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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,