The other major binding mode is that the ions lie across the narrow major groove, bridging the phosphate groups from the opposing strands due to the closeness of the two backbones in A- and Z-DNA (with 4.5 and 4.7 Å for closest P–P distances respectively).

The binding of neomycin to RNA may involve both binding modes. For example, we speculate that neomycin binds to RRE RNA using part of the tetrasaccharide to bridge across the major groove and another part to bind to the GGG[n=46–48] sequence (1). This remains to be confirmed by either NMR or crystallographic studies of the neomycin–RRE complex.

**CONCLUSIONS**

Our results here demonstrate that neomycin, spermine and Co(NH$_3$)$_6^{3+}$ are capable of inducing the A-DNA conformation in a (dG)$_n$·(dC)$_n$ sequence. Many DNA oligonucleotides having a (dG)$_n$·(dC)$_n$ sequence have been found to crystallize in the A-DNA conformation. Thus the GpG sequence seems to have a high propensity to adopt the A conformation, possibly due to its unique intrastrand base–base stacking interactions (11). It is likely that some factors may influence the B- to A-DNA transition. Here we have shown that three complex ions rich in hydrogen bond donors (amine functions), which are distributed over a rigid molecular frame and are highly positively charged, are potent in inducing the formation of A-DNA. Note that streptomycin is less effective in promoting the transition.

This characteristic may mimic the arginine-rich RNA binding α-helical peptides (27). This hypothesis may point out future directions for preparing more potent derivatives of simple ligands for RNA binding or the induction of A-DNA. For example, a bis-platinum(II)(triamine) complex with a butanediamine tether has been found to be very potent in promoting the B- to Z-DNA transition for the (dC·dG)$_n$ sequence (29). It is conceivable that a similar bis-cobalt(III)(pentaamine) ion may be equally or more potent. Such bis-metal ion complexes may be very effective in promoting the B- to A-DNA transition for (dG)$_n$·(dC)$_n$ sequences or in binding to certain RNA sequences. This can be tested and experiments are in progress.

**Table 2.** Chemical shifts (p.p.m.) for the Na-form (top) and Co-form (bottom) of d(ACCCGCCGGT)$_2$ at 2°C

<table>
<thead>
<tr>
<th></th>
<th>H2/5/Me</th>
<th>H8/6</th>
<th>H1'</th>
<th>H2'</th>
<th>H2''</th>
<th>H3'</th>
<th>H4'</th>
<th>H5'</th>
<th>H5''</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ade 1</td>
<td>8.10</td>
<td>8.31</td>
<td>6.29</td>
<td>2.76</td>
<td>2.89</td>
<td>4.88</td>
<td>4.30</td>
<td>3.78</td>
<td>3.78</td>
</tr>
<tr>
<td>Cyt 2</td>
<td>5.39</td>
<td>7.50</td>
<td>5.88</td>
<td>2.21</td>
<td>2.44</td>
<td>4.84</td>
<td>4.25</td>
<td>4.13</td>
<td>4.20</td>
</tr>
<tr>
<td>Cyt 3</td>
<td>5.57</td>
<td>7.53</td>
<td>6.00</td>
<td>2.15</td>
<td>2.48</td>
<td>4.86</td>
<td>4.21</td>
<td>4.12</td>
<td>4.12</td>
</tr>
<tr>
<td>Cyt 4</td>
<td>5.63</td>
<td>7.49</td>
<td>5.52</td>
<td>2.13</td>
<td>2.41</td>
<td>4.86</td>
<td>4.13</td>
<td>4.09</td>
<td>4.09</td>
</tr>
<tr>
<td>Gua 5</td>
<td>7.93</td>
<td>5.90</td>
<td>2.66</td>
<td>2.74</td>
<td>5.00</td>
<td>4.39</td>
<td>4.04</td>
<td>4.12</td>
<td>4.12</td>
</tr>
<tr>
<td>Gua 7</td>
<td>7.85</td>
<td>5.50</td>
<td>2.66</td>
<td>2.71</td>
<td>4.96</td>
<td>4.31</td>
<td>3.97</td>
<td>3.97</td>
<td>3.97</td>
</tr>
<tr>
<td>Gua 8</td>
<td>7.71</td>
<td>5.74</td>
<td>2.62</td>
<td>2.73</td>
<td>4.98</td>
<td>4.39</td>
<td>4.11</td>
<td>4.17</td>
<td>4.17</td>
</tr>
<tr>
<td>Gua 9</td>
<td>7.75</td>
<td>6.02</td>
<td>2.57</td>
<td>2.74</td>
<td>4.92</td>
<td>4.39</td>
<td>4.20</td>
<td>4.20</td>
<td>4.20</td>
</tr>
<tr>
<td>Thy 10</td>
<td>1.51</td>
<td>7.39</td>
<td>6.25</td>
<td>2.22</td>
<td>2.22</td>
<td>4.55</td>
<td>4.07</td>
<td>4.08</td>
<td>4.26</td>
</tr>
</tbody>
</table>

| Ade 1  | 8.11    | 8.46 | 6.34| 2.95| 3.02 | 4.89| 4.37| 4.02| 4.02 |
| Cyt 2  | 5.50    | 7.74 | 5.97| 2.49| 2.68 | 4.88| 4.40| 4.41| 4.28 |
| Cyt 3  | 5.65    | 7.83 | 6.05| 2.49| 2.68 | 4.90| 4.36| 4.27| 4.39 |
| Cyt 4  | 5.68    | 7.74 | 5.89| 2.41| 2.63 | 4.97| 4.33| 4.35| 4.26 |
| Gua 5  | 7.99    | 6.14 | 2.81| 2.81 | 5.04| 4.48| 4.29| 4.29| 4.29 |
| Gua 7  | 7.91    | 5.95 | 2.82| 2.82 | 4.97| 4.40| 4.41| 4.41| 4.28 |
| Gua 8  | 7.66    | 6.04 | 2.82| 2.82 | 4.89| 4.41| 4.39| 4.27| 4.27 |
| Gua 9  | 7.53    | 6.12 | 2.62| 2.82 | 4.80| 4.43| 4.37| 4.24| 4.24 |
| Thy 10 | 1.44    | 7.69 | 6.39| 2.37| 2.46 | 4.67| 4.24| 4.36| 4.25 |
ACKNOWLEDGEMENTS

This work was supported by NIH (GM-41612) and American Cancer Society (DHP-114) grants to AH-JW. The Varian VXR500 NMR spectrometer was supported in part from NIH shared instrumentation grant 1S10RR06243.

REFERENCES