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Solution Properties of Cu(II)–L-α-alaninehydroxamic Acid

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Abstract

In the aqueous solution of copper(II) ions, bidentate L-α-alaninehydroxamic acid ($CH_3CH(NH_2)$-CONHOH = HL) binds cupric ion forming of mono-, dimeric and bis(L-α-alaninehydroxamato)copper(II) complexes. These complexes were studied by potentiometric, ESR and spectrophotometric methods.

The ESR studies provide important evidence for the formation of different Cu(II) complexes with L-α-alaninehydroxamic acid, depending on pH. The ESR spectra can be used to follow the appearance of the individual complexes, to estimate the coordination sphere around Cu(II) and to observe the equilibria between different complexes.

The solution electronic spectra are reported. The experimental curve was resolved into precise-positioned absorption bands by Gaussian analysis for the bis(L-α-alaninehydroxamato)copper(II) species. These data were used in a weak tetragonal ligand field model to calculate ligand field parameters.

The distribution and the relevant stability constants of species present in aqueous solutions were obtained by analytical potentiometry.

Introduction

Hydroxamic acid and its metal complexes were found to play an important role in living systems as constituents of antibiotics, growth factors, tumor inhibitors, cell-division factors and pigments [1–3]. They are intimately associated with iron transport phenomena in the metabolism of microorganisms [4–6]. At the same time hydroxamic acids have a number pharmacological actions including antituberculous, antifungous and antileucemic activities [7, 8]. Some of iron(III) hydroxamates have been patented as metallotherapeutics [9]. Some aminohydroxamic acids have been applied with the aim of designing metal chelates as suitable sources of various trace elements essential in animal nutrition [10–12]. The formation of different complexes between Cu(II) and glycinehydroxamic acid was recently postulated on the basis of potentiometric titrations, absorbance and ESR spectra of liquid solutions [13].

In spite of the great interest in hydroxamic acid and metal hydroxamates and their biological function, the studies on aminohydroxamic acid metal complexes are rather scarce.

In this paper we report the data on the behaviour of bidentate L-α-alaninehydroxamic acid in the presence of Cu(II) ions in water solution, extending the studies on the ESR of frozen solutions, and on the numerical interpretation of the spectrum of the most stable CuL_2 complex.

Experimental

Reagents and Materials

L-α-alaninehydroxamic acid (HL) was prepared by mixing ice-cold methanol solutions of L-α-alanine methyl ester (0.1 mol) and hydroxylamine (0.1 mol). When the mixture was cooled, HL crystallized easily, and after washing with a small amount of cold water gave pure crystals: yield 50%. *Anal.* Calc.: C, 34.61; H, 7.74; N, 26.90. Found: C, 34.60; H, 7.44; N, 26.72%.

Bidistilled water was used throughout and all titrations were carried out under an atmosphere of purified argon. All reagents were of analytical grade. Stock solution of copper(II) perchlorate (0.1 mol) was prepared by dissolving a proper amount of copper(II) perchlorate hexahydrate in water. The exact concentration of the solution was determined by the iodometric method.

Potentiometric Titrations

Measurements of pH were carried out on OP-208 pH-meter (Radelkis) with a digital readout, equipped with a glass (OP 7183) and a saturated calomel

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TABLE I. Logarithmic Stability Constants (log β_n) of Complexes Species $M_pH_qL_r$ (M = Cu, L = L-α-alaninehydroxamate Ion). I = 0.1 ($NaClO_4$), T = 25 °C

p	q	r	Numerical method (SCOGS)	Bjerrum method
0	2	1	9.15 ± 0.01	9.13
0	1	1	16.34 ± 0.02	16.36
1	0	1	10.90 ± 0.08	11.30
2	−1	2	21.41 ± 0.03	
1	0	2	19.65 ± 0.02	19.85
1	−1	2	9.74 ± 0.03	

(OP 830) electrodes. The measuring system was thermostated at 25 ± 0.1 °C. Constant ionic strength (0.1) was maintained by means of $NaClO_4$. Small amounts of titrant were added with the use of a micropipette.

Ligand titrations were performed in the absence of copper ions (in this case, titration curves were obtained by varying the initial concentration of L-α-alaninehydroxamic acid at 1.0×10^{-2}, 2.5×10^{-2} and 5.0×10^{-2} M, respectively) and in the presence of copper ions (in this case, the concentration ratios of ligand to metal ions varied in the range of 2:1 and 10:1). Formation constants were calculated using Bjerrum method [14] and a modified version of the computer program [15]. The resulting formation constants are given in Table I.

ESR Measurements

The ESR spectra were obtained using a JES-ME X-band spectrometer, proton magnetometer and ESR standards, at 295 K and 170 K. The samples were prepared from a stock solution containing 4.0×10^{-3} M copper(II) ions and 1.0×10^{-2} M L-α-alaninehydroxamic acid in bidistilled water. The pH was regulated by addition of 1.0 M NaOH solution.

Spectrophotometric Measurements

Absorption in the region 400–900 nm were obtained on SPECORD M-40 (C. Zeiss, Jena) spectrophotometer and samples (4.0×10^{-3} M) were scanned at a series of pH values from pH 2.5–11.0 by using 1-cm quartz cells thermostated at 25.0 °C. Measurements of potential were done with the system used in the potentiometric titrations. The stock solution (0.1 M in $NaClO_4$, 4.0×10^{-3} M in Cu(II) and 1.0×10^{-2} M in L-α-alaninehydroxamic acid) was adjusted by adding 1.0 M NaOH solution.

Results and Discussion

Titrations

In the normal aqueous titration range, L-α-alaninehydroxamic acid can liberate two protons, one

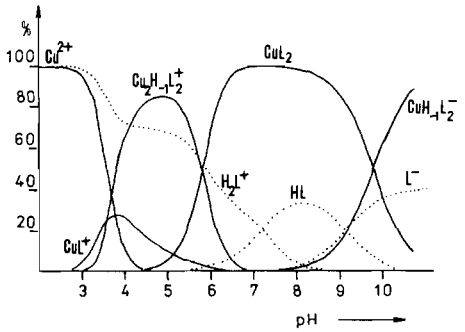

Fig. 1. Species distribution in the Cu(II)/L-α-alaninehydroxamic acid system as a function of pH, $c_M = 3.0 \times 10^{-3}$ M; $c_L = 1.0 \times 10^{-2}$ M. Percentages of the species refer to total metal except for the metal-free forms, which refer to total ligand.

from the protonated amino group (NH_3^+), pK_1^H and one from the OH^- group of the hydroxamic group (NHOH), pK_2^H. These show buffering behaviour in the pH range 7.5–9.5. The final refined values of pK_1^H and pK_2^H are 7.19 and 9.15, respectively. Evaluation of the β_{pqr} constants, using the computer program SCOGS, led us to include a fourth copper(II) species. The results are shown in Table I and the species distribution is given in Fig. 1. It is noteworthy that the formation of CuL^+ complex, suggested on the basis of graphical comparisons between experimental and simulation titration curves, is strongly supported experimentally by the ESR spectra of frozen solutions (see below).

ESR Spectra

The ESR spectra of liquid solution of Cu(II)–L-α-alaninehydroxamic acid as a function of pH are almost identical to those observed recently for Cu(II)–glycinehydroxamic acid [13]. The spectrum at pH 2.75 is characteristic for uncomplexed Cu(II) ions in water solution. However, the pH increase to 3.20 results in a new spectrum assigned to the CuL^+ complex; the spectrum of Cu(II) ions at pH 3.20 is still visible. At pH 3.46 the overlapping of two spectra is consistent with the presence of Cu(II) aquoions and of CuL^+ complex. The general disappearance of the ESR spectra observed at pH 4.36 is diagnostic for domination of the dimeric complex $Cu_2H_{-1}L_2^+$ in solution; the presence of weak signals corresponds to small admixture of CuL^+ and a similar form (see below). The distinct spectrum assigned to the CuL_2 complex appears at pH 5.67 and it becomes stronger when the pH increased to 10.35, without any change of its parameters.

The ESR spectra of frozen solutions collected in Fig. 2 give further information about the complexes. At the lowest pH (2.75) the parameters of the ESR spectrum (Table II) are typical for Cu(II) ions coordinated by H_2O molecules [16]. The same

TABLE II. The Parameters of the ESR Spectra

pH	2.75	4.32			5.67		6.64	10.35
Form	Cu(II)aquo	CuL^+	X[a]	$Cu_2H_{-1}L_2^+$	$Cu_2H_{-1}L_2^+$	CuL_2	CuL_2	$CuH_{-1}L_2^-$
$g_\parallel$	2.401	2.326	2.283	–	–	2.185	2.185	2.178
$\lvert A_\parallel \rvert$ $[10^{-4}\ cm^{-1}]$	146	167	168	–	–	213	213	215

[a]Additional N_2O_2 coordination sphere.

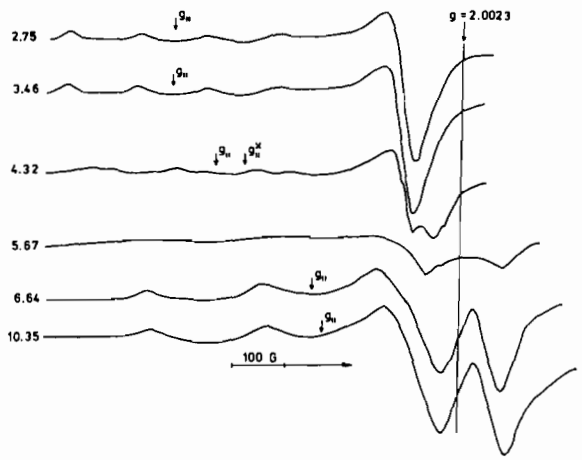

Fig. 2. ESR spectra of Cu(II)L-α-alaninehydroxamic acid as a function of pH, $c_M = 4.0 \times 10^{-3}$ M; $c_L = 1.0 \times 10^{-2}$ M, solvent: H_2O, temperature: 170 K, microwave frequency: 9.186 GHz.

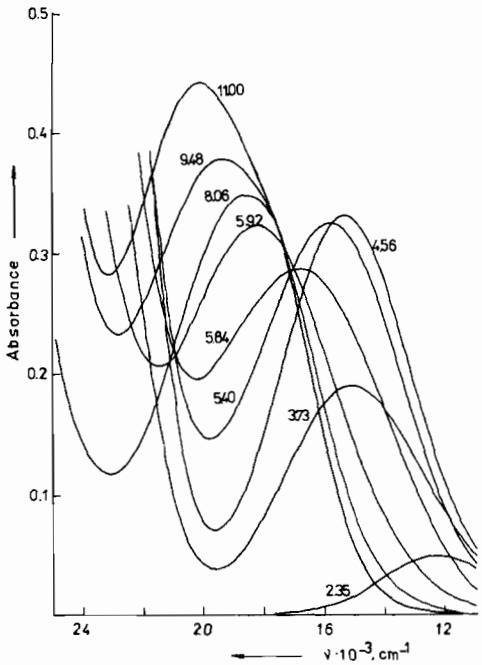

Fig. 3. Visible spectra of Cu(II)/L-α-alaninehydroxamic acid as a function of pH, $c_M = 4.0 \times 10^{-3}$ M; $c_L = 1.0 \times 10^{-2}$ M, (1 cm cells).

complex still exists at pH 3.46. At pH 4.32 the decrease of contribution of Cu(II) monomer gives rise to an ESR signal which corresponds to simultaneous formation of the dimer. At this pH two new sets of parallel copper hyperfine peaks are evidence for the formation of two copper(II) complexes. CuL^+ is the only complex established on the basis of potentiometric titration and liquid solution ESR measurements at this pH. The relatively small differences between $g_\parallel$ and $|A_\parallel|$ values of the two species suggest the same N_2O_2 coordination sphere around Cu(II) [17, 18] as for CuL^+. At pH 5.67 the spectrum practically disappears and only weak signals due to a small admixture of CuL^+ and CuL_2 complexes are observed. This is a further proof of existence of the dimeric $Cu_2H_{-1}L_2^+$ complex as a predominant form. The ESR parameters of a strong, well resolved spectrum at pH 6.64 (Table II) are typical for Cu(II) complexes with chelating ligands coordinated by four nitrogen atoms in the plane [17, 19] which supports the formation of CuL_2 complex. The small changes of the spectra with pH increase to 10.35 suggest the existence of the same coordination, in spite of the appearance of the species $CuH_{-1}L_2^-$, which is well characterized by the absorption spectra.

Electronic Spectra/pH Profile

Since the literature on the systems is very poor, we were forced to do the qualitative interpretation of the absorption spectra by comparing them with the well known spectra of Cu(II)–amino acids and dipeptides. The absorption spectra of Cu(II)–L-α-alaninehydroxamic acid exhibit, in general, approximately the same changes with increasing pH to those stated for the Cu–glycinehydroxamic acid system [13]. Some differences are observed in the energy of the characteristic absorption maxima.

The formation of the complexes between Cu(II) and the investigated ligand begins at low pH value (Fig. 3). The maximum of the broad spectrum near the infrared region shifts towards the visible region with increase of pH. At pH 4.56 the maximum occurring at 654 nm corresponds to the greatest concentration of the dimeric complex (Fig. 1). The characteristic isosbestic point seen at 595 nm is

TABLE III. Spectrophtoometric Data for CuL_2 Species as a Function of pH (pH range 6–11)

pH	Band maxima (nm)	Absorption
6.010	550	0.32
6.649	541	0.34
7.390	538	0.35
7.612	538	0.35
7.928	538	0.35
8.057	538	0.35
8.315	538	0.35
8.640	535	0.35
9.480	532	0.35
10.610	518	0.37

associated with an equilibrium between the $Cu_2H_{-1}L_2^+$ and CuL_2 complexes. The maximum at 538 nm does not change its energy from pH = 7.4 to 8.3 (Table III), which corresponds to the predominant concentration of CuL_2 complex in this pH range. The next shifting of the maximum with further increase in pH above 10 is consistent with the formation of new $CuH_{-1}L_2^{-1}$ complex.

According to Narain and Shulka [20] the position of the absorption maximum for Cu(II) complexes in water solution depends on the number of Cu–N bonds. The maximum at 620–630 nm is typical for Cu(II) coordinated by two nitrogen atoms of the amino- or amido-groups of the α-amino acids or peptides, respectively [20–22]. Since the dinuclear complex $Cu_2H_{-1}L_2^+$ exhibits λ_{max} at 654 nm, it is possible to conclude that one copper cation is coordinated by two nitrogen atoms of the ligand. The studies of the equilibria existing between Cu(II) ion and glycinehydroxamic acid has indicated the presence of an OH^- in the dinuclear species [13]. This one OH^- ion acts as a bridging group. The similar bridging of $[CuL^+]$ units by OH^- group is postulated in $Cu_2H_{-1}L_2^+$ complex. It is interesting to note that the same coordination (via two nitrogens) as in $[CuL^+]$ unit is determined for analogous CuL^+ complex on the basis of ESR spectra of frozen solution.

As follows from refs. 22, 23 the complexes of Cu(II) with dipeptides in the stoichiometric ratio 1:2 show the absorption maximum at 540–560 nm. Moreover, $[Cu(en)_2]^{2+}$ complex (en = ethylenediamine) with two chelate rings and four Cu–N bonds has the spectrum in the same region [24]. Consequently, the appearance of the absorption maximum at 538 nm in the case of the studied Cu(II)/HL system suggests the coordination of one copper cation by two ligands forming two five-membered rings and supports its CuL_2 formula. In this complex the coordination is realised by two nitrogens of α-amino- and -NHOH groups. In agreement with the predicted bidentate character of the ligand and hence its chelating effect is the greatest value of the stability constant for formed CuL_2 complex (Table I).

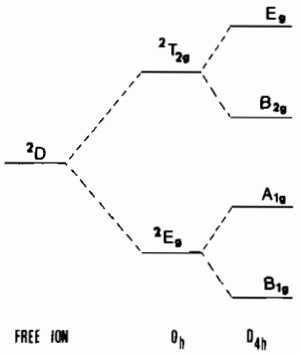

Fig. 4. Splitting of d^9 ^2D term in O_h and D_{4h} (elongated along z axis) ligand fields (not to scale).

Interpretation of Spectrum of the CuL_2 Species

Most octahedral and pseudo-octahedral complexes of Cu(II) yielded electronic spectra that are traceable to the doublet term system display one (in the case of octahedral) or more absorption bands. The theoretical energy level diagram [27] for a d^9 metal ion in O_h and D_{4h} symmetry is presented in Fig. 4. The diagram indicates that as many as three doublet-doublet transitions may be observed in the electronic spectra of complexes with D_{4h} symmetry. Observed spectra for such compounds rarely display more than two absorption bands. In some cases, however, all three absorption bands can be detected [28]. But they are never completely separated from other, and sometimes one band is totally hidden by the other two so that it does not even appear as shoulder. This results from splittings that are small compared to band widths. This complication requires careful and detailed analysis of the experimental spectra as an essential aspect of their assignment, *i.e.*, to determine the parameters of component bands and particularly of the band maxima positions. Gaussian analysis was performed on the electronic spectrum discussed herein using a nonlinear least-squares computer program [29]. All band maxima reported here are derived from Gaussian analysis of the experimental curve.

It is well known that any curve may be considered to be the sum of an infinite number of Gaussian components. However, it is conceivable that a unique fitting could be obtained if account was taken of the relative symmetry. This would require a sophisticated model. To be realistic the model must allow for possible reduction in the symmetry of the system. The possible effective symmetry of the investigated system are O_h and D_{4h}. The further reduction in symmetry (C_{2v}) theoretically provides additional

splittings and should, at best, result in band broadening. However, if the additional splitting (beyond D_{4h}) is small compared to the principal splittings that derive from the presence of four nitrogen donors in an equatorial plane and two different donor atoms on the axis perpendicular to that plane, then the assumption of D_{4h} symmetry may be adequate to account for the spectral band locations.

The experimental curve was resolved herein into precise-positioned absorption bands by Gaussian analysis which allowed O_h (one band) and D_{4h} symmetry (three bands). However, both analyses were different in terms of the root-mean-square deviation (RMS%) calculated from observed values. Fine detail of spectral shape is reproduced more faithfully for the following RMS% values: 1.56 for O_h and 0.56 for D_{4h} symmetry. Thus, it is apparent that D_{4h} symmetry should be taken into account. This result is in agreement with the axial character of ESR frozen solution spectra of the CuL_2 complex. Moreover, the X-ray crystal studies on similar Cu(II)–glycinehydroxamic acid complex [30] have proved essentially the same symmetry.

Finally, the assignments and calculated positions of the maxima are based on two assumptions:

(1) the effective symmetry about the metal ions is D_{4h};

(2) the ligand field model is adequate for the deduction of spectral parameters.

Figure 5 shows the experimental spectrum and component bands obtained from spectral resolution. Table IV summarizes the results of resolution, parameters of components bands, their oscillator strength values and root-mean-square deviations. On the basis of the well known rule that ligands exert a stronger field as they lie on the right side of the water in the spectrochemical series, in this compound the tetragonal distortion takes the form of an elongation of the octahedron, such that the two axial Cu–H_2O bonds are considerably longer than the four equatorial bonds. In this case, as shown in Fig. 4, the B_{1g} term will be ground state and the energy order of the excited states will be $^2A_{1g} < {}^2B_{2g} < {}^2E_g$ (weak distortion). Thus, three spin-allowed transitions from the $^2B_{1g}$ state to the other doublet states are

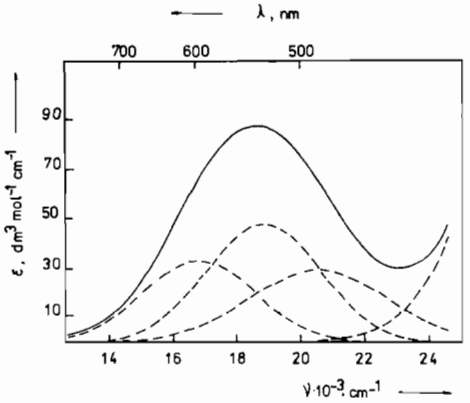

Fig. 5. Absorption spectrum of $CuL_2(H_2O)_2$: —— experimental spectrum; – – – composite bands.

to be expected and their energies in terms of Dq, Ds and Dt parameters are given by the expressions [26]:

$$\nu_1 = E(B_{1g} \longrightarrow A_{1g}) = -4Ds - 5Dt$$

$$\nu_2 = E(B_{1g} \longrightarrow B_{2g}) = 10Dq$$

$$\nu_3 = E(B_{1g} \longrightarrow E_g) = 10Dq - 3Ds + 5Dt$$

From the set of equations the formulae for the ligand field parameters Dq, Ds and Dt were derived to calculate these parameters from the positions of bands found in the spectrum:

$$Dq = \nu_2/10$$

$$Ds = (\nu_2 - \nu_1 - \nu_3)/7$$

$$Dt = (1/35)(4(\nu_3 - \nu_2) - 3\nu_1)$$

From the transition energies and band assignments included in Table IV, the ligand field parameters for $[CuL_2(H_2O)_2]$ complex were calculated. Derived values of ligand field parameters are as follows: Dq = 1880 cm^{-1}; Ds = −2642 cm^{-1}; Dt = −1246 cm^{-1}.

TABLE IV. Parameters of the Composite Bands Resulting from Gaussian Analysis of the Absorption Spectrum of $[CuL_2(H_2O)_2]$ (L = L-α-alaninehydroxamate ion)[a]

Assignment	ϵ (dm^3/mol cm)	ν (cm^{-1})	TW (cm^{-1})	f
$^2B_{1g} \rightarrow {}^2A_{1g}$	33.585	16799.2	4147.8	6.404×10^{-4}
$^2B_{1g} \rightarrow {}^2B_{2g}$	48.113	18824.0	4191.8	9.272×10^{-4}
$^2B_{1g} \rightarrow {}^2E_g$	29.558	20517.7	5066.8	6.855×10^{-4}
Unassigned band	712.121	31853.4	7212.0	2.360×10^{-2}

[a]RMS% = 0.56; region of spectrum: 12 000–25 000 cm^{-1}.

Conclusion

The above potentiometric, ESR and spectroscopic results have shown that L-α-alaninehydroxamic acid coordinates to Cu(II) via the α-amino nitrogen and the nitrogen atom of the -NHOH group and that aqueous solutions contain dimeric species in the pH range 4.0–6.0. In the pH range 7.4–8.3 only the CuL_2 species is suggested. On the basis of the results of Gaussian analysis of electronic spectrum, the diagram for the CuL_2 species of D_{4h} symmetry was assumed to be relevant for this species.

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