Pure & Appl. Chem., Vol. 70, No. 4, pp. 961–968, 1998.
Printed in Great Britain.

Complexes of lopsided *N*-donor heterocyclic bioligands: has the electrostatic effect of the N_2CH proton been overlooked in metallobiochemistry?

Luigi G. Marzilli[a], Patricia A. Marzilli[a], and Enzo Alessio[b]

[a]*Emory University, Atlanta, Georgia 30322, U.S.A. and Institute for Molecular Science, Okazaki National Research Institutes, Okazaki, Japan*
[b]*Dipartimento di Scienze Chimiche, Università di Trieste, 34127 Trieste, Italy*

Abstract: The orientation and fluxional motions of planar *N*-donor heterocyclic coordinated ligands (*L*'s), historically difficult to assess in solution, need to be evaluated because these properties influence the structure and function of many metallobiochemicals. Compelling NMR evidence on the solution conformer of *cis,bis* imidazole-ring-ligated untethered ligands indicates that orientation is dictated by the electrostatic attraction of the $N_2C^{\delta+}$ proton for the negative *cis* ligands (e.g. oxo, Cl). A powerful strategy in which complexes similar except that one has a lopsided *L* and the other has a C_2-symmetrical *L* are examined by using exchange-NOE cross-peaks afforded insightful information on *L* dynamics, including the extent and direction of rotation about the metal–N bond, and even the halves of C_2-symmetrical *L*'s that interchange during dynamic processes. Since metal centers make this imidazole proton more positive, it is likely that this electrostatic effect has some influence on both structure and function of all biological systems with metal sites ligated by imidazole rings (namely imidazole and benzimidazole bound to B_{12}, Pt drug adducts to G of DNA, and numerous metalloenzymes and metalloproteins).

INTRODUCTION

The interaction of metal species with non-macrocyclic *N*-heterocycles in nucleic acids, proteins/enzymes, and cofactors has many biological and biomedical consequences. With nucleic acids, such interactions are significant in cancer therapy, in metal toxicity, in stabilizing the structures of nucleic acids in their natural states, and in probe agents that are used as tools to gain insight into nucleic acid and nucleotide biochemistry. There is not yet a complete understanding of all of these interactions. This is true, not only because they are involved in so many systems, but also because the nucleotide models and the nucleic acids are quite flexible and fluxional. They are linked by many single bonds and thus adopt several conformations, each offering many points for metal interactions. Usually no single dominant feature determines the site of interaction or the conformations and properties of these important biomolecules. In contrast, in enzymes/proteins and cofactors normally the structure is designed by nature to be rather rigid (1). Indeed, in some cases, such as the Zn sites of viral zinc fingers (2), the metal-imidazole interactions contribute to stabilizing the conformation. In other cases, normally involving biocatalysis, it is likely that the *N*-heterocycle orientations and the metal interactions could change during the functional process since the M–N bonds are single bonds, accommodating many *N*-heterocycle orientations. Such a feature seems to occur in the recently discovered replacement of 5,6-dimethylbenzimidazole by a histidine imidazole in the two classes of B_{12} enzymes needed for human metabolism (3-5). Thus one heterocycle is replaced by another. In a related case in which the *N*-heterocycle can be viewed as pendant, we have speculated that the oxidation of Fe(II) to activated Fe(III) bleomycin could involve rearrangment of this anticancer antibiotic complex containing a bound imidazole (6).

Cis,bis adducts

This article will be restricted to the case in which there are two *N*-heterocyclic ligands (*L*) on a metal center in a *cis,bis* relationship. Using such models, we can explore many features of the properties of bound heterocyclic bioligands; many of these findings will apply equally well to other cases in which one, three, four, etc. *N*-heterocyles are bound. The bioligand heterocycles are lopsided, i.e. they lack C_2-symmetry.

Although studies of ligand orientation and dynamics are highly relevant and significant to metallo-biochemistry, such studies have not been performed extensively because it is difficult to find suitable systems. Another reason this area has been neglected is the contemporary trend toward large systems with biomolecules or supramolecular assemblies. However, such a modern approach in our own research (in the areas of Pt(II) and Ru(II) anticancer drug activity involving cross-linking and the construction of large molecular assemblies) has underlined for us the need for returning to fundamentals.

For two *cis* lopsided *N*-heterocyclic ligands (e.g., ***L*** = purines), the corresponding atoms of each ***L*** can be on the same or opposite sides of the N-M-N plane, giving the head-to-head (HH) and the head-to-tail (HT) orientations, respectively (Fig. 1). In a preliminary communication, we described the rarity of finding the HH orientation of two *cis* lopsided ligands (7). A HT arrangement is the most common solid-state conformation of *cis,bis*(ligand) complexes of purines with Pt(II), Co(III), Cu(II), and Zn(II) metal centers (8). Only four HH solid-state structures of untethered ligands had been reported, all of them square-planar Pt(II) complexes with 9-ethylguanine (9), until our recent brief report on the dynamic properties and stereochemistry of Ru- and Re-Me_3Bzm complexes (7) .

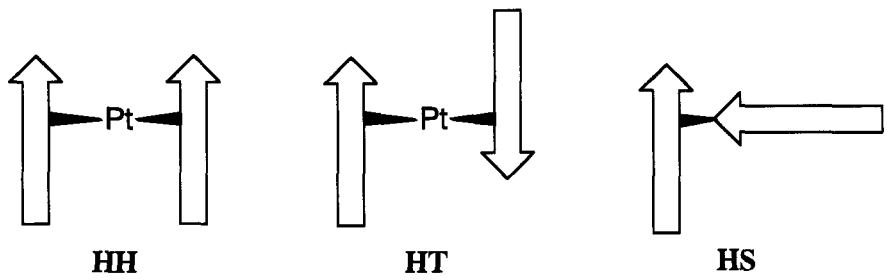

Fig. 1. Relative orientations of lopsided ligands.

We were motivated to develop our new approaches by our interest in understanding why HH complexes are present in some very simple species, even though related complexes are HT and clear cases of HH forms in solution had been found very rarely. Indeed, in the interim, there still have not been many examples found of such HH forms. In the case of the Pt complexes, the finding was a result of a rational design intended to control the stereochemical and conformational features of adducts (8, 10). For the Ru and Re derivatives (7, 11-13) the findings were serendipitous; however, it is just these latter cases which afforded an important clue as to some of the factors influencing lopsided ligand orientation.

Importance of cis,bis adducts, the anticancer drug case

An early and important finding with Pt anticancer complexes was the identification of an HH species in solution with *cis*-$PtA_2G^*_2$ [G* is a guanine derivative with Pt at N7, A_2 = a specially designed diamine] even with G*'s that were not linked (8). Since such a species had not been observed previously, it was a challenge to understand the nature of this atropisomer. One surprising finding about the HH adduct was that one base was canted toward the other. This canting is characteristic of intrastrand dG*pG* adducts (14-17), the major species formed when *cis*-type Pt anticancer drugs bind to DNA (18). However, since unlinked G* bases were ordinarily HT and only linked G*'s were known to be HH in solution, the canting was previously thought to result from restrictions on the bases imposed by the sugar-phosphate backbone. Our result showed that this canting will occur in the absence of a direct link between the bases. Clearly, other interactions within the coordination sphere influence the positioning of the bases; such interactions are thus an important contributor to overall *cis*Pt-DNA adduct structure and possibly to the structures of other metallobiochemicals.

Next we list why the metal-bound bases are key to the metal-DNA adduct structure. (Similar lists could be constructed illustrating the important role of metal-bound heterocyclic bases in peptides, cofactors, etc.) Metal-bound bases do the following:

- link the metal to the nucleic acids.
- control the conformation of the seventeen-membered chelate ring in intrastrand cross-links.
- interact with the complementary strand.
- interact with the flanking sequences on either side of the metal binding site.
- H-bond to the non-leaving ligands or avoid steric interactions with these ligands.
- cause mismatches responsible for mutations and eventually cancer.
- determine the ease and type of cross-linking after the initial base binds.

Despite the importance of these roles, little is known about the orientations adopted by the bases, the reasons for these orientations, and the consequences of the orientations on DNA-adduct structure. Some structures are beneficial, accounting for the anticancer activity of metallodrugs, whereas others are detrimental, causing mutations and eventually cancer. *Cis*-type Pt drugs form intrastrand HH cross-links and interstrand HT cross-links (14, 17, 19, 20). Thus it is essential to elucidate the factors that influence the orientation of the base with respect to the other ligands on the metal, including the other base, both during and after cross-link formation. Interaction of the ligands on the metal with the DNA is important to understand since distorted

structures are hypothesized to be recognized by the proteins responsible eventually for cancer cell cytotoxicity.

Since rotation about M–N bonds of coordinated nucleobases influences cross-linking ability, we are interested in studying dynamic properties of metal complexes containing *cis* lopsided bases. New methods for elucidating dynamic pathways have been developed in our work.

STUDIES WITH AN INFORMATIVE NUCLEOBASE ANALOG

In our studies, we chose to examine an ***L*** that is a lopsided nucleopurine analog, 1,5,6-trimethylbenzimidazole (Me_3Bzm, Fig. 2, *left*). We chose this ligand mainly to avoid one big problem with G derivatives, namely that there is only one nonexchangeable proton (H8, the "N_2CH") on G to use as an NMR probe. Our new approach of employing ligands with well-distributed uncoupled H signals, particularly Me_3Bzm, has afforded much clearer assessments of base orientation and dynamics. As in G, Me_3Bzm has only one relatively acidic proton (hence bearing a partial positive charge, $\delta+$), and this proton is in the imidazole ring. However, Me_3Bzm lacks the bulkier hydrogen-bonding G 6-oxo group and has instead a probe proton, H4, providing readily interpreted NMR shift information. The Me_3Bzm results allow us to understand other adducts. Although the properties of Me_3Bzm make it very useful, it does share one limitation with G*: one set of NMR signals per unique ligand will be found either if Me_3Bzm is not fluxional or if it is highly fluxional. One way to circumvent this problem is to study identical complexes except with symmetrical ligands like py or 3,5-lutidine (3,5-lut). These ligands also have the advantage of lacking a very acidic CH. Thus the pyridine-based ligands can be used as controls to test the influence on orientation of the $\delta+$ protons, such as H8 of G* or H2 of Me_3Bzm.

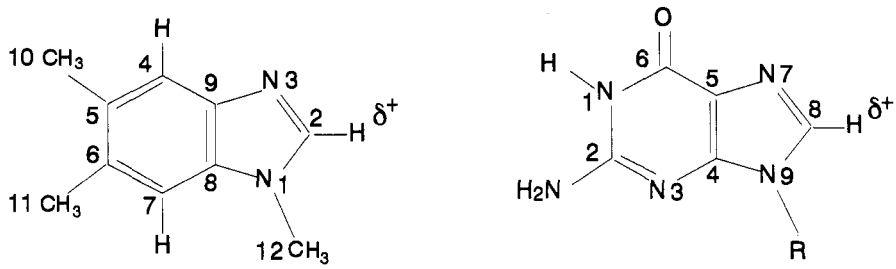

Fig. 2 Comparison of the nucleopurine analog, Me_3Bzm, and N9-alkylguanine.

NMR and canting

One consequence of the canting, first found for the two G*'s in a specially designed *cis*Pt analog (8), is that the two bases are influenced differently by the anisotropy of the other. In HH forms, the N_2CH of one ***L*** is shifted downfield, while that of the other ***L*** is shifted upfield. Each HH conformer has two N_2CH signals of equal intensity, and slow atropisomerization can be easily assessed. For *cis*Pt adducts with C_2 symmetry, there are two HT conformers of complexes with G nucleosides/nucleotides since the sugar is chiral. Each HT conformer has only one N_2CH signal.

The characteristic upfield, downfield H8 signal pattern for HH (due to the canting, Fig. 3) makes atropisomer recognition straightforward. Prior to our work with the *cis*Pt analog and the Ru and Re complexes to be described here, no one had reported an HH atropisomer in solution for any relevant untethered nucleopurine complex.

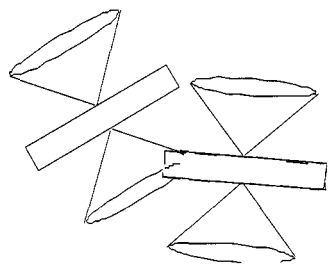

Fig. 3 Mutual effect of the anisotropy of canted ***L***'s.

Orientations (structure / equilibrium / dynamics)

In this subsection, we discuss ruthenium and then rhenium complexes.

Ruthenium examples

Ruthenium compounds attracted our interest as models for cross-link adducts of the Ru(II) anticancer drug, *cis*-$RuCl_2(Me_2SO)_4$ (21-23). In drug adducts, N donors occupy sites "a" and "b" (Fig. 4, *left*) displacing two different types of Me_2SO's, *trans* to Cl and *trans* to Me_2SO. The drug presents both a unique challenge and a unique opportunity, since two HH GpG cross-link adduct geometries are possible with the 3'G at "a" or "b", each with a diastereomer since the Ru is now chiral. Our use of non-chiral ***L***'s avoids the diastereomer problem.

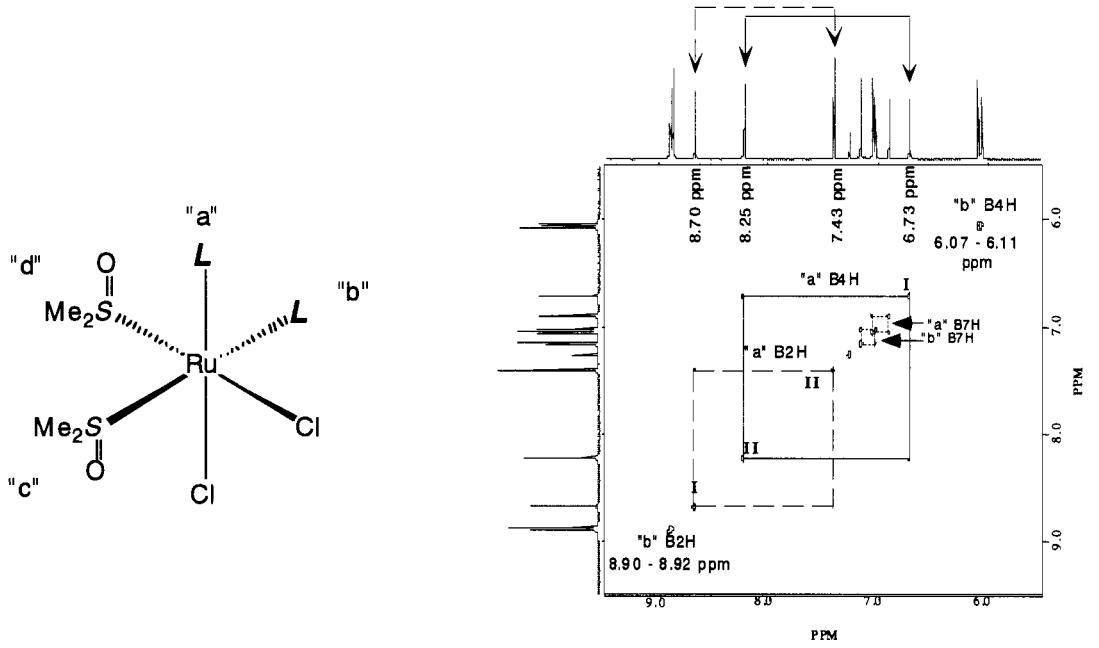

Fig. 4. *Left*: Labeling scheme for the octahedral Ru complex ligand positions. *Right*: Downfield region of 2D ^{1}H EXSY spectrum of *cis,cis,cis*-$RuCl_2(Me_2SO)_2(Me_3Bzm)_2$

For *cis,cis,cis*-$RuCl_2(Me_2SO)_2L_2$ (Fig. 4, Me_2SO = *S*-bonded DMSO), there are four potential atropisomers (2HT, 2HH) when ***L*** lacks a chiral group like a sugar. The atropisomer of *cis,cis,cis*-$RuCl_2(Me_2SO)_2(Me_3Bzm)_2$ that crystallizes is HH, with the five-membered ring "heads" pointing toward the Cl ligands. The ^{1}H NMR ROESY and EXSY spectra of this compound reveal the presence of only two species (I and II) and allow assignment of the four signals for each type of Me_3Bzm proton (7, 12). Three of four H4 signals (Fig. 4) were shifted markedly upfield and only one of the four H2 signals was upfield. This distinctive pattern can be explained only if II is the solid-state HH species and I is the HT species formed by flipping Me_3Bzm "a" *trans* to Cl. *cis,cis,cis*-$RuCl_2(Me_2SO)_2(Me_3Bzm)_2$ holds the distinction of being the first compound of its type found to be HH both in solution and in the solid state.

The two unobserved atropisomers (one HH and one HT) have the Me_3Bzm "b" (*trans* to Me_2SO) flipped; however, models suggest such flipping would lead to severe steric clashes. The base/metal coordination plane dihedral angles (B/M) range from 38-51°, compared with values closer to 90° for HH square-planar complexes, *cis*-$Pt(NH_3)_2$dpG*pG* (24, 25) and other HH derivatives, representative of the major DNA adduct formed by *cis*-$Pt(NH_3)_2Cl_2$. Modeling of octahedral Ru(II) dG*pG* complexes with such HH conformations (26) has predicted that severe steric clashes with axial ligands can occur. Since Me_3Bzm is relatively less bulky than G, the low B/M values support the modeling predictions of steric clashes between G* nucleobases and axial ligands of octahedral HH complexes. Such clashes may explain why octahedral anticancer drugs are less effective, in general, than square-planar ones.

The HH species found to be present can be rationalized if there is a significant attraction between the partially positively charged (δ^+) aromatic CH in the imidazole ring and the partially negatively charged Cl ligands.

Since the Cl's are coordinated in *cis* positions, this factor seems to account for the presence of this HH species. In the HH rotamer, Me_3Bzm "a" is canted, with the NC_2H pointed toward Me_3Bzm "b". Our NMR analysis established that atropisomerization involves rotation of Me_3Bzm "a"; Me_3Bzm "b" remains relatively fixed.

Rotamers of several *cis,cis,cis*-$RuCl_2(Me_2SO)_2(1,2\text{-}Me_2Imd)L$ complexes [L = 1,2-Me_2Imd (1,2-dimethylimidazole), a pyridine ligand, or Me_3Bzm] have been studied in $CDCl_3$. From 2D NMR data, *cis,cis,cis*-$RuCl_2(Me_2SO)_2(1,2\text{-}Me_2Imd)(Me_3Bzm)$ has 1,2-Me_2Imd in position "a" and Me_3Bzm in position "b". There are two stable atropisomers [HT, 84% and HH, 16%, defining the aromatic H of Ru-N-C-H as head for both ligands]. Me_3Bzm has the same orientation in both atropisomers. In this orientation, the unfavorable interligand steric interactions of Me_3Bzm with the Me_2SO and 1,2-Me_2Imd ligands appear to be countered by favorable electrostatic attraction between the δ^+ N_2CH moiety of Me_3Bzm and the δ^- *cis* Cl ligands. Thus, Me_3Bzm "b" adopts a standard orientation. The 1,2-Me_2Imd lacks a δ^+N_2CH group, and its orientation is dominated by steric effects of the 2-Me group.

Another complex, *cis,cis,cis*-$RuCl_2(Me_2SO)_2(1,2\text{-}Me_2Imd)(3,5\text{-lut})$, provides a rare example in which the solid state closely reflects the solution state, both in the structure of species involved in a conformational equilibrium and in the position of that equilibrium. Thus, we are able to gain structural information on both the less stable and the more stable rotamers in the same solid state environment. The N-Ru-N bond angles show that there is a displacement of 1,2-Me_2Imd toward 3,5-lut in the less abundant conformer in the solid, a clear feature that cannot normally be derived from solution results. This movement of the 1,2-Me_2Imd might be the result of the repulsion between the 2-Me of 1,2-Me_2Imd and the *cis* chlorides and/or favorable attractive forces between the heterocycles. Such forces would be worth understanding since *cis* arrangements of heterocycles occur widely in metallobiochemistry. Therefore, additional examples of crystallographically characterized unstable conformers would be valuable to study, especially if complementary data are available for the stable form.

In the two types of *cis,cis,cis*-$RuCl_2(Me_2SO)_2(1,2\text{-}Me_2Imd)L$ complexes, there are only two rotamers. In contrast, the NMR spectrum of *cis,cis,cis*-$RuCl_2(Me_2SO)_2(1,2\text{-}Me_2Imd)_2$ is consistent with four rotamers in restricted rotation about both Ru-N bonds: two HH and two HT. 2D NMR techniques (NOESY and ROESY) afforded complete proton signal assignments. The ligand disposition could be assessed from the large chemical shift dispersion of some 1,2-Me_2Imd ligand signals (Δ 0.86-1.52 ppm) arising from *cis*-1,2-Me_2Imd shielding modulated by deshielding influences of the *cis* halides. The relative stability of the four rotamers correlates best with steric interactions between the 2-Me groups and the Me_2SO ligands. The most favorable conformer (46%) is the HH rotamer with both 2-Me groups pointing away from the Me_2SO ligands. The least favorable conformer (14%) was also HH, but the methyl groups in this case point toward the Me_2SO ligands. In the HT conformers of intermediate stability (~20%), one 2-Me group is toward and the other is away from the Me_2SO ligands. Exchange cross-peaks in the 2D spectra are unusually informative about the dynamic processes in solution; the spectra provide evidence that the rotamers interchange in a definite pattern of succession. Thus, all conceivable exchange pathways are not available. 1,2-Me_2Imd "b" can rotate regardless of the orientation of 1,2-Me_2Imd "a". 1,2-Me_2Imd "a" can rotate only when "b" has the orientation with its 2-Me group directed away from "a". Thus, 1,2-Me_2Imd "b" can switch 1,2-Me_2Imd "a" rotation on or off.

Rhenium examples

The dimer $Re_2O_3Cl_4(Me_3Bzm)_4$ was characterized by NMR and X-ray analysis. The crystal structure showed that the Me_3Bzm dimer consists of a nearly linear O=Re-O-Re=O grouping with two *cis* Cl and two HH oriented *cis* Me_3Bzm ligands on each Re. Additionally, two Me_3Bzm's, one from each Re, are stacked upon one another in the HT orientation and are designated as Me_3Bzm^s (the terminal Me_3Bzm's = Me_3Bzm^t). In CD_2Cl_2 at 20 °C, only one set of Me_3Bzm ^1H NMR signals was observed, showing that the dimer has dynamic characteristics. At -90 °C, each signal had split into two equal intensity signals. For both sets, the chemical shifts have an interesting and unusual dispersion. This dispersion can be readily explained by the anisotropic effects expected from the X-ray structure, providing compelling evidence that the structure in solution is essentially identical to that in the solid. For example, an unusually large upfield shift (~1.5 ppm) of the Me_3Bzm^s NMe signal is caused by the combined anisotropy of the benzene ring of the Me_3Bzm^s partner (~1 ppm) and by the *cis*-Me_3Bzm^t ligand (~0.5 ppm). Inter-ligand NOE cross-peaks provide other compelling evidence that the predominant solution conformer of $Re_2O_3Cl_4(Me_3Bzm)_4$ has the HH,HT,HH structure.

This is the only case in which *cis,bis* imidazole-ring-ligated untethered ligands have been found to be essentially exclusively HH in solution. This predominance of the interesting extended HH,HT,HH arrangement of Me_3Bzm's can be attributed to the electrostatic attraction of the δ^+ N_2C protons for the

negative core of the molecule. There are several reasons why we believe this is the key interaction that dictates conformation. First, there are so many favorable interactions; for example, the δ^+ H on Me_3Bzm^t is attracted by four negative groups (bridging O, *cis* Cl on same Re, and two *cis* Cl's on the other Re). Second, the distribution of negative groups surrounding the stacked site is more symmetrical than for the terminal site. In the observed Me_3Bzm^s orientation, the bulky Me_3Bzm^s six-membered ring is directed away from the bulkiest ligand on the same Re, Me_3Bzm^t. The lower steric crowding favors the observed HT Me_3Bzm^s orientation compared to the orientation obtained by rotation of Me_3Bzm^s by 180° from that found in $Re_2O_3Cl_4(Me_3Bzm)_4$. These steric and electrostatic factors act in concert to favor the HH,HT,HH conformer observed in the solid. Third, further insight into base orientation came from a related mixed ligand dimer (27). The dimer, $[ReOCl_2(py)(Me_3Bzm)]O[ReOCl_2(py)(Me_3Bzm)]$, has two possible geometric isomers with one py and one Me_3Bzm on each Re. In the isomer we studied, there are two conformers, one with the py's and the other with the Me_3Bzm's as the stacked bases (L^s's), with the terminal bases (L^t's) being Me_3Bzm's and py's, respectively. Although Me_3Bzm is better at stacking, both the X-ray structure and the solution NMR data show that it is the py's that are stacked in the stable conformer. The Me_3Bzm^t's are oriented in the correct way for favorable electrostatic interactions in the mixed ligand dimer; this finding was the breakthrough that allowed us to rationalize all of our past results on base orientation in Ru(II) and Re(V)O chemistry in terms of electrostatic attraction. Finally, although Re(V) and Ru(II) are very different metal centers, the relative stability of the atropisomers found for Ru(II) complexes of lopsided ligands can best be rationalized by invoking this same type of electrostatic attraction of the $NC_2H\delta^+$ toward the coordinated Cl ligands.

Dynamics, a closer look

We introduced the use of exchange-NOE NMR data as a powerful method for defining features of L's such as the extent of rotation about the metal–N bond, the direction of rotation, and even the halves of C_2-symmetrical ligands that interchange during dynamic processes. The full value of the approach depended on a strategy in which the complexes studied are similar except that one has a lopsided L (e.g. Me_3Bzm) and the other has a C_2-symmetrical L (e.g. 3,5-lut). The two halves of C_2-symmetrical L's can be distinguished in chiral complexes by probing the respective NMR signals. The orientations of L's can be assessed with interligand NOE cross-peaks and anisotropic effects on chemical shift. Combined information gained from studying two similar compounds is much greater than that from the study of either separately.

The most striking results were obtained with dimers of the type $Re_2O_3Cl_4L_4$ (13). These dimers are chiral, and we examined the fluxional inversion of $[Re_2O_3Cl_4(Me_3Bzm)_4]$ and $[Re_2O_3Cl_4(3,5\text{-lut})_4]$. At ambient temperature, the latter has just one ^1H NMR signal for each class of 3,5-lut proton. Such a simple spectrum requires rapid interconversion of enantiomers with rotations of ~180° about the Re–O–Re bonds and of at least ~90° about all four Re-L bonds. The ^1H NMR spectrum of $Re_2O_3Cl_4(3,5\text{-lut})_4$ at -100 °C reveals two sets of five 3,5-lut signals assignable to the stacked ($3,5\text{-lut}^s$) and terminal ($3,5\text{-lut}^t$) ligands. As the temperature was raised slightly above -100 °C, the α-H and β-CH_3 signals of the $3,5\text{-lut}^t$ broadened, but the signal of the γ-H, which is on the Re–N rotation axis, did not. The signals for the $3,5\text{-lut}^s$ remained sharp. This result demonstrated that $3,5\text{-lut}^t$ rotates faster about its Re–N bond than does $3,5\text{-lut}^s$. Re–N bond rotation is slower than rotation about the Re–O–Re bonds since the exchange-NOE data showed preferential exchange between two halves of the 3,5-lut ligands. The exchange-NOE data show that the half of L^t away from the dimer center interchanges with the half of L^s close to the center, with the L plane rotating by 90° past the O=Re–O bonds, not the N–Re–Cl bonds. Thus, the exchange-NOE data help establish the direction of the rotation.

The spectral features of the Me_3Bzm dimer are most consistent with restricted rotation about the Re-N bonds, but very fast rotation cannot be ruled out since the same number of signals is expected for a lopsided ligand in either slow or fast exchange. No preferential broadening of the Me_3Bzm^t of that dimer was observed, a result indicating that there is only one rate-limiting process. This process has to be Re–O–Re rotation that is faster than Re–N bond rotation of Me_3Bzm. The latter rotation must occur and since the rate of the inversion process is similar for the two dimers, the Me_3Bzm must also rotate through 90°. Thus, we are able to elucidate the dynamic properties of a complex that has only lopsided ligands.

Conclusions from analog studies

In octahedral sites in biological systems, lopsided ligands can select among four staggered orientations. Rarely has more than one staggered conformer been identified for any site. In model chiral octahedral complexes, a symmetrical ligand can select between only two possible staggered positions; the ligand can move between the two orientations by two opposing routes of rotation. In our work, we showed how 2D exchange-NOEs can be used to distinguish these two routes of rotation. To our knowledge, this is the first

time preferred pathways of rotation have been demonstrated. Exchange-NOEs have not been widely used to assess dynamic pathways. By comparisons with the analogous complex containing a lopsided ligand, we showed that the lopsided ligand very probably rotates using the same pathway as the symmetrical ligand. Such rotational motion can be significant in many biological processes such as in the formation of DNA cross-links by metal anticancer drugs.

The characteristic HH shift pattern and the NOE data demonstrate that there is only one significant conformer of $Re_2O_3Cl_4L_4$ in solution and that this conformer has the HH,HT,HH structure found in the solid. This is the first case in which a very predominant HH form of a complex with this type of untethered ligand has been demonstrated in solution. The predominance of this form strongly suggests that the electrostatic attraction of the δ^+ aromatic proton in the imidazole ring of Me_3Bzm^t toward the negative central core of the dimer is responsible for these properties. This conclusion is supported by a mixed-ligand dimer. In this dimer, the Me_3Bzm ligands prefer the terminal position with an orientation that allows the $N_2CH\delta^+$ proton to interact closely with the greatest concentration of negative charge on the complex.

In Ru(II) models, we find that the "b" site is favored, and that the base at this site is less fluxional than the base at "a". This could be a reason why DNA cross-linking is so inefficient, if our hypothesis that ease of rotation influences cross-linking is correct. In Ru complexes with the Me_3Bzm ligand, the conformations found suggest again that the electrostatic attraction of the $N_2CH\delta^+$ proton for the negative ligands is important. In this case steric repulsion with neutral ligands on Ru opposes the electrostatic attraction, and the conformational preference is less than in the case of the Re dimers. The 1,2-Me_2Imd ligand lacks a $N_2CH\delta^+$ proton and the results of studies with this ligand provide indirect support for our hypothesis that Me_3Bzm orientation is strongly influenced by interligand electrostatic interactions. Furthermore, complexes with the 1,2-Me_2Imd ligand are useful for elucidating the influence of steric effects on dynamic pathways.

IMPLICATIONS FOR METALLOBIOSYSTEMS

There are several reasons why the $N_2CH\delta^+$ proton electrostatic interactions with other ligands have biomedical relevance. Once a G is attacked by a cationic Pt(II) species (aquated Pt(II) drugs usually attack DNA), the $N_2CH\delta^+$ proton becomes more positive because the metal withdraws electron density from the base. This $N_2CH\delta^+$ proton will be attracted toward centers of negative charge such as the phosphate groups in the DNA backbone. Even though this may be a small energy term, it still can influence structure, ease of base-pair breathing, and possibly interactions of Pt-DNA adducts with proteins. Above we mentioned the diversity of the X-ray and the NMR results with both small and large molecules. Within the ionic environment of some crystals and also in crystals of neutral complexes with charged groups, such as phosphate groups, the orientations found may be dictated by the location of the negative charge compared to the position of the G*.

The shift difference between the upfield and downfield N_2CH signals for *bis* G* Pt(II) complexes with stereochemically controlling ligands is greater than for dG*pG* adducts. Thus, our work suggests that the sugar-phosphate DNA backbone, rather than causing the canting, may actually decrease the canting. Other NMR results suggest that there are stabilizing forces in which the G* $N_2CH\delta^+$ proton favorably interacts with the π cloud of the *cis*-coordinated G* base (28). This finding leads to a third relationship, head-to-side (HS, Fig. 1). Of course, this is a novel concept and must be tested further.

Metal binding also increases the partial positive charge on H2 in imidazole. Nearby negative charge will affect the orientation of imidazoles in metalloproteins, influencing structure and reactivity of these biomolecules. In these more complicated molecules, it is difficult to determine the contribution of the electrostatic term, but it must have some influence on the properties of the biomolecule. In this report, we hope to stimulate metallobiochemists to consider this possibility in analyzing their data.

One additional implication concerns the influence of these electrostatic forces (balanced always by steric effects) on the dynamic processes in biological systems. Again, selecting nucleobase cross-linking of DNA as an example, these forces could influence the orientation of the metal in an initial monodentate adduct. Furthermore, the electrostatic effect could influence the rate and direction of rotation. The orientation and dynamic characteristics will in turn affect the rate and products of the cross-linking reactions. These in turn will affect the anticancer activity of the drug.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Grant GM29222, by Italian MURST (40% Grant), and by the Institute for Molecular Science. L.G.M. gratefully acknowledges support from a Japan Society for the Promotion of Science Fellowship.

REFERENCES

1. S. J. Lippard and J. M. Berg. *Principles of Bioinorganic Chemistry*. University Science Books, Mill Valley, CA (1994).
2. P. R. Blake and M. F. Summers. In *Advances in Inorganic Biochemistry* (G. L. Eichhorn, L. G. Marzilli, ed.), vol. 10, pp. 201. Elsevier, New York (1994).
3. J. M. Puckett, Jr., M. B. Mitchell, S. Hirota and L. G. Marzilli. *Inorg. Chem.* **35**, 4656 (1996).
4. C. L. Drennan, S. Huang, J. T. Drummond, R. G. Matthews and M. L. Ludwig. *Science (Washington, D.C.)* **266**, 1669 (1994).
5. F. Mancia, N. H. Keep, A. Nakagawa, P. F. Leadlay, S. McSweeney, B. Rasmussen, P. Bosecke, O. Diat and P. R. Evans. *Structure* **4**, 339 (1996).
6. A. M. Calafat, H. Won and L. G. Marzilli. *J. Am. Chem. Soc.* **119**, 3656 (1997).
7. L. G. Marzilli, M. Iwamoto, E. Alessio, L. Hansen and M. Calligaris. *J. Am. Chem. Soc.* **116**, 815 (1994).
8. Y. Xu, G. Natile, F. P. Intini and L. G. Marzilli. *J. Am. Chem. Soc.* **112**, 8177 (1990).
9. H. Schöllhorn, G. Raudaschl-Sieber, G. Müller, U. Thewalt and B. Lippert. *J. Am. Chem. Soc.* **107**, 5932 (1985).
10. D. Kiser, F. P. Intini, Y. Xu, G. Natile and L. G. Marzilli. *Inorg. Chem.* **33**, 4149 (1994).
11. M. Iwamoto, E. Alessio and L. G. Marzilli. *Inorg. Chem.* **35**, 2384 (1996).
12. E. Alessio, M. Calligaris, M. Iwamoto and L. G. Marzilli. *Inorg. Chem.* **35**, 2538 (1996).
13. E. Alessio, L. Hansen, M. Iwamoto and L. G. Marzilli. *J. Am. Chem. Soc.* **118**, 7593 (1996).
14. J. H. J. den Hartog, C. Altona, J. H. van Boom, G. A. van der Marel, C. A. G. Haasnoot and J. Reedijk. *J. Am. Chem. Soc.* **106**, (1984).
15. G. Admiraal, J. L. van der Veer, R. A. G. de Graaff, J. H. J. den Hartog and J. Reedijk. *J. Am. Chem. Soc.* **109**, 592 (1987).
16. J. Kozelka, M.-H. Fouchet and J.-C. Chottard. *Eur. J. Biochem.* **205**, 895 (1992).
17. P. M. Takahara, A. C. Rosenzweig, C. A. Frederick and S. J. Lippard. *Nature (London)* **377**, 648 (1995).
18. A. M. J. Fichtinger-Schepman, A. T. van Oosterom, P. H. M. Lohman and F. Berends. *Cancer Res.* **47**, 3000 (1987).
19. H. Huang, L. Zhu, B. R. Reid, G. F. Drobney and P. B. Hopkins. *Science (Washington, D.C.)* **270**, 1842 (1995).
20. F. Pacquet, C. Pérez, M. Leng, G. Lancelot and J.-M. Malinge. *J. Biomol. Struct. Dyn.* **14**, 67 (1996).
21. G. Sava, S. Pacor, M. Coluccia, M. Mariggio, M. Cocchietto, E. Alessio and G. Mestroni. *Drug Invest* **8**, 150 (1994).
22. F. Loseto, E. Alessio, G. Mestroni, G. Lacidogna, A. Nassi, D. Giordano and M. Coluccia. *Anticancer Res.* **11**, 1549 (1991).
23. P. Colucci, M. Coluccia, P. Montemurro, M. Conese, A. Nassi, F. Loseto, E. Alessio, G. Mestroni and N. Semeraro. *Int. J. Oncol.* **2**, 527 (1993).
24. S. E. Sherman, D. Gibson, A. H.-J. Wang and S. J. Lippard. *Science* **230**, 412 (1985).
25. M. Coll, S. E. Sherman, D. Gibson, S. J. Lippard and A. H.-J. Wang. *J. Biomol. Struct. Dyn.* **8**, 315 (1990).
26. G. Esposito, S. Cauci, F. Fogolari, E. Alessio, M. Scocchi, F. Quadrifoglio and P. Viglino. *Biochemistry* **31**, 7094 (1992).
27. E. Alessio, E. Zangrando and L. G. Marzilli. Unpublished work.
28. M. Iwamoto, S. Mukundan, Jr. and L. G. Marzilli. *J. Am. Chem. Soc.* **116**, 6238 (1994).