Kinetics and Mechanism of the Hydrolysis of α-Amino-acid Esters in Mixed-ligand Complexes with Copper(II) Ethylenediaminemonoacetate

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Amino-acid esters (EH^+) interact with $[Cu(edma)]^+$ to give mixed-ligand complexes in accordance with the equilibrium, $[Cu(edma)]^+ + EH^+ \rightleftharpoons [Cu(edma)E]^+ + H^+$, where EH^+ is the protonated ester $\overset{+}{N}H_3CH(R)CO_2R'$ and edma$^-$ = ethylenediaminemonoacetate. The ternary complexes are only formed over a narrow pH range (7.3—8.2) and 8.4—8.8 with methyl L-histidinate. At higher pH values there is kinetic evidence for the competing equilibrium, $[Cu(edma)E]^+ + OH^- \rightleftharpoons [Cu(edma)(OH)]^0 + E$. Rate constants have been obtained at I = 0.1 mol dm^{-3} and 25 °C for both base hydrolysis and solvolysis of the ester group of a series of α-amino-acid esters, while co-ordinated (probably chelated) in the ternary complexes. Base hydrolysis is some 10^3 times faster than for the unprotonated esters (E), and possible mechanisms for these reactions are considered.

TRANSITION-METAL ions and complexes have long been known to promote the hydrolysis of α-amino-acid esters, and the topic has been recently reviewed.[1] To various degrees these studies have been said to mimic the role played by metal ions in metalloenzymes. Angelici and co-workers have studied the base hydrolysis of α-amino-acid esters in mixed-ligand complexes of the type $[CuL\{NH_2CH(R)CO_2R'\}]^{n+}$ using ligands (L) such as iminodiacetate (ida),[2] nitrilotriacetate (nta),[3] 2,2′,2″-tris(aminoethyl)amine (tren),[4] terpyridyl (terpy),[4] diethylenetriamine (dien),[5] and bis(2-pyridylmethyl)-amine (bpma).[6] These authors [6] first noted a correlation between the formation constant K_{CuL} of the metal complex and the catalytic activity of the complex. It was found that large formation constants resulted in reduced Lewis-acid character of the metal complex and therefore lower base hydrolysis rates of the α-amino-acid esters in the ternary complex. A further paper dealing with correlations of this type has recently appeared.[4]

$$Cu^{2+} + L \xrightleftharpoons{K_{CuL}} CuL^{2+} \qquad (1)$$

The present paper deals with the base hydrolysis of a series of α-amino-acid esters in mixed-ligand complexes of the type $[Cu(edma)\{NH_2CH(R)CO_2R'\}]^+$ where edma = ethylenediaminemonoacetate. These complexes are positively charged and it is of interest to compare their reactivities with the corresponding $[Cu(nta)\{NH_2CH(R)CO_2R'\}]^-$ complexes which carry a negative charge. The K_{CuL} values are similar (edma = 13.29 and nta = 13.10) for these two systems.

EXPERIMENTAL

The ligand 1,2-diaminoethane-*N*-monoacetic acid dihydrochloride monohydrate (Hedma·2HCl·H_2O) was prepared by a modification of a previously reported method.[7] A solution of chloroacetic acid (94.5 g) in water (100 cm^3) was neutralised with an equimolar quantity (84 g) of solid sodium hydrogencarbonate. Ethylenediamine (60 g) was then added dropwise to the solution. On completion of the addition a solution of sodium hydroxide (40 g) in water (80 cm^3) was added. The mixture was then evaporated to dryness on a rotary evaporator and the residue obtained heated at *ca.* 90 °C in a vacuum oven for several hours to remove any unreacted ethylenediamine. The residual solid was repeatedly washed with ethanol and then dissolved in the minimum volume of hot water and the solution acidified to pH 3 with hydrochloric acid (12 mol dm^{-3}). The solvent was removed on a rotary evaporator, and the oily residue treated with concentrated hydrochloric acid (350 cm^3). At this stage some sodium chloride precipitated and was filtered off. Ethanol–diethyl ether (350 cm^3, 1 : 1 v/v) was added to the filtrate, which was then allowed to stand overnight in an ice-bath. The white *product* so obtained was filtered off and recrystallised from 50% (v/v) water–ethanol containing a few drops of concentrated hydrochloric acid. The compound gives off hydrogen chloride at *ca.* 89 °C and the residue has a sharp m.p. at 145 °C.[8] Potentiometric titration gave M = 206 (Calc. 209), the ligand has ν(CO) at 1 740 cm^{-1} (Found: C, 22.9; H, 6.7; N, 13.0. Calc. for $C_4H_{10}N_2O_2$·2HCl·H_2O: C, 23.0; H, 6.7; N, 13.4%).

The α-amino-acid esters used in the study were obtained from Fluka and used as received. All solutions were prepared with deionised water which was degassed before use. AnalaR potassium nitrate was used to maintain the ionic strength of solutions at 0.1 mol dm^{-3}. Metal salt solutions were prepared from $Cu[NO_3]_2 \cdot 3H_2O$. Sodium hydroxide solutions were prepared from B.D.H. CVS ampoules and diluted appropriately.

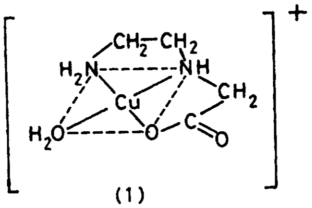
Kinetics and Measurements.—All kinetic measurements were carried out with a Radiometer TTT2 automatic titrator used as a pH-stat. A high-alkalinity glass electrode, type G202B, was used as indicator electrode and a saturated calomel electrode with diffusion filter, type K401, as reference electrode. The electrode system was standardised at 25 ± 0.1 °C with 0.01 mol dm^{-3} $Na_2[B_4O_7] \cdot 10H_2O$ (pH = 9.185) and 0.025 mol dm^{-3} phosphate buffer (pH = 6.86). The general technique employed in the kinetic measurements has been outlined.[9] All kinetic studies were carried out at I = 0.1 mol dm^{-3} ($K[NO_3]$) and 25 ± 0.1 °C. Values of the hydroxide-ion concentration were obtained from the pH using a molar activity coefficient of 0.775 [10] and a value of pK_W = 14.00 at 25 °C.[11] The solutions used in the kinetic measurements were 7×10^{-3} mol dm^{-3} in copper(II) ethylenediaminemonoacetate and 7×10^{-4} mol dm^{-3} in the α-amino-acid ester. A 10% excess of ethylenediaminemonoacetate over the copper(II) solution was employed to ensure complete complexation of the metal ion. Under the conditions of the experiments the α-amino-acid esters were not fully bound to $[Cu(edma)]^+$.

The percentage of mixed-ligand complex varied over the range 40—80%, the lowest percentage occurring with methyl glycinate (glyOMe) and the highest with methyl histidinate. Attempts to increase the amount of ternary complex in solution by increasing the ratio of $[Cu(edma)]^+$: ester to greater than 10 : 1 led to precipitation and irreproducible data. However, under the conditions of pH employed, the unbound ester does not undergo any measurable hydrolysis and the infinity values (V_∞) were quite stable and reproducible. A 5 : 1 ratio of $[Cu(edma)]^+$: α-amino-acid ester gave, within experimental error, the same results as 10 : 1 ratios.

Rate constants ($k_{obs.}$) were calculated from data obtained from plots of log ($V_\infty - V_t$) against time, where V_∞ is the final volume of base consumed and V_t that consumed at time t.

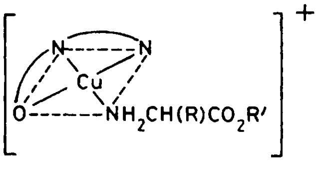
RESULTS AND DISCUSSION

Pilbrant [8] has determined the pK_a values of ethylenediaminemonoacetic acid ($pK_1 = 1.59$, $pK_2 = 6.67$, and $pK_3 = 9.82$) and the stepwise formation constants with copper(II) (log $K_1 = 13.29$ and log $K_2 = 7.76$) at 25 °C and $I = 0.1$ mol dm^{-3} ($Na[ClO_4]$). The potentiometric data are consistent with the formation of complex (1) in which the ligand is tridentate since three protons are liberated on formation of the 1 : 1 complex. The additional equatorial site is expected to be occupied by a water molecule.



(1)

α-Amino-acid esters can act as either unidentate ligands (*via* the α-NH_2 group) or bidentate ligands (*via* the α-NH_2 group and the carbonyl oxygen of the ester). As a result, ternary complexes with α-amino-acid esters could be formulated as either (2) or (3).



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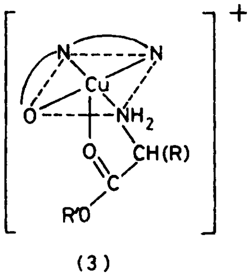

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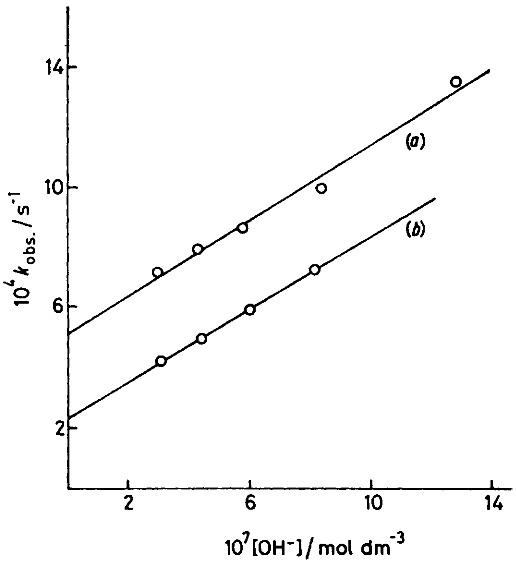


Plots of $k_{obs.}$ against the hydroxide-ion concentration for (*a*) ethyl glycinate and (*b*) ethyl L-β-phenylalaninate complexes

Formation of the ternary complex can be represented by the equilibria (2) and (3) where EH^+ is the protonated

$$[Cu(edma)]^+ + EH^+ \rightleftharpoons [Cu(edma)E]^+ + H^+ \quad (2)$$

$$[Cu(edma)]^+ + E \rightleftharpoons [Cu(edma)E]^+ \quad (3)$$

form of the amino-acid $[\overset{+}{N}H_3CH(R)CO_2R']$ and E is the unprotonated species. The pK_a for the equilibrium $EH^+ \rightleftharpoons E + H^+$ normally lies within the range 7—7.5.[1] At 10 : 1 ratios of $[Cu(edma)]^+$ to α-amino-acid ester (pH 7.4—8.2) significant concentrations of the ternary complex are present (40—80% of the theoretical amount). The percentage of ternary complex can be estimated from the final volume of base required to hydrolyse the ester ligand in the ternary complex [equation (4)] where $A^- = NH_2CH(R)CO_2^-$. The un-

$$[Cu(edma)E]^+ + OH^- \xrightarrow{k_{OH}} [Cu(edma)A]^0 + R'OH \quad (4)$$

complexed ester does not undergo any detectable hydrolysis in the pH range 7.4—8.2 and thus the base consumption data give a direct measure of the amount of ternary complex present in solution. The ternary complex is only formed over a narrow pH range. At higher pH values, the volume of base consumed decreases providing kinetic evidence for the competing equilibrium (5). The hydrolysis of methyl glycinate, ethyl glycinate,

$$[Cu(edma)E]^+ + OH^- \rightleftharpoons [Cu(edma)(OH)]^0 + E \quad (5)$$

methyl L-β-phenylalaninate, ethyl L-β-phenylalaninate, and ethyl L-α-alaninate were all studied in the pH range 7.4—8.2. Methyl L-histidinate was studied in the pH range 8.36—8.80.

Values of $k_{obs.}$, the observed first-order rate at constant pH for the hydrolysis of the mixed-ligand complexes [equation (4)], were obtained from plots of log ($V_\infty - V_t$). These plots were quite linear for at least three half lives. The kinetic data obtained are summarised in Table 1. Plots of $k_{obs.}$ against the hydroxide-ion concentration are linear with a positive intercept, Figure, indicating that pH-independent hydrolysis involving attack by water is also occurring. For all the systems studied $k_{obs.} = k_0 + k_{OH}[OH^-]$. Values of k_{OH} were obtained from the slopes of these plots and the rate constant k_0 from the intercept. The secondorder rate constants for water attack were obtained from the expression $k_{H_2O} = k_0/55.5$. The rate constants k_{H_2O} and k_{OH} for the hydrolysis of the various ester ligands are listed in Table 2. In addition to the reaction (4) the further hydrolytic reaction (6) also occurs in these systems. Both reactions are monitored by pH-stat

$$[Cu(edma)E]^+ + H_2O \xrightarrow{k_{H_2O}} [Cu(edma)A] + R'OH + H^+ \quad (6)$$

as reaction (4) consumes base, while reaction (6) liberates protons. The relative nucleophilicities of hydroxide ion and water as determined by the values of k_{OH}/k_{H_2O} are *ca.* 5×10^7 for these reactions, Table 2.

TABLE 1

Kinetic data for the hydrolysis of the ternary complexes $[Cu(edma)E]^+$ at 25 °C and $I = 0.1$ mol dm^{-3} (K[NO$_3$])

(*a*) [Cu(edma)]–ethyl glycinate

pH	$10^7[OH^-]$ *	$10^4k_{obs.}/s^{-1}$
7.35	2.92	7.20
7.51	4.22	7.87
7.64	5.69	8.57
7.81	8.42	9.60
7.99	12.75	13.57

% ester bound = 60—65

(*b*) [Cu(edma)]–methyl glycinate

pH	$10^7[OH^-]$	$10^3k_{obs.}/s^{-1}$
7.40	3.28	2.32
7.64	5.69	2.58
7.78	7.86	3.13
7.93	11.10	3.60

% ester bound = 40

(*c*) [Cu(edma)]–ethyl α-alaninate

pH	$10^7[OH^-]$	$10^4k_{obs.}/s^{-1}$
7.39	3.20	5.66
7.49	4.03	7.10
7.66	5.96	7.66
7.82	8.62	9.60
7.95	11.63	10.86

% ester bound = 75

(*d*) [Cu(edma)]–ethyl L-β-phenylalaninate

pH	$10^7[OH^-]$	$10^4k_{obs.}/s^{-1}$
7.37	3.06	4.21
7.53	4.42	4.95
7.66	5.96	5.95
7.79	8.04	7.28
8.16	18.85	10.43

% ester bound = 55—60

(*e*) [Cu(edma)]–methyl L-β-phenylalaninate

pH	$10^7[OH^-]$	$10^3k_{obs.}/s^{-1}$
7.37	3.06	1.92
7.56	4.74	1.98
7.64	5.69	2.06
7.70	6.54	2.21
7.87	9.67	2.54

% ester bound = 40—45

(*f*) [Cu(edma)]–methyl L-histidinate

pH	$10^6[OH^-]$	$10^4k_{obs.}/s^{-1}$
8.36	2.99	1.56
8.50	4.12	1.92
8.57	4.85	1.96
8.73	7.00	2.65
8.80	8.23	2.83

* In mol dm^{-3} throughout.

TABLE 2

Values of k_{OH} and k_{H_2O} for the hydrolysis of the mixed-ligand complexes $[Cu(edma)E]^+$ at 25 °C and $I = 0.1$ mol dm^{-3} (K[NO$_3$])

Ester	$10^5k_{H_2O}$ /dm^3 mol^{-1} s^{-1}	$10^{-3}k_{OH}$ /dm^3 mol^{-1} s^{-1}	$10^{-7}k_{OH}/k_{H_2O}$
Methyl glycinate	3.1	1.71	5.5
Methyl L-β-phenylalaninate	2.8	0.99	3.5
Ethyl glycinate	0.91	0.63	6.9
Ethyl L-α-alaninate	0.77	0.59	7.7
Ethyl L-β-phenylalaninate	0.63	0.38	6.0
Methyl L-histidinate	0.15	0.025	1.7

TABLE 3

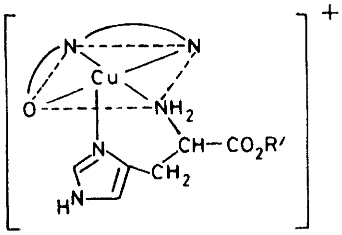
Catalytic effects for the hydrolysis of the mixed-ligand complexes $[Cu(edma)E]^+$ at 25 °C and $I = 0.1$ mol dm^{-3} (K[NO$_3$])

Ester	k_E [a] /dm^3 mol^{-1} s^{-1}	$10^{-3}k_{OH}$ /dm^3 mol^{-1} s^{-1}	$10^{-3}k_{OH}/k_E$
Methyl glycinate	1.28	1.71	1.34
Methyl L-β-phenylalaninate	0.55	0.99	1.18
Ethyl glycinate	0.64	0.63	1.0
Ethyl L-α-alaninate	0.55 [b]	0.59	1.1
Ethyl L-β-phenylalaninate	0.24	0.38	1.6
Methyl L-histidinate	0.62	0.025	0.04

[a] Rate constants for $NH_2CH(R)CO_2R' + OH^- \longrightarrow NH_2CH(R)CO_2^- + R'OH$. Data taken from ref. 1. [b] Approximate value obtained by halving the reported constant for the methyl ester (ref. 1).

With the exception of methyl L-histidinate the α-amino-acid esters undergo base hydrolysis at similar rates in the ternary complexes, with values of k_{OH} in the range 0.4×10^3—1.7×10^3 dm^3 mol^{-1} s^{-1} at 25 °C and $I = 0.1$ mol dm^{-3}. The methyl esters of α-amino-acids normally hydrolyse 2—3 times faster than the corresponding ethyl esters in base hydrolysis[1] and similar leaving-group effects occur in the ternary complexes. The ester ligands in the ternary complexes undergo base hydrolysis some 10^3 times faster than the free ester ligands $NH_2CH(R)CO_2R'$ although considerably lower catalytic effects occur with methyl L-histidinate where the value of k_{OH}/k_E is only 40, Table 3. The magnitude of the catalytic effects as determined by the values of k_{OH}/k_E suggest that the α-amino-acid esters may be bidentate as shown in structure (3) with a rather weak alkoxycarbonyl–metal ion interaction. In the case of the methyl histidinate complex, bidentate co-ordination could occur *via* the α-amino-group and the pyridine nitrogen of the imidazole ring as illustrated in structure (4), and this may well account for the lower reactivity of this ester in the ternary complex.

The present data do not exclude the possibility of intramolecular processes involving attack by co-ordinated hydroxide as illustrated in (5), however this process appears unlikely since the pK_a value of a co-ordinated water molecule on copper(II) is normally 6—7. A first-order dependence on the hydroxide-ion concentration would only occur if 10% of the aquo-complex were converted to the conjugate base as otherwise the concentration of the hydroxo-complex would not be directly proportional to the hydroxide-ion concentration. Most of the measurements were extended to pH 8 where there was no divergence from a first-order dependence on the hydroxide-ion concentration. As a result the co-ordinated water would have to have a pK_a > 9 to be consistent with the experimental observations. Angelici and co-workers[6] first noted a correlation between the formation constant K_{CuL} of the metal complex [see equation (1)] and the catalytic activity of the complex. Table 4 summarises the available literature data on these systems. The Lewis acidities of the CuL^{n+} complexes decrease as the formation constant for CuL^{n+} increases, and values of k_{OH} decrease as log K_{CuL} increases. The effects of charge on these reactions does not appear to be of great importance, thus $[Cu(edma)(glyOMe)]^+$ undergoes slower base hydrolysis than $[Cu(ida)(glyOMe)]^0$ while the hydrolysis of $[Cu(bmpa)(glyOMe)]^{2+}$ is some ten times slower than the edma derivative. The effectiveness of the various $[CuL]^{n+}$ complexes in promoting the hydrolysis of α-amino-acid esters depends primarily on the strength of the binding of the ligand L to copper(II), the stronger the binding the less active the ternary complex towards base hydrolysis.



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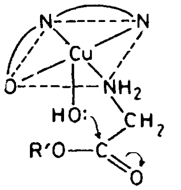

(5)

TABLE 4

Rate constants (k_{OH}) and equilibrium constants (log K_{CuL}) associated with the CuL promoted hydrolyses of methyl glycinate at 25 °C

[CuL(glyOMe)]	k_{OH}/ dm^3 mol^{-1} s^{-1}	log K_{CuL}	Ref.
$[Cu(ida)(glyOMe)]^0$	$7.6 \times 10^{3\,a}$	10.63	4
$[Cu(edma)(glyOMe)]^+$	$1.7 \times 10^{3\,a}$	13.29	This work
$[Cu(nta)(glyOMe)]^-$	$4.6 \times 10^{2\,b}$	13.10	3
$[Cu(terpy)(glyOMe)]^{2+}$	$2.2 \times 10^{2\,c}$	13.40	4
$[Cu(bmpa)(glyOMe)]^{2+}$	$1.7 \times 10^{2\,c}$	14.4	6
$[Cu(dien)(glyOMe)]^{2+}$	$1.4 \times 10^{2\,a}$	15.9	5
$[Cu(tren)(glyOMe)]^{2+}$	$1.3^{\,c}$	18.8	4

[a] I = 0.1 mol dm^{-3}. [b] I = 0.07 mol dm^{-3}. [c] I = 0.05 mol dm^{-3}.

The results are of some significance as far as metalloenzymes are concerned, since the binding constants for these systems will presumably influence their effectiveness as Lewis-acid catalysts. For the binding of Zn^{2+} to apocarboxypeptidase a value of log K = 10.5 [12] has been estimated and a similar value has been reported[13] for Zn^{2+} with apocarbonic anhydrase. Such considerations may be of importance in determining the metal-ion specificities of metalloenzymes since metal ions with high binding constants may be poor Lewis-acid catalysts.

One of us (P. B.) wishes to thank the Leverhulme Foundation and the S.R.C. for a Research Fellowship.

[9/1286 *Received, 13th August*, 1979]

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