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Proton Nuclear Magnetic Resonance Relaxation Study of the Manganese(II)–L-Histidine complex

Analysis of pH Dependence and Correlation Times

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Longitudinal and transverse relaxation rates of ligand nuclei have been measured and their pH dependence has been investigated in terms of the dynamics of the Mn^{II}–L-histidine equilibrium.

A careful analysis of the relative weights of dipolar and scalar contributions to the observed relaxation rates has been performed and the results related to changes in the correlation times.

Details of the electronic and molecular structure of paramagnetic metal complexes as well as their dynamic properties can be investigated by the analysis of nuclear relaxation rates.[1-3]

Much information on the metal–protein interaction has been obtained by studying metal binding to various protein constituents, among which histidine is known to play a major role. The metal–imidazole coordination has been identified in the structure of the active site in several enzymes, in biological redox processes and in the stabilization of macromolecular complexes.[4, 5]

This histidine–metal interaction has been thoroughly investigated[6-9] and the structures of different complexes in solution have been sketched.[10, 11] In this connection, the analysis of nuclear magnetic resonance parameters in the presence of a paramagnetic metal ion provides the most suitable approach,[2, 12] especially when Mn^{II} is used as paramagnetic relaxation probe.[13]

Since the use of Mn^{II} has been shown to be particularly useful in gaining information on the dynamics of solution equilibria in both binary and mixed ligand complexes,[14-17] we report here an investigation of the relaxation rates of both ligand and solvent nuclei in Mn^{II}–L-histidine complexes in the pH range 3-10. The proper use of the Solomon–Bloembergen theory allows the determination of the dominant relaxation mechanism provided an evaluation of the relevant correlation times is carried out.[18-21]

EXPERIMENTAL

N.m.r. spectra were obtained using a Bruker WH-90 spectrometer operating at 90 MHz and equipped with a Nicolet BNC-12 computer. Temperature was held constant by using a Bruker B-ST 100/700 temperature control unit (accuracy ± 1 K). The pH was adjusted with NaOD and DCl (Merck), and measured before and after every n.m.r. measurement.

Longitudinal relaxation times were calculated from partially relaxed spectra using the $(180\text{-}\tau\text{-}90\text{-}t)_n$ pulse sequence. Transverse relaxation times were measured from half-height linewidths of the Fourier-transform spectra, assuming a pure Lorentzian lineshape. T_1 and T_2 values were averaged over at least three measurements (accuracy $\pm 5\%$ for T_1 and $\pm 3\%$ for T_2). The paramagnetic contributions to the relaxation rates were calculated from:

$$T_{ip}^{-1} = T_{ip}^{-1}(\text{exp}) - T_i^{-1}(\text{blank}) \quad (i = 1,2).$$

L-histidine and D_2O (99.75%) were Merck chemicals. The source of manganese(II) was $Mn(ClO_4)_2 \cdot 6H_2O$ (Alpha Inorganic, analytical grade).

RESULTS AND DISCUSSION

For the Mn^{II}–L-histidine system in water the following complexes must be considered: MH_2L, MHL, ML and ML_2. The relative structures[2, 11] are reported in fig. 1. The molar concentration of each species is calculated from the acidity and stability constants[22] and is reported in fig. 2 as a function of pH. The pH dependence of the paramagnetic relaxation rates T_{ip}^{-1} of the H(2) and H(4) protons of histidine is shown in fig. 3.

The experimental results may be interpreted in terms of relative weights of the dipolar and scalar contributions to the observed relaxation rate; *i.e.* the interplay of different Mn^{II}–L-histidine complexes in solution determines a change in the correlation time modulating the magnetic interactions. From an analysis of fig. 3 it is apparent that at low pH $T_{1p}^{-1} \ll T_{2p}^{-1}$, while at high pH $T_{1p}^{-1} \simeq T_{2p}^{-1}$. Therefore, in acid solution the dipolar mechanism determines the longitudinal relaxation rate, while the scalar interaction dominates the transverse relaxation rate. Furthermore, the low values of T_{1p}^{-1} are consistent with a weak magnetic interaction between the metal ion and the proton nuclei, as seen from the predominance of MH_2L and MHL at these pH values (see fig. 2). The MH_2L and MHL complexes, because of the persistence of some symmetry elements in the Mn^{II} environment, are characterized by relatively low zero-field splitting values and therefore by relatively long electron spin relaxation times, τ_s, which are the dominant correlation times for the scalar interaction. In this case ($\omega_s^2\tau_s^2 \gg 1$) the paramagnetic contribution to the relaxation rates is given by the simplified Solomon–Bloembergen equations:[13]

$$T_{1p}^{-1} = K\sum_i f_i \frac{\tau_{ci}}{r_i^6}$$

$$T_{2p}^{-1} = K\sum_i \left(f_i \frac{\tau_{ci}}{r_i^6} + K_i' A_i^2 \tau_{ei} \right)$$

$$\tau_c^{-1} = \tau_s^{-1} + \tau_M^{-1}$$

where the summation Σ_i takes the different complexes into account. K is a constant for the dipolar term, K' is a constant for the scalar term, f_i is the molar fraction of metal complex, τ_c is the dipolar correlation time, τ_e is the scalar correlation time, r is the metal–ligand distance, A is the coupling constant, τ_M is the chemical exchange correlation time, and ω_s is the electronic Larmor precession frequency.

In the pH range 7-10 the paramagnetic contributions to the relaxation rates show a different trend; in fact $T_{1p}^{-1} \simeq T_{2p}^{-1}$ for both H(2) and H(4). At these pH values the equilibrium is shifted towards the formation of ML. The presence of a new ligand group in the first solvation sphere induces a further distortion of the cubic symmetry and a larger zero-field splitting occurs. As a consequence, τ_s is relatively shorter and the condition $\omega_s^2\tau_s^2 > 1$ no longer holds. When this takes place a larger scalar contribution to both the longitudinal and transverse relaxation rates occurs, so that T_{1p}^{-1} and T_{2p}^{-1} have similar values. The following equations must now to be considered:

$$T_{1p}^{-1} = K\sum_i \left(f_i \frac{\tau_{ci}}{r_i^6} + K_i' A_i^2 \tau_{ei} \right)$$

$$T_{2p}^{-1} = K\sum_i f_i \frac{\tau_{ci}}{r_i^6} + K'\sum_i A_i^2 \tau_{e_i}.$$

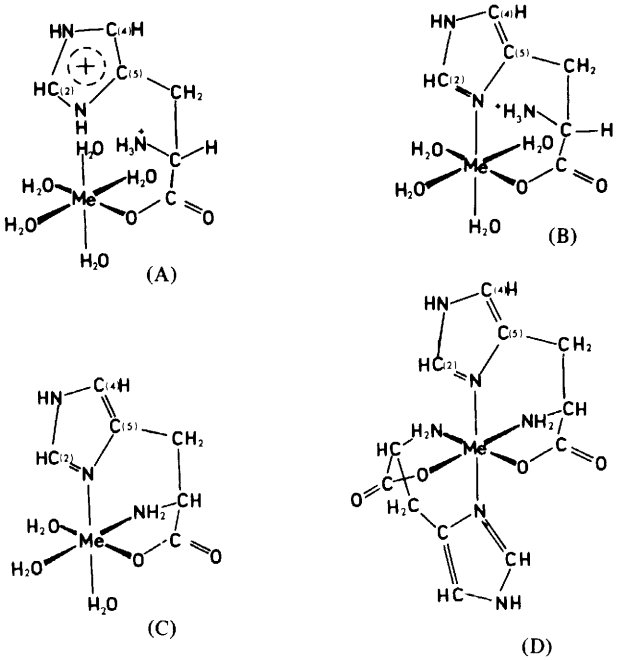

FIG. 1.—Schematic structures for Mn^{II}–L-histidine complexes. (A) MH_2L, (B) MHL, (C) ML, (D) ML_2.

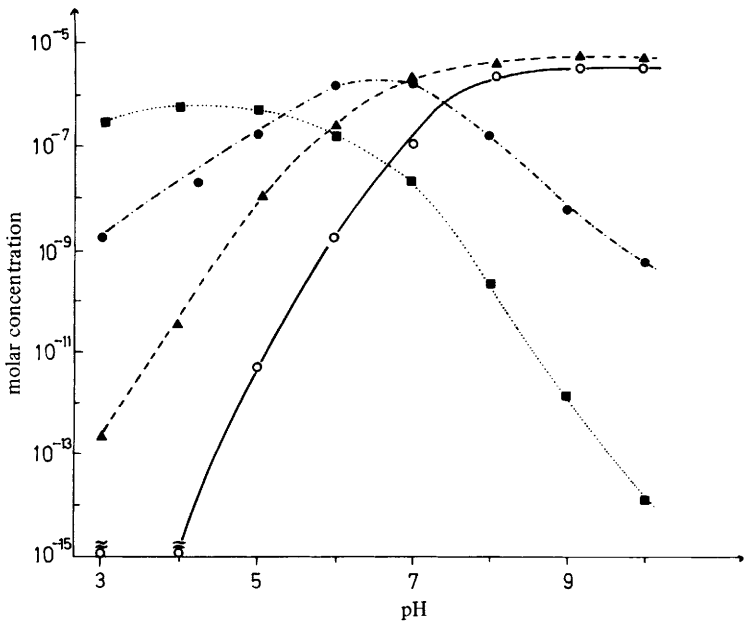

FIG. 2.—Molar concentrations of Mn^{II}–L-histidine complexes plotted against pH at 298 K. $[Mn^{2+}] = 10^{-5}$ mol dm^{-3}, [histidine] $= 5 \times 10^{-2}$ mol dm^{-3}. ■, $Mn(H_2L)$; ●, Mn(HL); ▲, MnL; ○, ML_2.

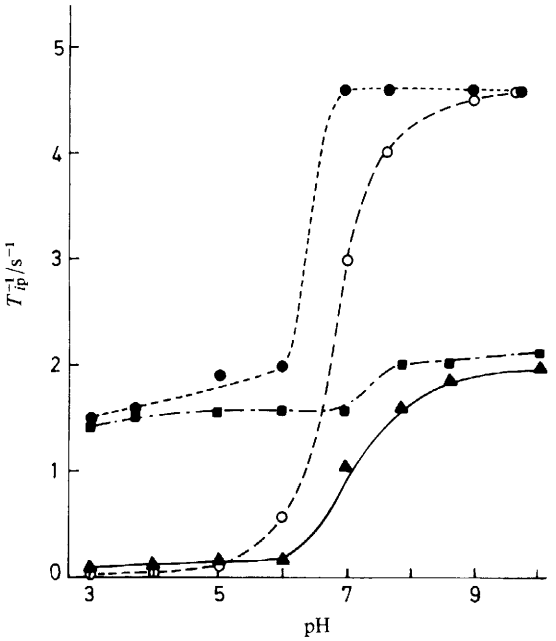

FIG. 3.—Paramagnetic contribution to relaxation rates for H (2) and H (4) protons in Mn^{II}–L-histidine complex plotted against pH at 298 K. $[Mn^{2+}] = 10^{-5}$ mol dm^{-3}, [histidine] $= 5 \times 10^{-2}$ mol dm^{-3}. ○, T_{1p}^{-1} [H (2); ●, T_{2p}^{-1} [H (2)]; ■, T_{1p}^{-1} [H (4)]; ▲, T_{2p}^{-1} [H (4)].

TABLE 1.—WATER-PROTON PARAMAGNETIC CONTRIBUTIONS TO THE LONGITUDINAL RELAXATION RATES IN Mn^{II}–L-HISTIDINE COMPLEX AS A FUNCTION OF pH AT 298 K $[Mn^{2+}] = 10^{-5}$ mol dm^{-3}, [histidine] $= 5 \times 10^{-5}$ mol dm^{-3}

pH	T_{1p}/s^{-1}
3	0.3
4	0.2
5	0.2
6	0.1
7	0.07
8	0.03
9	0.02
10	0.01

Table 1 shows the pH-dependent paramagnetic contributions to the water-proton relaxation rates in the Mn^{II}–L-histidine system. A continuous decrease of the water T_{1p}^{-1} with increasing pH is apparent which can be explained by a progressive increase in inner complexation by the histidine ligand groups. This is accompanied by a displacement of water molecules from the first solvation sphere, resulting in a reduction of the bound-water mole fraction, f, and, as a consequence, in a reduction of the solvent T_{1p}^{-1}.

A careful analysis of fig. 2 provides an understanding of the dynamics of the

Mn^{II}–L-histidine equilibrium. Whenever MH_2L, MHL and outer-sphere complexes represent the dominant species in solution, the symmetry of the metal environment is not sufficiently affected to cause a drastic change in the T_{1p}^{-1} value for the ligand nuclei. At higher pH values where ML and ML_2 became the major species, a scalar term is contributing to both T_{1p}^{-1} and T_{2p}^{-1} to the same extent. In the intermediate pH zone ($\simeq 7$), the metal–amino-acid side-chain binding is particularly labile, so that no complex behaves as a dominant species. These findings suggest that many reversible biochemical interactions may be interpreted in terms of the equilibria in solution.

The strong pH dependence makes the presence of outer-sphere complexes unlikely at high pH values (7-10), in agreement with previous results.[23]

The analysis of correlation times seems to be a straightforward method of understand the dynamic equilibria of different metal complexes simultaneously present in solution.

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