

## 2.5: Coordination Geometries

The binding of inhibitors is also pH-dependent. It is possible, however, to obtain fully inhibited systems by adjusting the inhibitor concentration and pH. In this manner the so-called limit spectra of CoCA derivatives are obtained. Many systems have been characterized, providing a variety of spectral characteristics<sup>59</sup> (Figure 2.10).

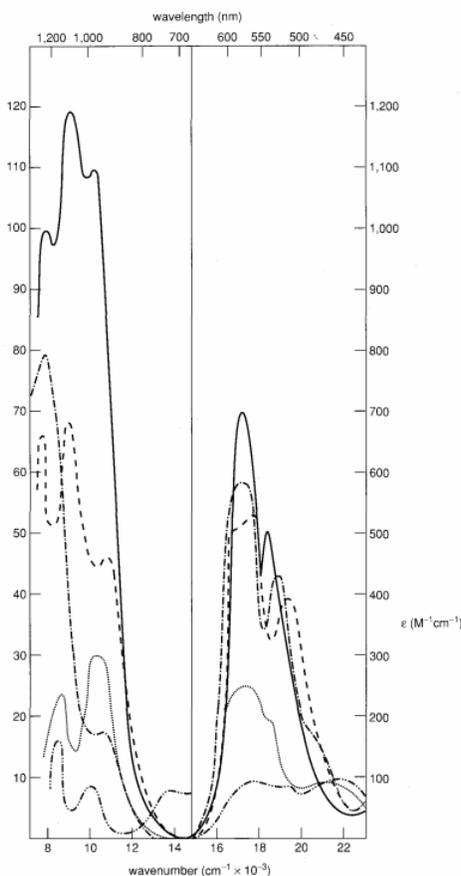


Figure 2.10 - Electronic spectra of cyanide (---), cyanate (-.-.-), acetazolamide (---), azide (•••••), and thiocyanate (-.-.-) adducts of cobalt(II)-substituted bovine carbonic anhydrase II.<sup>59</sup>

The differences in molar absorbance are larger than expected for changing only one coordinated atom. A rationalization of the experimental data came by applying a criterion, first suggested by Gray,<sup>60</sup> according to which four-coordinate species have larger maximal absorption than five-coordinate species. This property theoretically arises from greater mixing of p and d metal orbitals in the four-coordinate case, which makes the d-d transitions partially allowed, neglecting other factors such as the covalency of the coordination bond, nephelauxetic effects,\* or vicinity of charge transfer bands. Subsequent extension of the measurements to the near-infrared region was instructive:<sup>59</sup> the low-intensity spectra exhibited a weak absorption between 13,000 and 15,000  $\text{cm}^{-1}$ . The latter band was assigned to the highest in energy of the  $F \rightarrow F$  transitions, which increases in energy with the coordination number. † Therefore both the low intensity of the bands ( $\epsilon_{\text{max}} < 200 \text{ M}^{-1}\text{cm}^{-1}$ ) and the presence of the  $F \rightarrow F$  transition at high energy were taken as evidence for five coordination. Spectra showing high maximal absorption ( $\epsilon_{\text{max}} > 300 \text{ M}^{-1}\text{cm}^{-1}$ ) were assigned as arising from four-coordinate species. The corresponding chromophores are  $\text{CoN}_3\text{In}(\text{OH}_2)$  and  $\text{CoN}_3\text{In}$ , where In denotes inhibitor. Intermediate maximal absorptions may indicate an equilibrium between four- and five-coordinate species. In Table 2.6 some inhibitors are classified according to their behavior. Bicarbonate, which is a substrate of the enzyme, gives rise to an equilibrium between four- and five-coordinate species.<sup>48,59</sup>

Table 2.6 - Classification of inhibitors of bovine carbonic anhydrase II according to the electronic spectral properties of the adducts with cobalt(II) derivatives. <sup>a</sup> 48,59

\*Donor sets in parentheses

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Four-coordinate	Equilibria between four- and five-coordinate species	Five-coordinate
Sulphonamides (N <sub>4</sub> )	Bicarbonate (N <sub>3</sub> O—N <sub>3</sub> O <sub>2</sub> )	Carboxylates (N <sub>3</sub> O <sub>2</sub> )
Cyanide (N <sub>3</sub> C)	Chloride (N <sub>3</sub> Cl—N <sub>3</sub> OCl)	Thiocyanate (N <sub>4</sub> O)
Cyanate (N <sub>4</sub> )	Bromide (N <sub>3</sub> Br—N <sub>3</sub> OBr)	Nitrate (N <sub>3</sub> O <sub>2</sub> )
Aniline (N <sub>4</sub> )	Azide (N <sub>4</sub> —N <sub>4</sub> O)	Iodide (N <sub>3</sub> OI)
Phenol (N <sub>3</sub> O)		
Chlorate (N <sub>3</sub> O)		

\* Nephelauxetic (literally, cloud-expanding) effects are due to partial donation of electrons by the ligand to the metal, and are stronger for less electronegative and more reducing ligands

† By F → F transition we mean here a transition between two electronic states originating from the same F term (the ground term) in the free ion and split by the ligand field; the stronger the ligand field, the larger the splitting. For high-spin cobalt(II), the free-ion ground state <sup>4</sup>F (quartet F) is split in octahedral symmetry into <sup>4</sup>T<sub>2g</sub>, <sup>4</sup>T<sub>1g</sub>, and <sup>4</sup>A<sub>2g</sub> states, the <sup>4</sup>T<sub>2g</sub> lying lowest; in lower symmetries the T states are further split. The highest F → F transition is, therefore, that from the ground state <sup>4</sup>T<sub>2g</sub>, or the lowest of its substates in low symmetry, to the <sup>4</sup>A<sub>2g</sub> state. For the same type of ligands, e.g., nitrogens or oxygens, the ligand field strength, and therefore the energy of the F → F transition, increases with the number of ligands.

The differences in the electronic spectra outlined above also have been detected in both CD and MCD spectra. In the latter, pseudotetrahedral species give a sizably positive band in the high-energy region, whereas five-coordinate species show a much weaker positive band and six-coordinate complexes have only weak negative bands (Figure 2.11).<sup>21,61</sup> This additional empirical criterion may be helpful in assigning the coordination number. A further criterion is based on how much of the splitting of the S =  $\frac{3}{2}$  ground state is caused by spin-orbit coupling (zero-field splitting). This splitting can be indirectly measured from the temperature dependence of the electronic relaxation times of the cobalt complexes, in turn estimated from their ability to saturate the EPR lines of the complexes at low temperatures.<sup>62</sup> There are theoretical reasons to predict that the above splitting increases in the order four coordination < five coordination < six coordination.<sup>63</sup>

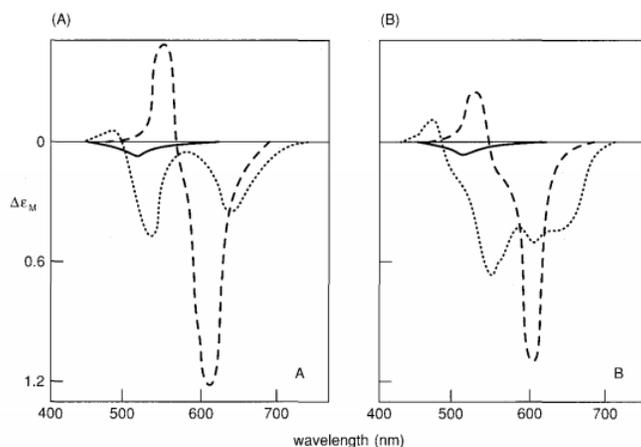


Figure 2.11 - (A) MCD spectra of model six-coordinate (Co(Gly-Gly)<sub>2</sub>, —), five-coordinate ((CoMe<sub>6</sub>tren)Br<sub>2</sub>, . . . . , and four-coordinate (Co(py)<sub>2</sub>Br<sub>2</sub>, ---) cobalt(II) complexes and (B) MCD spectra of the cobalt(II) derivatives of pyruvate kinase (—), alkaline phosphatase (••••), and carbonic anhydrase in the presence of acetazolamide (---).<sup>21,61</sup>

Three binding sites have been identified in the cavity of CA<sup>40,64-66</sup> (Figure 2.12). The OH<sup>-</sup> binding site, which provides a tetrahedral structure around the metal ion, is called the A site. The hydrogen interacts via hydrogen bonding with the oxygen of Thr-199. Thr-199 and Thr-200, together with their protein backbone, identify a hydrophilic region that probably plays a fundamental role in the energetic balance of ligand binding. On the back of the cavity there is a hydrophobic region formed by Val-143, Leu-198, and Trp-209. Although this cavity is hydrophobic, the x-ray structure shows evidence of a water molecule, H-bonded to the coordinated water. Ligands with a hydrophobic end could easily be located in this binding position, which is called B. The

coordinated water molecule would change its position in order to make reasonable angles between coordinated groups. The new position is labeled C. The x-ray structure of the thiocyanate derivative of HCA II<sup>40,64</sup> illustrates the latter case (see Figure 2.5). The NCS<sup>-</sup> ion is in van der Waals contact with Val-143, Leu-198, and Trp-209. The water interacts with the hydroxyl group of Thr-199. The geometry of the five-coordinate derivative can be roughly described as a distorted square pyramid with His-94 in the apical position (Figure 2.13A). This could be a typical structure for those derivatives that have spectra typical of five-coordinate adducts, like the carboxylate derivatives.

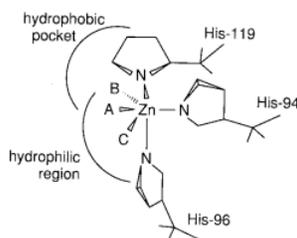


Figure 2.12 - Schematic drawing of the active cavity of HCA II showing the three possible ligand binding sites.<sup>64-66</sup> Site A is the site of the OH<sup>-</sup> ligand in the active form; site B is the binding site of NCS<sup>-</sup>, which gives rise to a five-coordinate adduct with a water molecule in the C site.<sup>64-66</sup>

In aromatic sulfonamide (Ar—SO<sub>2</sub>—NH<sub>2</sub>) derivatives, which probably bind as anions (see Section IV.C.4), the NH<sup>-</sup> group binds zinc in the A position,<sup>64-66</sup> giving rise to an H-bond with Thr-199. The oxygens do not interact with the metal; one of them sits in the hydrophobic pocket. The chromophore around zinc is pseudotetrahedral (Figure 2.13B). The energy involved in the coordination includes the coordination bond, the hydrophobic interactions of the aromatic sulfonamide ring, and the maintenance of the Zn-X-H-Thr-199 hydrogen bonding (X=N,O). It is interesting to note that cyanate, according to spectroscopic studies,<sup>48,59</sup> gives rise to tetrahedral derivatives, probably because the terminal oxygen can enter into H-bonds with the hydrophilic region of the cavity. <sup>13</sup>C NMR data on N<sup>13</sup>CO<sup>-</sup> interacting with CoBCA indicate that the anion interacts directly with the metal ion.<sup>67</sup> We do not have direct information on where it binds.<sup>212</sup>

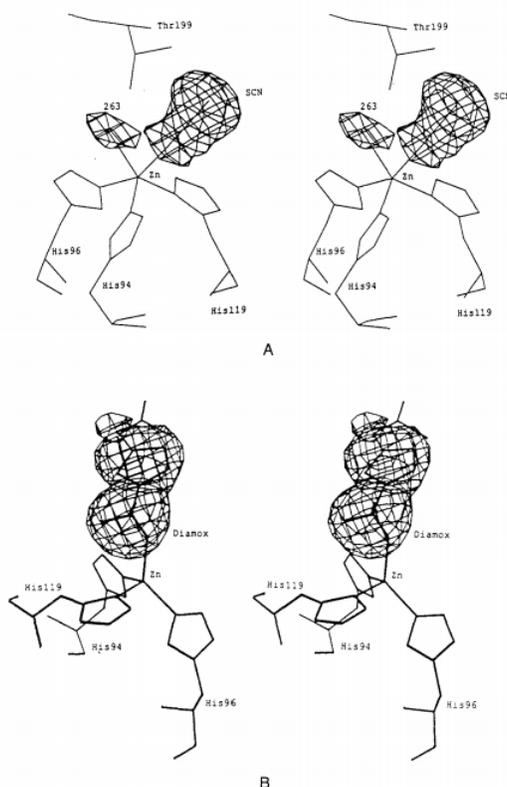


Figure 2.13 - Stereo views of the NCS<sup>-</sup> (A) and acetazolamide (B) adducts of HCA II.<sup>40</sup>

The fine balance between hydrophobic and hydrophilic interactions, as well as major steric requirements, play important roles in the binding of inhibitors. Cyanide is the only ligand that may bind in a 2:1 ratio.<sup>68</sup> It is likely that the bis-cyanide adduct has the same arrangement as the  $\text{NCS}^- \text{---} \text{H}_2\text{O}$  derivative. The spin state of the bis-cyanide adduct is  $S = \frac{1}{2}$ .<sup>68</sup>

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