2.4: Coordinated Water and NMR

It is quite relevant to know whether a water molecule is coordinated to the metal ion in a metalloenzyme, and whether it is still coordinated in the presence of substrates and inhibitors. The presence or absence of H_2O coordinated to a paramagnetic center can in principle be monitored by solvent water ^{1}H NMR,[69] by exploiting the occurrence of a magnetic interaction between the magnetic moments of the unpaired electrons and the nuclear magnetic moments of the water protons. When this interaction fluctuates with time, it causes a shortening of the water-proton relaxation times.*

* The nuclear longitudinal relaxation time, T_1 can be defined as the rate constant by which the populations of the $M_I = \frac{1}{2}$ and $M_I = -\frac{1}{2}$ (for protons) levels reach their equilibrium value after an external perturbation (e.g., a radiofrequency pulse in an NMR experiment). The transverse relaxation time, T_2, can be defined as the average lifetime of a hydrogen nucleus in a given spin state. The NMR linewidth is inversely proportional to T_2. The relation $T_2 \leq T_1$, always holds.

The longitudinal relaxation rate values, T_1^{-1}, of all the solvent water protons increase when even a single water molecule interacts with a paramagnetic center, provided that this bound water exchanges rapidly with free water molecules. To obtain the necessary experimental data, a methodology has been developed based on the measurement of water ^{1}H T_1^{-1} values at various magnetic fields (Nuclear Magnetic Relaxation Dispersion, NMRD).[69-71] The experimental data contain information on the correlation time, i.e., the time constant for the dynamic process that causes the proton-unpaired electron interaction to fluctuate with time; furthermore, under certain conditions, they may provide quantitative information on the number of interacting protons and their distance to the metal. The enhancement of T_1^{-1}, called T_{1p}^{-1}, is caused by the paramagnetic effect on bound water molecules and by the exchange time τ_m, according to the relationship

$$(T_{1p})^{-1} = f_M(T_{1M} + \tau_M)^{-1} \tag{2.11}$$

where f_M is the molar fraction of bound water and T_{1M} is the relaxation time of a bound water proton. Therefore we measure the water ^{1}H T_1^{-1}, subtract the diamagnetic effect (i.e., the water-proton relaxation rate measured in a solution of a diamagnetic analogue), obtain T_{1p}^{-1}, then check that τ_m is negligible with respect to T_{1M} For high-spin cobalt(II), T_{1M} is of the order of 10^{-3} s, whereas τ_m is about 10^{-5} s. Then the experimental T_{1p} can be safely related to T_{1M}. It is now important, in order to proceed with the analysis, to define the correlation time for the interaction between proton nuclei and unpaired electrons, τ_c. Its definition is important in order to obtain a physical picture of the system, and to quantitatively analyze the obtained T_{1M} values.[69] τ_c is defined by

$$\tau_c^{-1} = \tau_r^{-1} + \tau_s^{-1} + \tau_m^{-1} \tag{2.12}$$

where (\tau\)$_r$ is the rotational correlation time, (\tau\)$_s$ is the electronic relaxation time, and (\tau\)$_m$has been previously defined. (\tau\)$_r$ depends on the size of the molecule, which can be calculated rigorously if the molecule is spherical, or approximately if it is not. The appropriate expression is

$$\tau_r = \frac{4\pi\eta a^3}{3k_BT} \tag{2.13}$$

where η is the microviscosity of the solution, a is the radius (or approximate radius) of the molecule, k_B is the Boltzmann constant, and T is the absolute temperature. For CA, τ_r can be safely calculated to be $\simeq 10^{-8}$ s at room temperature. Since the correlation time τ_c in high-spin cobalt proteins varies between 10^{-11} and 10^{-12} s, it must therefore be determined by the electronic relaxation time.

Water ^{1}H NMRD profiles are often analyzed by using the classical dipolar interaction approach, as first described by Solomon:[72]

$$T_{1M}^{-1} = \frac{2}{15}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_I^2 g_e^2 \mu_B^2 S(S+1)}{r^6}\left(\frac{7\tau_c}{1+\omega_S^2\tau_c^2} + \frac{3\tau_c}{1+\omega_I^2\tau_c^2}\right) \tag{2.14}$$

where μ_0 is the permeability of vacuum, γ_I is the nuclear magnetogyric ratio, g_e is the electron g-factor, S is the electron spin quantum number, r is the electron-nucleus distance, and ω_S and ω_I are the electron and nuclear Larmor frequencies, respectively. This equation describes the dipolar interaction between the magnetic moment of nucleus $I(\hbar\gamma_I\sqrt{I(I+1)})$ and the magnetic moment of the electrons $S(g_e\mu_B\sqrt{S(S+1)})$as a function of the correlation time (τ_c) and of the magnetic field (expressed as (\omega\)$_I$ and ω_S). Neglect of the zero-field splitting of the $S = \frac{3}{2}$ manifold may introduce an error in the quantitative estimates within a factor of two. [73]

Fitting of the data for pseudotetrahedral complexes shows that they have τ_s of 10^{-11} s, whereas five-coordinate complexes have a shorter τ_s, on the order of 10^{-12} s. The latter derivatives also have exchangeable protons that could correspond to a water molecule in the coordination sphere, whereas the former do not.[25] The τ_s values are thus proposed as indicators of the coordination number in low-symmetry, four- and five-coordinate cobalt complexes. The shorter electronic relaxation times are related to low-lying excited states, which, independently of the particular mechanism, favor electron relaxation.[74]

Short electronic relaxation times in paramagnetic compounds cause only minor broadening of ^{1}H NMR lines, whereas the isotropic shifts (i.e., the shifts due to the presence of unpaired electron(s), usually very large) are independent of the value of the electronic relaxation times. For cobalt-substituted carbonic anhydrase, the ^{1}H NMR spectra have been recorded for several derivatives, and the proton signals of histidines coordinated to the metal were found to be shifted well outside the diamagnetic region (Figure 2.14).[75] Five-coordinate species give sharper signals than four-coordinate ones. The spectra in D_2O for both kinds of derivatives show three fewer isotropically shifted signals than in H_2O. These signals are assigned to histidine NH protons, which are replaced by deuterons in D_2O. Five-coordinate species provide ^{1}H NMR spectra with many signals slightly shifted from the diamagnetic position. It is believed that such complexes have relatively large magnetic anisotropy, which, summed up to the external magnetic field, provides further differentiation in shifts of the protons. Such shift contributions are called pseudocontact shifts. These shifts depend on the third power of the distance from the metal and on the position of the proton with respect to the molecular axes. These signals belong to protons of noncoordinated residues from 5 to 10 Å from the metal. Their assignment in principle provides further information on the structure in the vicinity of the metal ion. The ^{1}H NMR spectra of cobalt(II) enzymes thus afford a powerful method for monitoring structure and reactivity of the metal-bound residues. This is one task for future investigations of the enzyme.

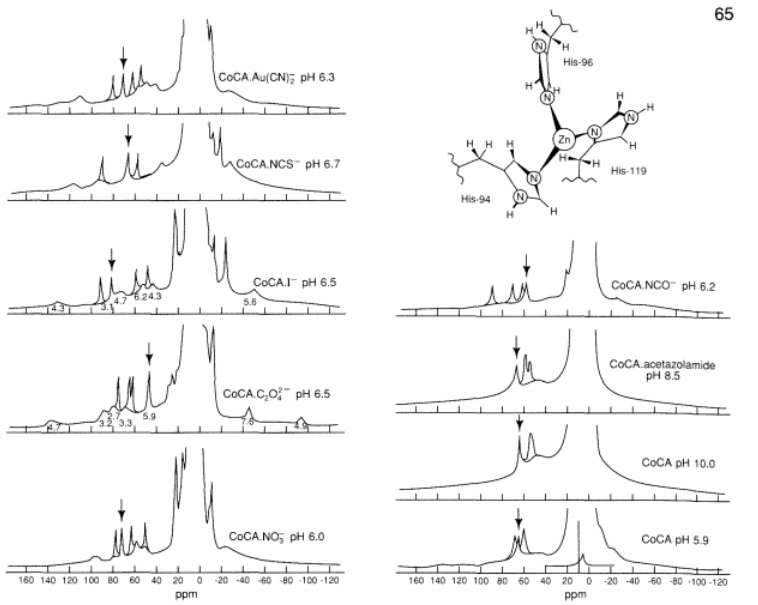

Figure 2.14 - ^{1}H NMR spectra of cobalt(II)-substituted bovine carbonic anhydrase II and some inhibitor derivatives. The three sharp downfield signals in each spectrum disappear in D_2O and are assigned to the exchangeable ring NH protons of the three coordinated histidines. The sharp signal labeled with an arrow is assigned to the Hδ2 proton of His-119, which is the only non-exchangeable ring proton in a meta-like rather than in an ortho-like position with respect to the coordinating nitrogen. The T_1 values (ms) of the signals for the I^- and $C_2O_4^{2-}$ derivatives are also shown.[25,75]