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Comparative EPR Study of Copper(II) Complexes with Threonine Derivatives

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Summary. In order to get better insight into the structural reasons for different properties of copper(II) complexes with *L*-threonine, *L-allo*-threonine, *L*-N,N-dimethyl-threonine, and *L*-N,N-dimethyl-*allo*-threonine, their EPR spectra were studied as a function of *pH* and temperature. At $pD \sim 9.4$, in all complexes a change in the copper(II) coordination sphere from the "glycine" to the "hydroxy" type was observed. In *bis*(*L*-threoninato)copper(II), the "hydroxy" type formed at $pD \sim 9.4$ was found to be stablized by increasing the temperature of the solution from 280 to 320 K. In all other copper(II) complexes, the conformational change is accompanied by the disruption of the Cu-N bond of one chelate ring.

Keywords. EPR; Copper(II); Amino acid; *L*-Threonine; *L-allo*-Threonine; N,N-Dimethyl-*L*-threonine; N,N-Dimethyl-*L-allo*-threonine.

Vergleichende EPR-Untersuchungen von Kupfer(II)-Threonin-Komplexen

Zusammenfassung. Um einen besseren Einblick in die Beziehungen zwischen Struktur und Eigenschaften von Kupfer(II)-Komplexen mit *L*-Threonin, *L-allo*-Threonin, *L*-N,N-Dimethyl-Threonin und *L*-N,N-Dimethyl-*allo*-Threonin zu gewinnen, wurden ihre EPR-Spektren in Abhängigkeit vom *pH*-Wert und von der Temperatur untersucht. Bei einem *pH*-Wert von 9.4 (in deuterierter Lösung) wurde eine Veränderung in der Kupfer (II)-Koordinationssphäre festgestellt, die von einer „Glycin"-ähnlichen Konformation in eine vermutlich „Hydroxy"-ähnliche Konformation übergeht. Der *bis*(*L*-Threoninato)-Kupfer (II)-„Hydroxy"-Komplex wird durch eine Temperaturerhöhung von 280 auf 320 K stabilisiert. Die Veränderung der Koordination vom „Glycin"-Typ wird von einem Bruch der Cu-N-Bindung eines Chelatrings begleitet.

Introduction

Copper(II) complexes with *L*-threonine, *L-allo*-threonine, and *L*-serine have been extensively studied by many authors [1–8]. The side chains of these amino acids (CH_2OH and $CH(CH_3)OH$, respectively) were found to have only a weak coordination tendency towards copper(II) ions [3]. In aqueous solutions, visible absorption measurements lead to the conclusion that at neutral *pH* ($6 < pH < 8$) the amino acids maintain a "glycine" like configuration in the copper(II) complexes [4, 5]. At $pH > 9$, copper(II) may promote ionization of the alcohol

hydrogens of the chelated threonines ($pK_1 = 10.3, pK_2 = 11.3$) [5]. This may be followed by a change of the "glycine" like structure (N-amino, O-carboxy) to the "hydroxy" type (N-amino, O-hydroxy) of the copper(II) coordination sphere [4, 5].

Differences in stability between Cu(II)-complexes with natural *L*-threonines and *L-allo*-threonines have been determined [6–8]. It has been proposed that in the "glycine" like configuration the higher stability of the complexes with *L*-threonine is due to the weak outer sphere coordination between amino and hydroxy groups through the apical water molecule [6–8]. This should stabilize the *trans* coordination of the 2N2O atoms of the copper(II) coordination sphere as determined for the copper(II) complex with *L*-threonine by X-ray crystallography. [9]. *Trans* coordination was also found in the crystalline copper(II) complex with N,N-dimethyl-*L*-threonine [10].

Recently, stability constants have been determined for the complexes of N,N-dimethyl-threonine with Cu^{2+}, Ni^{2+}, and Co^{2+} by potentiometric titrations [11]. The results suggest that dimethyl-threonine acts as a bidentate ligand toward copper(II) by engaging either amino and carboxyl groups, or upon dehydronation at high *pH*, the amino and hydroxy groups.

In order to get better insight into the structural reasons for the differences in the properties of the copper(II) complexes with *L*-threonine, *L-allo*-threonine, and their N,N-dimethylated counterparts, we studied EPR spectra of these copper(II) complexes as a function of the *pD* value in D_2O solutions.

EPR spectroscopy can provide detailed information on the nature of the copper(II) coordination sphere and the number of species in solution and in the frozen state [12, 13]. For a number of *bis*(aminoacidato)copper(II) complexes in aqueous solutions, the ^{14}N-superhyperfine (SHF) structure of the EPR spectra has confirmed the presence of *cis* and *trans* isomers (with respect to the 2N2O atoms of the amino and carboxy groups of the amino acids) in the copper(II) coordination sphere [14]. Thus, we believe that the possible coordination of the OH groups of the amino acid side chains in the complexes will be reflected in the equilibrium between the *cis* and *trans* isomers in solution.

Results and Discussion

In the interval $6 < pD < 9$, EPR spectra of the copper(II) complexes with *L*-threonine, *L-allo*-threonine, and *L*-serine were determined (Table 1). The spectra showed a resolved ^{14}N-superhyperfine (SHF) structure at the resonance transition $m_{Cu} = +3/2$ as displayed in Fig. 1 for the *bis*(*L*-threoninato)copper(II) complex in D_2O at $pD = 7.2$. The SHF lines (Fig. 1b) are due to the two overlapping quintets with an intensity ratio of 1:2:3:2:1 each, reflecting the two sets of the 2N donor atoms to the copper(II) coordination sphere, *i.e.* the *cis* and *trans* isomers in equilibrium as determined by *Goodman* and *McPhail* for the "glycine" type of copper(II) complexes with many other *L*-α-amino acids [14, 20]. Taking into account the reasons presented by *Goodman* and *McPhail* [20], we assigned the SHF lines at higher magnetic field to the *trans* isomer.

In the copper(II) complexes with *L*-threonine and *L-allo*-threonine examined here, the ratio between *cis* and *trans* isomers was found to vary with the *pD* of the

Table 1. EPR parameters of copper(II) complexes with amino acids

Ligand	*pH*	g_o	g_{II}	g_I	a_o (mT)	a_{II} (mT)	a_I (mT)	a_{oN} (mT)
L-α-serine	8.0	2.127	2.262	2.060	6.9	17.0	1.9	*cis-trans*
L-α-serine	12.0	2.114	2.238	2.057	8.4	19.0	3.1	1.0
L-α-threonine	8.0	2.128	2.257	2.063	7.0	17.7	1.6	*cis-trans*
L-α-threonine	12.0	2.113	2.235	2.051	8.5	19.5	3.0	1.0
L-α-*allo*-threonine	8.0	2.127	2.260	2.060	7.0	16.9	2.05	*cis-trans*
L-α-*allo*-threonine	12.0	2.113	2.241	2.054	8.4	18.9	3.5	/
L-α-N,N-dimethyl-threonine (ethanol)	8.0	2.117	2.254	2.044	8.4	18.2	3.5	/

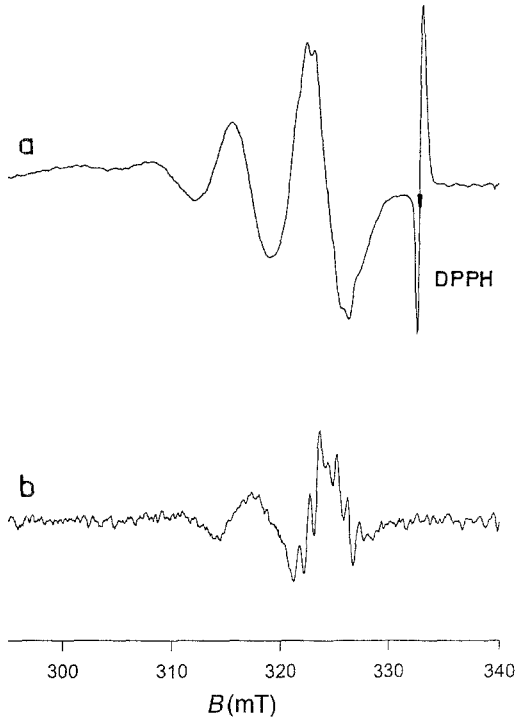

Fig. 1. EPR spectra of *bis*(*L*-threoninato)copper(II) dissolved in D2O + DCl at $pD = 7.2$ at 290 K; a: first, b: second derivative representation

D_2O solution. The SHF lines at higher magnetic field were more pronounced at lower *pD* of the solution and broadened above $pD > 8$.

The ^{14}N SHF structures of the EPR spectra of *bis*(*L-allo*-threoninato)copper(II) and *bis*(*L*-threoninato)copper(II) complexes compared at one particular *pD* value indicated that in *bis*(*L*-threoninato)copper(II) the equilibrium of the *cis-trans* isomers was shifted towards the *trans* isomer. This may be due to a copper(II) outer sphere coordination with two OH groups of the amino acid side chains through hydrogen bonds with the apical water. Such an arrangement is not favorable in the copper(II) complex with *L-allo*-threonine.

At $pD \sim 9.4$, a shift of g_o to 2.116 was detected in the copper(II) complexes with *L*-threonine and *L-allo*-threonine. At that *pD*, SHF lines of the EPR spectra of

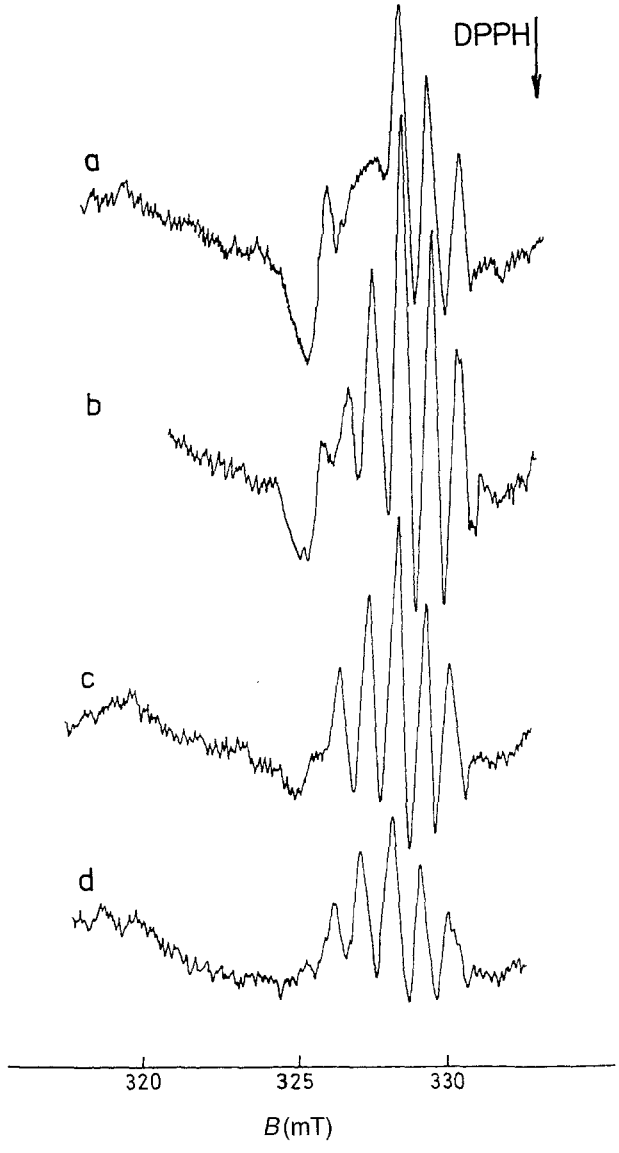

Fig. 2. Second harmonic display of the $m_{Cu} = +3/2$ resonance transition in the EPR spectra of *bis*(*L*-threoninato)copper(II) in D_2O + NaOD at $pD = 9.4$; a, 280 K; b, 290 K; c, 300 K; d, 320 K; the change in the spectra was irreversible, the five ^{14}N SHF lines remained unchanged upon cooling

bis(*L*-threoninato)copper(II) showed a complicated structure, indicating the presence of more than two species in solution (Fig. 2). The structure of the SHF lines changed with increasing temperature (Fig. 2a–d). At 320 K , the five sharp lines with an intensity ratio of 1:2:3:2:1 (Figure 2d) prevailed, and this structure remained unchanged upon recooling the sample. This suggested that-after a conformational change favored at higher temperature-, only one 2N2O isomer remained in solution, presumably in the "hydroxy" like copper(II) coordination.

Different results were obtained with *bis*(*L-allo*-threoninato)copper(II) at a *pD* of about 9.4 (Fig. 3a, b). Three SHF lines in an intensity ratio of 1:1:1 (Fig. 3b) suggested only one nitrogen donor to the copper(II) coordination sphere of the predominant complex in solution over the whole temperature range (280–320 K). In the complex with *L-allo*-threonine, the conformational change at *pD* about 9.4 causes the breakage of one Cu-N bond of the copper(II) coordination sphere.

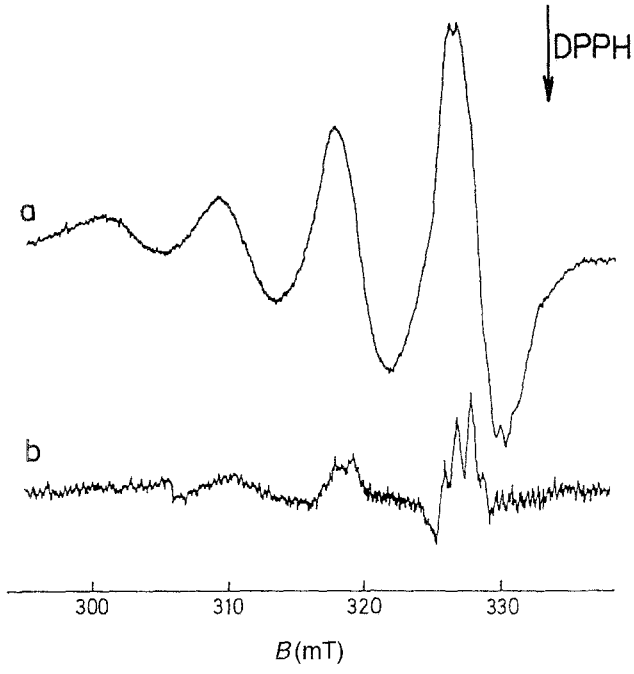

Fig. 3. EPR spectra of *bis*(*L-allo*-threoninato)copper(II) in D_2O at $pD = 9.4$ at 290 K; a, first harmonic; b, second harmonic

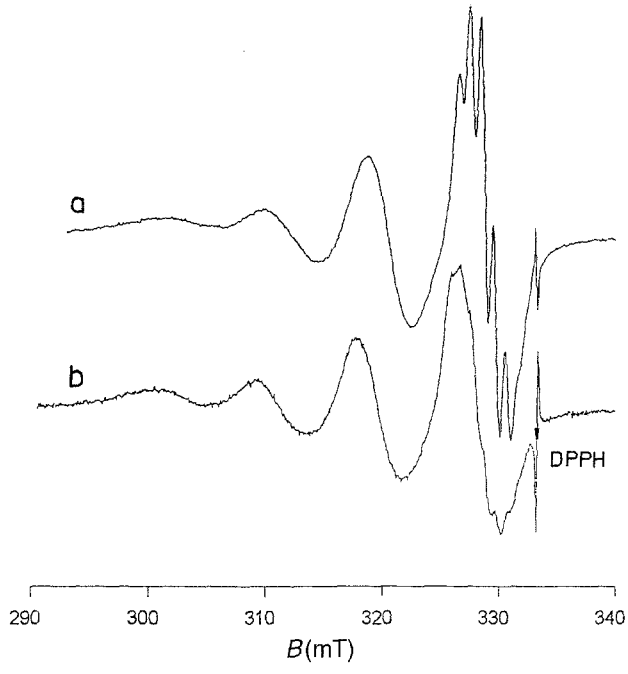

Fig. 4. EPR spectra of *bis*(*L*-threoninato)copper(II) (a) and *bis*(*L-allo*-threoninato)copper(II) (b) in D_2O at $pD = 12.5$ and 290 K

In Fig. 4, the EPR spectra of *bis*(*L*-threoninato)copper(II) (a) and *bis*(*L-allo*-threoninato)copper(II) (b) measured at $pD = 12.5$ and 290 K are compared. In *bis*(*L*-threoninato)copper(II), the SHF structure of the resonance transition at $m_{Cu} = +3/2$, *i.e.* the appearance of five well resolved lines in an intensity ratio of 1:2:3:2:1 (Fig. 4a) suggested the presence of only one 2N2O isomer in the

copper(II) coordination sphere of the complex in solution. In the spectra of *bis*(*L-allo*-threoninato)copper(II) (Fig. 4b), six SHF lines could be recorded in the second derivative, suggesting more than one isomer in solution.

The observed differences in the EPR spectra between *bis*(*L*-threoninato)copper(II) and *bis*(*L-allo*-threoninatio)copper(II) at $pD = 12$ are probably due to steric hindrance between the CH_3 groups at the C^*_β atoms and the COO^- groups at the C^*_α atoms in the chelate rings of the copper(II) complex. The hindrance is greater in the case of the complex with *L-allo*-threonine because COO^- and CH_3 are located at the same side of the chelate plane, destabilizing both *cis* and *trans* isomers. In the case of *trans-bis*(*L*-threoninato)copper(II), the two CH_3 groups of the two amino acid side chains in the axial position may form *van der Waals* contacts. This will stabilize the complex.

Methylation of the NH_2 groups in *L*-threonine and *L-allo*-threonine leads to a destabilization of the parent copper(II) complexes in D_2O. Only in ethanol at 77 K, *bis*(N,N-dimethyl-*L*-threoninato)copper(II) gave an EPR spectrum with the resolved ^{14}N SHF structure in the $g_\perp$ region (Fig. 5), suggesting 2N donor atoms to the copper(II) coordination sphere. The SHF structure was not resolved at room temperature. At $pH > 9.4$, the second derivative representation of the EPR spectrum of *bis*(N,N-dimethylthreoninato)copper(II) (Fig. 6a, b) showed three sharp SHF lines in an intensity ratio of 1:1:1 at the resonance transition $m_{Cu} = +3/2$ suggesting only one nitrogen donor to the copper(II) coordination sphere. A similar result was obtained with *bis*(N,N-dimethyl-*allo*-threoninato)-copper(II) (Figure 6c, d), although three SHF lines were broader. This suggests that in ethanol at $pH > 9.4$ a disruption of the Cu-N bond of the chelate ring occurred.

Recently, the crystal structure of *bis*(N,N-dimethyl-*L*-threoninato)copper(II) dihydrate has been determined [10]. The shape of the copper(II) coordination polyhedron was reproduced with molecular mechanics calculations, and the

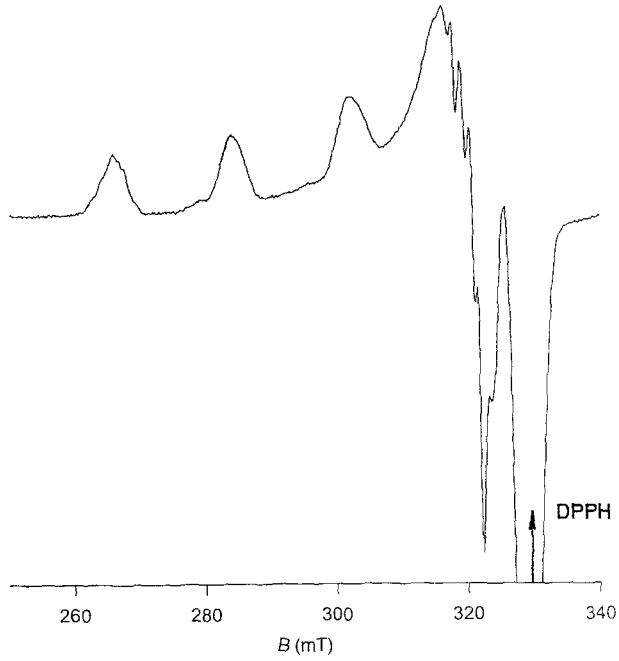

Fig. 5. EPR spectra of *bis*(N,N-dimethyl-*L*-threoninato)copper(II) dissolved in ethanol at 77 K

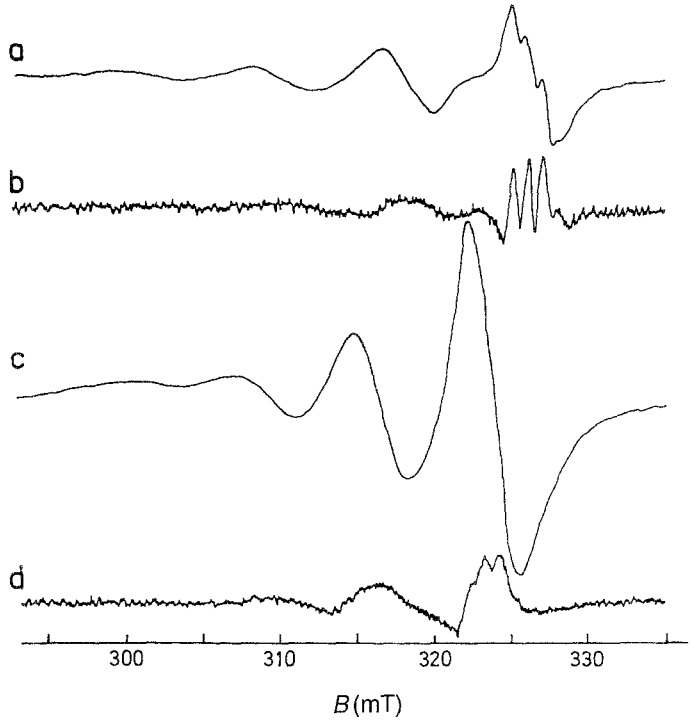

Fig. 6. EPR spectra of *bis*(*L*-N,N-dimethyl-threoninato)copper(II) (a, first harmonic; b, second harmonic) and *bis*(*L*-N,N-dimethyl-*allo*-threoninato)copper(II) (c, first harmonic; d, second harmonic) at 290 K in ethanol + NaOH ($pH = 10$)

calculated strain energy of the crystal conformation was found to be 21 $kJ \cdot mol^{-1}$ higher than the energy of the most stable conformer. This was tentatively attributed to the additional stabilization of the molecular conformation by intermolecular hydrogen bonds in the solid state. This is in accordance with our finding that the copper(II) complexes with both N,N-dimethylated threonines are stable only in ethanol solution and in the *pH* interval $7 < pH < 9$. In this *pH* interval, the complexes retain the "glycine" like copper(II) coordination. On the other hand, in the copper(II) complexes with N,N-dimethylated valine and N,N-dimethylated isoleucine the complexes were stable in both D_2O and in organic solutions and it was shown that water dissolved in organic solvents influenced the copper(II) electronic states and may perhaps induce the changes in conformation of the whole compex [21, 22]. This was not observed in the copper(II) complexes with N,N-dimethylated threonines.

Experimental

The EPR spectra of the *bis*(aminoacidato)copper(II) complexes consist of four $(2I + 1)$ resonance transitions arising from the hyperfine coupling of an unpaired electron with a copper nuclear spin of $I = 3/2$. The two isotopes ^{63}Cu (69%) and ^{65}Cu (31%) have slightly different magnetic moments. The g_0 values corresponding to the two isotopes were found to be similar, whereas there were slight differences between the copper(II) hyperfine splitting constants [14]. In the present investigation, we used complexes with natural abundance of copper isotopes in DCl/D_2O solutions.

The g factor was measured relative to diphenyl-picrylhydrazyl (*DPPH*) as an external standard ($g = 2.0036 \pm 0.0003$) [15].

The isotropic g_o factor and the isotropic hyperfine splitting constant a_o, shown in Table 1, are average values calculated from the EPR spectra recorded in the temperature interval of 300–330 K where the anisotropic parts of the g factor and of the copper hyperfine splitting constant are fully averaged out.

Anisotropic parameters ($g_\parallel$ and $a_\parallel$) of the glassy state were measured at 77 K. $g_\perp$ and $a_\perp$ were calculated according to Ref. [12]. In order to get a good glassy state, a drop of glycerol was added to the D_2O solution of the complex. EPR spectra were recorded on a Varian E-9 spectrometer equipped with a data acquisition program [16] and a Bruker variable temperature control unit.

All copper(II) complexes with amino acids (*p.a.*, Fluka, Germany) were prepared by two different methods: from $CuSO_4$ neutralized with $Ba(OH)_2$ [17] or from the sodium salt of the amino acid and freshly prepared $Cu(OH)_2$ [18]. The complexes were recrystallized from water-acetone solutions at controlled *pH*. N,N-Dimethyl-threonine and N,N-dimethyl-*allo*-threonine were synthesized by *Bowman*'s method [19].

pD values were measured with *pH* meter (Radiometer, Copenhagen) standardized with *pD* buffers; *pD* was adjusted with NaOD and/or DCl to the desired value.

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References

[1] Freeman HC, Martin RP (1969) J Biol Chem **224**: 4823
[2] Sarkar B, Kruck TPA (1979) In: The Biochemistry of Copper. Acad Press, New York, p 183
[3] Petit LD, Hefford RJW (1979) Stereoselectivity in the Metal Complexes of Amino Acids and Dipeptide. In: Sigel H (ed) Metal Ions in Biological Systems, vol 9, Decker, New York, p 182
[4] Tsangaris JM, Martin RB (1970) J Am Chem Soc **92**: 4255
[5] Grenouillet P, Martin RP, Rossi A, Ptak M (1973) Biochim Biophys Acta **322**: 185
[6] Kralj Z, Paulić N, Raos N, Simeon V (1982) Croat Chem Acta **55**: 337
[7] Ivičić N, Simeon V (1981) J Inorg Nucl Chem **43**: 2581
[8] Ivičić N, Simeon V (1978) Thermochimica Acta **25**: 299
[9] Amirthalingam V, Muralidharan KV (1975) Pramana **4**: 83
[10] Kaitner B, Ferguson G, Paulić N, Raos N (1993) J Coord Chem **30**: 233
[11] Blagović B, Paulić N, Raos N, Simeon V (1994) Monatsh Chem **125**: 1083
[12] Hyde JS, Froncisz W (1982) Ann Rev Biophys Bioeng **11**: 391
[13] Basosi R, Antholine WE, Hyde JS (1993) Biological Magnetic Resonance **13**: 103
[14] Goodman BA, McPhail DB, Powell HKJ (1981) J Chem Soc Dalton Trans 822
[15] Poole Jr CP (1983) In: ESR, A Comprehensive Treatise on Experimnetal Techniques. Wiley, New York
[16] Morse II PD (1987) Biophys J **51**: 440a
[17] van der Helm D, Franks WA (1969) Acta Cryst **B25**: 451
[18] Yasui T (1965) Bull Chem Soc Japan **38**: 1746
[19] Bowman RE, Stroud HH (1950) J Chem Soc **9**: 1342
[20] Goodman BA, McPhail DB (1985) J Chem Soc Dalton Trans 1717
[21] Noethig-Laslo V, Paulić N (1992) J Chem Soc Dalton Trans 2045
[22] Noethig-Laslo V, Paulić N (1995) Z anorg allg Chem **621**: 84

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