Supporting information

The Cu(II) complex of β40 peptide in ammonium acetate solutions. Evidence for ternary species formation.

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The meta-analysis of Cu(II) binding by acetate and ammonia

We used the IUPAC Stability Constant Database [S1] to collect sets of literature reports on acetate and ammonia protonation constants and Cu(II) complexes at 25 °C. Our goal was to identify or deduce the set of binding constants appropriate for ammonium acetate buffers used in experiments on Cu(II) binding to Aβ40. Two papers dealt with the acetic acid protonation at low ionic strengths, with the $\mathrm{p}K_\mathrm{a}$ values of 4.6 for $I = 0.1$ and 4.7 for lower I values, corresponding to 5, 16 and 30 mM ammonium acetate at pH 7.4 [S2, S3]. Other laboratories delivered the corresponding $\mathrm{p}K_\mathrm{a}$ values of 4.57-4.58 for $I = 0.1$ [S4-S7]. We therefore chose the consensus $\mathrm{p}K_\mathrm{a}$ values for acetic acid as 4.7 at low ionic strengths and 4.6 at $I \sim 0.1$.

The majority of studies on cupric acetate complexes reported two species: $\mathrm{Cu(AcO)^+}$ and $\mathrm{Cu(AcO)_2}$. Among the remaining ones, some reported just the $\mathrm{CuAcO^+}$ species, while two of them proposed a set of species including $\mathrm{CuAcO^+}$, $\mathrm{Cu(AcO)_2}$, $\mathrm{Cu(AcO)_3^-}$ and $\mathrm{Cu(AcO)_4^{2-}}$. The supplementary table S1 presents all relevant stability constants, measured in water solutions at various ionic strengths [S5, S6, S8-S23]. Complexes with stoichiometries higher than 1:1 were measured largely at high I values ≥ 1 M.

The studies performed at $I = 0.1$ M usually reported only the $\mathrm{CuAcO^+}$ complex, with the $\log K_1^\mathrm{A}$ value of ca. 1.8. The $\log K_1^\mathrm{A}$ values provided by other studies exhibit a much higher spread. Figure S1 presents a meta-analysis of these data. One can see a slight tendency for increase of both $\log K_1^\mathrm{A}$ and $\log \beta_{12}$ values with increasing I (Fig. S1A). The trend lines yielded values extrapolated to $I = 0$ M equal to 1.6 ± 0.2, and 2.4 ± 0.2, respectively. However, limiting this analysis to log K_2^A values, one obtains no dependence on I, with the average value of 0.9 ± 0.1 (Fig. S1B). Taking into account these results we chose the consensus log K_1^A and log K_2^A values for dilute ammonium acetate buffers as 1.7 and 2.6, respectively. The respective constant values at $I \sim 0.1$ are higher by 0.1 log units. We decided to ignore the formation of higher cupric acetate complexes, $Cu(AcO)_3^-$ and $Cu(AcO)_4^{2-}$, because they are obviously formed only in very highly concentrated acetate solutions.

The situation is simpler for ammonia and its complexes. There is very little variation in published logarithmic protonation constant values of ammonia, obtained at $I = 0.1$ M [S9, S24-S26] and 0 M [S27-S31], 9.35 ± 0.04 and 9.24 ± 0.02, respectively. We chose the former of these values for 60 and 100 mM ammonium acetate buffers, and the latter one for the remaining buffers.

The stability constants of cupric ammine complexes were recorded at high ionic strengths, $I = 1$ M and 2 M. There are no systematic differences among the results reported, with the uniform set of species: $Cu(NH_3)^{2+}$, $Cu(NH_3)_2^{2+}$, $Cu(NH_3)_3^{2+}$, $Cu(NH_3)_4^{2+}$ [S32-S36]. Therefore, we obtained the consensus values for all ionic strengths by a simple averaging of all respective log K values. The standard deviations of these averages were of the order of 0.1 log units. All source data are presented in Supplementary Table S2.

Table S1. Consensus values of protonation (pK_a) and Cu(II) (log K) binding constants of acetate and ammonia relevant for diluted ammonium acetate buffers.

Species	Acetate		Ammonia	
	5, 16 and 30 mM	60 and 100 mM	5, 16 and 30 mM	60 and 100 mM
HL	4.7	4.6	9.24	9.35
CuL	1.7	1.8	4.2	4.2
CuL_2	0.9	0.9	3.5	3.5
CuL_3	-	-	3.0	3.0
CuL_4	-	-	2.1	2.1

These data were supplemented by constants published for soluble Cu(II) hydroxides, $Cu(OH)^+$ (log β_{11} = –7.72), $Cu_2(OH)_2^{2+}$ (log β_{22} = –10.75), and $Cu_3(OH)_4^{2+}$ (log β_{34} = –21.38).[13] Of these, only the $Cu(OH)^+$ species had a significant contribution to Cu(II) speciation.

Spectroscopic search for ternary Cu(II)/acetate/ammonia complexes.

Two kinds of spectroscopic titrations were performed. According to the first one, solutions containing 1 mM Cu(II) and 1 M ammonium nitrate at pH values between 3.7 and 5.8 were titrated with solid sodium acetate at a constant pH, controlled with concentrated acetic acid and NaOH up to 1 M titrant concentrations. The positions of d-d absorption maxima were monitored. The aim of these experiments was to find out whether an acetate molecule may attach itself to Cu(II) ammine complexes, resulting in a shift of the absorption band envelope. The slight effects seen were coincident with acetate binding to Cu(II) aqua ion. In particular, no effect was seen at pH values above 5.0, when the Cu(II) aqua ion was superseded effectively by ammine complexes. In another series of measurements, 1 mM Cu(II) solutions in 100 mM sodium acetate at pH values between 4.8 and 5.5 were titrated in parallel with 1 M NH_4Cl and 1 M NaCl, up to 200 mM titrant concentrations. The aim of these experiments was to find out whether the presence of the acetate molecule attached to Cu(II) may enhance NH_3 binding. The net changes in the Cu(II) absorption band were negligible. Both series of experiments thus indicated an absence of ternary complexes in the ammonium acetate buffer. Therefore, the data presented in Tables S1, S2 and S3 were used to calculate conditional dissociation constants K_d^{cond} of the CuAβ40 complex from corresponding apparent constants K_d^{app} using equation S1 (the product $K_1^A K_2^A$ is equal, by definition, to β_{12} in Table S2):

$$K_d^{cond} = K_d^{app} \times (1 + K_1^A[AcO^-] + K_1^AK_2^A[AcO^-]^2 + K_1^N[NH_3] + K_1^NK_2^N[NH_3]^2 + K_1^NK_2^NK_3^N[NH_3]^3 + K_1^NK_2^NK_3^NK_4^N[NH_3]^4 + K_1^O[OH^-]) \quad (S1)$$

Table S2. Logarithms of stability constants of cupric acetate complexes derived from the IUPAC Stability Constants Database (SCD) [S1].

Method[a]	background	I [M]	Log K_1^A	Log β_{12}	Log β_{13}	Log β_{14}	SCD code	Reference
gl	oth/un	0.0	2.23	3.63	-	-	1964AMa	S8
gl	$NaNO_3$	0.10	1.81	-	-	-	1981BKb	S9
gl	KNO_3	0.10	1.85	-	-	-	1985SMf	S6
gl	KNO_3	0.10	1.85	-	-	-	1984DHa	S5
gl	oth/un	0.10	1.8	-	-	-	1960YYa	S10
gl	KCl	0.10	1.75	2.43	-	-	1983LTa	S11
kin	$NaClO_4$	1.00	1.72	-	-	-	1973HHb	S12
ISE	$NaClO_4$	1.00	1.43	2.25	-	-	1990VKb	S13
gl	$NaClO_4$	1.00	1.59	2.09	-	-	1994FGa	S14
cal	$NaNO_3$	1.00	1.33	2.31	-	-	1974ARd	S15
EMF	$NaClO_4$	1.00	1.71	2.71	-	-	1966GEa	S16
ISE	KNO_3	1.00	-	2.58	-	-	1980NWa	S17
ISE	KNO_3	1.00	-	2.58	-	-	1975NWa	S18
vlt	$NaClO_4$	1.00	1.30	2.04	-	-	1965TSb	S19
sp	$NaClO_4$	2.00	2.11	2.86	-	-	1970GFa	S20
vlt	$NaClO_4$	2.00	1.70	2.65	2.60	2.54	1968FPa	S21
ISE	$NaClO_4$	3.00	1.74	2.79	-	-	1969WAa	S22
EMF[b]	$NaClO_4$	3.00	1.87	3.12	3.58	3.33	1966GEa	S16
gl	$NaNO_3$	4.00	2.52	3.33	-	-	1963SWb	S23

[a] gl – glass electrode; kin – kinetic; ISE – ion selective electrode; cal – calorimetry; EMF – electromotoric force; vlt – voltammetry; sp – spectroscopy

[b] quinhydrone electrode

Table S3. Logarithms of stability constants of cupric ammine complexes derived from the IUPAC Stability Constants Database (SCD) [S1].

Log K_1^N	4.18	4.14	4.16	4.27	4.27
Log K_2^N	3.52	3.47	3.31	3.59	3.55
Log K_3^N	2.76	2.87	3.38	3.00	2.90
Log K_4^N	2.06	2.04	2.20	2.19	2.18
SCD code	1984NDa	1969ESb	1965MBb	1958PAa	1954LLa
Reference	S32	S33	S34	S35	S36

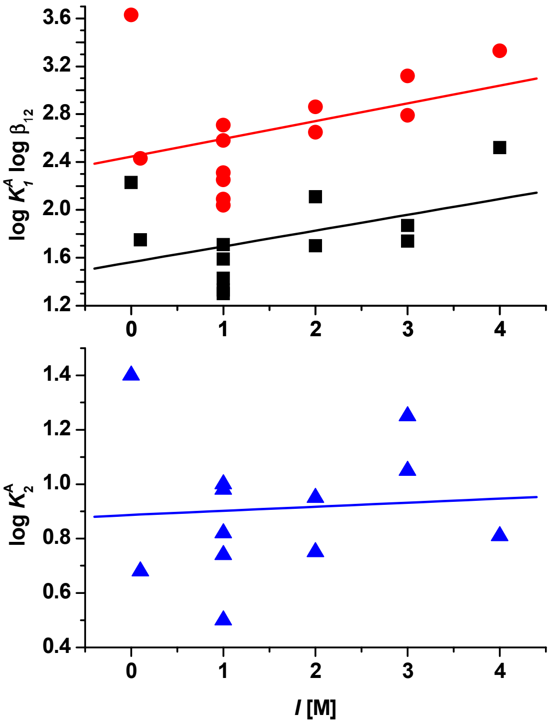

Fig. S1. Meta-analysis of literature data on stability constants of Cu(II) acetate complexes in water solutions of various ionic strengths, according to Table S2. Top, values of log K_1^A (black squares) and log β_{12} (red dots); bottom, values of log K_2^A, equal to (log β_{12} – log K_1^A). Lines represent trends obtained by linear regression.

Details of solutions used in spectrofluorimetric titrations

The total of 29 titrations were, covering five ammonium acetate buffer concentrations, from 5 mM to 100 mM, all at pH 7.4, were used to obtain apparent dissociation constants K_d^{app}. Table S4 presents concentrations of Aβ40 samples and the number of independent spectrofluorimetric titrations, performed for each sample.

Table S4. Concentrations of reagents used in spectrofluorimetric titrations

ammonium acetate concentration (mM)	Aβ40 concentration (μM)	No. of titrations
5	8.2	2
5	11.3	5
16	8.1	2
16	10.9	2
30	12.2	4
30	9.7	1
60	10.3	3
60	15.4	4
100	7.4	4
100	8.3	2

Supporting material references

[S1] IUPAC Stability Constants Database, V. 4.11, IUPAC and Academic Software, **2003**.

[S2] de Robertis, A.; de Stefano, C.; Rigano, C.; Sammartano, S.; Scarcella, R. *Chem. Res.* **1985**, 42.

[S3] de Robertis, A.; de Stefano, C.; Rigano, C.; Sammartano, S. *J. Sol. Chem.* **1990**, *19*, 569.

[S4] Daniele, P.; de Robertis, A.; de Stefano, C.; Rigano, C.; Sammartano, S. *Ann. Chim.(Rome)*, **1983**, *73*, 619.

[S5] Dubler, E.; Haering, U. K.; Scheller, K. H.; Baltzer, P.; Sigel, H. *Inorg. Chem.* **1984**, *23*, 3785.

[S6] Sigel, H.; Malini-Balakrishnan, R.; Häring, U. K. *J. Am. Chem. Soc.* **1985**, *107*, 5137.

[S7] Liang, G.; Tribolet, R.; Sigel, H. *Inorg. Chem.* **1988**, *27*, 2877.

[S8] Archer, D. W.; Monk, C. B. *J. Chem. Soc.* **1964**, 3117.

[S9] Banerjea, D., Kaden, T., Sigel, H. *Inorg. Chem.* **1981**, *20*, 2586.

[S10] Yasada, M.; Yamasaki, K.; Ohtaki, H. *Bull. Chem. Soc. Jpn.* **1960**, *33*, 1067.

[S11] Linder, P. W.; Torrington, R. G.; Seemann, U. A. *Talanta* **1983**, *30*, 295.

[S12] Hutchinson, M. H.; Higginson, W. C. E. *J. Chem. Soc., Dalton Trans.* **1973**, 1247.

[S13] Vasilev, V.; Khochenkova, T. *Zhur. Neorg. Khim.* **1990**, *35*, 2581.

[S14] Foti, C.; Gianuzza, A.; Licastro, F. *Ann. Chim. (Rome)* **1994**, *84*, 295.

[S15] Aruga, R. *Ann. Chim. (Rome)* **1974**, *64*, 659.

[S16] Gerding, P. *Acta Chem.Scand.*, **1966**, *20*, 2664.

[S17] Nakagawa, G.; Wada, H.; Sako, T. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 1303.

[S18] Nakagawa, G.; Wada, H.; Hayakawa, T. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 424.

[S19] Tanaka, N.; Saito, Y.; Ogino, H. *Bull. Chem. Soc. Jpn.* **1965**, *38*, 984.

[S20] Grabaric, B.; Filipovic, I. *Croat. Chem. Acta* **1970**, *42*, 479.

[S21] Filipovic, I.; Piljac, I.; Medved, A.; Savic, S.; Bujak, A.; Bach-Dragutinovic, B.; Mayer, B. *Croat. Chem. Acta* **1968**, *40*, 131.

[S22] Warnke, Z. *Rocz. Chem.*, **1969**, *43*, 1939.

[S23] Swinarski, A.; Wojtczakowa, J. *Z. Phys. Chem.* **1963**, *223*, 345.

[S24] Saha, N.; Sigel, H. *J. Am. Chem. Soc.* **1982**, *104*, 4100.

[S25] Saha, A.; Sigel, H. *Inorg. Chim. Acta* **1982**, *66*, L29.

[S26] Paoletti, P.; Stern, J. H.; Vacca, A. *J. Phys. Chem.* **1965**, *69*, 3759.

[S27] Binder, A.; Ebel, S. *Zhur. Anal. Khim.* **1974**, *272*, 16.

[S28] Komar, N.; Zung, N. *Zhur. Neorg. Khim.* **1967**, *12*, 1265.

[S29] Everett, D.; Landsman, D. *Trans. Faraday Soc.* **1954**, *50*, 1221.

[S30] Bates, R.; Pinching, G. *J. Res. Nat. Bur. Stand.* **1949**, *42*, 419.

[S31] Everett, D.; Wynne-Jones, W. *Proc. Roy. Soc. (A)* **1938**, *169*, 190.

[S32] Nagypal, I.; Debreczeni, F. *Inorg. Chim. Acta*, **1984**, *81*, 69.

[S33] Evtimova, B.; Scharff, J.; Paris, M. *Bull. Soc. Chim. Fr.* **1969**, 81.

[S34] Mercier, R.; Bonnet, M.; Paris, M. *Bull. Soc. Chim. Fr.* **1965**, 3577.

[S35] van Panthaleon, C. Thesis, Leiden University **1958**.

[S36] Lloyd, R. Thesis, Temple University, Microf. 12401 **1954**.