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Metal–Metal Interactions in Enzymes: EPR and NMR Investigations

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Our recent work has dealt with an evaluation of metal–metal interactions in enzymes that require more than one divalent cation as active site components. Two enzymes we have extensively investigated are glutamine synthetase and inorganic pyrophosphatase.

Glutamine synthetase catalyzes the formation of glutamine from glutamate, ATP and NH_3 as shown below.

$$ATP + NH_3 + Glutamate \overset{2M^{2+}}{\rightleftharpoons} ADP + P_i + Glutamine$$

The enzyme requires two divalent cations for catalysis. An EPR method was utilized to calculate the distance between metal ions bound at the two metal ion sites. β,γ-Cr^{3+} ATP was used to study the interaction between Mn(II) at the n_1 site and Cr(III) of the nucleotide complex bound at the n_2 site. Addition of a saturating amount of β,γ-CrATP produced a decrease in the EPR spectrum of enzyme-bound Mn(II). This dipolar spin–spin interaction was analyzed at 35 GHz to calculate the distance between Mn(II) and Cr(III) (~7 Å). Similarly, the EPR signal amplitude of enzyme–$Mn(n_1)$ was diminished by addition of Mn(II) to the n_2 site. Analysis of the data indicated that this phenomenon was due to a dipolar spin-spin interaction. NMR results also corroborated this conclusion. Distances between the two metal ion sites were calculated from both sets of data. The Mn(II)–Mn(II) distances were found to be 8.1–11.2 Å with nucleotide and 11.5–13 Å without nucleotide. Thus, nucleotide binding induced a conformational change which brings the two metals closer together. The two metal ions are in close enough proximity to be involved in substrate binding, orientation, and activation.

The distances measured for Mn(II) at both the n_1 and n_2 sites are larger than those measured for Mn(II) at n_1 and Cr-nucleotides at n_2. This suggests that the Cr(III) moiety of a Cr-nucleotide does not bind directly to the n_2 metal ion site, but is several angstroms displaced from the 'true' metal ion active site.

NMR and EPR studies were conducted to evaluate the number of metal ion binding sites on yeast inorganic pyrophosphatase (PPase). Apo-PPase binds two Mn^{2+} per subunit and these metal ions are in close enough proximity to magnetically interact. Analysis of the NMR and EPR data in terms of dipolar relaxation mechanism between Mn^{2+} ions provides an estimate of the distance between them (10–14 Å). When the diamagnetic substrate analogs $Co(NH_3)_4$-PNP or $Co(NH_3)_4$PP are bound to PPase, two Mn^{2+} ions still bind to the enzyme and their magnetic interaction increases. In the presence of these Co^{3+} substrate analogs the Mn^{2+}–Mn^{2+} separation decreases to 7–9 Å. Several NMR and EPR experiments were conducted at low Mn^{2+} to PPase ratios (~0.3), where only one Mn^{2+} ion binds per subunit, in the presence of Cr^{3+} or Co^{3+} complexes of PNP or PP. Analysis of the Mn^{2+}–Cr^{3+} dipolar relaxation evident in NMR and EPR data resulted in calculation of Mn^{2+}–Cr^{3+} distances. When the substrate analog PNP was present, the Mn^{2+}–Cr^{3+} distance was ~7 Å whereas when the product complex formed from PP was bound to PPase the Mn^{2+}–Cr^{3+} distance was ~5 Å. These results strongly support a model for the catalytic site of PPase that involves three metal ions in binding and catalysis.

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Intramolecular Ligand–Ligand Interactions in Mixed Ligand Complexes

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One of the most important properties of mixed ligand complexes is direct and metal ion-mediated ligand–ligand interactions, the latter being known as π-acid–π-base interactions around the central metal ion. Examples of direct intramolecular ligand-ligand interactions are ionic and hydrogen bonds between charged and/or polar side groups and hydrophobic interactions between coordinated aromatic rings and side chain aliphatic groups or aromatic rings (aromatic ring stacking). Ionic interactions were found to exist in aqueous solutions of low-molecular-weight mixed ligand complexes, *e.g.*, Cu(A)(B), where A refers to acidic amino acids (aspartate or glutamate) and B to monoprotonated basic amino acids (zwitterionic arginine, lysine, or ornithine) [1]. The ligand–ligand interactions in the M(L-A)-(L-B) systems (M = Cu(II) or Pd(II)) give rise to a CD spectral magnitude anomaly which is dependent on the solvent polarity and the ionic strength, and the rotamer populations calculated from the NMR coupling constants are affected by the interactions in the Pd(II) complexes [1, 2]. On the other hand,

the hydrogen bonding between the carboxylate group of histidinate (His) and the polar side group of asparaginate (Asn), serinate (Ser), threoninate (Thr), *etc.* (referred to as AA) in the ternary systems M(AA)(His) was confirmed by evidence from synthetic and CD and NMR spectral studies [3]. The infrared spectra of the isolated complexes indicated the existence of geometric isomerism in the diastereomers of M(A)(B) and M(AA)(His). On the basis of the steric requirements for the intramolecular ligand–ligand interactions, the geometry of M(L-A)(L-B) and M(D-AA)(L-His) has been inferred to be *trans* and that of M(D-A)(L-B) and M(L-AA)(L-His) *cis* with respect to the amine nitrogens. This is further supported by our recent infrared spectral study of the crystalline ^{63}Cu- and ^{65}Cu(AA)(L-His) complexes (AA = L-Asn, D-Asn, L-Thr, L-Ser, or D-Ser), which shows that only Cu(L-AA)(L-His) exhibits a metal isotope shift (*ca.* 1 cm^{-1}) of the Cu–NH_2 symmetric stretching band. The finding is reasonably explained by the *cis* structure for Cu(L-AA)(L-His) as established for AA = Asn andThr by X-ray analysis and the *trans* structure for Cu(D-AA)(L-His) where the isotope shift is not observed because the symmetric stretching mode does not involve the displacement of Cu.

Possibilities of ligand–ligand interactions in mixed ligand heteronuclear complexes will also be discussed.

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Chemistry and Molecular Biology of Anticancer Platinum Drugs

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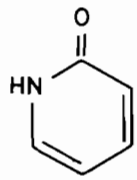
The anticancer platinum drug *cis*-diamminedichloroplatinum(II) (*cis*-DDP) binds avidly to DNA both *in vitro* and *in vivo*. The mechanism by which *cis*-DDP kills tumor cells is thought to involve DNA–Pt interactions [1], but further experimental work to establish this point would be valuable. Accordingly, in joint studies with M. Poirier and her group at the National Cancer Institute, we have developed an antibody to *cis*-DDP-DNA adducts formed *in vitro* which reacts specifically with *cis*-DDP–DNA adducts extracted from leukemia cells of mice treated with the platinum drug [2]. Though these antibody studies and other work using nucleases, especially restriction enzymes and exonuclease III, to probe the nature of the *cis*-DDP–DNA binding stereochemistry we find substantial evidence to support the hypothesis that the *cis*-$(NH_3)_2Pt^{2+}$ unit forms an intrastrand crosslink between two adjacent or nearby guanine bases on DNA [3]. The stereochemistry of this adduct has been deduced by NMR studies of a model complex formed betwcen the self-complementary hexanucleoside pentaphosphate d(ApGpGpCpCpT) and *cis*-DDP. In this complex the two adjacent guanine bases are coordinated to the *cis*-diammineplatinum(II) moiety through their N-7 nitrogen atoms [4].

When *cis*-DPP is administered in chemotherapy it is invariably used in combination with other anticancer drugs. How do these drugs affect the chemistry of *cis*-DDP within the cell? Moreover, agents within the cell such as glutathione are known to form complexes with *cis*-DDP. Will these intracellular reactions affect the DNA-*cis*-DDP interaction? With these questions in mind we have studied the binding of *cis*-DDP to DNA plasmids and restriction fragments. Our results demonstrate that the minor groove intercalating drug ethidium bromide switches the nuclease sensitive binding sites of *cis*-DDP [5] and that glutathione also alters the DNA binding properties of the antitumor drug.

Finally, an investigation has been conducted using ^{195}Pt NMR spectroscopy which demonstrates the kinetic mobility of diammineplatinum(II) adducts of the nucleobase analog α-pyridone [6]. In particular, the head-to-head isomer of $[(en)Pt(C_5H_4NO)]_2^{2+}$



α-pyridone

rearranges thermally to the head-to-tail isomer by a process that is reversible, intramolecular, and dissociatively activated. The possible relevance of this isomerization reaction to *cis*-DPP–DNA adducts will be discussed.

Acknowledgements. This work was supported by PHS grant number CA 34992 awarded by the National Cancer Institute, DHHS. We thank Englehard Industries for a generous loan of K_2PtCl_4 from which all platinum compounds were prepared.