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Effectiveness and Limits of Magnetic Resonance Techniques in the Identification of Different Coordination Pathways of the Mn(II) in the Presence of Biomacromolecules

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SUMMARY

Magnetic resonance techniques yield important information on different coordination behaviour of Mn(II) in the presence of biomacromolecules. A preliminary study on model systems is necessary to have a deeper insight on dynamics and structural aspects.

The EPR technique is not able to identify a binding interaction between Trp and manganous ion when the metal is bonded to the Imidazole. Proton shift NMR studies on the Mn(II)-5'ATP-Imid complex evidenziate a ternary system formation and a stacking interaction between the imidazole-ring and the nucleotide purine-moiety.

An EPR and NMR combined analysis on the Mn(II)-5'ATP-Trp system points out a stacking interaction and the simultaneous presence of a covalent binding between the metal ion and the biomolecules. The role of the metal ion to favour this coordination is suggested.

INTRODUCTION

In the last years, the detection of mixed-ligand metal ion compplexes assumed particular relevance since the ternary coordination plays a predominant role in many biological reactions ("Metal ions in biological systems", 1973-79, B.J.Bulkin et al.,1973, M.Dixon et al., 1970). In the nucleic acid-proteins interactions (C.Helene,1976, M. Durand et al., 1975) various model systems have been proposed in order to allow inferences on the "in vivo" processes.
The metal ion bridged adducts between two different ligands which non-covalently interact through their aromatic-moieties have been demonstrated by means of different techniques (C.F.Naumann et al.,1974, H.Siegel et al., 1976a, I.Muro et al., 1971, R.Basosi et al.,1979a, F.Laschi et al., 1979a). The use of the magnetic resonance spectroscopies lead to an interesting check on the characterization of the structure and the stability of binary and ternary systems in solution.

The combined analysis of EPR and NMR data is particularly useful whenever a paramagnetic metal ion as Mn(II) is involved (H.Siegel 1980b, F.Laschi et al. 1978b). In fact the manganous ion is a suitable paramagnetic relaxation probe and it is involved in some enzymatic processes (R.A.Dwek,1975, Y.F.Lam et al., 1974). The aim of this paper is to clarify the effectiveness and the limits of the ma-

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gnetic resonance techinques in the study of some ternary systems.

EXPERIMENTAL

Imidazole, Tryptophan and 5'Adenosine triphosphate disodium salt were Merck reagent grade. $Mn(ClO_4)_2.6H_2O$ (Alpha Inorganic) has been used in order to minimize ionic complexation. EPR spectra were recorded with a Bruker ER 200tt spectrometer operating at X-band; temperature was controlled by means the Bruker B-ST 100/70 unit, with accurancy of $\pm$ 1 °K.

The pH values in the EPR solution was obtained by adding KOH or $HClO_4$ to the solution and checked by means of a metrohm model E-388 potentiometer.

NMR spectra were obtained by using a Bruker WH-90 spectrometer operating at 90 MHz and 22.63 MHz for ^1H and ^{13}C respectively. Transverse relaxation times were measured from half-height linewidth assuming lorentzian lineshape. Longitudinal relaxation times were calculated from partially relaxed spectra by using the pulse sequence $(180°- \tau - 90° - t)_n$.

The NMR solution were prepared by dissolving weighted ammounts of the various ligands in 99.75% D_2O (Merck). pD values were measured by means of a metrohm model E-388 potentiometer after adding of suitable ammount of KOD or DCl (Merck).

RESULTS AND DISCUSSION

Fig.1 shows the X-band EPR intensity versus molar ratios for the Mn(II)-Imid and Mn(II)-Imid-Trp systems. The two systems evidentiate the same trend, in this conditions the EPR techniques is not able to distinguish between binary and ternary coordination.

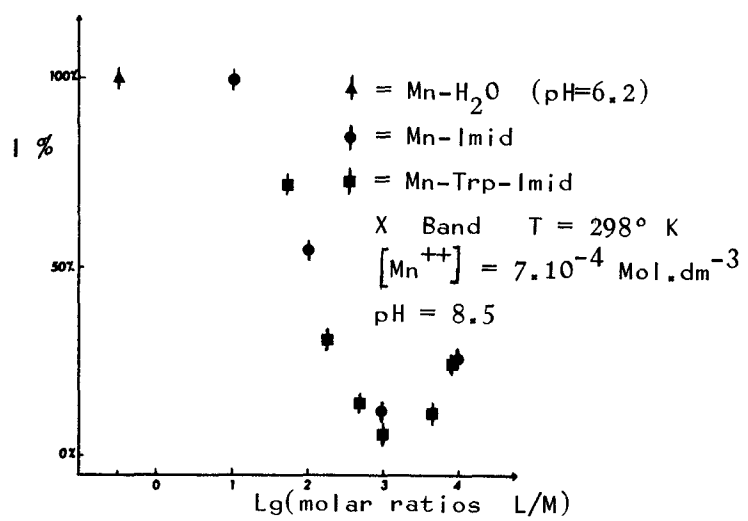

Fig. 1: X-band EPR intensity vs.molar ratios

In fact the Mn(II)-Trp and Mn(II)-Imid-Trp EPR spectra are undetectable due to the asimmetry in the molecular structure (F.Laschi et al. 1979a). Furthermore the Mn(II)-Imid EPR spectrum evidenziate intensity variations only at highest Trp molar ratios. These findings can be confirmed by studying hyperfine coupling constants at different molar ratios (fig.2). The Mn(II)-Imid binary and the Mn(II)--Imid-Trp ternary systems evidenziate again the same trend.

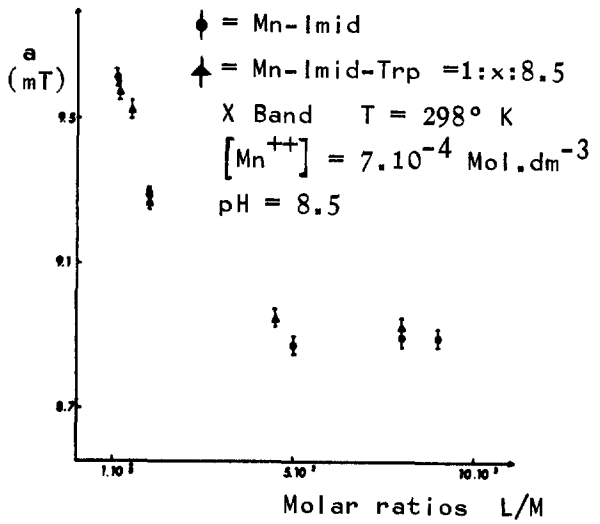

Fig.2: hyperfine coupling constants vs. molar ratios, for Mn(II)--Imid and Mn(II)-Imid-Trp systems.

Therefore it is possible to propose a dynamic model where the presence of Trp does not substantially alter the chemical equilibrium between Mn(II)-H_2O and Mn(II)-Imid systems:

$$Mn(II)\text{-}H_2O + Imid \underset{}{\overset{K^{Mn(II)\text{-}H_2O}_{Mn(II)\text{-}Imid}}{\rightleftarrows}} Mn(II)\text{-}Imid + H_2O \qquad (1)$$

Fig.3 shows (a) the ^{1}H-NMR spectra of the 5'ATP-Imid system at various molar ratios and (b) the same system in the presence of manganous ion. In the part (a), the H_2 - H_8 (5'ATP) and H_2 (Imid) peaks are shifted respect to the $H_{4,5}$ (Imid), when the 5'ATP concentration is increased. This effect is due to the "non-covalent" interaction between the aromatic moieties of the biomolecules in the diamagnetic system (J.L.Dimicoli et al. 1974, F.Brun et al. 1975).

The addition of small amounts of metal ion noticeably increase these shift effects.

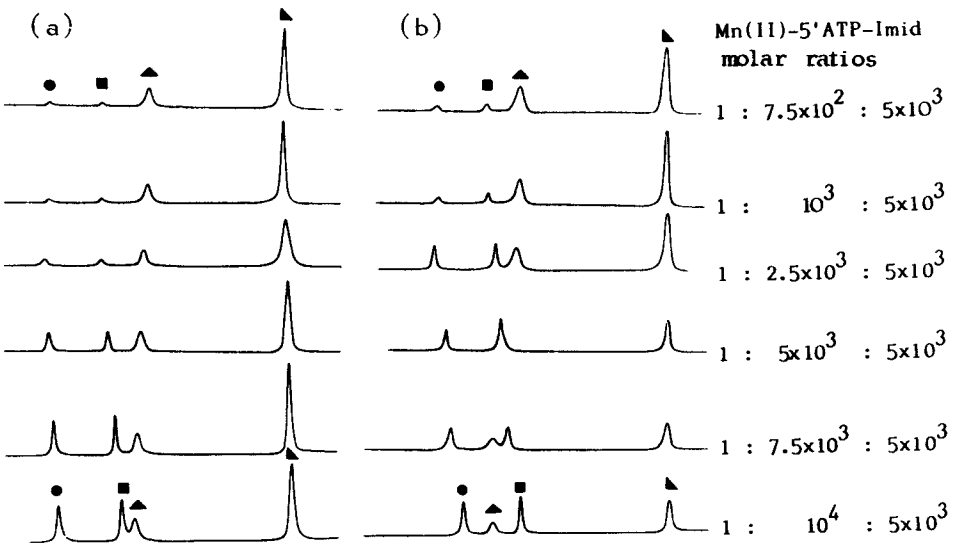

Fig.3: proton NMR spectra. (a) 5'ATP-Imid, (b) Mn(II)-5'ATP-Imid. T = 298 °K; pH = 7. (●),(■) H_8 and H_2 protons of 5'ATP. (▲),(◣) H_2 and $H_{4,5}$ protons of Imid. $[Mn^{++}] = 10^{-5}$.

These findings underline the role of the metal ion in the stacking interaction. In fact the Mn^{++} strongly binds to the 5'ATP phosphate groups and, neutralizing the ionic charges, favours non-covalent stacking interactions.

Figure 4 shows the EPR lineshape of Mn(II)-Trp (a), Mn(II)-5'ATP (b), and Mn(II)-Trp-5'ATP (c) systems at 1:8.5; 1:1; 1:8.5:1 molar ratios. EPR lineshape of spectrum (a) of Fig.4 is characteristic for the Mn(II)-H_2O system, since the Mn(II)-Trp complex is undetectable. The presence of Mn(II)-Trp complex can be pointed out by the intensity decrease, observed at higher molar ratios and pH's. Spectrum (b) of Fig.4, strongly evidenziate the metal ion-nucleotide interaction: the lineshape substantially differs from that of Mn(II)-H_2O species, at any metal-ligand molar ratios. The intensity decrease confirms the quantitative formation of the binary complex (R.Basosi et al. 1975b). Spectrum (c) of Fig.4 shows an additional intensity decrease and an hyperfine structure loss due to the formation of molecular species characterized by larger size and structural asymmetry as the Mn(II)--Trp-5'ATP ternary complex.

As shown in Fig.5 the ΔH_{exp} of Mn(II)-Trp slightly increases at higher molar ratios, while the ΔH_{exp}'s of Mn(II)-5'ATP binary and Mn(II)-Trp-5'ATP ternary systems increase even for small metal--ligand ratios. This is due to the presence of molecular species with structural parameters different from the highly symmetric Mn(II)-H_2O system.

In the ternary system Mn(II)-Trp-5'ATP, the interaction between

the nucleotide and the metal-amino acid complex is particularly favoured as suggested by the drastic ΔH_{exp} change.

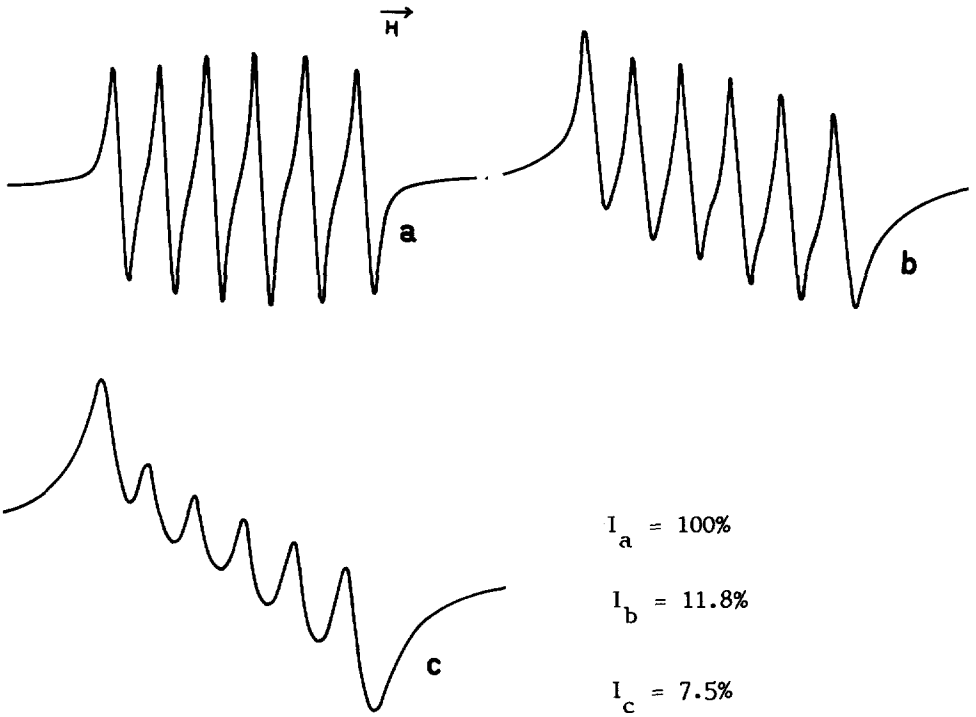

Fig.4: EPR spectra of Mn(II)-Trp, Mn(II)-5'ATP, Mn(II)-Trp-5'ATP systems at 1:8.5 (a); 1:1 (b); and 1:8.5:1 (c) molar ratios. T = 298°K, pH = 7. $[Mn^{++}] = 10^{-3}$.

This fact indicates that the chemical equilibrium:

$$\text{Mn(II)-Trp} + \text{5'-ATP} \underset{}{\overset{K_{\text{Mn(II)-Trp-5'ATP}}}{\rightleftarrows}} \text{Mn(II)-Trp-5'ATP} \quad (2)$$

is strongly shifted towards the formation of the Mn(II)-Trp-5'ATP, while at higher 5'ATP concentrations Mn(II)-$(5'ATP)_x$ complexes with $x > 1$ are the major species in solution.

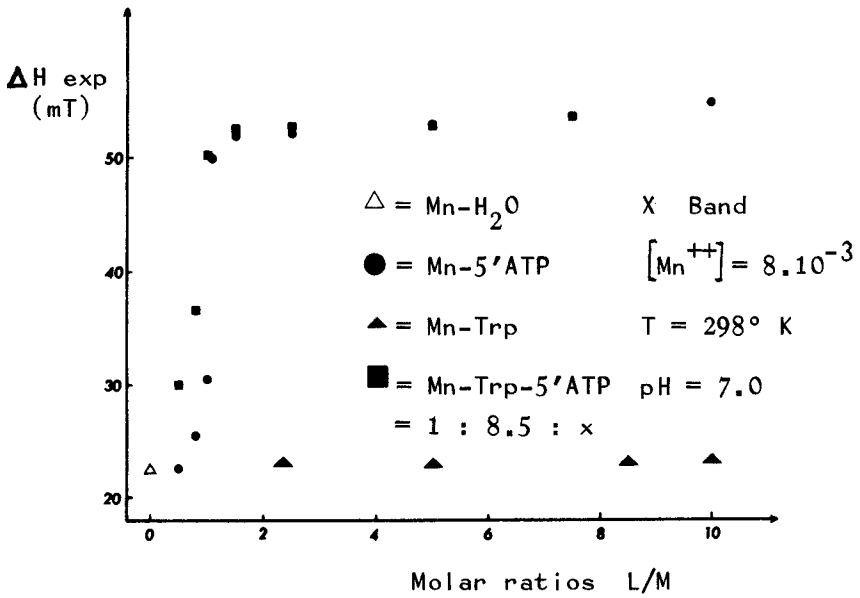

Fig. 5: EPR ΔH_{exp} vs. molar ratios.

Table 1 shows ^{13}C T_{1p}/T_{2p} ratios for the 5'ATP in the binary and Mn(II)-5'ATP-Trp ternary systems. These ratios calculated for binary and ternary complexes are completely different. These data suggest a new spatial rearrangement when the third ligands as Trp, is added to the binary complex.

TABLE 1

^{13}C 5'ATP T_{1p}/T_{2p} ratios, in Mn(II)-5'ATP and Mn(II)-5'ATP-Trp systems.

	Mn(II)-5'ATP(°)	Mn(II)-5'ATP-Trp
C(2)	-	1.0
C(4)	18.1	9.0
C(5)	11.9	5.4
C(6)	3.7	6.5
C(8)	3.7	4.5

$[Mn^{++}] = 5x10^{-5}$; [5'ATP] = $5x10^{-1}$; [Trp] = $5x10^{-2}$; pH = 7; T=300°K

(°) from Y.F.Lam et al. 1974.

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