Copper(II)-Histidine-Containing Dipeptide Systems: An EPR Study

JACK HUET

Laboratoire de Chimie de Coordination Bioorganique, C.N.R.S. Université de Paris XI, 91405 Orsay, France

and ERNA VILKAS*

Laboratoire de Chimie Organique Biologique, Université de Paris XI, 91405 Orsay, France

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The copper(II) complexes of some dipeptides containing the L-histidine residue at N– or C–terminal positions (models for superoxide dismutase) have been studied by the epr technique in frozen and liquid states, at near physiological pH.

The analysis of the spectra indicates that in the Cu–His–X system the chelation is essentially glycine-like (without the imidazole ring) while in the Cu–X–His system three nitrogen atoms: imidazole, amine and peptide interfere (histamine like mode). These results are consistent with potentiometric titrations reported previously.

Introduction

Histidine is frequently found as part of the active site of metalloproteins [1]. The knowledge of the mode of coordination between the metal and histidine-containing peptides could serve as a suitable model for studies on the interaction between the metal and the protein.

Recently, one of us reported the catalytic reactivity of some copper–histidine containing dipeptide complexes towards the dismutation of O_2^- produced by the pulse radiolysis technique [2]. The complexes behave in different ways according to the N– or C– terminal position of the histidyl residue. Indeed, the potentiometric and spectroscopic measurements show two different kinds of chelation of the copper ion by the dipeptide [2, 3].

The X-ray diffraction analysis of the crystalline Cu–Ala–His complex shows that this peptide is chelated to copper by the amine, amide and imidazole nitrogens; the fourth coordination site of the metal is occupied by the carboxyl oxygen of another peptide unit [4]. For the other copper complexes tested as catalysts in the work cited above, no evident proof of structure was obtained [3].

Electron paramagnetic resonance (epr) is known to be a valuable tool for assessing the number of nitrogen atoms chelated to the central ion in metallic complexes, *i.e.* the copper proteins and their low molecular weight models [5]. In the present paper we report some epr results concerning the copper chelates of the histidine-containing dipeptides studied previously by potentiometric and other spectroscopic methods.

Experimental

The dipeptides Ala-His, His-Ala and His-Phe were obtained from commercial sources (Sigma). Val-His and His-Val were synthetized according to [6]. The copper chelates were obtained as described previously [2]. The solutions for epr measurements were prepared in H_2O or D_2O at a ligand to copper ratio of 2:1 at pH ≅ 6 for the Cu–X–His system (X = alanyl or valyl residue) and at pH ≅ 7 for the Cu–His–X system (X = alanine, valine or phenylalanine residue).

X-band epr spectra were recorded on JEOL-ME3X spectrometer modulated at 100 KHz and equipped with an accessory to perform low-temperature experiments at −140 °C. The magnetic field was measured with a NMR proton probe and the microwave frequency with a wavemeter giving an accuracy of ±1 MHz. Quartz tubes of 1 mm internal diameter were used.

*Author to whom correspondence should be addressed.

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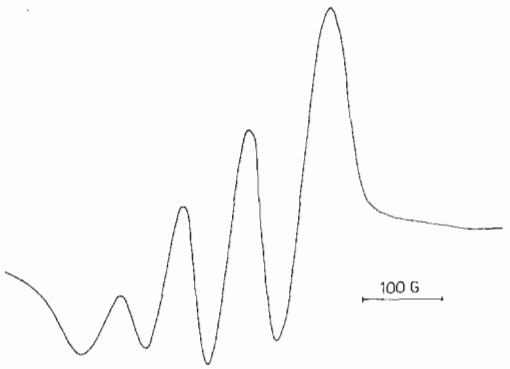

Fig. 1. Cu–Ala–His complex at room temperature (modulation amplitude: 20 gauss).

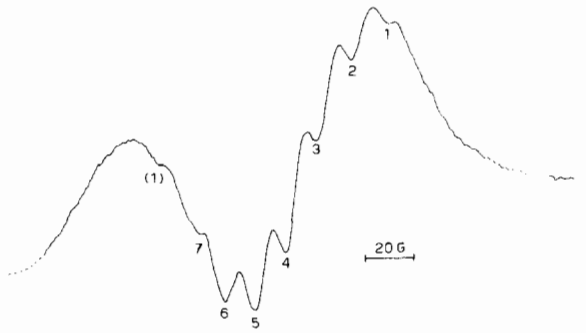

Fig. 2. Cu–Ala–His complex at room temperature: the shfs of the high-field line (modulation amplitude: 2 gauss).

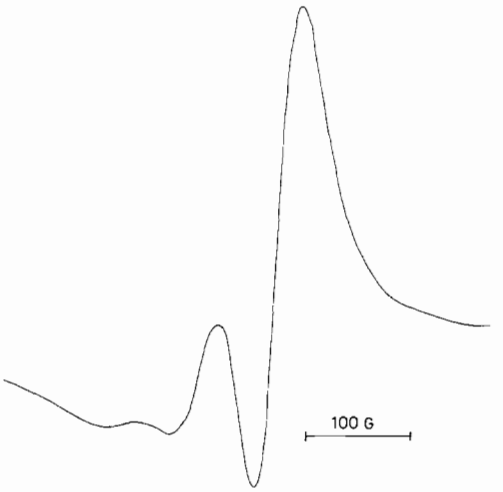

Fig. 3. Cu–His–Val complex at room temperature.

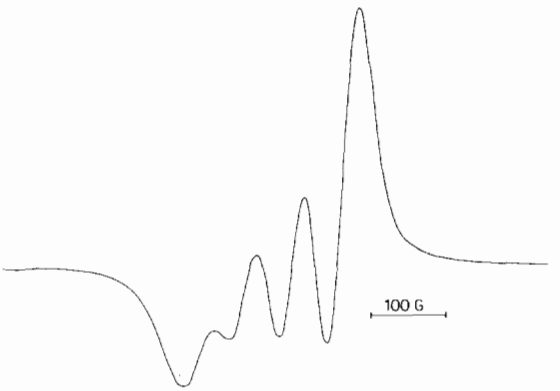

Fig. 4. Cu–His–Val complex at 90 °C.

Results and Discussion

Liquid-state epr

The potentiometric measurements which have been previously reported for Cu–X–His and Cu–His–X systems (X = alanine, valine and phenylalanine) pointed out the predominance of the binary complex Cu H_{-1} L (L = X–His peptide) among a mixture of several compounds at pH ≅ 6 and a metal concentration of $C_M = 2 \cdot 10^{-3}$. In contrast, at pH ≅ 7 the ternary complex CuL_2 (L = His–X peptide) is almost the single compound of the second system [3]. Some selected epr spectra of the two systems, recorded in the same conditions as the potentiometric measurements, are given in Figs. 1, 2 and 3. It is well recognized that the line shape of epr spectra is strongly dependent on the tumbling of molecules [7]. According to Kivelson's theory [8], linewidth is a function of the anisotropy of g and A tensors and the viscosity of the medium but depends primarily on the bulk of the molecule. The greater it is, the broader is the linewidth. Because of the nuclear spin of the copper atom (I = 3/2), a four-line hyperfine structure is expected. Moreover, the linewidth of a hyperfine line is proportional to M_I, being narrower for $M_I = 3/2$ than for $M_I = -3/2$. Clearly, only the high-field lines could be detected for the Cu–His–X system. The low-field line is broadened to the extent that it cannot be observed (Fig. 3). In contrast, the four-line hyperfine structure could be seen for the Cu–X–His system (Fig. 1). The fact that the rotational rate of the Cu–His–X species markedly determined the line shape at room temperature is in agreement with the observation that an increase in temperature improves resolution (Fig. 4). The drastic difference in the dynamic behaviour of the Cu–X–His and Cu–His–X systems indicates that the size of the former complexes is much smaller than that of the latter. This confirms the potentiometric data: only one dipeptide ligand chelated the copper atom in the binary complexes (Cu–X–His system) whereas two dipeptide ligands do so in the ternary complexes (Cu–His–X system). A further

TABLE I. EPR Parameters Discussed in the Text.

System	X	$g_{\parallel}$	$A_{\parallel}$ (gauss)	A_{oN} (shfs) (gauss)
Cu–X–His	Ala	2.205 (±0.05)	202 (±4)	13–16
	Val	2.210 (±0.05)	197 (±4)	13–16
Cu–His–X	Ala	2.241 (±0.02)	174 (±2)	–
	Val	2.243 (±0.02)	175 (±2)	–
	Phe	2.230 (±0.02)	177 (±2)	–

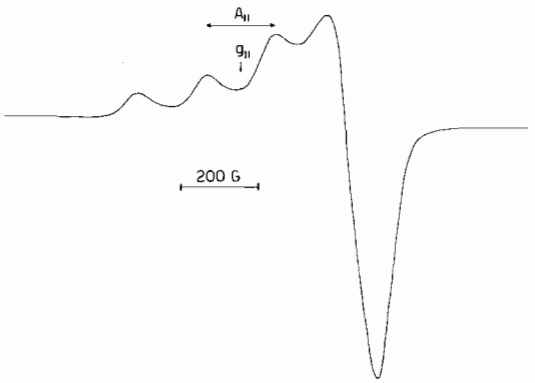

Fig. 5. Cu–His–Val complex at –140 °C.

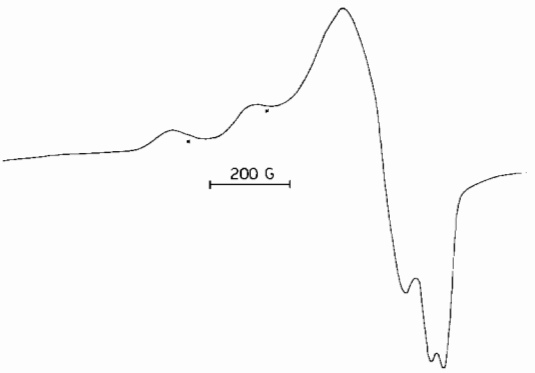

Fig. 6. Cu–Ala–His complex at –140 °C, (x: peaks of other compounds, see ref. 3).

characteristic of importance in the spectra is the additional superhyperfine structure (shfs) of the more high-field line for the Cu–X–His system at room temperature (Fig. 2). Seven shf lines are observed with a 13–16 gauss splitting. However, owing to the large degree of overlap in these lines, no assignment of intensities can be made from the spectrum. Further improvements in resolution using D_2O as solvent failed. Although not conclusive, the presence of seven shf lines (^{14}N: I = 1) is consistent with three nitrogen nuclei, giving a N_3O equatorial ligation. Moreover, the 13–16 gauss splitting indicates that the nitrogen atoms are magnetically non equivalent. However, coordination by four nitrogen nuclei cannot be ruled out. No shf could be detected for any line of the spectrum of the Cu–His–X system in H_2O or D_2O. Contrary to the Cu–X–His system, epr measurements on the Cu–His–X system cannot provide direct evidence for the number of nitrogen atoms coordinated to copper(II). For other ternary entities, such as the bis(histidinato)–copper complex, no nitrogen shfs have been reported [9].

Frozen-state epr

In agreement with the potentiometric titrations [3], epr spectra of the Cu–X–His and Cu–His–X systems recorded at –140 °C indicate the presence of a major complex (Figs. 5 and 6). On the other hand, the frozen solution spectra exhibit parallel and perpendicular features typical of a Cu^{2+} ion in an axial ligand field with tetragonal distortion by elongation along the axial direction [10]. For a Cu^{2+} ion in such a distorted octahedron, two sets of lines are expected, centered at $g_{\parallel}$ and $g_{\perp}$. Figure 5 shows this effect at $g_{\parallel}$ where three of the four lines are resolved, the fourth being obscured by the absorption around $g_{\perp}$ (in Fig. 6 only two lines at $g_{\parallel}$ are evident). The four hyperfine lines at $g_{\perp}$ are not resolved since $A_{\perp}$ is usually much smaller than $A_{\parallel}$ in Cu^{2+} complexes [11]. For a tetragonal field with axial elongation (the limit being a square-planar stereochemistry), the $g_{\parallel}$ and $A_{\parallel}$ values are the pertinent parameters to provide structural assignments. It is well established that $g_{\parallel}$ decreases and $A_{\parallel}$ increases as the strength of the equatorial ligand-field increases (*i.e.* any increase in the number of the nitrogen equatorial ligations would lead to a decrease of $g_{\parallel}$ and increase of $A_{\parallel}$). An axial symmetry is assumed for all our complexes. In Table I are reported $g_{\parallel}$ and $A_{\parallel}$ data respectively attributed to the Cu H_{-1} L complex for the Cu–X–His system and to the CuL_2 complex for the Cu–His–X system. Comparison of these values with those of

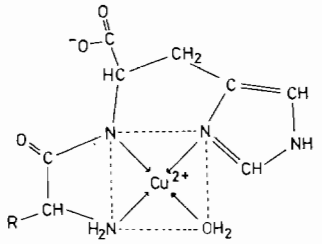

Fig. 7. Structure of the binary species: Cu–X–His system.

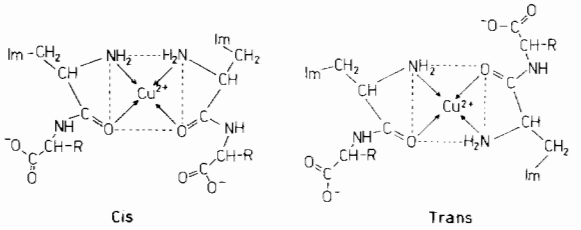

Fig. 8. Proposed structures of the ternary species: Cu–His–X system (Im = Imidazole).

numerous well characterized reliable complexes [11–15] suggests that three or four nitrogen atoms are coordinated to the copper as equatorial ligands in the binary specues Cu $H_{-1}L$ whereas one or two nitrogen atoms coordinate the copper ion in the ternary species CuL_2. Furthermore, simulation of the frozen solution spectra for CuL_2 (L = His–Val) compound, with the help of the 'Repelec' computer program, devised by M. Henry [16] shows that the linewidth for the parallel region is greater than for the perpendicular region. This means that an unresolved splitting contributes to line broadening for $g_{\parallel}$ and, as a consequence, involves the presence of one or more nitrogen atom(s) in the basal plane around the central ion.

The $g_{\parallel}$ values for the CuL_2 (L = His–Phe) compound are significantly lower than the corresponding values for the other two complexes of the Cu–His–X system. According to some authors [17], aromatic groups in Cu-dipeptide systems can operate a weak association over the ionic center of the complex. However, the relationship between such an interaction and the $g_{\parallel}$ parameter is not clear.

No evidence of binuclear Cu^{2+} coordination was found as described in other related systems [18]. If a binuclear Cu^{2+} complex was formed, new resonances, especially near g = 4, or a decrease in signal intensity due to spin-spin coupling would have been observed [19]. However, such was not the case.

Epr correlation between liquid and frozen solutions: proposal for structural assignments

Taking into account the results reported above, we assume that the structure of the Cu H_{-1} L compound for the Cu–X–His system is the same in aqueous solution as in the solid state [4] except for a water molecule occupying the fourth ligand position of the square planar arrangement, according to the geometry indicated in Fig. 7.

So among the two modes of bonding of amino-acids or peptides [20–23] the chelation of the Cu H_{-1} L entities is thought to be histamine-like for the Cu–X–His system. As far as the parent complex for the Cu–His–X system is concerned, it would be of considerable interest to obtain some structural features in order to understand its reactivity towards the superoxide anion [2]. Unfortunately, the concentration at which this species becomes the major component of the system is much too low for the epr technique [3]. Nevertheless the fact that shfs is observed in the epr spectrum of the Cu–H_{-1}L complex denotes a tighter copper–ligand binding which can perhaps explain its low activity [2].

In spite of numerous studies of ternary complexes, few papers reported proofs for structural assignments [11–15, 20–24]. The $g_{\parallel}$ and $A_{\parallel}$ values which fall within the range of $Cu–N_2–O_2$ or $Cu–N–O_3$ in plane chromophores for ternary species of the Cu–His–X system tend to demonstrate a rather glycine-like chelation mode [25]. At any rate, an histamine-like chelation, as encountered in other systems [21, 22] is ruled out here. Furthermore, it is quite possible that the lack of shfs in the room-temperature epr spectrum may be due to a weak coordination of the nitrogen atoms on the metal. It may also be taken as an indication of a fast equilibrium, on the epr time scale, of two geometric isomers. Such a geometric isomerism was pointed out for the bis(glycinato)copper system [26]. Therefore it is reasonable to propose for the Cu L_2 complex both structures indicated in Fig. 8. Besides, these structures take into account the greater stability associated with a five membered ring [25, 27].

In conclusion, we believe that our epr study shows that the imidazole ring enters the chelation mode of the binary species Cu H_{-1} L for the Cu–X–His system but does not do so for the neutral ternary species CuL_2 for the Cu–His–X system.

Investigation of this topic on other compounds and their assignments is in progress.

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