Inorganica Chimica Acta, *92* (1984) 47–50

Competition Equilibria of Metal Ions with Nucleobases: an ESR Study

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Received September 13, 1983

An Electron Paramagnetic Relaxation study of the competitive equilibria of Cu(II) and Mn(II) towards imidazole and methyl-imidazole was carried out. ΔH, a, I and lineshape variations were studied in order to define the extent and the limits of the metal ions interaction with nucleobases. The EPR evidence of a major affinity of Cu(II) with respect to Mn(II) in binding to nucleobases was demonstrated.

Introduction

Organic nucleobases such as substituted purines and pyrimidines constitute important units for the activity of biomolecules such as proteins, enzymes and nucleic acids [1].

The activity of these biomolecules is often strictly related to the presence of metal ions, divalent metal ions particularly play a fundamental role in many biochemical reactions [1–3]. The specificity of the metal–ligand interaction and the competition of different metal ions in binding to bioligands are important in defining the extent and the limits of complexation equilibria [3–5].

The Electron Paramagnetic Resonance analysis yields interesting information on these complex systems whenever paramagnetic metal ions such as Cu(II) and Mn(II) are involved. The different behaviour of the EPR parameters shown by Cu(II) and Mn(II) complexes clarifies the structure of the metal–ligand complexes and shows the different affinity of the metal ions towards the ligands used [6, 7]. The aim of this paper is to contribute to the interpretation of the dynamic and the structural features of the metal ions–nucleobase interactions in aqueous solutions.

Experimental

Solutions of copper(II) and manganous(II)-imidazole and methyl-imidazole complexes were prepared by dissolving the appropriate amounts of $Cu(ClO_4)_2 \cdot 6H_2O$ (Aldrich) and $Mn(ClO_4)_2 \cdot 6H_2O$ (Alpha Inorganics) solid salts in aqueous solutions of imidazole and methyl-imidazole. Imidazole and methyl-imidazole were from Merk. The pH values were obtained by adding $HClO_4$ to the solutions and measuring with a metrohm model E-388 potentiometer.

EPR spectra were recorded with a Bruker ER 200tt spectrometer operating at the X-Band. Temperatures were controlled with the Bruker B-ST 100/70 unit (accuracy ±1 K). DPPH was used as field marker.

Results and Discussion

Cu(II)–Nucleobase Systems

The Cu(II)–nucleobase interaction is analyzed in terms of the X-band EPR hyperfine structure of the cupric spectra.

Due to the interaction of $S_{Cu(II)} = 1/2$ with $I_{Cu(II)} = 3/2$, four lines of equal intensity are observed in the presence of imidazole and methyl-imidazole ligands. The Hamiltonian of this interaction is [8]:

$$\mathcal{H}_{Cu(II)} = -\beta_o[g_\parallel H_z S_z + g_\perp(H_x S_x + H_y S_y)] + A_\parallel I_z S_z + A(I_x S_x + I_y S_y) \qquad (1)$$

For Cu(II)–imidazole molar ratios = 1:120 (pH = 9.4) we observe the best hyperfine resolution and highest EPR intensity (Fig. 1a). Further ligand addition causes a slight intensity decrease. It has to be underlined that for Cu(II)–imidazole = 1:30 also the hyperfine structure is well resolved. These findings suggest a very strong metal–nucleobase interaction as well as a quantitative right-shift of the equilibrium (2) at very low metal–ligand molar ratios:

$$Cu(II)(H_2O)_6^{++} + 4imid \xrightleftharpoons{K_4^{Cu(II)-imid}} Cu(II)(imid)_4(H_2O)_2^{++} + 4H_2O \qquad (2)$$

The Cu(II)–methyl-imidazole system exibits a similar trend. At lower pH's, the lineshape analysis reveals an interesting feature: the spectrum of the solution at molar ratios 1:10 (pH = 6.8) shows a high-field superhyperfine splitting of nine lines, consistent with the interaction of the cupric ion with four equivalent N-atoms: $4x(2I_N) + 1$ lines (Fig. 1b).

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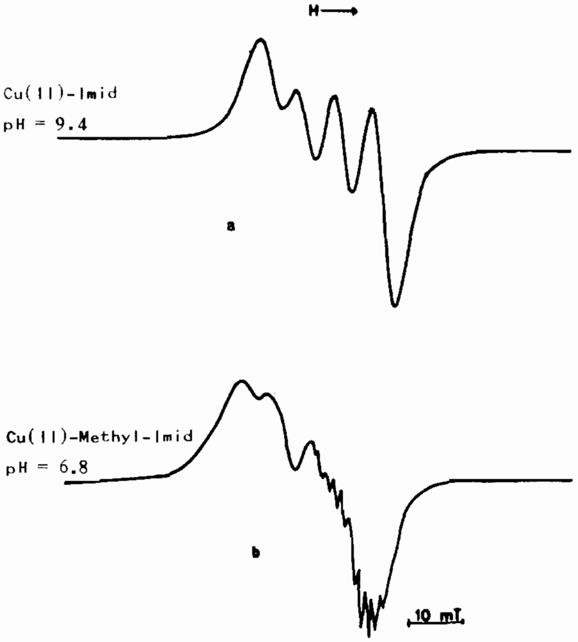

Fig. 1. X-band ($\omega = 5.8 \times 10^{10}$ rad/s) EPR spectra of: (a) Cu(II)-imid = 1:120 system. $[Cu^{++}] = 10^{-3}$ mol dm^{-3}. $a_{Cu(II)-imid}$ = 7.26 mT, g = 2.131. (b) Cu(II)–methyl-imid = 1:10 system. $[Cu^{++}] = 10^{-3}$ mol dm^{-3}. Super hyperfine splitting value Cu(II)–methyl-imid = 1.52 mT. Temperature = 298 K.

Both the Cu(II)–imidazole and Cu(II)–methyl-imidazole systems show in the same experimental conditions the same g value. In Fig. 2 a tentative structure of the Cu(II)–methyl-imidazole complex is shown.

Mn(II)–Nucleobase Systems

The presence of different molecular species in a manganous solution can be detected by analysing the EPR parameters such as a, ΔH, I, lineshape. The total spin Hamiltonian for high spin Mn(II) complexes in solution is [9, 10]:

$$\mathcal{H}_{Mn(II)} = g\beta HS + aIS + D[S_z^2 - \frac{1}{3}S(S+1)] + 2E(S_x^2 - S_y^2) \qquad (3)$$

aIS is the hyperfine energy term and a is the coupling constant, strictly related to the ligand electronegativity. The third and fourth terms in (3) refer to the Zero Field Splitting Energy. The dynamic ZFS modulation is the fundamental relaxation mechanism for the manganous ion. The variation of a, ΔH and I (spectrum intensity) indicate consistent variations in the molecular structure and dynamics of the manganous ion environment [9].

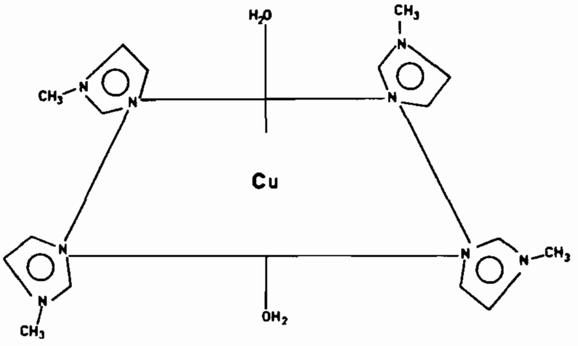

Fig. 2. Tentative and simplified structure of Cu(II)–methyl-imidazole complex.

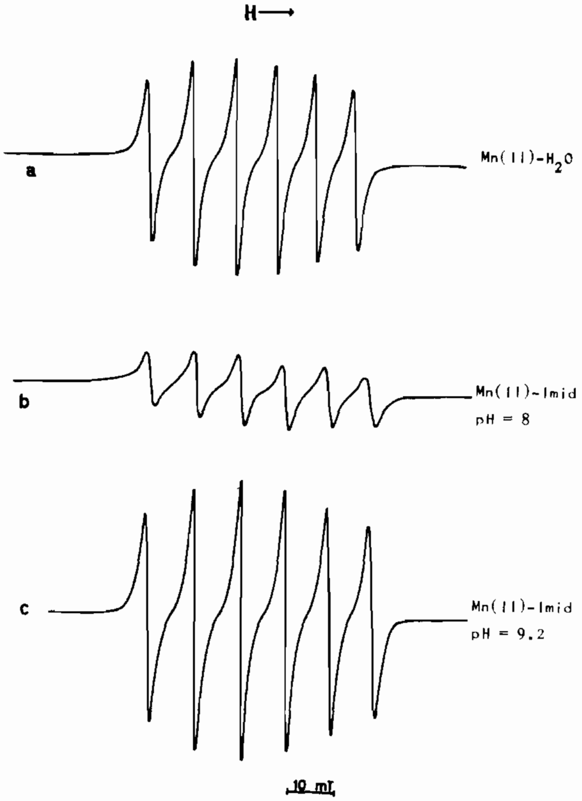

Fig. 3. X-band ($\omega = 5.8 \times 10^{10}$ rad/s) EPR spectra of: (a) Mn(II)–H_2O system. $[Mn^{++}] = 10^{-3}$ mol dm^{-3}. (b) Mn(II)–imid = 1:900 system. $[Mn^{++}] = 10^{-3}$ mol dm^{-3}. (c) Mn(II)–imid = 1:5000 system. $[Mn^{++}] = 10^{-3}$ mol dm^{-3}. Temperature = 298 K.

Figure 3a shows the Mn(II)–H_2O spectrum characterized by $a_{Mn(II)-H_2O} = 9.8$ mT and ΔH = 1.50 mT (at room temperature). For molar ratios Mn(II)–imid = 1:900, $a_{Mn(II)-imid} = 9.60$ mT and $\Delta H_{Mn(II)-imid} = 2.10$ mT (Fig. 3b) and for molar ratios 1:5000 $a_{Mn(II)-imid} = 8.92$ mT and $\Delta H_{Mn(II)-imid} = 1.40$ mT (Fig. 3c).

The methyl-imidazole system shows a similar trend: for molar ratios 1:1000, $a_{Mn(II)-Me\text{-}imid}$ = 9.50 mT at pH = 8 and for molar ratios 1:6200,

$a_{Mn(II)-Me\text{-}imid} = 8.90$ mT at pH = 9.8. The variation of a and ΔH at increasing ligand concentrations is interpreted by a change in the first coordination sphere of the metal ion [9].

At highest ligand concentrations, the a and ΔH values suggest a tetrahedral arrangement [11, 12] of the ligands around the metal ion, *i.e.* the equilibrium (4) is totally right-shifted:

$$Mn(II)(H_2O)_6^{++} + 4N\text{-base} \underset{}{\overset{K_4^{Mn(II)-N\text{-base}}}{\rightleftharpoons}} Mn(II)(N\text{-base})_4^{++} + 6H_2O \qquad (4)$$

From the EPR analysis of the Cu(II)–nucleobase and Mn(II)–nucleobase systems, it is apparent that under the same experimental conditions, the manganous ion has a much lower affinity toward nucleobases than Cu(II) species.

Cu(II)–Mn(II)–Nucleobase Systems

When dilute solutions of Cu(II) and Mn(II) are mixed together (Me(II) < 10^{-2} mol dm^{-3}), the exchange interactions are so weak that the formation of mixed metal-dimers is ruled out [7]. In these conditions it is a good approximation to consider that the EPR spectra of Cu(II)–Mn(II)–L systems are derived by the simple superimposition of Cu(II)–L and Mn(II)–L absorptions. Figure 4a shows the Cu(II)–Mn(II)–H_2O spectrum at molar ratios Cu(II): Mn(II) = 15:1. This molar ratios value was chosen in order to minimize the effects of the Mn(II) high sensitivity. Figure 4b shows the Cu(II):Mn(II):imid = 15:1:1000 spectrum. The lineshape analysis reveals that the noticeable contribution of the Cu(II)–imidazole hyperfine structure considerably affects the manganous lines. Here, the $a_{Mn(II)-imid}$ value is characteristic of the manganous-exaquo ion system and the intensity is 15% of the spectrum 4a. These findings suggest that the cupric ions are totally involved in the binding with imidazole ligands while manganous ions experience a mixed coordination:

$$Cu(II)_{aq} + Mn(II)_{aq} + imid \rightleftharpoons Cu(II)\text{–}imid + Mn(II)\text{–}H_2O\text{–}imid + H_2O \qquad (5)$$

Figure 4c shows the Cu(II)–Mn(II)–imid = 15:1:5000 spectrum. The a value is characteristic of the Mn(II)–imidazole binary system ($a_{Mn(II)-imid}$

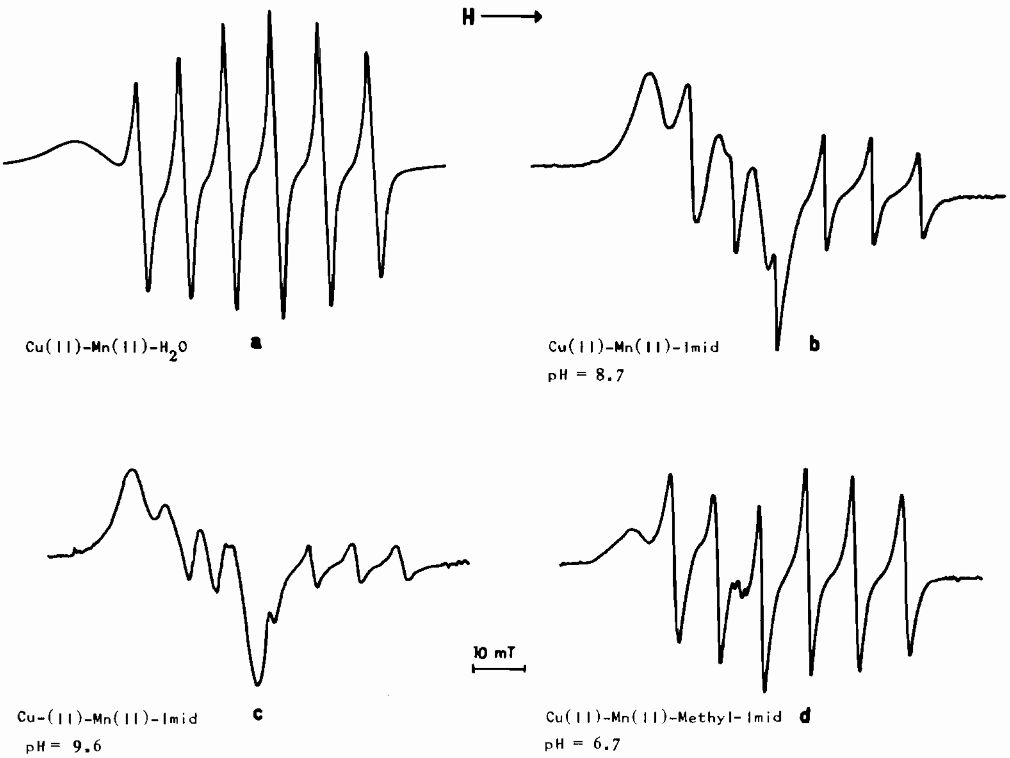

Fig. 4 X-band (ω = 5.8×10^{10} rad/s) EPR spectra of: (a) Cu(II)–Mn(II)–H_2O = 15:1 system. [Mn^{++}] = 6×10^{-4} mol dm^{-3}. (b) Cu(II)–Mn(II)–imid = 15:1:1000 system. [Mn^{++}] = 6×10^{-4} mol dm^{-3}. (c) Cu(II)–Mn(II)–imid = 15:1:5000 system. [Mn^{++}] = 6×10^{-4} mol dm^{-3}. (d) Cu(II)–Mn(II)–methyl-imid = 15:1:150 system. [Mn^{++}] = 6×10^{-4} mol dm^{-3}. Temperature = 298 K.

= 8.94 mT) and the intensity is 60% of the intensity of the spectrum shown in Fig. 4a. These experimental data confirm that the equilibrium (5) is quantitatively right-shifted and a further ligand addition does not alter the overall lineshape.

The presence of methyl-imidazole in the competitive Cu(II)–Mn(II)–L equilibrium shows, at molar ratios Cu(II):Mn(II):Me-imid = 15:1:150 (pH = 6.7), the Cu(II)–methyl-imidazole superhyperfine structure (Fig. 4d) according to the stronger Cu(II)–N interaction. The above analysis shows the competitive binding of Cu(II) and Mn(II) towards the imidazole and methyl-imidazole ligands and it is shown that Cu(II) has a stronger affinity than Mn(II) in the interaction with these nucleobases. On the basis of the above results a further application can be suggested, that Cu(II) and Mn(II) ions can be used as NMR paramagnetic relaxation probes acting with their own characteristic relaxation mechanisms in the ^{1}H and ^{13}C NMR studies of metal–ligand complexation.

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