BINUCLEAR COMPLEXES OF AMINOACETOHYDROXAMIC ACID WITH COPPER(II) AND IRON(III)

Rolf KARLÍČEK and Miroslav POLÁŠEK

Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, 501 65 Hradec Králové

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Aminoacetohydroxamic acid (glycinehydroxamic acid, $H_2NCH_2CONHOH$) (HL) forms the complex species $FeHL^{3+}$, $Cu_2H_{-1}L^{2+}$ and $CuFeH_{-1}L^{3+}$ at $c_M > c_L$. The two binuclear complexes were proved to be formed with the elimination of two hydrogen ions from one hydroxamic functional group, the amide nitrogen atom of the hydroxamic group taking part in the coordination. The spectrophotometric characteristics of the complexes and the equilibrium constants of the reactions involved are given.

The chemistry and, in particular, biochemistry of hydroxamic acids are increasingly attracting interest owing to the fact that the oxidized peptidic —CON(OH)— group is present in a number of substances produced by molds, fungi and yeasts[1]. The hydroxamic group is the most important functional group in natural substances that bind iron and thus play a significant role in the transport of iron in living organisms. In addition to iron, other trace metals such as Mn, Co, Ni, Cu, Mo, and V, whose biological role is not yet fully understood, are also present in organisms; all of them can form complex compounds with hydroxamic acids.

The formation and stability of complex hydroxamates and their biological effects are affected appreciably by the occurrence of additional functional groups in the molecule of the hydroxamic acid. Natural hydroxamic acids of the type of desferrioxamine B contain, in addition to the hydroxamic group, an amino group. For this and other reasons, particular attention is being paid to aminoacetohydroxamic acid and its derivatives as suitable model substances with simple structures. For a series of N-acyl derivatives of aminoacetohydroxamic acid, evidence has been gained that these substances inhibit the activity of urease both *in vitro* and *in vivo*. This inhibiting effect has found therapeutic use in the prevention and curing of urolithiasis. Moreover, aminoacetohydroxamic acid has a capacity of inhibiting the enzyme RNA reductase, thus exhibiting some antitumor activity[2].

The preparation of aminoacetohydroxamic acid was described as early as 1913 by Lay and Mänchen[3], who also isolated its copper(II) complexes with the compositions Cu : L = 1 : 1 and 1 : 2. The formation of copper(II) and iron(III) complexes of aminoacetohydroxamic acid in solution was examined polarographically by Cieleszky

and coworkers[4], who established the formation of complexes with the ratio Cu : L = = 1 : 1 and 1 : 2 and determined their stability; they give no data as to the composition and stability of the iron complexes. The copper(II), nickel(II), and iron(III) complexes were studied in more detail by Majer and coworkers[5]. More recently, the composition, stability and structure of the copper(II) complexes in solutions were investigated by Paniago and Carvelho[6]. The iron(III) complexes were dealt with by Biruš and coworkers[7] and by Brown and coworkers[8]; Brown and Rochee[9] also described the nickel complex.

In all studies except ref.[7], the solution contained the ligand in an excess with respect to the metal ion ($c_L > c_M$).

In the present work the properties of complexes of aminoacetohydroxamic acid are investigated in solutions with excess copper(II) or iron(III) ions or both; the complexes formed are found to differ in composition and structure from those reported by the previous authors.

EXPERIMENTAL

Apparatus and Chemicals

The pH was measured with a PHM-64 pH-meter equipped with a GK 2401 B combined electrode (Radiometer, Denmark). Prior to measurements, the system was calibrated using standard buffers: potassium tetraoxalate, $c = 0{\cdot}05$ mol l^{-1}, pH 1·675; potassium hydrogen phthalate, $c = 0{\cdot}05$ mol l^{-1}, pH 4·002; a mixture of equal volumes of equimolar solutions ($c = 0{\cdot}025$ mol . $.l^{-1}$) of KH_2PO_4 and Na_2HPO_4, pH 6·881; sodium tetraborate, $c = 0{\cdot}01$ mol l^{-1}, pH 9·225 at 20°C. The pH adjustment of the solutions examined was performed with 0·1M or 1M-NaOH or $HClO_4$ solutions using an OP 930 plunger microburette (Radelkis, Hungary). Analytical concentration of hydrogen ions was calculated, for solutions of pH < 1·5, from the volume and concentration of the perchloric acid added; for the remaining solutions, from the observed activity of hydrogen ions using the experimental value of $\gamma_{H^+} = 0{\cdot}803$. All pH data are given in the concentration scale. The measurements were conducted at 20°C with nitrogen flushing; the ionic strength of the solutions was adjusted to $I = 0{\cdot}1$ with sodium perchlorate.

Spectrophotometric measurements were performed on an SP 8-200 instrument (Unicam, U.K.) interfaced to a printer (T-100 teletype) using standard cells $l = 1$ cm and special 100 ml cells $l = 3{\cdot}48$ cm allowing for simultaneous pH and absorbance adjustment and measurement[10].

Aminoacetohydroxamic acid was synthesized following the procedure by Safir and Williams[11]; its identity was verified by melting point measurements (140–141°C; ref.[11] 142°C) and elemental analysis. The purity of the acid was tested by potentiometric neutralization titration and found to be 99·2%. Fresh solutions of this compound were prepared daily. The stock solutions of $Cu(ClO_4)_2$ and $Fe(ClO_4)_3$, $c = 0{\cdot}1$ mol l^{-1}, were prepared from the respective chemicals (Fluka, Switzerland) and their actual concentrations were determined chelatometrically.

The composition of the complexes was determined by the modified molar ratios method. The molar absorptivities of the complexes, numbers of protons eliminated during the complex formation reactions and their equilibrium constants were determined by graphical analysis of the spetrophotometric data[12,13] employing homemade programs for the TI-59 calculator. The dissociation constants of the substance in the overlapping buffering regions were calculated

by the homemade program DISCO-2-POT for the TI-59, which is based on the multiple linear regression[14]; the data are reported as averages of eight replicate determinations along with the respective confidence intervals.

RESULTS AND DISCUSSION

Aminoacetohydroxamic Acid

Titration of the protonated form (H_2L^+) of aminoacetohydroxamic acid with hydroxide in aqueous solution is associated with the elimination of two protons, with an overlap of the buffering regions.

The dissociation constants obtained in this work and those found by other authors are given in Table I. The course of the acid-base titration, shape of the UV absorption curves and a comparison with aminoacetohydroxamic acid derivatives substituted at the nitrogen atom[15] give unambiguous evidence that the first proton is split off from the protonated amino group (K_{a0}), the second proton, from the hydroxamic functional group (K_{a1}), hence, in the reverse order with respect to that reported by Biruš and coworkers[7].

Which of the two hydrogen ions splits off from the hydroxamic functional group in aqueous medium will depend to a great extent on the nature of the substituents in the radical R of the hydroxamic acid RCONHOH; some hydroxamic acids behave as O-acids, others, as N-acids[16]. Elimination of both protons from a single hydroxamic functional group during acid-base titrations has not yet been observed for any hydroxamic acid.

TABLE I
Dissociation constants of aminoacetohydroxamic acid

t °C	Medium	log K_a for equilibrium		Ref.
		$[H_2L^+]/[HL][H^+]$	$[HL]/[L^-][H^+]$	
20	0·1M-$NaClO_4$	7·50 ± 0·01[a]	9·26 ± 0·05[a]	this work
20	0·004M-KNO_3	7·10	9·10	4
25	0·15M-NaCl	7·52	9·18	8
25	0·1M-$NaClO_4$	7·37	9·12	6
25	water	7·81	—	17
25	water	7·80	—	18

[a] Average of eight replicate determinations with the confidence interval at the significance leve 0·05.

Cu(II)–*Aminoacetohydroxamic Acid System*

The formation of the copper(II) chelates was examined spectrophotometrically over the region of 500–800 nm in solutions at pH 2·0–4·5 in the presence of excess copper(II) ions. All absorption curves displayed a single absorption band with the maximum at 660 nm. As follows from the evaluated A–pH curve (Fig. 1), a reaction product is created whose formation is complete at pH $\geqq$ 4·1. The molar ratios method gives evidence that the composition of the chelate is Cu : L = 2 : 1 (Fig. 2).

The analysis of the A–pH curves over the region of pH 3·1–4·0 indicates the formation of a single complex associated with the elimination of three hydrogen ions (Fig. 3). Since in this pH region, aminoacetohydroxamic acid occurs in the protonated form, the chelation can be expressed by the equation

$$2\,Cu^{2+} + H_2L^+ \rightleftarrows Cu_2H_{-1}L^{2+} + 3\,H^+ . \qquad (A)$$

The total number of three hydrogen ions released from one molecule of the protonated ligand during the coordination of copper(II) also follows from the difference of the titration curves for the potentiometric acid-base titrations of the reagent in the absence and in the presence of excess copper ions.

It is clear that in solutions with excess copper ions, the formation of the binuclear chelate is accompanied by the liberation of one hydrogen ion from the protonated amino group and two hydrogen ions from a carbohydroxamic functional group. Thus, similarly as with aliphatic peptides[17,19], the coordination of copper(II) is

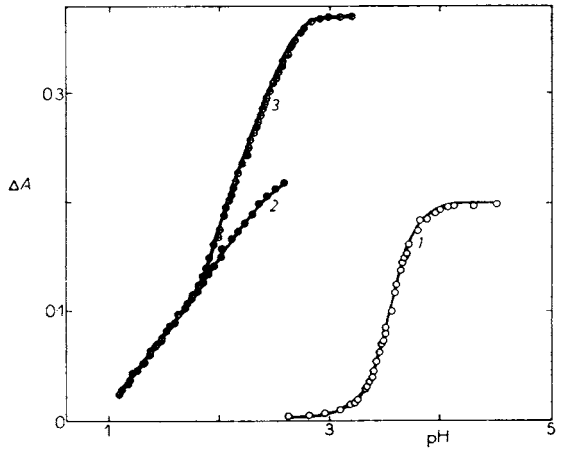


FIG. 1

A–pH curves of aminoacetohydroxamic acid complexes. c_{Cu}, c_{Fe}, c_L (mmol l^{-1}): *1* 21·1, 0, 2·22 (660 nm); *2* 0, 1·97, 0·431 (490 nm); *3* 40·0, 1·97, 0·430 (540 nm). $\Delta A = A - \varepsilon_M c_M$; l = 1 cm

associated with the liberation of the hydrogen ion of the amide group, a phenomenon that practically does not occur with the reagent alone during acid-base titration.

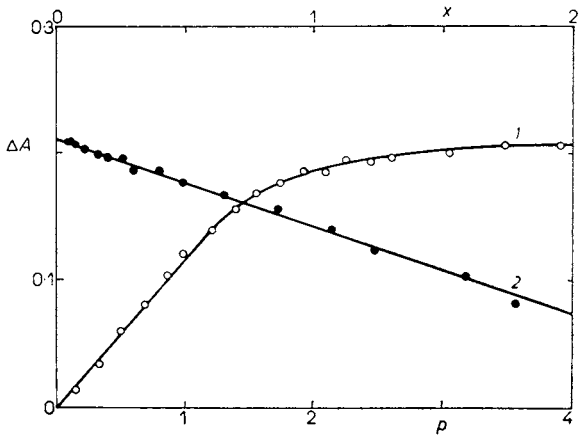


FIG. 2

Molar ratios and graphical analysis for Cu(II)–aminoacetohydroxamic acid system; pH 4·07, 660 nm, $l = 1$ cm. $\Delta A = A - \varepsilon_M c_M$. Curve *1*: $p = c_{Cu}/c_L$, $c_L = 2{\cdot}29$ mmol l^{-1}. Curve 2: $x = \Delta A/\{c_M - 2(\Delta A/\varepsilon_1 - \varepsilon_M)\}^2 . 10^{-5}$

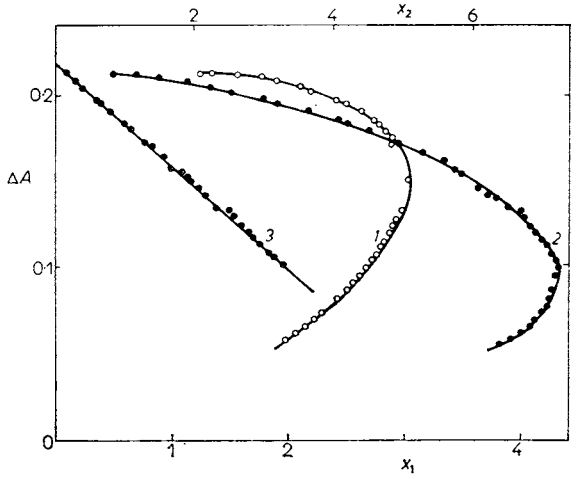
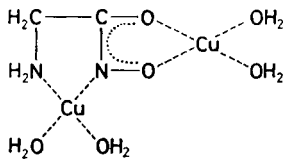


FIG. 3

Graphical analysis of the *A*–pH curves for the Cu(II)–aminoacetohydroxamic acid system; $c_{Cu} = 20{\cdot}0$ mmol l^{-1}, $c_L = 2{\cdot}39$ mmol l^{-1}, 660 nm, pH 3·35—3·98; $\Delta A = A - \varepsilon_M c_M$. Curves: *1* $x_1 = \Delta A[H^+] . 10^5$; *2* $x_2 = \Delta A[H^+]^2 . 10^9$; *3* $x_1 = \Delta A[H^+]^3 . 10^{12}$

For the binuclear $Cu_2H_{-1}L^{2+}$ chelate in question, a structure arrangement can be assumed where the nitrogen atom of the deprotonated amino group and the deprotonated nitrogen atom of the hydroxamic group take part in the coordination of one cupric ion, and the two oxygen atoms of the hydroxamic group, in the coordination of the other central ion (Structure *I*).



I

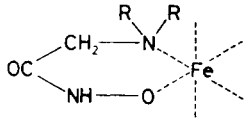
Thus, in solutions with excess copper the chelate formed is of a type different from that occuring in solutions with excess ligand[5,6] (Tables II and III).

Fe(III)–*Aminoacetohydroxamic Acid System*

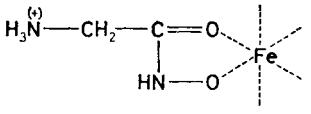
In conditions of excess iron(III) over ligand at pH < 2·3, a single chelate with the absorption maximum at 490 nm range is formed; its composition is Fe : L = 1 : 1. It also follows from the evaluated *A*–pH curve (Fig. 1, curve 2) that only one proton is liberated during the complex formation:

$$Fe^{3+} + H_2L^+ \rightleftarrows FeHL^{3+} + H^+ . \qquad (B)$$

Spectral properties of this complex and the equilibrium constant of the reaction are given in Tables II and III; partial hydrolysis of iron(III) ions was taken into account, using the value[20] of $\log K_h = -2{\cdot}63$ at $I = 0{\cdot}1$ for $K_h = [Fe(OH)][H]/[Fe]$. A chelate of the same composition and properties has been identified by Biruš and coworkers[7] in solutions containing a 300-fold excess of iron(III) ions. It has been shown[8] that the same hydrogencomplex, $FeHL^{3+}$, is formed at pH < 2·4 also in the presence of excess ligand and that in complexes of aminoacetohydroxamic acid of the compositions FeL^{2+} and FeL_3, the nitrogen atom of the amino group takes part in the coordination of the central ion (Structure *II*) similarly as is the case with N-derivatives of this acid[15]. In the case of the $FeHL^{3+}$ complex, however, the



II



III

amino group clearly remains protonated and the complex has the same structure arrangement as iron(III) complexes of simple aliphatic hydroxamic acids, *i.e.*, the two oxygen atoms are engaged in the coordination (Structure *III*).

Cu(II)–Fe(III)–*Aminoacetohydroxamic Acid System*

Studying the spot tests for hydroxamic acid, we observed that if copper(II) and iron(III) ions are simultaneously added to solutions of some hydroxamic acids, an intense blue-violet to blue colour different from that induced by iron(III) or copper(II) ions alone appears. This colour reaction occurs only with aliphatic or aromatic hydroxamic acids that contain additional functional groups with suitable donor atoms, particularly the amine nitrogen atom; aminoacetohydroxamic acid is among them.

TABLE II

Spectrophotometric characteristics of aminoacetohydroxamic acid complexes

Complex	Optimum pH range	c_{Cu}	c_{Fe}	c_L	λ_{max}	ε
		mmol l^{-1}			nm	1 mol^{-1} cm^{-1}
$Cu_2H_{-1}L^{2+}$	3·1—4·0	20·0	—	2·42[a]	660	91·7
		6·2—12·4	—	2·29	660	92·1
$FeHL^{3+}$	1·2—2·3	—	1·97	0·431[b]	490	730
	1·2—1·8	40·0	1·97	0·430[c]	490	915
$CuFeH_{-1}L^{3+}$	2·0—2·7	40·0	1·97	0·430[d]	540	980

[a–d] Data valid also for Table III.

TABLE III

Equilibrium constants for aminoacetohydroxamic acid complexes at 20°C, $I = 0{\cdot}1$ ($NaClO_4$

Equilibrium	Constant	log *K*
$[Cu_2H_{-1}L][H]^3/[Cu]^2[H_2L]$	$^*K_{21}$	−6·76[a]
$[FeHL][H]/[Fe][H_2L]$	$^*K_{1H}$	0·85[b]
$[FeHL]/[Fe][HL]$	β_{1H}	8·35
$[FeHL][H]/[Fe][H_2L]$	$^*K_{1H}$	0·85[c]
$[CuFeH_{-1}][H]^2/[FeHL][Cu]$	$^*K_{11}$	−3·40[d]

[a–d] Concentrations as in Table II.

In solutions of aminoacetohydroxamic acid containing both copper(II) and iron(III) ions in an excess with respect to the ligand, a new type of complex forms whose properties are markedly different from those of the binary copper and iron chelates, respectively (Fig. 4). The formation of this complex was examined spectrophotometrically over the 400–700 nm region at pH 1–4 in solutions with different metal-to-ligand ratios, $c_M/c_L = 1$ to 100. Solutions of pH < 1·5 are red in colour and they exhibit an absorption maximum at 490 nm that is shifted to 540 nm with increasing pH. One of the *A*–pH functions (Fig. 1, curve 3) shows that at the concentrations used, the formation of the blue-violet chelate is complete at pH 2·7. The molar ratios method shows that the complex composition is Cu : Fe : L = = 1 : 1 : 1.

The analysis of the *A*–pH curves gives evidence that at pH < 1·8, similarly as in the simple Fe(III)–aminoacetohydroxamic acid system, the hydrogencomplex $FeHL^{3+}$ is first formed according to Eq. (*B*) (Tables II and III). At pH > 1·8, the formation of the complex exhibiting the absorption maximum at 540 nm is associated

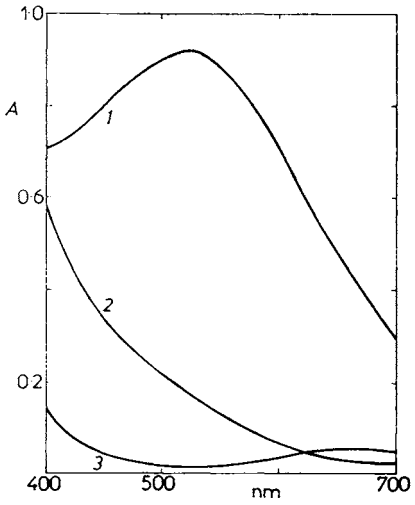


FIG. 4

Absorption curves of aminoacetohydroxamic acid complexes. $c_{Cu} = 5{\cdot}0$ mmol l^{-1}, $c_{Fe} =$ $= 0{\cdot}51$ mmol l^{-1}, $c_L = 0{\cdot}49$ mmol l^{-1}; pH 3·7, $l = 4{\cdot}00$ cm. Curves: *1* system with Cu and Fe, *2* system with Fe, *3* system with Cu

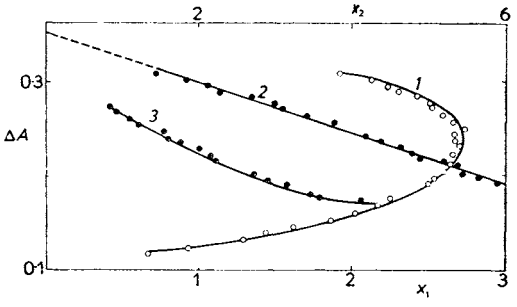


FIG. 5

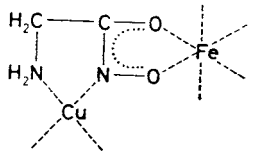
Graphical analysis of the *A*–pH dependence of the Cu(II)–Fe(III)–aminoacetohydroxamic acid system; $c_{Cu} = 40{\cdot}0$ mmol l^{-1}, $c_{Fe} =$ $= 1{\cdot}97$ mmol l^{-1}, $c_L = 0{\cdot}430$ mmol l^{-1}; 660 nm, pH 1·93 to 2·71. Curves: *1* $x_2 = (\Delta A - A_{01})\,[H^+] \cdot 10^4$; *2* $x_1 = (\Delta A - A_{01})\,[H^+]^2 \cdot 10^6$; *3* $x_1 = (\Delta A - A_{01})\,[H^+]^3 \cdot 10^8$

with the elimination of two hydrogen ion (Fig 5), so that the corresponding reaction it resumably

$$FeHL^{3+} + Cu^{2+} \rightleftarrows CuFeH_{-1}L^{3+} + 2H^{+}. \qquad (C)$$

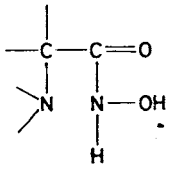
This complex is only formed upon increasing the pH of a strongly acid solution containing both copper(II) and iron(III) ions and/or in solutions of pH > 1·8 if first a solution of cupric ions and thereafter a solution of ferric ions are added to the reagent.

This gives an indirect evidence that the nitrogen atom of the amino group and the oxygen atom of the hydroxamic group take part in the coordination of iron in the "normal" iron complex, FeL^{2+} (Structure *II*). The stability of this chelate is so high that an addition of copper ions cannot induce structure changes and the formation of a heteronuclear complex is not possible. It is also clear that both oxygen atoms of the hydroxamic group are engaged in the coordination of iron in the hydrogen-complex $FeHL^{3+}$ (Structure *III*), owing to which the noncoordinated nitrogen atom of the amino group and the amidic nitrogen atom of the hydroxamic group can consecutive coordinate copper (with the elimination of the proton from the amidic nitrogen) to form the $CuFeH_{-1}L^{3+}$ binuclear complex (*IV*).



IV

This is a case of a heterobinuclear complex where the amide nitrogen atom of the hydroxamic group, from which the two hydrogen ions have split off, participates in the coordination. Hence it can be understood that the analogous heteronuclear chelates are formed neither by acyl derivatives of N-methylhydroxylamine[22] nor by any aliphatic or aromatic hydroxamic acid that is not derived from α-amino acids. It can be inferred that a prerequisite for the formation of heteronuclear chelates of hydroxamic acids of the type in question is the functional arrangement of the ligand shown in Structure *V*.



V

It follows from the study of nickel complexes of aminoacetohydroxamic acid that similarly as copper, nickel is coordinated *via* the nitrogen atom of the amino group and the nitrogen atom of the hydroxamic group giving rise to square planar complexes while the oxygen atoms of the hydroxamic group do not participate in the coordination[9]. This is consistent with our preliminary observation that nickel(II) and iron(III) ions form with aminoacetohydroxamic acid a heterobinuclear complex analogous to $CuFeH_{-1}L^{3+}$.

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