

indicated. Implications of these results for the interpretation of spectroscopic data on *cis*-DDP-oligonucleotide adducts are discussed.

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REFERENCES

- [1] a) B. ROSENBERG, L. VAN CAMP, J.E. TROSKO, V.H. MANSOUR, *Nature (London)*, **222**, 385-386 (1969);
b) Review:
S.J. LIPPARD, *Science*, **218**, 1075-1082 (1982).
- [2] a) P.J. STONE, A.D. KELMAN, F.M. SINEX, *Nature (London)*, **251**, 736-737 (1974);
b) T.D. TULLIUS, S.J. LIPPARD, *J. Am. Chem. Soc.*, **103**, 4620-4622 (1981).
- [3] G.L. COHEN, J.A. LEDNER, W.R. BAUER, H.M. USHAY, C. CARAVANA, S.J. LIPPARD, *J. Am. Chem. Soc.*, **102**, 2487-2488 (1980).
- [4] G.L. COHEN, W.R. BAUER, J.K. BARTON, S.J. LIPPARD, *Science*, **203**, 1014-1016 (1979).
- [5] a) J.H.J. DEN HARTOG, C. ALTONA, J.H. VAN BOOM, G.A. VAN DER MAREL, C.A.G. HAASNOOT, J. REEDIJK, *J. Am. Chem. Soc.*, **106**, 1528-1530 (1984);
b) B. VAN HEMELRYCK, E. GUITTET, G. CHOTTARD, J.P. GIRAULT, T. HUYNH-DINH, J.Y. LALLEMAND, J. IGOLEN, J.C. CHOTTARD, *J. Am. Chem. Soc.*, **106**, 3037-3039 (1984).
- [6] D. CREMER, J.A. POPLER, *J. Am. Chem. Soc.*, **97**, 1354-1358 (1975).



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CHIRAL DISCRIMINATION IN THE COVALENT BINDING OF BIS(PHENANTHROLINE)RUTHENIUM(II) COMPLEXES TO DNA

Isomers of bis(phenanthroline)dichlororuthenium(II) display striking enantioselectivity in binding covalently to right-handed B-DNA. Incubation of racemic (phen)₂RuCl₂ with calf thymus DNA yields ruthenium-bound complexes having a maximum binding ratio of 0.06 ruthenium per nucleotide. Much as for the analogous dichlorodiammineplatinum(II) complexes, binding studies with various synthetic polymers reveal a preference for guanine. Circular dichroism of the supernatants following ethanol precipitation of the ruthenium-DNA complexes show substantial optical activity and, importantly, a sequence dependence in the level of enrichment. Interestingly, in contrast to stereoselective intercalation, covalent binding of (phen)₂Ru²⁺ to simple B-DNA appears to favor the lambda isomer. Coordination of the (phen)₂Ru²⁺ moiety to the helix seems to require a structure of complementary symmetry. Curiously, incubation with poly d(GC) or Z-form polymers leads to the preferential covalent binding of the opposite (delta) ruthenium isomer. The conformation and sequence specificity of these chiral octahedral complexes suggests the possible utility of chiral bis(amine) complexes in DNA site-specific drug design.



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BIOCHEMICAL EFFECTS OF BINDING

$[(\text{H}_2\text{O})(\text{NH}_3)_5\text{Ru}^{\text{II}}]^{2+}$ AND $[(\text{NH}_3)_5\text{Ru}^{\text{III}}]$ TO DNA

The reaction of $[(\text{H}_2\text{O})(\text{NH}_3)_5\text{Ru}^{\text{II}}]^{2+}$ with calf thymus and salmon sperm DNA has been studied over a wide range of transition metal ion concentrations in order to determine the binding sites of the metal ion to the heterocyclic bases. Kinetic studies revealed a biphasic reaction with an initial fairly rapid coordination of the metal ion being followed by slower reactions. Under these conditions a true equilibrium could not be reached; however, it was possible to study metal ion coordination under pseudo-equilibrium conditions after the initial reaction was complete and before subsequent reactions had progressed substantially. For covalently bound Ru levels up to 0.26 Ru per DNA nucleotide, $(\text{Ru}_{\text{DNA}}/\text{P}_{\text{DNA}})$, the predominant binding site on helical DNA is in the major groove at the N-7 of guanine. Spectra of $[(\text{NH}_3)_5\text{Ru}^{\text{III}}]_n$ -DNA prepared by incubation of $[(\text{H}_2\text{O})(\text{NH}_3)_5\text{Ru}^{\text{II}}]^{2+}$ with DNA (where $[\text{P}_{\text{DNA}}] = 1.5 \text{ mM}$ and reactant $[\text{Ru}^{\text{II}}]/[\text{P}_{\text{DNA}}]$ ratios are in the range 0.1 to 0.3) followed by air oxidation yielded intense charge transfer bands which could only be attributed to $[(\text{NH}_3)_5\text{Ru}^{\text{III}}]$ coordination to N-7 dG sites. HPLC of acid-hydrolyzed samples of $[(\text{NH}_3)_5\text{Ru}^{\text{III}}]_n$ -DNA, which had been prepared by this method with helical DNA, also revealed the significant presence of only $[(\text{Gua})(\text{NH}_3)_5\text{Ru}^{\text{III}}]$ for

$0.1 < [\text{Ru}^{\text{II}}]/[\text{P}_{\text{DNA}}] < 0.5$, which was verified by UV-Vis identification of the isolated chromatographic band. An earlier X-ray molecular structure determination has established the coordination site as the imidazole N-7 of dG, which is exposed in the major groove of the DNA.

At $[\text{Ru}^{\text{II}}]/[\text{P}_{\text{DNA}}] \leq 0.5$ T_m values for the DNA decreased linearly with increasing ruthenium concentration and an increase in the intensity of the 565 nm charge transfer band was noted upon melting. The UV and CD spectra of these samples indicated no extensive destacking or alteration in geometry (B family) compared to unsubstituted DNA. At $[\text{Ru}^{\text{II}}]/[\text{P}_{\text{DNA}}] > 0.5$ or when single stranded DNA was used, increased absorbance at 530 nm and 480 nm suggested additional binding to the exocyclic amine sites of adenine and cytosine residues. HPLC and individual spectrophotometric identification of the products derived from hydrolysis of these species yielded both $[(\text{Gua})(\text{NH}_3)_5\text{Ru}^{\text{III}}]$ and $[(\text{Ade})(\text{NH}_3)_5\text{Ru}^{\text{III}}]$. Earlier crystallographic, spectroscopic and electrochemical studies have established the adenosine binding site of $[(\text{NH}_3)_5\text{Ru}^{\text{III}}]$ to be the exocyclic amine (N-6). Coordination to the exocyclic amines of adenine and cytosine, is indicative of double helix disruption since these amines are involved in hydrogen bonding on the interior of B-DNA. A highly metallated ($\text{Ru}_{\text{DNA}}/\text{P}_{\text{DNA}} = 0.26$) DNA sample found to be rapidly sedimenting and unable to electrophorese into an agarose gel appeared to have undergone counterion induced collapse, which is a phenomenon that has not been previously demonstrated for DNA covalently coordinated by a transition metal ion.

Agarose gel electrophoresis of superhelical PBR322 plasmid DNA after exposure to various amine complexes of $[(\text{NH}_3)_5\text{Ru}^{\text{III}}]$ in the presence of a reductant and air generally revealed moderately efficient cleavage of the DNA, presumably due to the generation of hydroxyl radical via Fenton's chemistry. However, similar studies involving $[(\text{NH}_3)_5\text{Ru}^{\text{III}}]$ directly coordinated to the DNA showed no strand cutting. Polyacrylamide gel electrophoresis of a 381 bp, $3'$ - ^{32}P -labeled fragment of PBR322 plasmid DNA containing low levels of bound $[(\text{NH}_3)_5\text{Ru}^{\text{III}}]$ further indicated negligible DNA cutting by the coordinated metal ion.