Molecular Features of Co^{III} Tetra- and Pentammines Affect Their Influence on DNA Structure

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The polyamines spermine and spermidine cause a range of structural effects on DNA. $[Co(NH_3)_6]^{3+}$ is known to mimic many of these effects and we have shown previously that (+)-$[Co(en)_3]^{3+}$ and (–)-$[Co(en)_3]^{3+}$ (en = ethylenediamine) do so as well. In this paper the effects of mono- and disubstitution of the Co^{III} ammines by aqua, halo and nitro groups is explored. The overall effectiveness of the amines in conferring thermal stability on the ct-DNA proceeds in the order: spermine > (+)-$[Co(en)_3]^{3+}$ ≈ (–)-$[Co(en)_3]^{3+}$ ≈ $[Co(NH_3)_6]^{3+}$ ≈ spermidine > $[Pt(NH_3)_4]^{2+}$ > $[Co(NH_3)_5H_2O]^{3+}$ ≈ $[Co(NH_3)_5Cl]^{2+}$ ≈ $[Co(NH_3)_5Br]^{2+}$ > *cis*-$[Co(en)_2NH_3Cl]^{2+}$ > $[Co(NH_3)_5(NO_2)]^{2+}$ ≈ *trans*-$[Co(NH_3)_4(H_2O)_2]^{3+}$; whereas the B→Z transition-induction ordering is: spermine ≈ $[Co(NH_3)_6]^{3+}$ > (+)-$[Co(en)_3]^{3+}$ ≈ $[Co(NH_3)_5(H_2O)]^{3+}$ > (–)-$[Co(en)_3]^{3+}$ > $[Co(NH_3)_5Br]^{2+}$ > $[Co(NH_3)_5Cl]^{2+}$, with the other amines failing to cause the transition under the 11 mM salt concentration and temperature ramp conditions used; the DNA bending ranking is: spermine > $[Co(NH_3)_6]^{3+}$ > (+)-$[Co(en)_3]^{3+}$ > (–)-$[Co(en)_3]^{3+}$ > $[Co(NH_3)_5(H_2O)]^{3+}$ > *trans*-$[Co(NH_3)_4(H_2O)_2]^{3+}$ > spermidine, with the other 2+ amines (the aquo complexes will be 2+ at neutral pH), especially the bromo and nitro compounds, having comparatively little effect. While the presentation of NNN triangular faces is found to be important in the B→Z transition, the fact that the halo ligands are probably substituted by the DNA phosphates plays a role, especially in the DNA bending, where deep penetration of the major groove is concluded to be required. A favourable interaction between the backbone and the aquo ligands may enhance the B→Z transition.

Introduction

The two DNA-binding polycations spermidine $[H_3N(CH_2)_3NH_2(CH_2)_4NH_3]^{3+}$ and spermine $[H_3N(CH_2)_3NH_2(CH_2)_4NH_2(CH_2)_3NH_3]^{4+}$ are present in all cells.[1] The precise cellular functions of polyamines are unclear but they are known to neutralise over 40% of chromosomal DNA and to inhibit damage by reactive oxygen species,[2] radiation,[3,4] and alkylating agents.[5] Furthermore, polyamines stabilise DNA to heat and induce and stabilise A, B, Z (usually observed with alternating G-C sequences) and collapsed DNA structures.[6–8] We have recently shown that the cobalt(III) ammine complexes $[Co(NH_3)_6]^{3+}$, (+)-$[Co(en)_3]^{3+}$, and (−)-$[Co(en)_3]^{3+}$ (en = ethylenediamine) mimic different aspects of the DNA interaction of the small polyamine molecules spermidine and spermine.[8,9]

Our molecular modelling study[9] of $[Co(NH_3)_6]^{3+}$, (+)-$[Co(en)_3]^{3+}$, and (−)-$[Co(en)_3]^{3+}$ with DNA shows that these cobalt ammines do not bind in the minor groove of any sequence, and their residence times in the major groove decrease in the order spermine > $[Co(NH_3)_6]^{3+}$ > (+)-$[Co(en)_3]^{3+}$ > (−)-$[Co(en)_3]^{3+}$. In addition, while the spermine binding was found by Yuki et al. to be cooperative,[11] in analogous simulations[9] the others were found not to be. These amines all reduce the linear dichroism (LD) signal of DNA by bending calf thymus (ct) and micrococcus lysodeikticus (ml) DNAs. Since the molecules are major groove binders, we concluded that their behaviour supports the amine-major-groove bending model.[12] The comparative effectiveness of the cobalt ammines at bending DNA suggests that the presentation of a triangular NNN face to the DNA optimises the bend-causing interaction — $[Co(NH_3)^{3+}]$ has a high number of pre-formed NNN triangles, whereas spermine does not. The ethylenediamine complexes are somewhat less effective as they have fewer such faces. A similar geometric argument is required for the induction of the B→Z transition. Crystal structure data are consistent with the formation of five hydrogen bonds between $[Co(NH_3)_6]^{3+}$ and the major-groove convex surface of Z-DNA, specifically to guanine O6 and N7 and the phosphate oxygens.[13]

In order to probe the role of NNN triangular faces further, we have investigated the interaction of a set of cobalt(III) tetra- and pentaamines with DNA. In this work we have used temperature-dependent normal absorption to probe the stabilising effect of the ammines on duplex DNA; circular dichroism (CD), which depends on the asymmetric character of an absorbing chromophore, to probe ligand binding; and linear dichroism (LD) to probe the effect of the ammines on DNA length and flexibility.

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Results and Discussion

ct-DNA Absorbance Temperature Dependence

The absorbance spectra of a set of substituted cobalt ammines are illustrated in Figure 1. A wavelength of 280 nm, rather than the usual 260 nm, was chosen to follow the temperature dependence of ct-DNA absorbance in the presence of these ammines (Figure 2) to minimise the ligand contribution to the changes. The DNA melting curves show unusual pre- and post-melting transitions, most of which are consistent with the temperature dependence of the free ligand (data not shown), although the negative pre-melting transitions are all enhanced in the presence of the DNA. There was no evidence of light scattering above 300 nm in either temperature-dependent absorbance (data not shown) or CD spectra (Figure 4). The ligand effects were largest for the bromine-substituted compound (a 40 μM solution had a change of −0.02 absorbance units from baseline at 60 °C), so it is likely, at least in this case, that a substitution reaction removes a ligand-to-metal charge-transfer transition and that the DNA influences the kinetics of such reactions. Whether the ligands end up covalently bound to the DNA or their aquation is catalysed is not clear from these data.

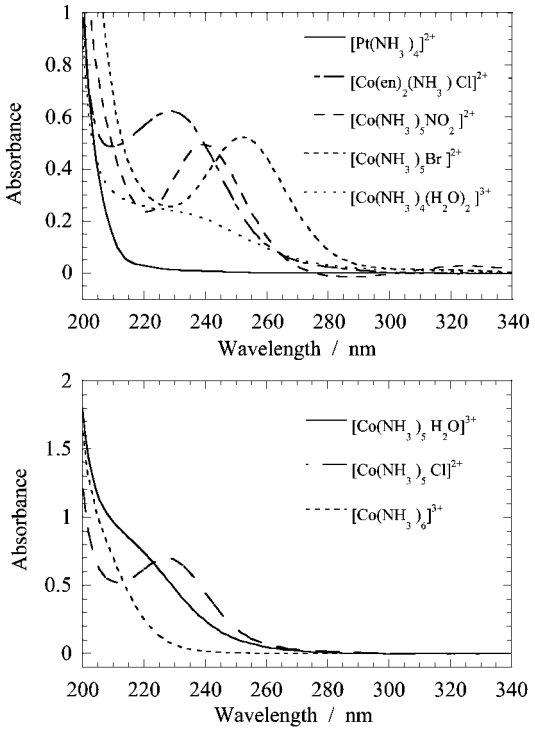

Figure 1. Normal absorption spectra of DNA binding ammines (40 μM in 10 mM NaCl and 1 mM cacodylate); $[Co(NH_3)_4(H_2O)_2]^{3+}$ is the *trans* isomer and $[Co(en)_2NH_3Cl]^{2+}$ (en = ethylenediamine) the *cis* isomer

Taking derivatives of the main hyperchromic denaturing transitions, thus assuming that the pre- and post-melting transitions could be ignored, the stabilization conferred upon the DNA by the amines is summarized in Figure 3. Substituting an amine group in $[Co(NH_3)_6]^{3+}$ by a Cl, Br, NO_2 or H_2O ligand, seriously impedes the metal complex's ability to stabilize DNA. The post-melting transition of $[Co(en)_2NH_3Cl]^{2+}$ makes it impossible to determine T_m for this system accurately after the first few ammine concentrations. The values would lie below the other halo species.

Effect of Ammines on poly[d(G-C)$_2$] Structure

The pentammine $[Co(NH_3)_5(H_2O)]^{3+}$ induces Z-form DNA as effectively as (+)-$[Co(en)_3]^{3+}$ (data not shown). $[Co(NH_3)_5Br]^{2+}$ and $[Co(NH_3)_5Cl]^{2+}$ also induce the Z-form DNA of poly[d(G-C)]$_2$ (Figure 4) to some extent. Concentrations of 20 μM spermidine,[9] *cis*-$[Co(en)_2NH_3Cl]^{2+}$, $[Pt(NH_3)_4]^{2+}$, $[Co(NH_3)_5(NO_2)]^{2+}$, and *trans*-$[Co(NH_3)_4(H_2O)_2]^{3+}$ do not promote the B→Z transition in poly[d(G-C)]$_2$ (60 μM in 10 mM NaCl) (Figure 4). The CD changes observed with these ligands, with the exception of *trans*-$[Co(NH_3)_4(H_2O)_2]^{3+}$, are the same as those induced by increasing the temperature of poly[d(G-C)$_2$].[9] $[Co(NH_3)_5(NO_2)]^{2+}$ does, however, promote the B→Z transition at 0 mM NaCl, 1 mM cacodylate buffer which is the behaviour previously observed for spermidine.[9]

LD of ct-DNA with Cobalt Tetra- and Pentaammines

Figure 5 summarises the LD of ct-DNA at 260 nm with added cobalt ammine. The initial addition of each ammine results in a small decrease in DNA orientation. We previously found that ≈20 μM spermine, ≈60 μM $[Co(NH_3)_6]^{3+}$, ≈80 μM $[Co(en)_3]^{3+}$, and 240 μM of spermidine are required to cause the LD signal to be sharply reduced. The monoaquo complex requires ≈140 μM and the diaquo complex begins to bend the DNA at ≈ 200 μM, and requires ≈400 μM to complete it.

Comparison of the Amines

The overall effectiveness of the amines in conferring thermal stability on the ct-DNA proceeds in the order:

spermine > (+)-$[Co(en)_3]^{3+}$ ≈ (−)-$[Co(en)_3]^{3+}$ ≈ $[Co(NH_3)_6]^{3+}$ ≈ spermidine > $[Pt(NH_3)_4]^{2+}$ > $[Co(NH_3)_5H_2O]^{3+}$ ≈ $[Co(NH_3)_5Cl]^{2+}$ ≈ $[Co(NH_3)_5Br]^{2+}$ > *cis*-$[Co(en)_2NH_3Cl]^{2+}$ > $[Co(NH_3)_5(NO_2)]^{2+}$ ≈ *trans*-$[Co(NH_3)_4(H_2O)_2]^{3+}$

whereas the B→Z transition induction ordering is:

spermine ≈ $[Co(NH_3)_6]^{3+}$ > (+)-$[Co(en)_3]^{3+}$ ≈ $[Co(NH_3)_5(H_2O)]^{3+}$ > (−)-$[Co(en)_3]^{3+}$ > $[Co(NH_3)_5Br]^{2+}$ > $[Co(NH_3)_5Cl]^{2+}$

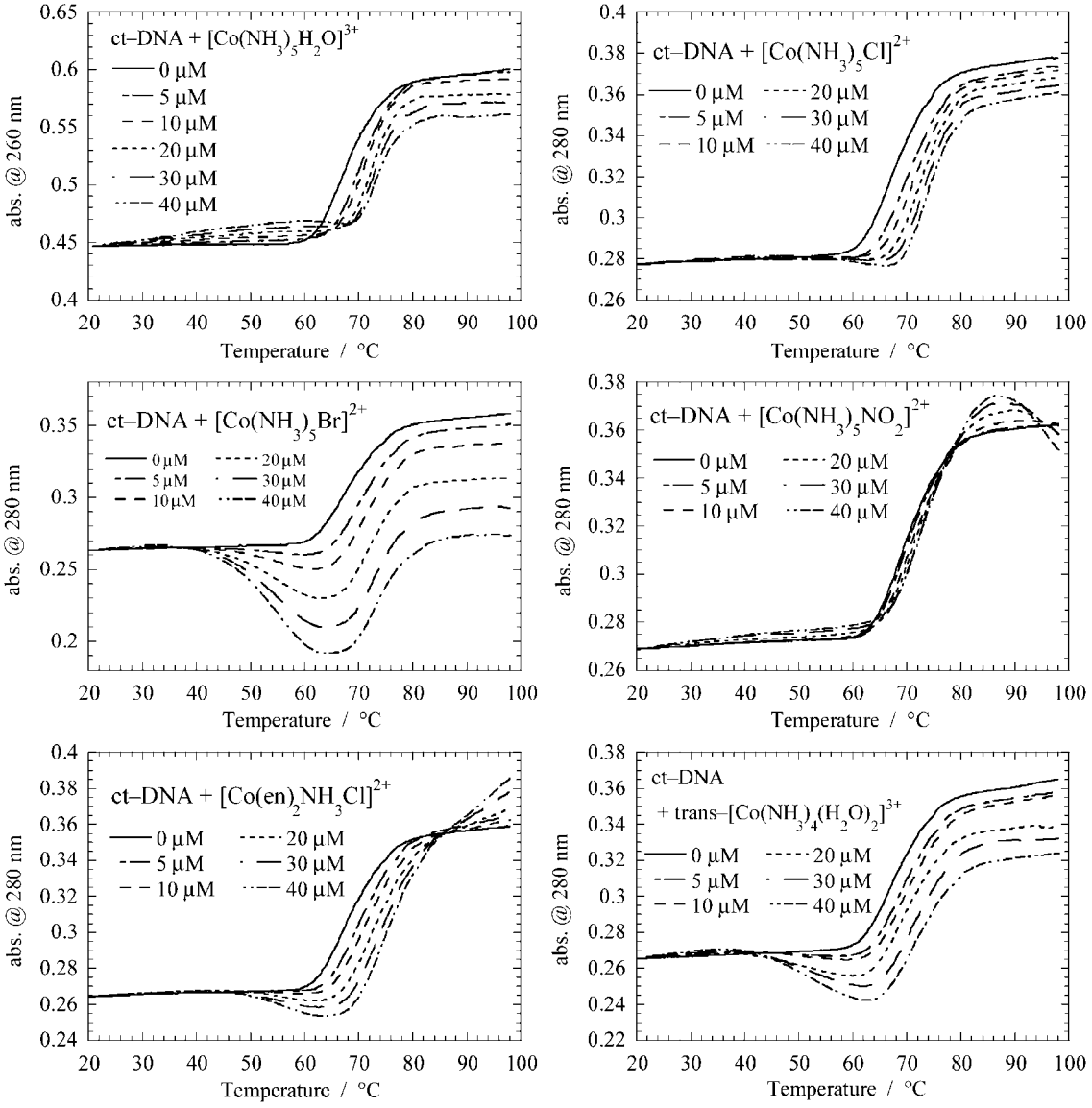

Figure 2. Absorbance versus temperature plot for ct-DNA (60 μM, 10 mM NaCl, 1 mM cacodylate) with addition of cobalt ammines at various concentrations as indicated in the figure

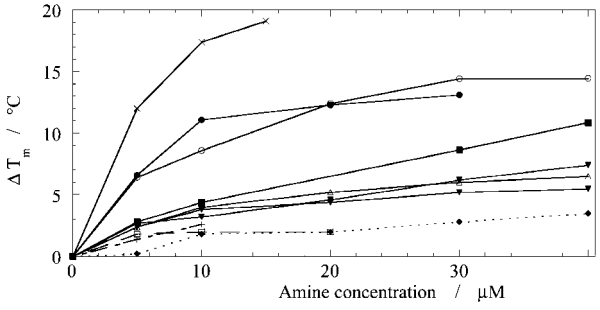

Figure 3. Summary of the change in T_m of ct-DNA (60 μM, 10 mM NaCl, 1 mM cacodylate) as a function of the amine concentration (concentrations are indicated in the figure); spermine, spermidine and $[Co(NH_3)_6]^{3+}$ data are taken from ref.[9]; the order of the melting temperature plots is, from top to bottom at 10 μM: spermine > $[Co(NH_3)_6]^{3+}$ > spermidine > $[Pt(NH_3)_4]^{2+}$ > $[Co(NH_3)_5Cl]^{2+}$ > $[Co(NH_3)_5Br]^{2+}$ > $[Co(NH_3)_5H_2O]^{3+}$ > *cis*-$[Co(en)_2NH_3Cl]^{2+}$ > $[Co(NH_3)_5(NO_2)]^{2+}$

with the other amines failing to cause the transition under the 11 mM salt concentration and temperature ramp conditions used; the DNA bending ranking is:

spermine > $[Co(NH_3)_6]^{3+}$ > (+)-$[Co(en)_3]^{3+}$ > (−)-$[Co(en)_3]^{3+}$ > $[Co(NH_3)_5(H_2O)]^{3+}$ > *trans*-$[Co(NH_3)_4(H_2O)_2]^{3+}$ > spermidine

with the other ammines having comparatively little effect. The most notable things about the above lists is that, with the exception of spermine, the ranking of the molecules varies. There is a general trend of reducing efficacy with reducing charge (the aquo complexes will be deprotonated at pH = 6.8[16]). The two enantiomers of $[Co(en)_3]^{3+}$ behave in a similar fashion, but with the possible exception of the thermal stability, where they have a very similar effect, the (−)-enantiomer affects the DNA structure less than the (+)-enantiomer. This correlates with the previous simula-

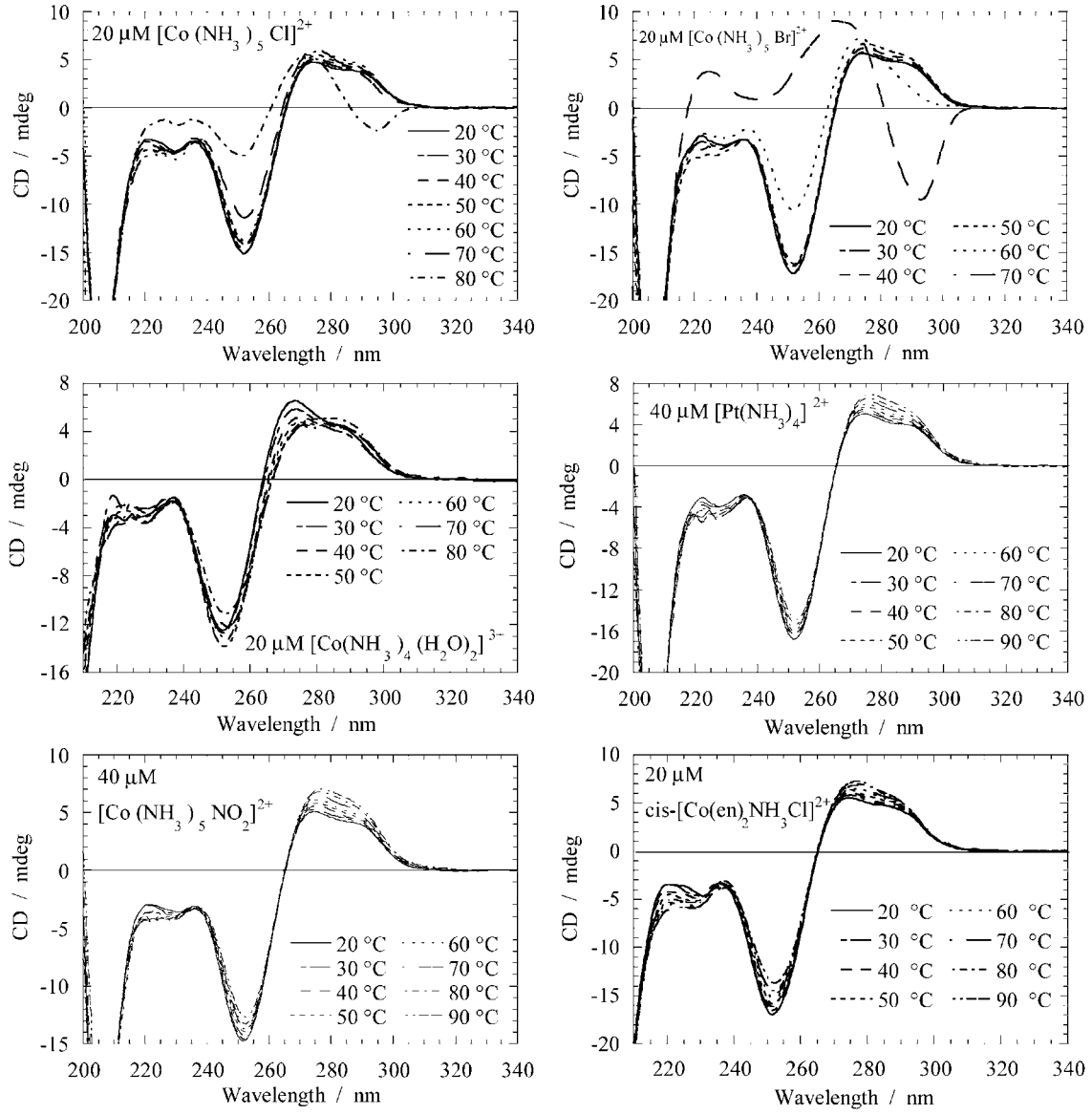

Figure 4. CD spectra of poly[d(G-C)]$_2$ (100 μM in 10 mM NaCl and 1 mM cacodylate buffer) in the presence of a fixed concentration of various amines as the temperature is raised at 4 °C/minute; amine concentration and temperatures are indicated in each figure

tion studies[8] that showed the (+)-enantiomer spending more time localised on the DNA than the (−)-enantiomer.

The DNA thermal stability gives an indication of binding strength, assuming the binding site size and binding modes are similar,[17] so we can conclude that water, halo and especially nitro substitution reduces the DNA affinity of a molecule or significantly changes the binding mode.

The changes in the absorbance of the cobalt tetra- and pentammines observed at elevated temperatures suggest that substitution reactions may be occurring (the change is not reversed upon temperature reduction). The halo-substituted complexes all lose absorbance intensity consistent with the loss of ligand (in this case the halogen ligand)-to-metal charge-transfer transitions. Strong direct cobalt−phosphate interactions have previously been observed by FTIR spectroscopy between $[Co(NH_3)_5Cl]^{2+}$ and the phosphates in ct-DNA with H-bonding interactions observed with the bases.[18] In contrast, no direct bonding was evident with $[Co(NH_3)_6]^{3+}$.[18] This was interpreted to mean that the chloro group was displaced, facilitating the direct bonding of the cobalt to the DNA backbone. Direct cobalt phosphate binding has also been observed between $[Co(NH_3)_5Cl]^{2+}$ and purine nucleotides.[19] From a DNA-charge neutralisation viewpoint these 2+ cobalt ammines thus effectively become 3+ when they loose the Cl^-; in addition they cannot be located particularly deeply in the major groove. It is reasonable to suppose that *cis*-$[Co(en)_2NH_3Cl]^{2+}$ behaves analogously but with lower affinity due to the steric hindrance of the ethylenediamines. The lower thermal stability of DNA in the presence of the

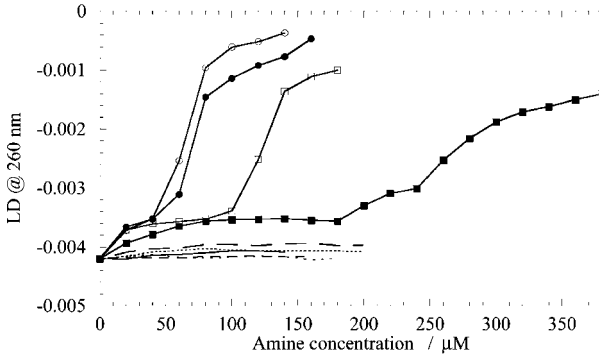

Figure 5. Summary of the LD signals of ct-DNA (60 μM in 10 mM NaCl, 1 mM cacodylate buffer) in the presence of ammines (concentration are indicated in the figure); experiment performed with the same rotation speed; order of LD plots is, from top to bottom at 50 μM: (+)-$[Co(en)_3]^{3+}$ > (−)-$[Co(en)_3]^{3+}$ > $[Co(NH_3)_5H_2O]^{3+}$ > $[Co(NH_3)_4(H_2O)_2]^{3+}$ > *cis*-$[Co(en)_2NH_3Cl]^{2+}$ > $[Pt(NH_3)_4]^{2+}$ > $[Co(NH_3)_5Cl]^{2+}$ > $[Co(NH_3)_5Br]^{2+}$ > $[Co(NH_3)_5(NO_2)]^{2+}$

halo compounds compared with $[Co(NH_3)_6]^{3+}$ may thus be deduced to correlate with a different binding site and a smaller binding affinity.

By way of contrast, the bulky negatively charged NO_2^- ligand will not have a good affinity for the backbone. Its groove binding preferences will also differ from those of an NH_3 ligand since its hydrogen bonding requirements are as an acceptor not a donor.

Conclusion

The inability of the nitro and halo compounds to bend the DNA significantly thus leads us to the conclusion that the bend-inducing binding mode requires deep penetration of the groove by hydrogen-bonding donors. In this context the low hydrogen bonding energy of $[Co(NH_3)_6]^{3+}$ compared with, for example, Mg^{2+} determined by Black and Cowan[20] is not necessarily significant — the hydrogen bonding energy just has to be large enough to position and orient the metal complex in the mode that favours DNA bending once the dominant electrostatic binding energy has assured DNA−metal complex binding.

The halo compounds creep up the ranking order for the B→Z transition, suggesting that the induction of this transition is favoured by some backbone interaction as well as groove binding. It has previously been concluded[9,10] that a triangular face of amines facing the DNA that could interact with the N7/O6 sites of intrastrand guanine bases is required to induce the B→Z transition. In agreement with this hypothesis, *trans*-$[Co(NH_3)_4(H_2O)_2]^{3+}$ and $[Pt(NH_3)_4]^{2+}$,[21] which have no triangular arrangements of amine groups, are incapable of inducing the B→Z transition (even in the absence of NaCl). Substitution of one amine on the $[Co(NH_3)_6]^{3+}$ reduces the transition induction and also reduces the number of triangular faces from 8 to 4. This is consistent with there being fewer effective binding modes to promote the B→Z transition. Neither spermidine nor the weaker binding NO_2 induces a transition in the presence of NaCl, however, they do so at 0 mM NaCl. This presumably relates to the need to reduce the competition for DNA sites from any other positively charged species. In the case of the ethylenediamine ligands, their bulk requires that an effective NNN face must not include the backbone of the ethylenediamine, so the number of NNN faces is reduced again resulting in the slip down the ranking order of the tris-ethylenediamine complexes, with the enantiomer that fits better into the major groove, according to our molecular modelling studies,[9] being able to optimise its interaction. The halo-ethylenediamine complex cannot present a triangular face to the groove while binding to the phosphates, and therefore cannot induce the transition. The mono-aquo complex ends up between the two tris-ethylenediamine enantiomers in its effectiveness, showing a balance of the number of NNN faces (which is higher for the mono-aquo complex) and DNA binding strengths (which is lower for the mono-aquo complex).

Experimental Section

Materials: Ultra pure water (18.2 MΩ) was used in all experiments. ct-DNA was purchased from Sigma/Aldrich Chemical Co. Ltd. Poly[d(G-C)]$_2$ was obtained from Pharmacia Biochemicals. All polynucleotides were dissolved in water without further purification (this required overnight stirring for ct-DNA) and kept frozen until the day of the experiment. DNA solutions were prepared by diluting in 1 mM cacodylate buffer (made up from the sodium salt of cacodylic acid, $C_2H_6AsO_2Na$, 98% purchased from Sigma/Aldrich buffered with hydrochloric acid to a pH of 6.8) with the required NaCl (>99.5% purity, Sigma/Aldrich). The concentration of DNA was determined spectroscopically using ε_{247nm} = 8400 $M^{-1}cm^{-1}$ for poly[d(G-C)]$_2$ and ε_{258nm} = 6600 $M^{-1}cm^{-1}$ for ct-DNA, expressed as the molarity of the phosphate groups.

Methods

Absorbance: Normal absorption spectra were obtained using a Cary 1E spectrometer equipped with a multicell cell changer and Peltier heating unit accessories. Rectangular quartz cuvettes with a reduced volume (4 mm width) and 1 cm path length were used for the absorbance versus temperature melting curves. The cuvettes were filled almost completely (approximately 1300 μL) and the top of the cuvette was sealed with PTFE thread seal tape to ensure no leakage occurred during heating. Samples were heated from 20−98 °C with a ramp rate of 0.1 °C/minute to eliminate any lag between the temperature of the solution and the heating block. Melting temperatures were determined by the derivative method in the Cary software with a data interval equal to the data collection interval (0.2 °C).

CD: Circular dichroism (CD), the difference in absorption of left and right circularly polarised light, was used to follow the transition of poly[d(G-C)]$_2$ into the Z-form in the presence of the ammines. Spectra were collected in 1 cm rectangular path length cuvettes using a Jasco J-715 spectropolarimeter equipped with a Peltier temperature-control device. The samples were heated from 20 °C at a ramp rate of 40 °C/hour and a CD spectrum collected every 10 °C. No equilibration time was allowed prior to the collection of the first spectrum. Since CD is uniquely sensitive to the symmetry of a system, even the achiral transition metal complexes will gain an induced signal at wavelengths where they absorb light. This be-

comes a particular issue for the brominated complex, which has significant absorbance at 260 nm (see Figure 1).

Flow LD: Linear dichroism (LD) is the difference in absorption of light linearly polarised parallel, $A_{\parallel}$, and perpendicular, $A_{\perp}$, to an orientation axis:

$$LD = A_{\parallel} - A_{\perp}$$

In this work, DNA was oriented by shear flow of the DNA sample between a fixed outer cylinder and a rotating solid quartz inner cylinder separated by a 0.5 mm wide gap.[14,15] The linearly polarised light was incident radial to the flow cell. A Jasco J-715 spectropolarimeter adapted for LD measurements was used. The LD of DNA is dominated by the $\pi-\pi^*$ transitions of the DNA bases. Thus, DNA LD probes the average orientation of the DNA bases. On a qualitative level, the LD of DNA can indicate changes in length and variations in the conformation of DNA induced by a binding drug. If the DNA is made more flexible or shortened then it will orient less effectively and the LD signal will be reduced.

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