Stereoselectivity in the Binding of Transition-Metal Chelate Complexes to Nucleic Acid Constituents: Bonding and Nonbonding Effects

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During the past few years, there has been a dramatic increase in interest in the binding of metal ions and metal complexes to nucleic acids and nucleic acid constituents.[1-3] The in vivo role of metal ions in nucleic acid chemistry—DNA replication, transcription, and translation; DNA denaturation and renaturation; RNA conformational properties; depolymerization of RNA; enzyme–metal–nucleic acid ternary species—has long been recognized.[1] Furthermore, the recent discovery that certain transition-metal complexes, e.g., *cis*-[Pt-$(NH_3)_2Cl_2$], are effective antineoplastic agents and that these complexes are thought to bind to nucleic acids in vivo has contributed significantly to the increase in the activity in this area. Additionally, a diversity of metal ions and metal compounds has been employed to probe into the function of nucleic acids and nucleic acid constituents and as aids in the separation, purification, and structural elucidation of these biologically important molecules.[1-3]

Selective coordination underlies all these aspects of the interaction of inorganic species with nucleic acids. There are two broad types of bonding selectivity. First, polynucleotides present to a metal center three major types of ligating regions: (1) the ribose moiety; (2) the phosphate oxygens of the phosphodiester linkages; (3) the heterocyclic ring nitrogen atoms and the exocyclic functional groups of the purine and pyrimidine bases. Metal compounds are often selective as to which of these ligating regions (or combination of regions) will form the strongest bond. Second, in many instances, metal ions or complexes will coordinate preferentially to one of the four common bases in DNA or RNA (Figure 1).

Many phenomena can be understood as arising from one of these two types of selectivity. For example, some metal ions (Cu^{2+}) destabilize whereas others (Mg^{2+}) stabilize double-helical DNA and influence the midpoint temperature (T_m) of the thermally induced double-helix → random-coil transition. This specificity can be understood as arising from Cu^{2+} ion to heterocyclic base binding which disrupts the Watson–Crick base pairing and from Mg^{2+} ion to phosphate binding which minimizes repulsion between the negatively charged phosphate backbones.[1] Neither the structural details of the binding nor the effectiveness of metal ions in promoting recoiling is completely understood. Replication of DNA, which has been shown several times to require zinc metalloenzymes, exhibits extremely high fidelity ($\sim 10^9$).[1,2] Such base selectivity greatly exceeds that found for simple metal ions and complexes, although it is not known whether the zinc(II) atom binds directly to the base portion of the nucleic acid.

In recent years, we have probed the underlying chemistry of selectivity by examining the interaction of chelate complexes with nucleic acid components. By systematically varying the geometry of the metal and the size and hydrogen-bonding potential of chelate ligands, we have attempted to define those factors which control selectivity toward the heterocyclic bases.

At the outset, relatively little work had been reported into the syntheses of pertinent complexes, and even fewer x-ray structural investigations had been performed.[1-3] Many of these early studies utilized the free bases, which normally coordinate through N.9 for purines and N.1 for pyrimidines. Owing to the presence of the glycosyl bond, these sites are unavailable in nucleic acids. Two important exceptions were an N.3-bound copper(II)–cytosine complex[4] and an N.7-bound zinc(II)–adenine complex.[5] Insufficient experience existed to overcome the generally great instability of nucleoside complexes.

It has now become clear through efforts in this laboratory that the exocyclic groups of the purine and pyrimidine rings are a key to selectivity. These exocyclic groups can function either to hinder coordination via steric factors or alternatively to stabilize coordination by participating in favorable hydrogen-bonding interactions with other ligands coordinated to the metal center. The importance of the influence of these exocyclic groups is augmented by the low basicity of the ring nitrogens, the transition-metal binding sites of overwhelming predominance. These binding sites will now be discussed in detail.

Thomas J. Kistenmacher received his B.Sc. degree from Iowa State University and his Ph.D. degree from the University of Illinois, the latter under the direction of Galen D. Stucky. After 2 years as an NSF postdoctoral fellow at California Institute of Technology with Richard E. Marsh, he moved to Johns Hopkins where he is now Associate Professor. His research interests include the study of the structure and bonding in transition-metal complexes of nucleic acid components, the structure of organic metals, the structural chemistry of small biologically interesting molecules, and x-ray diffraction techniques.

Luigi G. Marzilli first came to the editor's attention as a talented student in the educationally innovative Brown University freshman organic chemistry course which we happened to be teaching. While an undergraduate, he was initiated into research in transition-metal chemistry in work with David A. Buckingham. After taking his B.Sc., he followed Buckingham to the Australian National University in Canberra, where he took his Ph.D., working jointly with him and Alan M. Sargeson. In 1970, subsequent to a postdoctoral year with Jack Halpern at the University of Chicago, he joined the Chemistry Department at The Johns Hopkins University, where he is Associate Professor. Besides bioinorganic chemistry, Dr. Marzilli's research interests include synthesis, NMR, reaction mechanisms, reactions of coordinated ligands, and complexes of phosphorus donor ligands.

(1) G. L. Eichhorn in "Inorganic Biochemistry", G. L. Eichhorn, Ed., Elsevier, Amsterdam, 1973, Chapters 33 and 34.
(2) L. G. Marzilli in "Progress in Inorganic Chemistry", S. J. Lippard, Ed., Wiley, New York, N.Y., 1977.
(3) D. J. Hodgson "Progress in Inorganic Chemistry", S. J. Lippard, Ed., Wiley, New York, N.Y., 1977.
(4) M. Sundaralingam and J. A. Carrabine, *J. Mol. Biol.*, 61, 287 (1971).
(5) L. Srinivasan and M. R. Taylor, *Chem. Commun.*, 1668 (1970).

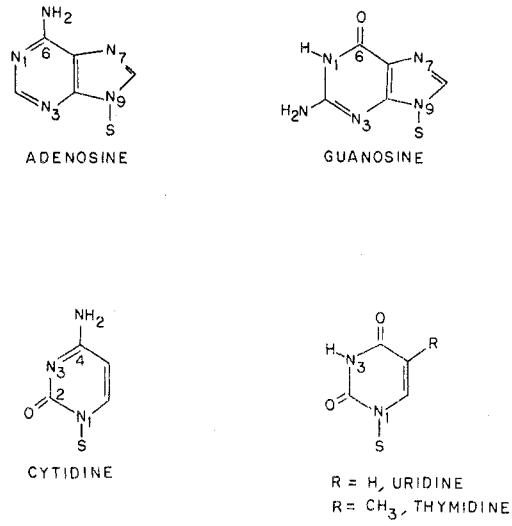

Figure 1. The common purine and pyrimidine ribonucleosides (S = the ribosyl group).

Base Binding Sites

Purines. In neutral solution, N.7 is usually the predominant binding site for N.9-substituted purines such as adenosine and guanosine. However, N.1 in adenine derivatives is only slightly less favorable than N.7.[6] A substituent at N.9, as is the case for the ribosyl moiety in nucleosides, hinders coordination at N.3 for both adenine and guanine derivatives. Coordination at N.1 of guanine or at the amino groups of adenine or guanine requires deprotonation. The lone pair in the amino group is delocalized into the ring π system.

However, the amino group at position 6 of the pyrimidine ring of adenosine is favorably placed in N.7- or N.1-bonded complexes to donate a hydrogen bond to an appropriate acceptor group on other ligands coordinated to the metal center. If such an acceptor is absent, as in a square-planar complex, enough space is available so as not to interfere with coordination. However, if a non-hydrogen-bond-acceptor ligand is present, particularly for octahedral complexes, the amino group sterically interferes with coordination.

Likewise, the exocyclic oxo group at position 6 of the pyrimidine ring of guanosine will favor bonding at N.7 in octahedral complexes if the other ligands are hydrogen-bond donors and will interfere with bonding at N.7 if these ligands can act only as hydrogen-bond acceptors. In square-planar systems, the oxo group has sufficient space so as not to interfere with bonding or it can bind weakly to the metal.

It is of some interest to note that the ligand water can either donate to or accept a hydrogen bond from the common exocyclic groups of the purines or pyridines. The widespread use of aquated metal ions may well account for the failure to observe binding selectivity.

Pyrimidines. N.1-Substituted pyrimidines (cytidine, uridine, thymidine) offer only one favorable ring bonding site at N.3. In cytidine, this site, which is not protonated at neutral pH, is flanked by an exocyclic oxo and an exocyclic amino group. Uridine and thymidine, which behave similarly, must be deprotonated at N.3 before coordination can occur. For these pyrimidines, the hydrogen-bonding and steric factors are similar to those discussed above for the purines. Cytidine is of special interest because it has hydrogen-bond donor and acceptor exocyclic groups and, for Cu^{2+} complexes at least, the exocyclic oxo group has proven to be a moderately strong donor to a metal center. Again, the lone pair of electrons on the amino group is delocalized into the ring.

(6) P. C. Kong and T. Theophanides, *Inorg. Chem.*, 13, 1981 (1974).

Chelate Ligands

In our studies, we have employed a variety of chelate ligands. For the purposes of this Account, these ligands are divided into three major categories: (a) Ligands with only hydrogen-bond donor sites near the metal center—ethylenediamine (en), diethylenetriamine, triethylenetetramine (trien); (b) ligands with only hydrogen-bond acceptor sites near the metal center—acetylacetonate (acac), dimethylglyoximate (dmg); (c) ligands with both hydrogen-bond donor and acceptor groups near the metal center—glycylglycinate, *N*-salicylidene-*N'*-methylethylenediamine, *N*-salicylidene-*N'*,*N'*-dimethylethylenediamine, *N*-3,4-benzosalicylidene-*N'*-methylethylenediamine, *N*-3,4-benzosalicylidene-*N'*,*N'*-dimethylethylenediamine.

In many of the complexes, solvent molecules, notably water, may reside in the primary coordination sphere, and in many cases they also interact with the coordinated nucleic acid bases.

Metals

Most of our results, which have involved Cu(II) and Co(III) systems, have been confirmed or are in accord with studies involving other metals. For example, we note the following representative complexes: (1) the Pt(II)–N.7 bonded complexes of inosine 5′-monophosphate[7a] and guanosine[7b] which serve as models for the platinum antineoplastic drugs; (2) the aquated Ni(II) complex of adenosine 5′-monophosphate,[8] where the metal is bonded through N.7 of the base and the phosphate group is connected via intramolecular hydrogen bonds to the coordinated water molecules; (3) the O^2-bound Mn(II)[9] and the N.3-bound Co(II)[10] and Cd(II)[10] complexes of cytosine 5′-monophosphate; (4) the $AgNO_3$ complex of 1-methylcytosine,[11] which shows metal binding through N.3 and O^2; (5) the chelated, N.7 and S^6, Pd(II) complex of 6-mercapto-9-benzylpurine;[12] (6) the N.7-bonded Cd(II) complex of the 8-azahypoxanthine monoanion;[13] and (7) the Os(VI) ester of 1-methylthymine.[14]

Molecular Conformational Properties

Binding Interactions in Adenosine Derivatives. As noted above, adenosine derivatives have the largest number of unprotonated, heterocyclic donor-nitrogen atoms (N.1 and N.7) available for metal coordination. Described below are the molecular conformational

(7) (a) D. M. L. Goodgame, I. Jeeves, F. L. Phillips, and A. C. Skapski, *Biochim. Biophys. Acta*, 378, 153 (1975); (b) R. W. Gellert and R. Bau, *J. Am. Chem. Soc.*, 97, 7379 (1975).

(8) A. D. Collins, P. de Meester, D. M. L. Goodgame, and A. C. Skapski, *Biochim. Biophys. Acta*, 402, 1 (1975).

(9) K. Aoki, *J. Chem. Soc., Chem. Commun.*, 748 (1976).

(10) G. R. Clark and J. D. Orbell, *J. Chem. Soc., Chem. Commun.*, 697 (1975).

(11) L. G. Marzilli, T. J. Kistenmacher, and M. Rossi, *J. Am. Chem. Soc.*, 99, 2797 (1977).

(12) H. I. Heitner and S. J. Lippard, *Inorg. Chem.*, 13, 815 (1974).

(13) L. G. Purnell, E. D. Estes, and D. J. Hodgson, *J. Am. Chem. Soc.*, 98, 740 (1976).

(14) T. J. Kistenmacher, L. G. Marzilli, and M. Rossi, *Bioinorg. Chem.*, 6, 347 (1976).

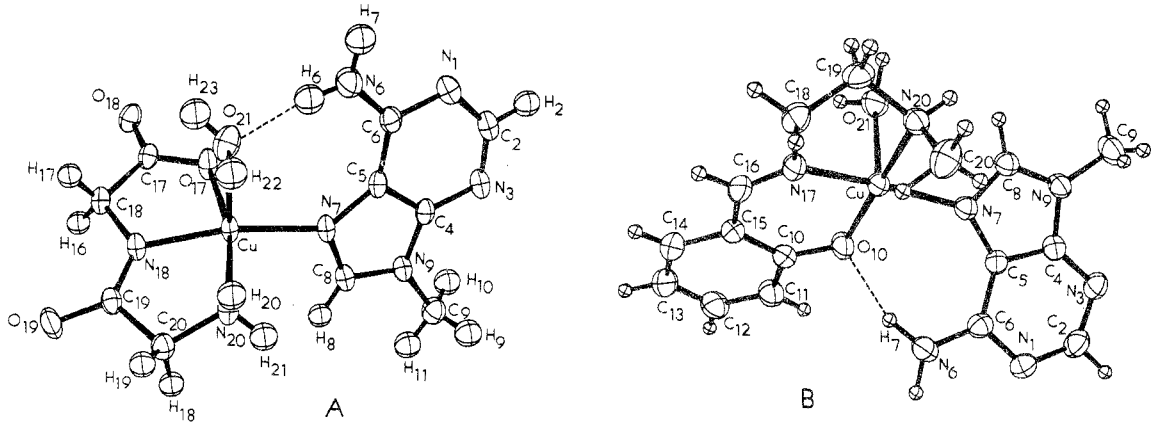

Figure 2. (A) (Glycylglycinato)(aqua)(9-methyladenine)copper(II); (B) (*N*-salicylidene-*N'*-methylethylenediamine)(aqua)(9-methyladenine)copper(2+) cation.

properties of three N.9-substituted adenine complexes. In each case, there are two important features to note: (a) the site of the metal attachment to the purine base, and (b) the interligand hydrogen-bond formation from the exocyclic amino group of the purine to various donor sites on the chelate or monodentate ligands.

(Glycylglycinato)(aqua)(9-methyladenine)copper(II). This complex[15] exists in the crystal in a slightly distorted square-pyramidal geometry (Figure 2A). The four equatorial sites are occupied by the tridentate glycylglycine dianion and N.7 of the 9-methyladenine ligand, while one of the axial positions is occupied by a water molecule. In the observed conformation of the complex, the exocyclic amine on the 9-methyladenine ligand forms an interligand hydrogen bond with the coordinated water molecule, the interligand hydrogen bond being denoted by a dashed line in Figure 2A. The parameters in this hydrogen bond are in accord with a strong hydrogen bond.[15]

[N-Salicylidene-N'-methylethylenediamine)-(aqua)(9-methyladenine)copper(2+)]$^+$. This complex cation[16] shows shows approximately square-pyramidal coordination geometry in the solid (Figure 2B). The four equatorial positions are occupied by the tridentate Schiff-base chelate and N.7 of the 9-methyladenine ligand. The primary coordination sphere is completed by the occupation of one of the axial positions by a water molecule. The most interesting aspect of the structure is that an interligand hydrogen bond is formed between the exocyclic amine of the 9-methyladenine and the equatorial oxygen atom at the 10 position of the salicylidene ring. The parameters in this interligand hydrogen-bond system are again consistent with a strong hydrogen bond.

Bis(acetylacetonato)(nitro)(deoxyadenosine)cobalt(III). The molecular conformation of this cobalt(III)–deoxyadenosine complex[17] is illustrated in Figure 3. The coordination sphere is approximately

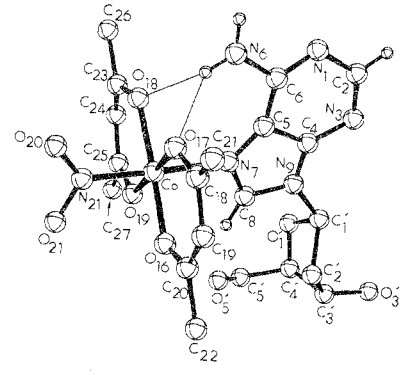

Figure 3. Bis(acetylacetonato)(nitro)(deoxyadenosine)cobalt(III).

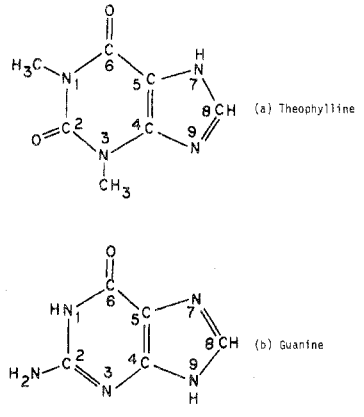

Figure 4. A comparison of the molecular structures of theophylline and guanine.

octahedral, with the equatorial plane defined by the two acac ligands in trans positions and the two axial positions occupied by the nitro and the deoxyadenosine ligands. The nucleoside is coordinated to the metal through N.7, and this complex represents the first structural study of a nucleoside coordinated to Co(III). The conformation of the complex is such that the plane of the adenosine ligand approximately bisects the O–Co–O bonds of two of the acac oxygen atoms; in the adopted conformation the exocyclic amine forms a bifurcated hydrogen bond system to two of the acac oxygens (Figure 3). This system has proved to be especially rich in information concerning selective interactions, and we will return to it.

Binding Interactions in Guanosine Derivatives. As discussed above, N.7 is the most likely metal binding

(15) T. J. Kistenmacher, L. G. Marzilli, and D. J. Szalda, *Acta Crystallogr., Sect. B*, **32**, 186 (1976).

(16) D. J. Szalda, T. J. Kistenmacher, and L. G. Marzilli, *Inorg. Chem.*, **14**, 2623 (1975). It was originally concluded from ^{1}H NMR line-broadening data (T_2) that the complex (chloro)(*N*-salicylidene-*N'*-methylethylenediamine)copper(II) showed a preference for binding at N.1 of adenosine. More conclusive T_1 data showed that the Schiff-base complex actually preferred N.7 slightly over N.1 [see L. G. Marzilli, W. C. Trogler, D. P. Hollis, T. J. Kistenmacher, C. H. Chang, and B. E. Hanson, *Inorg. Chem.*, **14**, 2568 (1975)].

(17) T. Sorrell, L. A. Epps, T. J. Kistenmacher, and L. G. Marzilli, *J. Am. Chem. Soc.*, **99**, 2173 (1977).

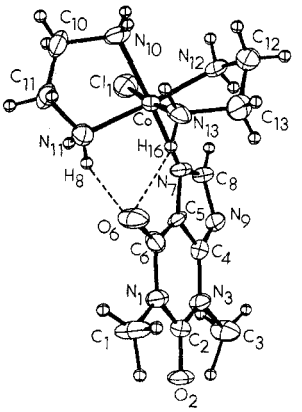

Figure 5. *cis*-(Chloro)(theophyllinato)bis(ethylenediamine)-cobalt(3+) cation.

site in guanosine derivatives. In all of our structural studies to date, we have employed the monoanion of the substituted purine theophylline as a model for metal binding to the guanine class of purines, i.e., those purines with a carbonyl group at position 6 of the pyrimidine ring. Figure 4 illustrates the molecular structures of both theophylline and guanine.

We describe below four chelate complexes containing the theophylline monoanion which show the various types of interactions we have encountered.

cis-[(*Chloro*)(*theophyllinato*)*bis*(*ethylenediamine*)-*cobalt*(3+)]$^+$. We have isolated and determined the molecular structure of both the cis[18] and the trans[19] isomers of the theophylline monoanion complex of $[(en)_2(Cl)Co^{III}]^{2+}$. The molecular conformations and bonding characteristics of the cis isomer are presented in Figure 5. The metal binding site is N.7 on the theophylline monoanion, and there are two interligand hydrogen bonds from different ethylenediamine ligands to O^6 on the substituted purine.

(*N-3,4-Benzosalicylidene-N'-methylethylenediamine*)(*theophyllinato*)(*aqua*)*copper*(*II*). The molecular structure of this Schiff-base copper(II) complex of the theophylline monoanion[20] is presented in Figure 6A. The copper(II) is in a square-pyramidal environment with the tridentate Schiff-base chelate and N.7 of the theophylline anion in equatorial positions and a water molecule in one of the axial positions. There are two interligand hydrogen bonds to O^6 of the purine ring, one from the secondary amine of the ethylenediamine terminal of the Schiff-base chelate and one from the axially bound water molecule.

(*N-3,4-Benzosalicylidene-N',N'-dimethylethylenediamine*)(*theophyllinato*)*copper*(*II*). A perspective view of this complex[21] is presented in Figure 6B. In this complex, the above Schiff-base chelate has been modified by the addition of a second methyl group to the *N*-methylethylenediamine terminal; thus this chelate has no hydrogen-bond donor near the metal binding site. The theophylline anion complex of this system has square-pyramidal geometry about the Cu(II) with the Schiff-base chelate occupying three of the four equatorial sites and N.7 of the purine anion in the fourth equatorial site. The axial position on one side of the equatorial plane is occupied by O^6 of the coordinated theophylline monoanion. The Cu(II)–O^6 bond length of 2.919 (3) Å is by far the shortest we or other workers have observed.[3] It is clear from this study that, in the absence of interligand hydrogen bonding and with the steric crowding of the axial positions by the two methyl substituents, O^6 will also bind to the metal center, making theophylline a bidentate ligand. This is the first instance in which chelation by a 6-oxopurine has been observed.

Bis(*theophyllinato*)(*diethylenetriamine*)*copper*(*II*). The molecular conformation of the bis(theophyllinato)(diethylenetriamine)copper(II) complex[22] is illustrated in Figure 7. The primary coordination sphere about the copper(II) center is approximately square pyramidal. The tridentate chelate diethylenetriamine, with its terminal amine nitrogen atoms in the commonly observed trans positions, occupies three of the four equatorial coordination sites. The coordination sphere is completed by a strongly bound equatorial theophylline monoanion, Cu(II)–N.7 bond length = 2.007 (3) Å, and a less tightly bound axial theophylline anion, Cu–N.7 bond length = 2.397 (3) Å. The binding of both of the purine anions through the imidazole ring nitrogen N.7 is of particular interest. As in the above complexes, the carbonyl O^6 of the equatorial purine anion is hydrogen bonded to one of the primary amine groups on the chelate ligand. The binding of two purine ligands to the Cu(II) center is interesting in view of the unusual role of Cu(II) in nucleic acid chemistry.[1,2]

Binding Interactions in Cytosine and Cytidine. As indicated above, N.3 is the most likely binding site for transition-metal complexes of cytidine.

(*Glycylglycinato*)(*cytidine*)*copper*(*II*). The molecular structure of the cytidine complex[23] of glycylglycinatocopper(II) is illustrated in Figure 8A. The coordination geometry about the copper(II) center is approximately square planar, with the glycylglycine dianion and N.3 of cytidine occupying the four equatorial coordination sites. Furthermore, the exocyclic carbonyl O^2 on the cytidine ring approximately occupies one of the axial positions, Cu–O^2 bond length = 2.74 (1) Å [averaged over the two independent molecules in the unit cell], extending the coordination geometry to square pyramidal.

The presence of the dipeptide and nucleoside ligand in the same coordination sphere makes this complex a good model for ternary enzyme–metal–nucleic acid systems. The related cytosine complex has a similar molecular conformation.[24]

[(*N-Salicylidene-N'-methylethylenediamine*)(*cytosine*)*copper*(2+)]$^+$. The copper binds at N.3, and there is significant axial interaction between O^2 on the cy-

(18) T. J. Kistenmacher and D. J. Szalda, *Acta Crystallogr., Sect. B*, **31**, 90 (1975).

(19) L. G. Marzilli, T. J. Kistenmacher, and C. H. Chang, *J. Am. Chem. Soc.*, **95**, 7507 (1973); T. J. Kistenmacher, *Acta Crystallogr., Sect. B*, **31**, 85 (1975).

(20) D. J. Szalda, T. J. Kistenmacher, and L. G. Marzilli, *Inorg. Chem.*, **15**, 2783 (1976). We have also studied the structure of the closely related complex (*N*-salicylidene-*N'*-methylethylenediamine)(theophyllinato)-copper(II) (T. J. Kistenmacher, D. J. Szalda, and L. G. Marzilli, *Inorg. Chem.*, **14**, 1686 (1975)). This complex is four-coordinate, and only the interligand hydrogen bond from the amino group of the Schiff base to the carbonyl oxygen O^6 remains.

(21) D. J. Szalda, T. J. Kistenmacher, and L. G. Marzilli, *J. Am. Chem. Soc.*, **98**, 8371 (1976).

(22) T. Sorrell, L. G. Marzilli, and T. J. Kistenmacher, *J. Am. Chem. Soc.*, **98**, 2181 (1976).

(23) D. J. Szalda, L. G. Marzilli, and T. J. Kistenmacher, *Biochem. Biophys. Res. Commun.*, **63**, 601 (1975); D. J. Szalda and T. J. Kistenmacher, *Acta Crystallogr.*, in press.

(24) T. J. Kistenmacher, D. J. Szalda, and L. G. Marzilli, *Acta Crystallogr., Sect. B*, **31**, 2416 (1975).

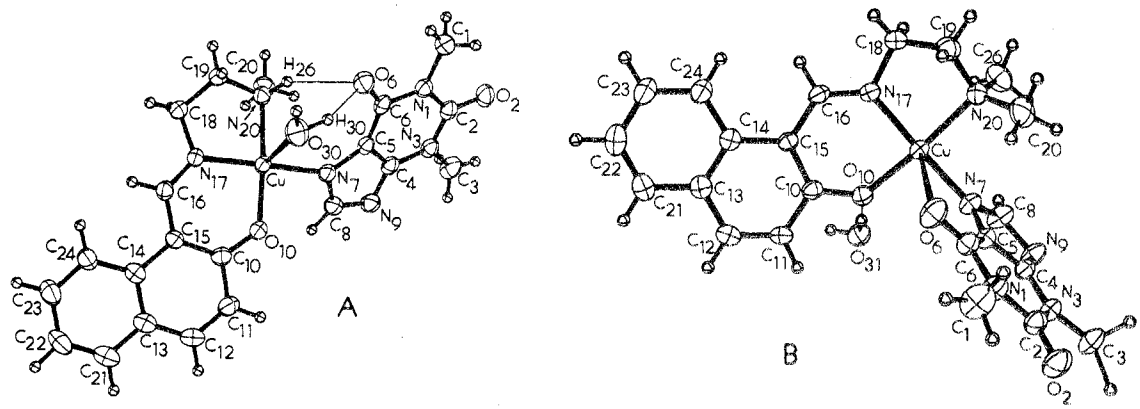

Figure 6. (A) (*N*-3,4-Benzosalicylidene-*N'*-methylethylenediamine)(theophyllinato)(aqua)copper(II); (B) (*N*-3,4-benzosalicylidene-*N',N'*-dimethylethylenediamine)(theophyllinato)copper(II).

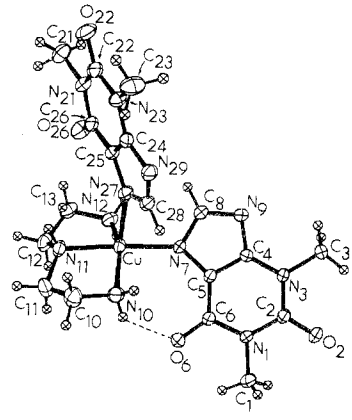

Figure 7. Bis(theophyllinato)(diethylenetriamine)copper(II).

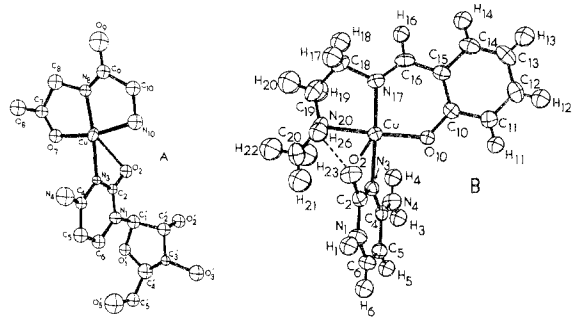

Figure 8. (A) (Glycylglycinato)(cytidine)copper(II); (B) (*N*-salicylidene-*N'*-methylethylenediamine)(cytosine)copper(2+) cation.

tosine ring and the Cu(II), Cu–O^2 distance = 2.772 (1) Å (Figure 8B).[25] The coordination sphere is completed via an axial interaction with an oxygen atom of the nitrate counterion, Cu–ONO_2^- distance = 2.806 (1) Å. Furthermore, in this complex there is an interligand hydrogen bond formed between O^2 and the hydrogen atom at the *N*-methylethylenediamine terminal of the Schiff-base chelate (Figure 8B).

Binding Interactions in Thymine and Thymidine. Thymidine (thymine) is unique among the nucleic acid constituents in that there are no free lone pairs available for metal complexation at neutral or low pH. In fact, solution and preparative studies indicate that, except for Hg^{2+} and RHg^+, the interaction between transition-metal complexes and thymidine is relatively weak.[2] Deprotonation of 1-alkylthymine derivatives leaves a lone pair at N.3, and Stewart[26] has shown that Hg(II) will bind to N.3 of deprotonated 1-methylthymine. If the free-base thymine deprotonates at N.1, Cu(II) binds at N.1.[27]

Summary. These crystallographic studies lead to several comments about metal–ligand and ligand–ligand interactions.

Adenosine Residues. The important molecular features of the above three 9-substituted adenine systems are that in each case the metal binding site is N.7 and in each instance the exocyclic amine group on the adenine framework is involved in an interligand hydrogen bond with an acceptor group on another ligand in the coordination sphere.

Guanosine Residues. Three important features emerge from the above and other studies on guanine derivatives: (1) in all of the complexes N.7 is the metal binding site; (2) in those complexes where a hydrogen-bond donor group exists on the chelate ligand, an interligand hydrogen bond is formed to the O^6 acceptor group on the coordinated purine; (3) in those complexes where no hydrogen-bond donor group exists on the chelate ligand, a significant—but weak—Cu–O^6 axial interaction is indicated from one crystallographic study.[21]

Cytidine Residues. The principal features which carry over among all the known cytosine or cytidine complexes of copper(II) are: (a) binding of the pyrimidine or pyrimidine nucleoside through N.3; (b) formation of an intramolecular, axial, Cu–O^2 interaction. The essentially constant geometric features exhibited in these Cu–O^2 interactions suggest that the Cu–O^2 axial interaction is important in copper–cytosine(cytidine) complexes.[4,23-25]

Solution Studies

In our work, a variety of solution studies have served to indicate that ligand–ligand interaction in chelate metal complexes can induce selectivity in the binding of such complexes to nucleic acid constituents.

It is well known that steric interactions are of paramount importance in determining the nature and type of complexes which can be formed with cobalt(III). The reaction of *cis*-β-$[Co^{III}(trien)Cl_2]^+$ with ^{14}C-labeled

(25) D. J. Szalda, L. G. Marzilli, and T. J. Kistenmacher, *Inorg. Chem.*, 14, 2076 (1975).

(26) L. D. Kosturko, C. Folzer, and R. F. Stewart, *Biochemistry*, 13, 3949 (1976).

(27) T. J. Kistenmacher, T. Sorrell, and L. G. Marzilli, *Inorg. Chem.*, 14, 2479 (1975).

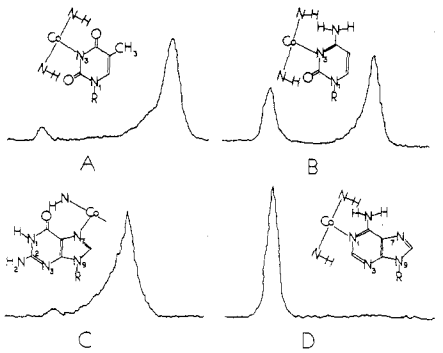

Figure 9. Scans of the radioactivity of electrophoretic charts of the reaction of [dichloro(triethylenetetramine)cobalt(3+)] cation with the common deoxynucleosides: (a) deoxythymidine, (b) deoxycytidine, (c) deoxyguanosine, (d) deoxyadenosine. In each scan, the slower moving peak (on the left) moves as the control deoxynucleoside.

Table I
Percent Reaction of Deoxyribonucleosides with [Co(trien)Cl_2]Cl

Nucleoside	1 Day	3 Days
	[Co] = 0.025 M	
dT	65	65
dG	45	50
dC	10	10
dA	0	0
	[Co] = 0.1 M	
dT	85-90	90-95
dG	65	>95
dC	10	65-70
dA	0	0

deoxythymidine, deoxycytidine, deoxyguanosine, and deoxyadenosine has been examined.[28] A representation of the possible cis interactions between the NH or NH_2 groups of coordinated trien and the deoxynucleoside bases is shown in Figure 9. If thymidine coordinates at N.3 (deprotonated), the two contiguous exocyclic carbonyl oxygens can hydrogen bond to the NH_2 or the NH groups of trien. Coordination of cytidine via N.3 could produce a favorable hydrogen bond to trien via O^2, but a repulsive interaction would occur via the amino group at C.4. Coordination of the purine nucleosides through N.7 leads to only favorable interligand hydrogen bonding with guanosine and to only repulsive interactions with adenosine (Figure 9). Coordination of adenosine through N.1 would also lead to only repulsive interactions.

Detailed results of an electrophoretic study of the reaction of *cis*-β-$[Co^{III}(trien)Cl_2]^+$ with the above four deoxynucleosides are presented in Table I. It can be seen that dT, with its capability of forming two interligand hydrogen bonds, reacts most rapidly, and dG, with its capability of forming one hydrogen bond, reacts second most rapidly. Given sufficient time at [Co] = 0.1 M, both these nucleosides react almost completely. Cytidine, with its favorable hydrogen-bonding capability via O^2 and its repulsive steric interaction through its 4-amino group, ranks third in order of reactivity. Adenosine, which cannot form interligand hydrogen bonds in this system, does not react at all. The relative affinity for binding of deoxynucleosides to the trien complex of cobalt(III) is thus: dT > dG > dC >> dA. This ordering is consistent with our expectations based on the interligand interactions observed in the crystallographic studies and reiterated above. Unfortunately, although the results illustrate the importance of interligand interactions between nucleoside ligands and other coordinated ligands, the nucleoside complexes are relatively unstable.

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Table II
Stability Constants for Substituted Purines and Pyrimidines with Bis(acetylacetonato)(nitro)cobalt(III) in Me_2SO

Compound	K, M^{-1}	Probable binding site(s)
9-Methyladenine	144	N.7, N.1
Purine ribonucleoside	109	N.7, N.1
Adenosine N^1-oxide	104	N.7
Adenosine	93	N.7, N.1
Deoxyadenosine	79	N.7, N.1
6-(γ,γ-Dimethylallyl-amino)purine ribonucleoside	55	N.7
1-Methyladenosine	30	N.7
Guanosine	0	
2-Aminopyrimidine	27	N.3, N.1
Cytosine	6	N.3
Cytidine	1	N.3
Uridine	0	

We have also reported a detailed study of the reaction of $Na[Co(acac)_2(NO_2)_2]$ (where acac = acetylacetonate) with a large variety of purines, pyrimidines, and nucleosides to yield products of the type $[Co(acac)_2(NO_2)B]$.[17] The interligand interactions between the nucleic acid constituents and the $[Co(acac)_2(NO_2)]$ moiety are expected to be substantially different from those encountered in the trien system. The acac complex presents to an incoming ligand the four coordinated oxygen atoms, potential hydrogen bond acceptors, of the acac chelates, while the trien chelate interacts with incoming ligands via its coordinated primary and secondary amino groups which are potential hydrogen-bond donors. Consistent with these expectations, the reaction of $Na[Co(acac)_2(NO_2)_2]$ with the four common nucleosides is reverse in trend [A >> C > U ~ G] to that of the trien system.[17]

Furthermore, the adenosine and deoxyadenosine complexes are sufficiently stable to isolate. The molecular structure of the deoxyadenosine complex[17] is shown in Figure 3.

Concentration studies obtained in Me_2SO-d_6 indicated the presence of at least two complexes, and it was established that the two complexes are the solvato complex and the nucleoside complex which are in equilibrium according to eq 1. The equilibrium

$$[Co(acac)_2(NO_2)(\text{deoxyadenosine})] \underset{K^{-1}}{\rightleftharpoons} [Co(acac)_2(NO_2)(Me_2SO)] + \text{deoxyadenosine} \quad (1)$$

constants for a variety of purine and pyrimidine derivatives are given in Table II.

Of particular interest is what differentiation is observed among the common nucleic acid constituents, and if differentiation has occurred, what are the sources of the interactions which provide the discrimination. The stability constants for adenosine, guanosine, cytidine, and uridine are 93, 0, 1, and 0, respectively. Thus, there is clearly a differentiation among the common nucleosides. We suggest that the observed

stability constant trend can be rationalized on the basis of interligand interactions and reference our discussion to the structural features found in the deoxyadenosine complex (Figure 3).

In the deoxyadenosine complex, the purine ribonucleoside is coordinated through N.7 and the exocyclic amino group at C.6 forms a bifurcated hydrogen-bond system with the equatorial acac ligands (Figure 3). The interligand hydrogen bonding is clearly a favorable interaction, and there are no unfavorable steric interactions in the primary coordination sphere of the complex.

For guanosine (or any N.9-blocked xanthine or hypoxanthine derivative), it has now been established that N.7 of the imidazole ring is the preferred transition-metal binding site. With reference to Figure 3, it is clear that the exocyclic oxo group at C.6 of guanosine in an N.7-bonded complex can partake only in nonbonded, repulsive interactions with the coordinated oxygens of the equatorial acac ligands. We attribute our inability to find any evidence for the binding of guanosine to $[Co(acac)_2(NO_2)]$ (Table II) to the repulsive interactions of the exocyclic oxo group and the equatorial plane.

A similar situation would exist for N.3-bonded uridine monoanion. The two oxo groups contiguous to the potential metal binding site will also lead to severe nonbonded repulsive interactions with the acac oxygen atoms of the equatorial plane.

Cytidine presents a somewhat different collection of potential interligand interactions. Binding of $[Co(acac)_2(NO_2)]$ to cytidine through N.3, the preferred binding site for transition metals, leads to a favorable interaction with the equatorial acac oxygens via the exocyclic amino group at C.4 and to a repulsive interaction via the exocyclic oxo group at C.2 of the ring system. Consistent with this collection of interligand interactions, we find evidence for the binding of cytidine (cytosine) to $[Co(acac)_2(NO_2)]$, although the formation constants are very small (Table II).

Complementary results were obtained in studies of the reaction of purine and pyrimidine bases with the *trans*-$[((n\text{-}Bu)_3P)Co^{III}(dmg)_2]^+$ cation.[29] The steric requirements of the *trans*-$[((n\text{-}Bu)_3P)Co^{III}(dmg)_2]^+$ moiety are greater than those for the $[Co^{III}(acac)_2NO_2]$ system. Only completely unhindered purine derivatives will react with this complex and, thus, it was not possible to prepare a complex with the theophylline anion, normally an excellent ligand. Adenine did form a complex, but coordination was probably via N.9. The complexes *trans*-$[((n\text{-}Bu)_3P)Co^{III}(dmg)_2(xanthinato)]$ and *trans*-$[((n\text{-}Bu)_3P)Co^{III}(dmg)_2(hypoxanthinato)]$[2] were isolated, and the structure of the former complex established bonding at the sterically unhindered site N.9. Some of these *trans*-$[((n\text{-}Bu)_3P)Co^{III}(dmg)_2$-(purinato)] complexes proved to be useful intermediates for the synthesis of N.7-alkylated purines.

Conclusions

Strong hydrogen-bonding interactions involving exocyclic groups have been found in numerous studies in these and other laboratories. The solution studies described here provide strong evidence that such interactions, coupled with repulsive nonbonded interactions, can influence the stability of complexes between nucleic acid derivatives and metal complexes. We believe such interactions will be widespread in solution, and their importance in biological systems remains to be demonstrated. It is clear that, in future, studies such as those described here must be extended to larger nucleic acid fragments and to nucleic acids themselves.

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