Kinetics of Base Hydrolysis of α-Amino-acid Esters in Mixed-ligand Complexes with [Glycylglycinato(2−)]copper(II)

By **Robert W. Hay** * and **Pradyot Banerjee**, Chemistry Department, University of Stirling, Stirling FK9 4LA

Amino-acid esters (E) interact with [Cu(gly-glyO)] to give mixed-ligand complexes according to equilibria (i) and

$$[Cu(gly\text{-}glyO)] + E \rightleftharpoons [Cu(gly\text{-}glyO)E] \quad (i)$$

$$[Cu(gly\text{-}glyO)] + HE^+ \rightleftharpoons [Cu(gly\text{-}glyO)E] + H^+ \quad (ii)$$

(ii), where HE^+ represents the protonated ester $\overset{+}{N}H_3CH(R)CO_2R'$ and gly-glyO^{2-} is the dianion of glycylglycine, $NH_2CH_2CONCH_2CO_2^-$. The ternary complex is only formed over a narrow pH range (8.5—10) since at higher pH there is evidence for the following competing equilibrium. Rate constants have been determined by pH-stat at

$$[Cu(gly\text{-}glyO)E] + OH^- \rightleftharpoons [Cu(gly\text{-}glyO)(OH)]^- + E$$

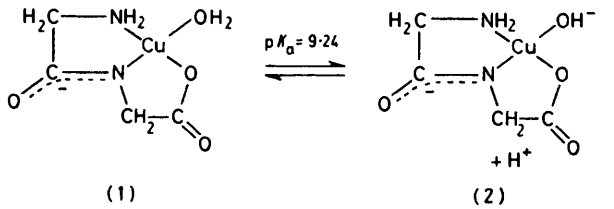
25 °C and I = 0.1 mol dm^{-3} ($K[NO_3]$) for the processes (iii) and (iv) where A^- is the anion $NH_2CH(R)CO_2^-$ and

$$[Cu(gly\text{-}glyO)E] + H_2O \xrightarrow{k_{H_2O}} [Cu(gly\text{-}glyO)A]^- + R'OH + H^+ \quad (iii)$$

$$[Cu(gly\text{-}glyO)E] + OH^- \xrightarrow{k_{OH}} [Cu(gly\text{-}glyO)A]^- + R'OH \quad (iv)$$

E = gly-OMe, gly-OEt, L−α−ala-OEt, or L−phe-OEt (ala = alanine, phe = phenylalanine). The ester ligands in the ternary complexes undergo base hydrolysis *ca.* 50 times faster than the free esters E, consistent with the formation of a mixed-ligand complex involving a unidentate ester species.

In recent years there has been considerable interest in the metal-ion-promoted hydrolysis of amino-acid esters and peptides, and the topic has been reviewed.[1] Angelici and co-workers have studied the hydrolysis of amino-acid esters in mixed-ligand complexes of copper(II) with ligands such as H_3nta (nitrilotriacetic acid) [2] and H_2ida (iminodiacetic acid).[3] Such systems can be regarded as biomimetic models for certain metalloenzymes, as the metalloenzyme–substrate complex can be considered as a special type of mixed-ligand complex. It is therefore of considerable interest to extend these investigations to mixed-ligand complexes involving peptide ligands.

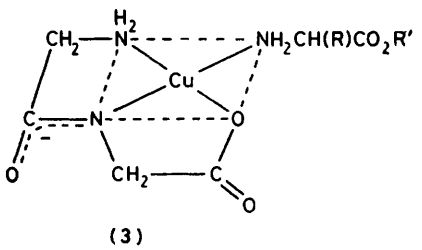


(1) (2)

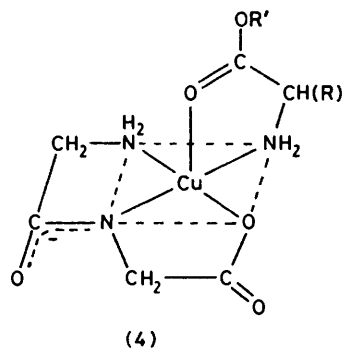
The deprotonated complex (1),[4] [Cu(gly-glyO)], is known to form mixed-ligand complexes with unidentate ligands such as imidazole [5,6] and bidentate ligands such as 1,10-phenanthroline [7] and 2,9-dimethyl-1,10-phenanthroline.[8] The last complex has been shown by *X*-ray crystallography to have a distorted square-pyramidal stereochemistry with the peptide ligand occupying three of the four equatorial sites.[8] Possible mixed-ligand complexes with α-amino-acid esters are shown in (3) and (4) where the ester ligand can be uni- or bi-dentate. Formation of these complexes should lead to accelerated rates of base hydrolysis, and the present paper describes kinetic investigations on the mixed-ligand complexes.

EXPERIMENTAL

Glycylglycine (AnalaR grade) from Koch-Light was used as received. Amino-acid ester hydrochlorides were obtained from Fluka. Copper(II) solutions were prepared from copper(II) nitrate trihydrate, and the ionic strength of solutions was maintained with potassium nitrate (AnalaR grade).



(3)



(4)

Kinetics.—Hydrolysis rates were monitored by pH-stat using a Radiometer 26 pH meter and a TTT11 titrator. Measurements were made using the procedures previously described.[9] A high-alkalinity type G202B glass electrode was used as indicator electrode and a type K401 saturated calomel electrode with diffusion filter as reference electrode. The electrode assembly was standardised at 25 ± 0.1 °C with N.B.S. phosphate buffer (pH 6.86) and borate buffer (pH 9.18).

Preliminary measurements established that a 10 : 1 ratio of [Cu(gly-glyO)] to amino-acid ester gave low titre values on complete hydrolysis when compared with the total ester concentration. At 50 : 1 ratios, the total base consumption was within 5% of the theoretical value. In each kinetic run the total solution volume was 75 cm³ with [Cu(gly-glyO)] = 3.5×10^{-2} mol dm^{-3} and [α-amino-acid ester] = 7×10^{-4} mol dm^{-3}. Sufficient K[NO_3] was present to give a total ionic strength of 0.1 mol dm^{-3}. In all the runs a 10% excess of glycylglycine was used with respect to copper(II) to ensure that no free copper(II) was present in solution. To initiate the reaction a weighed amount of the solid ester hydrochloride was added to the reaction vessel which contained the requisite concentrations of [Cu(gly-glyO)] and potassium nitrate. The pH was adjusted to the appropriate value by the addition of a few drops of concentrated Na[OH] solution. Reactions were normally followed to completion, but for some slower reactions only three half-lives were monitored. Values of the observed first-order rate constant ($k_{obs.}$) were obtained from the slopes of plots of $\ln(V_\infty - V_t)$ against time, where V_∞ is the final volume of base consumed and V_t is the volume consumed at time t. These plots were linear for at least two half-lives. Values of the hydroxide-ion concentration were obtained from the pH using $pK_w = 14.00$ at 25 °C,[10] and an activity coefficient of 0.774.[11]

Base hydrolysis could only be studied over a relatively narrow pH range (normally 8.5—10). At higher pH, values of V_∞ tended to decrease, suggesting that the following competing equilibrium was occurring:

$$[\text{Cu(gly-glyO)E}] + OH^- \rightleftharpoons [\text{Cu(gly-glyO)(OH)}]^- + E$$

RESULTS AND DISCUSSION

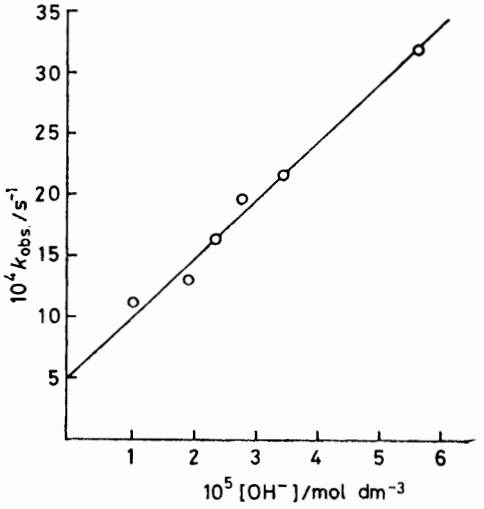
Amino-acid esters (E) and their N-protonated species (HE^+) are expected to interact with [Cu(gly-glyO)] according to equilibria (i) and (ii). At 10 : 1 ratios of

$$[\text{Cu(gly-glyO)}] + E \rightleftharpoons [\text{Cu(gly-glyO)E}] \quad \text{(i)}$$

$$[\text{Cu(gly-glyO)}] + HE^+ \rightleftharpoons [\text{Cu(gly-glyO)E}] + H^+ \quad \text{(ii)}$$

[Cu(gly-glyO)] to E the total base consumption for ester hydrolysis is considerably less (*ca.* 50—60%) than the theoretical value. However, at 50 : 1 ratios the theoretical value is consumed. At these latter ratios the equilibria (1) and (2) lie far to the right so that all the ester is bound in the mixed-ligand complex. Ester hydrolysis in the mixed-ligand complex was normally monitored in the pH range 8.5—10. Under these conditions the hydrolysis of E and HE^+ is extremely slow.[9]

Values of $k_{obs.}$ obtained from the slopes of plots of $\ln(V_\infty - V_t)$ against time are shown in Table 1, for gly-OMe, gly-OEt, L-α-ala-OEt, and L-phe-OEt (ala = alanine, phe = phenylalanine). Plots of $k_{obs.}$ against the hydroxide-ion concentration are linear, with a positive intercept, Figure, indicating that $k_{obs.} = k_0 + k_{OH}[OH^-]$. The rate constant k_0 represents the 'spontaneous' or water hydrolysis (iii) and k_{OH} the base-hydrolysis path-



Plot of $k_{obs.}$ against the hydroxide-ion concentration for the hydrolysis of [Cu(gly-glyO)(gly-OMe)] at 25 °C and I = 0.1 mol dm^{-3} (K[NO_3])

way (iv). Here A^- represents the amino-acidate anion, $NH_2CH(R)CO_2^-$. Values of k_{OH} were obtained from the

$$[\text{Cu(gly-glyO)E}] + H_2O \xrightarrow{k_{H_2O}} [\text{Cu(gly-glyO)A}]^- + R'OH + H^+ \quad \text{(iii)}$$

$$[\text{Cu(gly-glyO)E}] + OH^- \xrightarrow{k_{OH}} [\text{Cu(gly-glyO)A}]^- + R'OH \quad \text{(iv)}$$

slopes of plots of the type shown in the Figure, and values of k_0 from the intercept. These constants are summarised in Table 2.

TABLE 1

Kinetics of base hydrolysis of the mixed-ligand complexes at 25 °C and I = 0.1 mol dm^{-3} (K[NO_3])

Ester	pH	10^5[OH$^-$]/ mol dm^{-3}	$10^4 k_{obs.}/s^{-1}$
L-phe-OEt	8.99	1.26	3.52
	9.09	1.60	5.14
	9.42	3.39	7.67
	9.55	4.57	8.60
	9.81	8.32	14.62
	10.00	13.04	21.57
gly-OEt	9.10	1.64	6.31
	9.37	3.06	10.12
	9.65	5.83	14.58
	9.89	10.13	26.22
	10.01	13.37	36.35
L-α-ala-OEt	8.83	0.88	7.87
	9.00	1.30	9.88
	9.36	2.99	12.5
	9.55	4.58	16.93
	9.72	6.85	21.37
	9.82	8.62	21.67
	9.97	12.00	30.7
gly-OMe	8.90	1.04	10.27
	9.17	1.93	12.83
	9.26	2.37	16.43
	9.33	2.79	19.68
	9.43	3.51	21.83
	9.64	5.69	31.8

TABLE 2

Derived rate constants for base and water hydrolysis of amino-acid ester ligands in mixed-ligand complexes with [Cu(gly-glyO)] at 25 °C and I = 0.1 mol dm^{-3} ($K[NO_3]$)*

Ester	$10^6 k_{H_2O}$/ dm^3 mol^{-1} s^{-1}	$10^{-1} k_{OH}$/ dm^3 mol^{-1} s^{-1}	$10^{-6} k_{OH} : k_{H_2O}$
gly-OMe	9.18	4.75	5.17
gly-OEt	2.85	2.52	8.81
L-α-ala-OEt	12.55	1.93	1.54
L-phe-OEt	4.02	1.48	3.69

* For the base hydrolysis of the unprotonated esters (E) the values of k_{OH} are 1.28 (gly-OMe), 0.64 (gly-OEt), 0.55 (L-α-ala-OEt), and 0.24 dm^3 mol^{-1} s^{-1} (L-phe-OEt) at 25 °C and I = 0.1 mol dm^{-3}. For the protonated esters (HE^+) the reported [1] values of k_{OH} are 28.3 (gly-OMe) and 22.9 dm^3 mol^{-1} s^{-1} (gly-OEt) at 25 °C and I = 0.1 mol dm^{-3}.

The k_0 values were converted into second-order rate constants using the expression $k_{H_2O} = k_0/55.5$ where 55.5 mol dm^{-3} is the molar concentration of water. Values of the ratio $k_{OH} : k_{H_2O}$ fall within the range 1×10^6—5×10^6: which is somewhat lower than is generally observed for the relative reactivities of hydroxide ion and water in ester hydrolysis.[12] As normally occurs [6,9] with α-amino-acid esters, the methyl ester undergoes base hydrolysis at roughly twice the rate of the ethyl ester. Base hydrolyses of the esters in the mixed-ligand complex with [Cu(gly-glyO)] are *ca.* 50 times faster than the unprotonated esters E. This relatively small effect is consistent with the formation of the complex (3) in which the α-amino-acid ester is unidentate. The formation of the bidentate species (4) would lead to substantial rate accelerations by a factor of $\leqslant 10^6$.[1] Unidentate complexes of α-amino-acid esters normally undergo base hydrolysis at somewhat similar rates to those of the protonated esters HE^+, where $k_{OH}^{HE^+}/k_{OH}^{E}$ is *ca.* 30 (Table 2). Thus for glycine ethyl ester k_{OH}^{E} = 0.64 dm^3 mol^{-1} s^{-1} and $k_{OH}^{HE^+}$ = 22.9 dm^3 mol^{-1} s^{-1} at 25 °C and I = 0.1 mol dm^{-3}. Somewhat similar results have been obtained for the base hydrolysis of unidentate esters on cobalt(III) when intramolecular effects are absent. Thus for base hydrolysis [13] of *cis*-$[Co(en)_2(OH)\{NH_2(CH_2)_4CO_2Me\}]^{2+}$ k_{OH} = 0.72 dm^3 mol^{-1} s^{-1} which may be compared with the rate constants k_{OH}^{E} = 0.1 dm^3 mol^{-1} s^{-1} and $k_{OH}^{HE^+}$ = 2.08 dm^3 mol^{-1} s^{-1}. In addition, gly-glyO^{2-} binds strongly to copper(II) and as a result reduces the Lewis acidity of the metal centre.

We thank the Leverhulme Foundation and the S.R.C. for the award of a Research Fellowship (to P. B.).

[9/1575 *Received*, 3rd *October*, 1979]

REFERENCES

[1] R. W. Hay and P. J. Morris in 'Metal Ions in Biological Systems,' vol. 5, ed. H. Sigel, Marcel Dekker, New York, 1976, p. 173.
[2] R. J. Angelici and D. Hopgood, *J. Amer. Chem. Soc.*, 1968, **70**, 2514.
[3] B. E. Leach and R. J. Angelici, *Inorg. Chem.*, 1969, **8**, 907.
[4] M. K. Kim and A. E. Martell, *Biochemistry*, 1964, **3**, 1170; 1975, **4**, 137.
[5] R. Driver and W. R. Walker, *Austral. J. Chem.*, 1968, **21**, 671.
[6] R. E. Viola, C. R. Hartzell, and J. J. Villafranca, *J. Inorg. Biochem.*, 1979, **10**, 293.
[7] M. C. Lim, E. Sinn, and R. B. Martin, *Inorg. Chem.*, 1976, **15**, 807.
[8] C. J. Simmons, M. Lundeen, and K. Sett, *Inorg. Chem.*, 1978, **17**, 1429.
[9] R. W. Hay, L. J. Porter, and P. J. Morris, *Austral. J. Chem.*, 1966, **19**, 1197.
[10] R. A. Robinson and R. H. Stokes, 'Electrolyte Solutions,' 2nd edn., Butterworths, London, 1965.
[11] C. W. Davies, *J. Chem. Soc.*, 1938, 2093.
[12] R. W. Hay and K. B. Nolan, *J.C.S. Dalton*, 1975, 1348.
[13] R. W. Hay, R. Bennett, and D. P. Piplani, *J.C.S. Dalton*, 1978, 1046.