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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, ILLINOIS INSTITUTE OF TECHNOLOGY]

The Kinetics and Oxygen Exchange of the Cupric Ion-catalyzed Hydrolysis of α-Amino Esters[1]

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The cupric ion-catalyzed hydrolysis of glycine ethyl ester and DL-phenylalanine ethyl ester in aqueous solution at *p*H 7.3 using glycine as buffer proceeded rapidly at 25° and closely followed a first order rate law. However, the hydrolysis of glycine esters with cupric ion in tris-(hydroxymethyl)-aminomethane buffer under similar conditions gave decreasing first-order rate constants because the product of the reaction, glycine, is a better complexing agent for the metal ion than the reactant or buffer. Carbonyl oxygen exchange was found during the cupric ion-catalyzed hydrolysis of DL-phenylalanine ethyl ester-*carbonyl*-O^{18} at *p*H 7.3 using glycine as buffer. On the basis of the kinetic and oxygen exchange evidence, a mechanism of the hydrolysis is postulated involving the transient formation of a chelate between the metal ion and the α-amino ester which facilitates the attack of a nucleophilic species at the carbonyl carbon atom. The relationship of this model system to the mechanism of action of metal ion-containing peptidases is discussed.

Introduction

A number of enzymes of the exopeptidase class have been shown to require a polyvalent metal ion as a co-factor for their action.[3] An example of such an enzyme is leucine aminopeptidase which requires magnesium or manganous ion for activity.[4] On the basis of studies of the effect of structural variations and of the metal ion content of the enzyme, Smith has formulated a mechanism of this enzymatic hydrolysis involving the formation of a chelate of the metal ion on the enzyme surface with the two nitrogen atoms of the substrate, the amino and amide nitrogens. On the basis of studies of the binding of small molecules to proteins through metal ion bridges, Klotz has proposed that the metal ion functions rather as a non-chelate (linear) bridge between enzyme and substrate with binding of the metal ion to the carbonyl oxygen of the substrate.[5]

In the present paper an attempt has been made to provide evidence concerning the mechanism of exopeptidase action by the study of a model (non-enzymatic) system. A detailed kinetic and isotopic investigation of the cupric ion-catalyzed hydrolysis of several α-amino esters is presented here. The formation constants of copper(II) complexes of α-amino esters have been determined[6] and the kinetics of hydrolysis of such systems have been investigated previously.[7] Several features of the latter investigation have had to be re-examined in the course of the present work.

(1) This investigation was supported by research grant G-3787 of the National Institutes of Health. Paper VII in the series, "The Mechanism of Action of Hydrolytic Enzymes."

(2) Eastman Kodak Co. research fellow, 1955–1956.

(3) For general surveys of metal-enzymes and peptidases in particular, see A. L. Lehninger, *Physiol. Rev.*, **30**, 393 (1950), and E. L. Smith, *Advances in Enzymol.*, **12**, 191 (1951).

(4) D. H. Spackman, E. L. Smith and D. M. Brown, *J. Biol. Chem.*, **212**, 255 (1955); E. L. Smith and D. H. Spackman, *ibid.*, **212**, 271 (1955); E. L. Smith, N. C. Davis, E. Adams and D. H. Spackman, "The Mechanism of Enzyme Action," W. D. McElroy and B. Glass, editors, The Johns Hopkins Press, Baltimore, Md., 1954, pp. 291–318.

(5) I. M. Klotz and W. C. Loh Ming, THIS JOURNAL, **76**, 805 (1954); I. M. Klotz, "The Mechanism of Enzyme Action," W. D. McElroy and B. Glass, editors, The Johns Hopkins Press, Baltimore, Md., 1954, pp. 257–290.

(6) J. M. White, R. A. Manning and N. C. Li, THIS JOURNAL, **78**, 2367 (1956).

(7) H. Kroll, *ibid.*, **74**, 2036 (1952).

Experimental

Materials.—DL-Phenylalanine ethyl ester was prepared by refluxing a solution of DL-phenylalanine in absolute ethanol containing anhydrous hydrogen chloride, m.p. 125–127°. Glycine methyl ester was prepared from glycine in the same manner, m.p. 175–177°. Glycine ethyl ester hydrochloride was a Matheson Co. product, m.p. 142–145°. Tris-(hydroxymethyl)-aminomethane was a Matheson Co. product, m.p. 171–172°. The metal halides were C.P. products. Sodium hydroxide was standardized with potassium acid phthalate using phenolphthalein as indicator. Beckman *p*H meters, models G and H, equipped with external electrodes were used.

DL-Phenylalanine Ethyl Ester Hydrochloride-*carbonyl*-O^{18}.—DL-Phenylalanine (50 g., 0.30 mole) was equilibrated as the hydrochloride with water containing 1.5 atom % oxygen-18 and 0.5 ml. of 6 *N* hydrochloric acid. Carbobenzoxy-DL-phenylalanine-O^{18} was prepared from the above DL-phenylalanine-O^{18} (45 g., 0.223 mole) and carbobenzoxy chloride (39 g., 0.230 mole)[8]; 31 g. of product was obtained after recrystallization from carbon tetrachloride, m.p. 96–98°. Carbobenzoxy-DL-phenylalanine-O^{18} (10 g., 0.034 mole) was converted to the acid chloride by treatment with phosphorus pentachloride in ether.[8,9] Fifty ml. of absolute ethanol was added to the unpurified acid chloride and the mixture allowed to remain at 0° for 1 hr. and at room temperature overnight. Two such runs were concentrated *in vacuo* and the residue was hydrogenated with 1 g. of palladium black in 150 ml. of ethanol, 10 ml. of acetic acid and 1 ml. of water for 2 hr. at 35 p.s.i. Recrystallization of the product from anhydrous ethanol–ether gave 7.3 g. of DL-phenylalanine ethyl ester hydrochloride-*carbonyl*-O^{18}, m.p. 125–127°. A mixed melting point with an authentic sample of the unlabeled ester hydrochloride was not depressed.

Reactions Occurring in the System Ester–Metal Ion–Buffer.—During the hydrolysis of phenylalanine ethyl ester with cobaltous chloride using tris-(hydroxymethyl)-aminomethane as buffer, the difference in titrant required to reach *p*H 4.0, the end-point reported by Kroll,[7] at zero time and at subsequent times was larger than that required for the stoichiometric amount of ester present. A solution consisting only of cobaltous ion and buffer, titrated over a period of time in the same manner as above, showed a behavior similar to the "hydrolysis reaction," possibly indicating a slow reaction between cobaltous ion and buffer. The same phenomenon occurred to a lesser extent with manganous chloride–buffer systems.[10,11] These results are

(8) C. S. Smith and A. E. Brown, *ibid.*, **63**, 2605 (1941).

(9) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

(10) Private communication from Dr. I. M. Klotz indicates that Mr. Benedict Campbell of the Chemistry Department, Northwestern University, has made similar observations.

(11) Private communication from Dr. H. Kroll suggests that the instability of the cobaltous and manganous buffer systems, observed by us and by Mr. Campbell, was due to oxidation of the metal ion complex by atmospheric oxygen which was not present under the conditions of his reactions (nitrogen atmosphere and oxygen-free water).

TABLE I

REACTIONS OF METAL HALIDES WITH α-AMINO ESTERS IN TRIS-(HYDROXYMETHYL)-AMINOETHANE SOLUTION[a]

Ester	(Ester), *M*	Metal ion	*p*H	Time, min.	Ml. of titrant End-point *p*H 4.0[b] Found	Calcd.[d]	End-point *p*H 3.3[c] Found	Calcd.[d]
Phenylalanine ethyl ester	0.016	Co^{++}	7.9	1250	4.20	1.61		
	.00	Co^{++}	7.9	1440	2.26			
	.00	Mn^{++}	7.9	1550	0.53			
	.00	Cu^{++}	7.3	1550	0.00			
Glycine ethyl ester	.016	Cu^{++}	7.3	1440	2.64	1.64	2.89	2.89
	.016	Cu^{++}	7.3	1200	2.50	1.72	3.01	2.89
	.016	Cu^{++}	7.5	1200			2.83	2.89

[a] Aqueous solution, 25.04°, buffer 0.139 *M*. [b] 0.0495 *N* HCl used as titrant. [c] Aliquots placed in excess HCl and back titrated with 0.03 *N* NaOH. [d] Calculated for stoichiometric hydrolysis of the ester.

summarized in Table I. For succeeding kinetic determinations, cupric ion was chosen as catalyst.

Kinetics and Stoichiometry Using Tris-(hydroxymethyl)-aminomethane as Buffer.—As mentioned previously, the end-point chosen by Kross for the analytical titration was *p*H 4.0 which is the inflection point in the titration of hydrochloric acid in the presence of cupric ion, ester and buffer as shown in curve A of Fig. 1. While such an end-point is valid to determine initial rates (Kroll permitted the reactions to proceed to a maximum of 35% completion), it does not suffice for following the rate over the entire reaction. For example, curves B and C of Fig. 1 are titrations of the

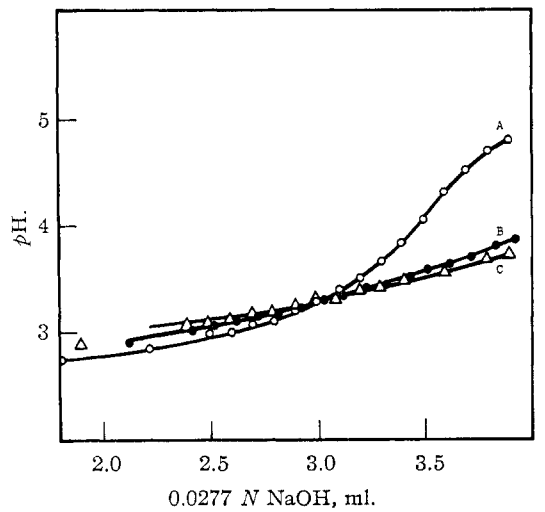


Fig. 1.—Titration of tris-(hydroxymethyl)-aminomethane, glycine ethyl ester and glycine in the presence of cupric ion: A, 5.0 ml. of a solution 0.016 *M* in glycine methyl ester and 0.133 *M* in tris-(hydroxymethyl)-aminomethane, 10.0 ml. of 0.044 *N* HCl and 1.0 ml. of 0.08 *M* cupric chloride; B, same as A with 3.0 ml. of 0.01431 *M* glycine added; C, same as A with 5.0 ml. of 0.01431 *M* glycine added.

same system as curve A in the presence of increasing amounts of glycine, the product of the hydrolysis reaction. Although there is a disparity in the curves at *p*H 4, the curves intersect near *p*H 3.3, and thus the amount of alkali required to reach this *p*H is the same regardless of the concentration of glycine. The difference in the amount of alkali required to reach *p*H 3.3 for a blank solution (at time zero) and at any time during the reaction is a direct measure of the number of carboxylic acid groups liberated. Thus, it is *p*H 3.3 which can be used as an end-point for the entire reaction. When *p*H 4.0 was used as the end-point of the cupric ion-catalyzed hydrolysis of glycine ethyl ester, the stoichiometry of the hydrolysis reaction was incorrect; when *p*H 3.3 was used as end-point, the stoichiometry was correct within experimental error as shown in Table I.

The following is the procedure of a typical kinetic determination in which the titration end-point was *p*H 3.3. Twenty-five ml. of a solution 0.666 *M* in tris-(hydroxymethyl)-aminomethane and 0.318 *N* in hydrochloric acid, 10.0 ml. of 0.16 *M* amino ester hydrochloride and 10.0 ml. of 0.16 *M* cupric chloride solution were mixed and diluted to 100.0 ml. All solutions were previously thermostated at 25.04 ± 0.02°. Five-ml. aliquots were pipetted into 11.0 ml. of 0.0442 *N* hydrochloric acid to quench the reaction and titrated to *p*H 3.3 with 0.0277 *N* sodium hydroxide.

Kinetics Using Glycine as Buffer.—In these determinations, titration to a constant *p*H was used.[12] The following example is typical. Twenty-five ml. of 0.016 *M* cupric chloride, 5.0 ml. of a solution 0.140 *M* in glycine and 0.0371 in sodium hydroxide, 6.0 ml. of 0.0998 *N* sodium hydroxide and 10.0 ml. of water were mixed and allowed to attain thermal equilibrium. The *p*H of the solution was usually between 7.30 and 7.35. Five ml. of 0.016 *M* amino ester hydrochloride solution was added and a null point of *p*H 7.25 was used. Titration was carried out with 0.0998 *N* sodium hydroxide of which about 0.7 ml. was added for an entire run. In such titrations the initial substrate concentration is unknown and must be determined by graphical means. It should be pointed out that in spite of the results obtained in the present case, the method of titration to a constant *p*H does not appear to be generally valid for the study of the kinetics of hydrolysis of amino esters. This is due to the difference of the *pK* values of the amino groups on the acid and ester which should have an effect on the *p*H of the solution in addition to the effect produced by the liberation of carboxylic acid groups.

Oxygen Exchange.—A hydrolysis mixture composed of 70.0 ml. of 0.40 *M* cupric chloride solution, 70.0 ml. of 0.70 *M* glycine solution, 151.2 ml. of 0.364 *N* sodium hydroxide, 70.0 ml. of water and 1.282 g. of phenylalanine ethyl ester hydrochloride-*carbonyl*-O^{18} (0.00558 mole) was maintained at *p*H 7.2–7.3 by the periodic addition of 0.75 *N* sodium hydroxide. Approximately 50-ml. samples were removed at one- and two-minute intervals and quenched in 1.5 ml. of 6 *N* hydrochloric acid. The acidic solutions were treated with hydrogen sulfide and the copper sulfide removed by filtration. After being brought to *p*H 9.5 with 3 *N* sodium hydroxide, the ester was extracted quickly with ether. The ethereal solutions were dried with anhydrous magnesium sulfate and treated with gaseous hydrogen chloride; the precipitated ester hydrochloride was recrystallized from ethanol–ether, m.p. 125–127°. A blank solution of ester and glycine was treated in the same manner except that the cupric chloride was added after the mixture was acidified. The recovered ester hydrochlorides were converted to carbon dioxide by the method of Doering and Dorfman.[13] The carbon dioxide samples were analyzed for oxygen-18 in a Consolidated–Nier Isotope Ratio Mass Spectrometer.

Results and Discussion

The use of the analytical method described for the hydrolysis of glycine esters with cupric ion in tris-(hydroxymethyl)-aminomethane solution gave the first-order velocity coefficients shown in Table II as calculated from the usual first-order equation. These results indicate decreasing first-order hydro-

(12) G. W. Schwert, H. Neurath, S. Kaufman and J. E. Snoke, *J. Biol. Chem.*, **172**, 221 (1948).

(13) W. E. Doering and E. Dorfman, THIS JOURNAL, **75**, 5595 (1953); *cf.* M. L. Bender and K. C. Kemp, *ibid.*, **79**, 116 (1957).

TABLE II

THE KINETICS OF HYDROLYSIS OF α-AMINO ESTERS WITH CUPRIC CHLORIDE[f]

Ester	(Ester), M	Cu^{++}, M	Buffer	(Buffer), M	pH	$k_{obsd} \times 10^3$, $sec.^{-1}$
Glycine methyl ester	0.016	0.016	Tris[g]	0.139	7.3	1.71[a]
						1.24[b]
						0.91[c]
	.016	.016	Tris	.139	5.8	.15[a,d]
Glycine ethyl ester	.016	.016	Tris	.139	7.3	.834[a]
						.460[b]
						.387[c]
	.016	.016	Tris	.139	7.5	.900[a]
						.407[b]
	.0015	.00785	Glycine	.0137	7.3	1.53 ± 0.02[e]
						1.72 ± .06
						1.71 ± .04
						1.68 ± .03
						1.44 ± .02
						1.59 av.
DL-Phenylalanine ethyl ester	.0138	.0775	Glycine	.136	7.3	2.63 ± .06
						2.71 ± .19
						2.67 av.

[a] 10–20% hydrolysis. [b] 50–60% hydrolysis. [c] 90–100% hydrolysis. [d] Approximate. [e] Average arithmetic deviation from the mean, 10–80% hydrolysis. [f] Aqueous solution, 25.04°. [g] Tris-(hydroxymethyl)-aminomethane.

lytic rate constants with tris-(hydroxymethyl)-aminomethane as buffer.[14]

The probable explanation for the decrease in rate constant in the tris-(hydroxymethyl)-aminomethane buffer systems is connected with the interaction of the various complexing agents with cupric ion. It will be assumed that the cupric ion reaction occurs by formation of a complex of ester and metal ion followed by hydrolysis of this complex. If establishment of the equilibrium for ester–copper complex formation is rapid as expected by analogy with the rapidity of cupric ion complex formation with other amines, the rate of reaction should then be determined by the decomposition of the complex.

$$\text{Ester} + (\text{Buffer–Cu}^{++}) \underset{k_2}{\overset{k_1}{\rightleftharpoons}} (\text{Ester–Cu}^{++}) \xrightarrow[H_2O]{k_3} \text{Products} \quad (1)$$

$$-\text{d(ester)}/\text{d}t = k_3(\text{Ester–Cu}^{++}) = k_3K(\text{Ester})(\text{Buffer–Cu}^{++}) \quad (2)$$

where $K = k_1/k_2$. In order that the observed first-order rate constant, $k_{obsd} = k_3K(Cu^{++})$, remain constant throughout the reaction, the buffer–copper complex concentration must remain constant. The concentration of buffer–copper complex certainly remains constant; however, the liberation of amino acid during the hydrolysis reaction results in a new and strong buffer complex of cupric ion with the amino acid which then gives rise to a new equilibrium constant, K', and a drift in the first-order rate constant.

In order to justify the statements made above in a semi-quantitative manner, a simplified picture of the reaction utilizing only 1:1 complexes will be given. If the cupric ion functions as a catalyst, it must be returned from the product amino acid to the buffer after one unit of reaction according to equation 3 where BNH_3^+ is the ammonium form of the buffer

$$BNH_3^+ + ANH_2Cu \rightleftharpoons ANH_3^+ + BNH_2Cu \quad (3)$$

and ANH_3^+ is the zwitterion form of the amino acid. The equilibrium constant for this reaction may be determined from the known or estimated equilibrium constants of the primary reactions as

$$(BNH_2)(H^+)/(BNH_3^+) = 7.9 \times 10^{-9}\ ^{15} \quad (1)$$

$$(BNH_2Cu)/(BNH_2)(Cu) = 1.4 \times 10^{4}\ ^{17} \quad (2)$$

$$(ANH_2)(H^+)/(ANH_3^+) = 2.2 \times 10^{-10}\ ^{16} \quad (3)$$

$$(ANH_2Cu)/(ANH_2)(Cu) = 1.69 \times 10^{8}\ ^{17} \quad (4)$$

The equilibrium constant for equation 3 is then equal to $K_1K_2/K_3K_4 \cong 10^{-3}$. Thus the transfer of the cupric ion from the liberated amino acid to the buffer takes place only to a small extent. The cupric ion in such a system is not catalytic in the strict sense of the word since it remains complexed with the product and a decreasing first-order rate constant should be observed. The order of magnitude found for the hydrolysis of glycine methyl ester, $k_{obsd} \cong 1.3 \times 10^{-3}$ $sec.^{-1}$, is much different from the value reported by Kroll,[7] for similar conditions, 42.5×10^{-3} $sec.^{-1}$.

When glycine was used as buffer, constant first-order velocity constants were obtained for the cupric ion-catalyzed hydrolysis as shown in Table II and Fig. 2. Glycine, the product of the reaction, was selected as buffer because then the small increase in glycine concentration due to the hydrolytic reaction should not change the effective concentration of cupric ion complexes. The hydrolysis reaction with glycine as buffer can be represented as

$$(\text{Glycine})_2\text{Cu} + \text{Ester} \rightleftharpoons \text{Glycine} + (\text{Glycine})(\text{Ester})\text{Cu}^{++} \quad (4)$$

(14) Private communication from Dr. H. Kroll indicates that decreasing rate constants have been observed in this reaction by him.

(15) B. M. Iselin and C. Niemann, *J. Biol. Chem.*, **182**, 821 (1950).

(16) E. J. Cohn and J. T. Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943, p. 80.

(17) J. Bjerrum, *Chem. Revs.*, **46**, 381 (1950). The formation constant of the buffer–cupric ion complex was taken as that for ammonia.

$$(\text{Glycine})\text{Cu}^{+} + \text{Ester} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} (\text{Glycine})(\text{Ester})\text{Cu}^{+} \quad (5)$$

$$(\text{Glycine})(\text{Ester})\text{Cu}^{+} + \text{H}_2\text{O} \xrightarrow{k_3} (\text{Glycine})_2\text{Cu} + \text{ROH}^{18} \quad (6)$$

Reaction 4 probably contributes little to the formation of the (Glycine)(Ester)Cu^+ complex since the formation constant of amino ester and cupric ion is much smaller than that of glycine and cupric ion (10^4 compared to 10^8). Furthermore, in the presence of diglycine–cupric ion complex, where less monoglycine–cupric ion complex was introduced than ester, the rate of hydrolysis fell to zero when all the monoglycine–cupric ion complex had reacted. If the effective reactions are then (5) and (6), the rate of hydrolysis is given by equation 7 where K is the equilibrium constant for reaction 5. Equilibrium 5 is essentially quantitatively to the right (log K = 3.83 for glycine methyl ester and cupric ion[6] and equation 5 would be expected to have a similar value, although there is admittedly a different charge and environment of the copper ion in (Glycine)Cu^+ as compared to $Cu(H_2O)_4^{++}$. If the term ((Glycine)Cu^+) remains constant throughout the reaction, the first-order rate constant should remain constant and the reaction would then be truly catalytic with respect to cupric ion. Table II demonstrates that constant rate constants are obtained for the cupric ion-catalyzed hydrolysis of glycine and DL-phenylalanine ethyl esters in aqueous glycine solution. It should be pointed out that the observed rate constants for these reactions in Table II are composite constants, equaling k_3K((Glycine)Cu^+). Calculations show that the concentration of glycine–cupric ion complex before and after reaction of glycine ethyl ester are essentially the same. The mechanistic scheme illustrated in equations 5 and 6 is thus consistent with the observed kinetics.

$$\text{d(Acid)}/dt = k_3((\text{Glycine})(\text{Ester})\text{Cu}^{+}) = k_3K((\text{Glycine})\text{Cu}^{+})(\text{Ester}) \quad (7)$$

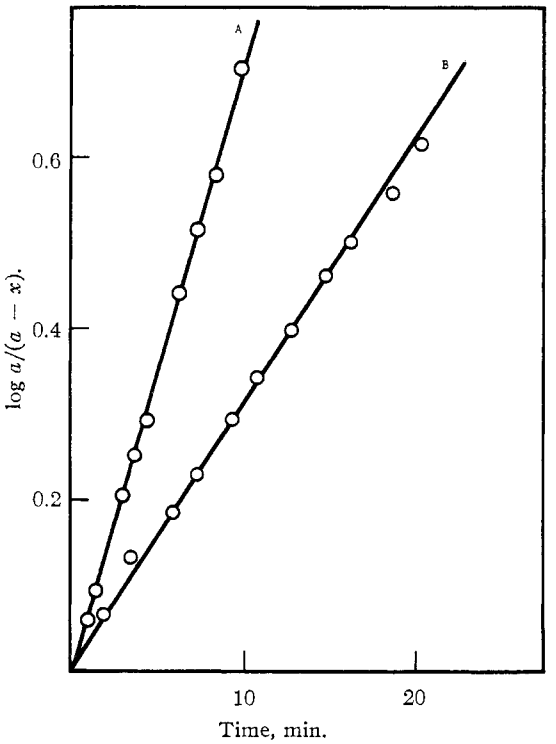


Fig. 2.—Hydrolysis of phenylalanine ethyl ester (A) and glycine ethyl ester (B) with cupric chloride, using glycine as buffer, pH 7.3, 25.04°.

The carbonyl oxygen exchange during the cupric ion-catalyzed hydrolysis of phenylalanine ethyl ester-*carbonyl*-O^{18} was determined in the usual manner[19] using the kinetic data given in Table II. It was found that oxygen exchange did occur and that the ratio k_h/k_e was 3.9 ± 0.4. The oxygen exchange data are presented in Table III and Fig. 3.

TABLE III

OXYGEN EXCHANGE DURING THE CUPRIC ION-CATALYZED HYDROLYSIS OF PHENYLALANINE ETHYL ESTER-*Carbonyl*-O^{18}[a]

Time, min.	Atom % excess O^{18}	k_{hydrol}/k_{exch}
0	0.896	
3.1	.774	3.3
5.3	.716	3.8
6.4	.680	3.6
9.4	.635	4.3
10.1	.629	4.5
Blank[b]	.896	

[a] (Ester) 0.0154 M, (Cupric ion) 0.0774 M, (Glycine) 0.136 M, pH 7.3. [b] The ester was treated for 10 min. with 0.136 M glycine at pH 7.3 and then isolated.

Although it has been pointed out by Li, *et al.*,[6] that the metal ion is bound solely to the amino group of the ester in the ground state, it seems unlikely that the cupric ion catalysis occurs only because of an indirect (electrostatic or inductive) interaction of the positively charged metal ion with the reaction center, the ester group. The reaction cannot be due to an attack of hydroxyl ion on a positively charged α-amino ester since Westheimer[20] has shown that the introduction of a positive charge two atoms away from the carbonyl group of an ester increases the rate constant of alkaline hydrolysis by a factor of 10^3, whereas there is a difference of approximately 10^6 between the cupric ion-catalyzed and alkaline hydrolyses of DL-phenylalanine ethyl ester as shown in Table IV. It should be pointed out that the effective charge on the cupric ion–glycine–ester complex is plus one so that the factor of 10^6 cannot be explained by an increase in charge over that present in the betaine case. Furthermore, the reaction cannot be due to attack by a water molecule on a positively charged α-amino ester since it has been shown that the rate constant of the acidic hydrolysis of phenylalanine ethyl ester is very small[21] (Table IV). It thus seems reasonable to assume that the rapid cupric ion-catalyzed hydrolysis of α-amino esters at pH 7.3 is due to an interaction of the metal ion with the reaction center, the ester group. The necessity of the α-amino group for

(18) At pH 7.3 where most of the kinetic experiments were carried out, glycine exists to a large extent in the anionic form.

(19) M. L. Bender, THIS JOURNAL, **73**, 1626 (1951).

(20) F. H. Westheimer and M. W. Shookhoff, *ibid.*, **62**, 269 (1940).

(21) M. L. Bender and B. W. Turnquest, *ibid.*, **77**, 4271 (1955).

TABLE IV

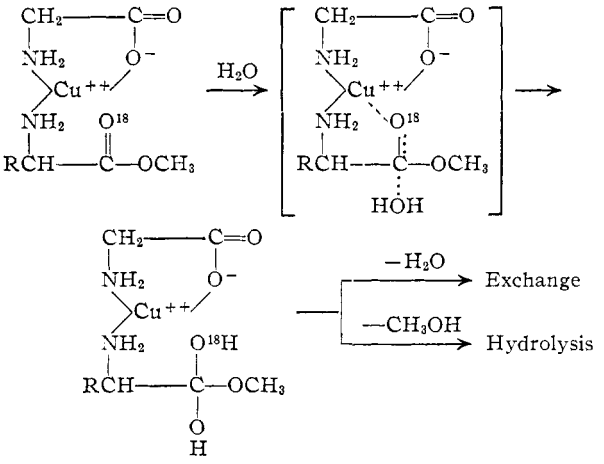
ACIDIC, BASIC AND CUPRIC ION-CATALYZED HYDROLYSES OF SOME ESTERS

Ester	Catalyst	Temp., °C.	k_2, l./mole sec.	k_1,[d] sec.$^{-1}$	Ref.
$N(CH_3)_2CH_2CO_2C(CH_3)_3$	OH^-	10.02	3.72×10^{-4}		20
$Cl^-{}^+N(CH_3)_3CH_2CO_2C(CH_3)_3$	OH^-	10.02	3.70×10^{-1}		20
DL-Phenylalanine ethyl ester	H^+	25.04		1.46×10^{-14}	21[a]
	OH^-	25.04	2.97×10^{-2}	5.8×10^{-9}	21[b]
	Cu^{++}	25.04		2.67×10^{-3}	[c]

[a] 70% dioxane–water. [b] 85% ethanol–water. [c] This investigation. [d] These first-order rate constants are those calculated or observed under the conditions of the cupric ion-catalyzed hydrolysis given in Table II. The tabulated rate constant for the cupric ion catalysis equals $k_3K((\text{Glycine})Cu^+)$ (see equation 7). To obtain k_3 for cupric ion catalysis, approximations must therefore be made for K and for $((\text{Glycine})Cu^+)$. It appears reasonable that $K/((\text{Glycine})Cu^+)$ is greater than one under the present conditions so that the tabulated rate constant is a lower limit for the cupric ion catalytic constant.

hydrolysis is indicated by the complete lack of reaction between ethyl acetate and cupric ion.[22]

The results of the oxygen exchange experiment indicate that an addition intermediate is formed in this reaction which is symmetrical with respect to the isotopic oxygen atom. Such an intermediate would be glycine–Cu^+–$NH_2CH(R)C(OH)(O^{18}H)(OR)$ and would be similar both in structure and in rate of oxygen exchange with proposed intermediates in the basic and acidic hydrolyses of esters.[18] A mechanism of reaction 6 which is consistent with both the kinetic and oxygen exchange evidence is



A question may arise concerning the arbitrary choice of the oxygen atom for transient interaction with the metal ion. By analogy with the acid-catalyzed hydrolysis of ordinary esters where protonation is believed to occur on the carbonyl oxygen atom, it is reasonable to postulate that the super-acid metal ion would complex at the same position. However, an alternative mechanism could be written in which the metal ion complexed with the alkoxyl oxygen atom, by analogy with the proposal of Smith for the hydrolysis of amides by leucine aminopeptidase.[4] Such a mechanism would indeed be expected for the metal ion-catalyzed hydrolysis of amides since in this case the basicity of the nitrogen may cause protonation (and metal ion complex formation) to occur at the nitrogen atom rather than at the carbonyl oxygen atom.[23]

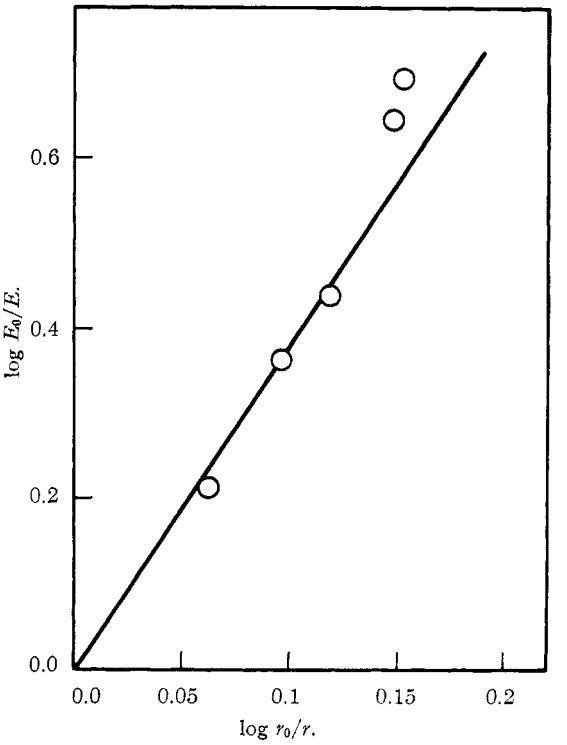


Fig. 3.—Oxygen exchange *versus* hydrolysis for the cupric ion catalyzed hydrolysis of phenylalanine ethyl ester, 25.04°.

Acknowledgments.—The authors gratefully acknowledge valuable discussions with Drs. F. H. Westheimer, who originally suggested this problem, I. M. Klotz, N. C. Li and R. G. Pearson and H. Kroll. The authors wish to express their appreciation to Dr. H. Taube of the University of Chicago through whose courtesy the mass spectrometer under AEC Contract At(11-1)92 was made available.

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(22) M. L. Bender and B. W. Turnquest, THIS JOURNAL, **79**, 1652 (1957).

(23) M. L. Bender and R. D. Ginger, *ibid.*, **77**, 348 (1955).