[CONTRIBUTION OF THE DEPARTMENT OF CHEMISTRY OF CLARK UNIVERSITY, WORCESTER, MASS.]

A Kinetic Study of the Copper(II) Chelate Catalyzed Hydrolysis of Diisopropyl Phosphorofluoridate[1]

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The catalytic effects of 1:1 Cu(II) chelates of N,N'-dimethylethylenediamine (DMEN), N,N,N',N'-tetramethylethylenediamine (TMEN), N-hydroxyethylethylenediamine (HEN), N,N'-dihydroxyethylethylenediamine (2-HEN), α,α'-dipyridyl (DIPY), 1,10-phenanthroline (PHEN), glycylglycine (GG) and diglycylglycine (GGG) on the hydrolysis of diisopropyl phosphorofluoridate (DFP) have been studied over a range of *p*H and metal chelate concentration. Detailed studies of the aqueous metal complex equilibria were correlated with rate measurements in order to calculate specific rate constants for catalysis by the 1:1 diaquo $CuL(H_2O)_2$ or monohydroxo ($Cu[OH]L(H_2O)$) chelates. The specific rate constants for catalysis by chelates of the various ligands decreased in the order DIPY > TMEN > PHEN > DMEN > HEN > 2-HEN. The specific rate constant for the interaction of DFP with hydroxyl ion was found to be 0.31 ± 0.03 liter $mole^{-1}$ $sec.^{-1}$. The protonated cupric chelate of glycylglycine was shown to have slight catalytic activity whereas the observed rates for the Cu(II)–GGG system were the result of catalysis by the aquo cupric ion which was present in equilibrium with the chelate. The heat and entropy of activation of the Cu(II)–dipyridyl catalyst are reported.

Introduction

Following the preliminary report of Wagner-Jauregg, *et al.*,[3] the role of metal ions and metal chelates as catalysts in the hydrolysis of cholinesterase inhibitors such as isopropyl methyl phosphonofluoridate (Sarin) and diisopropyl phosphorofluoridate (DFP) has been described by several workers. Wagner-Jauregg, *et al.*,[3] reported the use of copper(II) complexes of α,α'-dipyridyl, imidazole, histidine and other ligands as catalysts for the hydrolysis of DFP and have outlined some possible mechanisms. Courtney, *et al.*,[4] showed that chelates of Cu(II), La(III), Fe(III), Cr(III), Ti(IV), Sn(IV), UO_2(VI), VO(IV), MoO_2(VI) and ZrO(IV) have considerable catalytic activity in the hydrolysis of Sarin, and also studied the effect of various copper(II) chelates on the hydrolysis of DFP. Fowkes, *et al.*,[5] carried out a detailed study on the interaction of Cu(II)-dipyridyl with DFP, while Epstein, *et al.*,[6] investigated the catalytic hydrolysis of Sarin in the presence of cerous, cupric and manganous ions. Gustafson and Martell[7] reported in detail the catalytic effects of six copper(II) chelates upon the hydrolysis of Sarin and proposed a mechanism for the catalytic hydrolysis reaction.

It is the purpose of this investigation to extend the scope of the Sarin studies to include the reactions of the analogous compound, DFP. As in the previous case,[7] the ligands chosen were N,N'-dimethylethylenediamine (DMEN), N,N,N',N'-tetramethylethylenediamine (TMEN), N-hydroxyethylthylenediamine (HEN), N,N'-dihydroxyethylthylenediamine (2-HEN), α,α'-dipyridyl (DIPY) and 1,10-phenanthroline (PHEN). Also, the catalytic effects of two copper(II) chelates containing peptide ligands, Cu(II)-glycylglycine and Cu(II)-diglycylglycine, are investigated.

Experimental

Reagents.—The DFP was obtained from the Colgate-Palmolive Co. and had the following physical properties: b.p. 60–61° (8.2 mm.), d^{25} 1.055, $n^{25}D$ 1.3792. A stock solution of 0.06 molar concentration was prepared in spectrograde anhydrous isopropyl alcohol. Standardization was carried out by titration of the acid produced during catalytic hydrolysis by the cupric chelate of TMEN at $-\log [H^+]$ values of 6.9 and 8.9 with a Beckman automatic titrator. Cupric nitrate and the various ligand solutions were prepared and standardized as described in previous publications,[8,9] Standard potassium hydroxide was prepared by the method of Schwarzenbach and Biedermann.[10] Samples of glycylglycine and diglycylglycine were obtained from F. Hoffman LaRoche and Co., and aqueous solutions of these compounds were standardized by potentiometric titration with standard potassium hydroxide.

Kinetic Measurements.—The formation of acid during the course of DFP hydrolysis was measured as a function of time with a Beckman automatic titrator, which maintained the experimental solution at constant *p*H by automatic addition of standard potassium hydroxide from a microburet. The reaction was carried out in a multinecked flask, described previously,[4,7] which accommodated a mercury seal stirrer, gas inlet and outlet tubes, glass and calomel electrodes and microburet delivery tubes for DFP and potassium hydroxide solutions.

In a typical run an aliquot of copper chelate solution was introduced into the titration cell and the volume was adjusted to 100-ml. with potassium nitrate solution and distilled water such that the final ionic strength was 0.10. Presaturated nitrogen was passed through the solution to exclude carbon dioxide and the hydrogen ion concentration was adjusted by means of the automatic unit. An anticipation setting of 9 was used in all experiments. An aliquot of DFP was added by means of an automatic buret and time measurements were begun when half of the DFP had been added. Readings of time *vs.* corresponding standard base delivered were recorded over the course of the titration. In nearly all cases, linear plots of time *vs.* $\log [a/(a-x)]$ (where a is the amount of base required for complete hydrolysis of DFP and x is the amount of base added at time t) were obtained indicating first order kinetics.

Catalytic hydrolysis of DFP in the presence of the Cu(II) chelates of DMEN, TMEN, HEN, 2-HEN, DIPY and PHEN were carried out at $-\log [H^+]$ values of 6.90, 7.40, 7.90, 8.40 and 8.90 with several concentrations of catalyst at 25.0°. Several experiments were also performed at 0.3 and 42.5° with each of the chelates mentioned above. The catalytic effects of the copper(II) chelates of glycylglycine (GG) and diglycylglycine (GGG) were also measured at $-\log [H^+]$ values of 6.90 and 8.90 at 25.0°, and the reaction of DFP with hydroxyl ion was studied in the *p*H range 10.0–11.5 at 25.0°.

Calculations

A plot of time *vs.* $\log[a/(a-x)]$ resulted in a straight line, indicating first-order kinetics, since copper chelate and hydroxyl ion concentrations were maintained at constant values during the course of each hydrolysis experiment. First-order constants were calculated according to the relationship

$$k_{obs} = 0.693/t_{1/2} \qquad (1)$$

where k_{obs} is the first-order rate constant and $t_{1/2}$ is the experimental half-time.

Since hydroxyl ion alone will react with the DFP, rate measurements were carried out in the absence of a metal chelate catalyst. The measured hydrolysis rate may be summarized by the expression

$$k_{obs} = k_{OH}[OH] + c \qquad (2)$$

where k_{OH} is the rate constant assigned to the hydroxyl

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(3) T. Wagner-Jauregg, B. E. Hackley, Jr., J. A. Lies, O. O. Owens and R. Proper, *J. Am. Chem. Soc.*, **77**, 922 (1955).

(4) R. C. Courtney, R. L. Gustafson, S. Westerback, H. Hyytiainen, S. Chaberek, Jr., and A. E. Martell, *ibid.*, **79**, 3030 (1957).

(5) F. M. Fowkes, G. S. Ronay and L. B. Ryland, *J. Phys. Chem.*, **62**, 867 (1958).

(6) J. Epstein and D. H. Rosenblatt, *J. Am. Chem. Soc.*, **80**, 3596 (1958).

(7) R. L. Gustafson and A. E. Martell, *ibid.*, **84**, 2309 (1962).

(8) R. L. Gustafson and A. E. Martell, *ibid.*, **81**, 525 (1959).

(9) R. C. Courtney, R. L. Gustafson, S. Chaberek, Jr., and A. E. Martell, *ibid.*, **81**, 519 (1959).

(10) G. Schwarzenbach and W. Biedermann, *Helv. Chim. Acta*, **31**, 331 (1948).

ion and c is a constant which allows for the spontaneous hydrolysis of DFP in the solvent system employed. A plot of $[OH^-]$ as abscissa *vs.* k_{obs} as ordinate should give a straight line of slope k_{OH}, while the intercept at $[OH^-]$ equal to zero should give a value of the constant c.

The possible catalytic copper(II) species are: 1, the diaquo chelate, CuL^{2+}; 2, the monohydroxo chelate $Cu[OH]L^+$; 3, dihydroxo chelate, $Cu[OH]_2L$; 4, a dimer, $(Cu[OH]L)_2^{2+}$; and 5, the unbound or aquo copper(II) ion, Cu^{2+}. The calculation of the distribution of the above species as a function of *p*H and total concentration has been described in detail in previous publications.[8,9] Correlation of the equilibrium data with kinetic data obtained upon catalytic hydrolysis of DFP permitted the calculation of rate constants in the manner described previously.[7] The total observed rate, k_{obs}, may be defined in terms of thev arious catalytic and reacting species as

$$k_{obs} = k_L [CuL^{2+}][OH^-] + k_B[Cu[OH]_2L] + k_M[Cu^{2+}][OH] + k_{OH}[OH^-] \quad (3)$$

Here k_L is a rate constant which represents catalysis by both the diaquo chelate and the monohydroxo compound or either one alone, and k_B, k_M and k_{OH} are the rate constants assigned to the dihydroxo chelate species, unchelated copper(II) species and hydroxyl ion, respectively. Equation 3 assumes that the dimer is catalytically inactive and that there is negligible spontaneous hydrolysis of DFP due to interaction with the solvent water.

In *p*H regions where the concentration of dihydroxo chelate species is negligible, eq. 3 may be rearranged to give the slope intercept equation

$$\frac{k_{obs}^*}{[Cu^{2+}][OH^-]} = k_L \frac{[CuL^{2+}]}{[Cu^{2+}]} + k_M \quad (4)$$

where $k_{obs}^* = k_{obs} - k_{OH}[OH^-]$. Thus a plot of $k_{obs}^*/[Cu^{2+}][OH^-]$ as ordinate *vs.* $[CuL^{2+}]/[Cu^{2+}]$ as abscissa will give a straight line of slope k_L and intercept k_M.

Results and Discussion

Interaction of Hydroxyl Ion with DFP.—The results of experiments in which the hydrolysis of DFP was carried out in the absence of a copper(II) chelate catalyst in the $-\log [H^+]$ range 9.9–11.4 are shown in Table I. A plot of k_{OH} *vs.* $[OH^-]$ using these data resulted in a straight line with an intercept at or near the origin indicating that the constant c in eq. 2 is small. The rate of spontaneous hydrolysis of DFP due to interaction with the solvent medium alone may therefore be said to be negligible with respect to the effects observed when either copper(II) chelates or high hydroxyl ion concentrations are present.

TABLE I

REACTION OF DFP WITH HYDROXYL ION

DFP = 1800 μmoles/liter, t = 25.0°

$-\log [H^+]$	$[OH^-]$, moles/liter	k_{obs}, sec.$^{-1}$	$k_{obs}/[OH^-]$, l./mole sec. × 10^1
9.86	1.17×10^{-4}	4.49×10^{-5}	3.8
10.12	2.12×10^{-4}	6.08×10^{-5}	2.9
10.22	2.67×10^{-4}	9.0×10^{-5}	3.4
10.23	2.73×10^{-4}	9.6×10^{-5}	3.5
10.26	2.93×10^{-4}	9.0×10^{-5}	3.1
10.40	4.04×10^{-4}	1.14×10^{-4}	2.8
10.60	6.41×10^{-4}	1.93×10^{-4}	3.0
10.63	6.87×10^{-4}	2.14×10^{-4}	3.1
10.80	1.02×10^{-3}	2.75×10^{-4}	2.7
11.08	1.94×10^{-3}	5.9×10^{-4}	3.0
11.40	4.04×10^{-3}	7.1×10^{-4}	1.8

Average: k_{OH} in $-\log [H^+]$ range 9.86–11.08 = 3.1 ± 0.3×10^{-1} liters/mole sec.

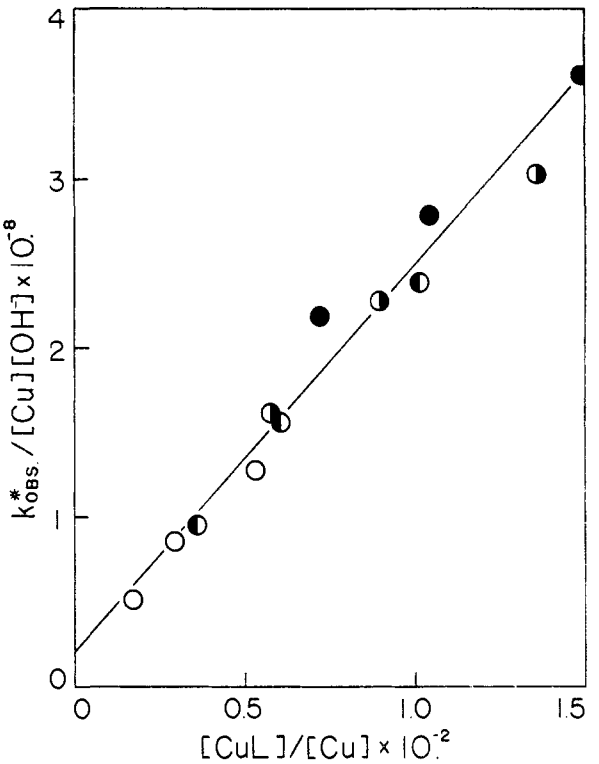


Fig. 1.—Graphical evaluation of the third-order rate constants for catalysis of DFP hydrolysis by the Cu(II) ion and by the 1:1 Cu(II)–DMEN complex; $k^*_{obs} = k_{obs} - k_{OH}[OH^-]$; slope = k_L and intercept = k_M.

Catalytic Effect of Cu(II) Chelates.—The results obtained on the catalytic hydrolysis of DFP in the presence of copper(II) chelates are shown in Tables II and III, in which the experimental half-times are given as a function of $-\log [H^+]$ and total metal concentration. On the basis of these data and the equilibrium data for the various copper(II) chelate systems employed,[8] rate constants for the diaquo (and/or monohydroxo) chelate, k_L, and for the dihydroxo (and/or monohydroxo) chelate, k_B, were calculated and are listed in Table IV. Similar constants calculated for the copper chelate catalyzed reaction of Sarin[7] are also tabulated for comparison. The results of typical calculations based on eq. 4 are shown in Fig. 1 for the Cu(II)–DMEN–DFP system.

Although the values of k_L obtained for hydrolysis of Sarin in the presence of Cu(II) chelates of TMEN, DMEN, HEN and 2-HEN are 13 to 15 times those obtained for DFP hydrolysis, the values of k_L calculated for Cu(II)-DIPY and Cu(II)-PHEN catalyzed hydrolysis of Sarin are only greater than those of the analogous DFP case by a factor of four. This is somewhat surprising since one might expect a larger difference in the relative reactivities of DIPY and PHEN with Sarin and DFP due to possible steric effects which might be encountered in the latter compound because of the presence of an additional isopropyl substituent.

Based on data for Cu(II)-DIPY catalyzed DFP hydrolysis shown in Table V, in which it was assumed that no unchelated copper(II) species were present, a heat of activation, $\Delta H^{\ddagger}$, of 5.1 ± 0.6 kcal./mole was obtained for the aquo or monohydroxo chelate catalyzed hydrolysis reaction, the rate constant of which is represented by k_A'. For the purpose of comparative calculations at $-\log [H^+] = 6.90$ it was assumed that

$$k_A' = k_{obs}/[CuL^{2+}][OH^-]$$

since the concentration of the dihydroxo chelate was negligible. The value of $\Delta H^{\ddagger}$ calculated previously for

TABLE II

CATALYTIC HYDROLYSIS OF DFP BY Cu(II) CHELATES

$t = 25.0°$, $\mu = 0.10$ (KNO_3); results given as half-times in seconds

Cu(II)-HEN, DFP = 4360 μmoles/liter				Cu(II)-2-HEN, DFP = 1750 μmoles/liter			
−log [H⁺]	5540 μM/l.	1910 μM/l.	576 μM/l.	−log [H⁺]	5560 μM/l.	3020 μM/l.	1280 μM/l.
6.90	2340	5700	18500	6.90	3660	6480	14400
7.40	1440	3480	9120	7.40	2100	3720	8220
7.90	1260	2520	5940	7.90	1860	2940	5940
8.40	..	2280	5280	8.90	1800	2940	6240
8.90	1080	2100	4860				

Cu(II)-TMEN, DFP = 3480 μmoles/liter

−log [H⁺]	711 μM/l.	430 μM/l.	216 μM/l.	72 μM/l.
6.90	1090	1440	2880	8340
7.40	740	770	1110	4080
7.90	520	550	850	2820
8.40	330	390	640	1980
8.90	320	360	580	1860

Cu(II)-DMEN, DFP = 1750 μmoles/liter				Cu(II)-DIPY, DFP = 1750 μmoles/liter			
−log [H⁺]	1340 μM/l.	410 μM/l.	137 μM/l.	−log [H⁺]	1140 μM/l.	345 μM/l.	115 μM/l.
6.90	1930	5160	14900	6.90	1380	3360	8520
7.40	880	2250	6120	7.40	1110	2220	4800
7.90	650	1290	2820	7.90	1050	1930	3660
8.90	500	920	1690	8.90	1000	1810	3300

Cu(II)-PHEN, DFP = 3460 μmoles/liter

−log [H⁺]	998 μM/l.	330 μM/l.	198 μM/l.	100 μM/l.
6.90	2700	5280	7920	13600
7.40	2220	3720	5100	8280
7.90	2100	3360	4380	6360
8.90	2040	3120	4020	5640

TABLE III

CATALYTIC HYDROLYSIS OF DFP WITH COPPER(II) CHELATES AT 0.3 AND 42.5°

Ligand	t, °C.	−log [H⁺]	Catalyst concn., μmoles/liter	$t_{1/2}$, sec.
DIPY	0.3	6.90	1150	14,300
TMEN	.3	7.90	74.4	13,900
2-HEN	.3	8.90	2010	17,800
DIPY	42.5	6.90	1140	530
DIPY	42.5	6.90	1140	500
PHEN	42.5	7.90	1000	650
TMEN	42.5	7.90	74.4	850
2-HEN	42.5	8.90	2980	1,030

TABLE IV

RATE CONSTANTS ASSIGNED TO COPPER(II) CHELATE SPECIES AT 25.0°

	DFP		Sarin	
Ligand	k_L, l.² mole⁻² sec.⁻¹	k_B, l. mole⁻¹ sec.⁻¹	k_L, l.² mole⁻² sec.⁻¹	k_B, l. mole⁻¹ sec.⁻¹
TMEN	7.0×10^{6}[a]	.....	1.0×10^{8}	.....
DMEN	2.3×10^{6}	.....	3.2×10^{7}	2.2×10^{2}
DIPY	7.4×10^{6}	.....	3.10×10^{7}	.....
PHEN	4.9×10^{6}	.....	1.92×10^{7}	.....
HEN	6.4×10^{5}[a]	5×10^{-1}[a]	9.3×10^{6}	5.5
2-HEN	4.0×10^{5}[a]	3×10^{-1}[a]	5.2×10^{6}	9.0×10^{-1}

[a] Assumes $k_M = 1.0 \times 10^{7}$ liters² mole⁻² sec.⁻¹.

the Cu(II)-DIPY catalyzed hydrolysis of Sarin was 2.4 kcal./mole and that for the hydroxyl ion reaction, 10.0 kcal./mole. The entropy and enthalpy of activation given in Table V differ somewhat from those reported for Sarin[7] ($\Delta H^{\ddagger}$ 2.4 and $\Delta S^{\ddagger}$ −16) catalysis by the same metal chelate. Because of the difference in the electronic interaction expected for Sarin and DFP, however, the observed differences in activation parameters are not unreasonable. These results, therefore, are compatible with the assumption that metal chelate catalysis of Sarin and DFP solvolysis occur *via* the same mechanism. Experimentally, it is found that whereas there is an eighty-fold difference in the values of k_{OH} obtained for Sarin and DFP hydrolysis, only a four- to fifteen-fold change in k_L is observed relative to the two substrates.

TABLE V

DATA USED FOR CALCULATION OF THE ENTHALPY OF ACTIVATION FOR THE Cu(II)-DIPY CATALYZED HYDROLYSIS OF DFP AT −LOG [H⁺] 6.90

t, °C.	T_M[a] × 10³	[CuL²⁺] × 10⁴	k_{obs}, sec.⁻¹	[OH⁻]	k_A'
0.3	1.150	8.60	4.85×10^{-5}	1.49×10^{-8}	3.76×10^{6}
25.0	1.147	4.89	5.02×10^{-4}	1.28×10^{-7}	8.02×10^{6}
42.5	1.144	2.04	1.35×10^{-3}	4.45×10^{-7}	1.49×10^{7}

[a] T_M = total metal ion concentration; $\Delta H^{\ddagger} = 5.1 \pm 0.6$ kcal./mole, $\Delta S^{\ddagger} = -9.8$ cal./mole deg. at 25°.

Catalysis by Cu(II)-glycylglycine (GG) and Cu(II)-diglycylglycine (GGG).—The results of catalytic hydrolysis of DFP by Cu(II)-GG and Cu(II)-GGG are shown in Table VI. Two interesting results may be noticed. First the catalytic effect of the chelate of the quadridentate ligand, GGG, is greater than that of the terdentate ligand, GG, at −log [H⁺] 6.90 and, second, the rate observed in the presence of Cu(II)-GGG is greater at −log [H⁺] 6.90 than at 8.90.

Consideration of the distribution of chelate species under the conditions employed makes it possible to explain the effects noted above. From data published by Murphy and Martell[11] the equilibrium constants for the following reactions were interpolated to the following values at 25°

$$H_2GG \overset{k_2}{\rightleftharpoons} HGG^{1-} + H^+;\ pk_2 = 8.14$$

$$Cu^{2+} + HGG^{1-} \overset{K_1}{\rightleftharpoons} CuHGG^{1+};\ \log K_1 = 7.10$$

$$CuHGG^{1+} \overset{K_{1a}}{\rightleftharpoons} CuGG + H^+;\ pK_{1a} = 5.38$$

$$H_3GGG \overset{k_2}{\rightleftharpoons} H_2GGG^{1-} + H^+;\ pk_2 = 7.86$$

$$Cu^{2+} + H_2GGG^{1-} \overset{K_1}{\rightleftharpoons} CuH_2GGG^{1+};\ \log K_1 = 5.52$$

$$CuH_2GGG^{1+} \overset{K_{1a}}{\rightleftharpoons} CuHGGG + H^+;\ pK_{1a} = 7.00$$

$$CuHGGG \overset{K_{2a}}{\rightleftharpoons} CuGGG^{1-} + H^+;\ pK_{2a} = 5.60$$

By the use of these constants it was possible to calculate the species distribution as illustrated in Fig. 2 and 3 for the Cu(II)–GGG and Cu(II)–GG systems, respectively. Correlation of rate data with equilibrium data for the Cu(II)–GGG system, assuming that [CuHGGG] and [$CuGGG^{1-}$] have negligible catalytic effects relative to those of [CuH_2GGG^{1+}] and the unchelated copper species, shows that the entire catalytic effect is caused by the unchelated metal ion

$$k_M = 8.2 \times 10^{6};\ k_{MH_2GGG} \sim 0$$

A summary of Cu(II)–GGG–DFP data showing the effect of the unbound cupric ion concentration on the reaction rate is shown in Table VII. Description of the observed rate of hydrolysis by the equation

$$k_{obs}^{*} = k_M[Cu][OH^-]$$

is in agreement with the experimental data within ±4%, which is roughly equivalent to an error of only ±0.02 pH unit in the instrument setting. It may be

(11) C. B. Murphy and A. E. Martell, *J. Biol. Chem.*, **226**, 37 (1957).

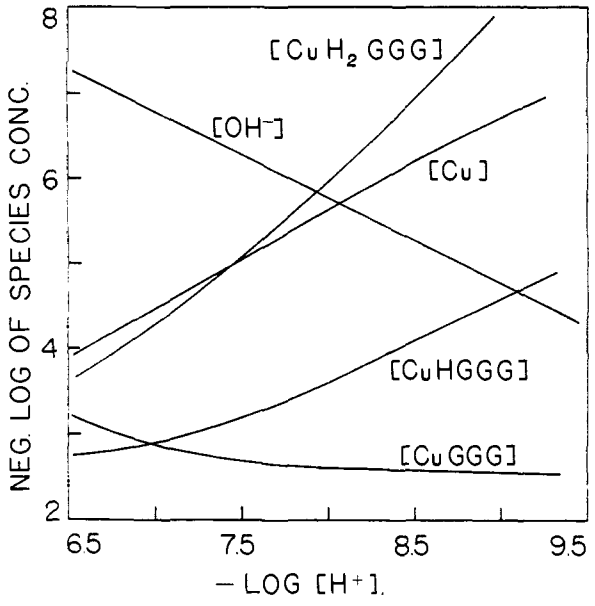


Fig. 2.—Concentrations of Cu(II)-glycylglycylglycine complex species as a function of hydrogen ion concentration; 1:1 molar ratio of Cu(II) to ligand; H_3GGG = glycylglycylglycine.

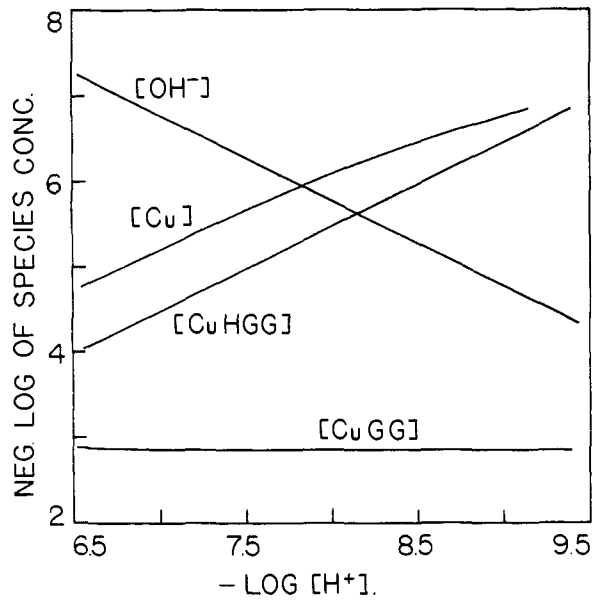
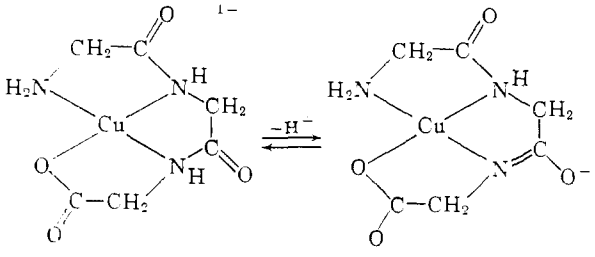


Fig. 3.—Concentrations of Cu(II)-glycylglycine complex species as a function of hydrogen ion concentration; 1:1 molar ratio of Cu(II) to ligand; H_2GG = glycylglycine.

seen in Fig. 2 that the quantity $[Cu^{2+}]$ decreases more rapidly than $[OH^-]$ increases, resulting in a smaller value of $[Cu][OH^-]$ as the pH increases. This is the reason for the decrease in the observed rate with increase in pH for the Cu(II)–GGG–DFP system.

The catalytic inactivity of the diglycylglycine chelate species suggests that formation of the chelate initially involves coördination of all four donor groups, followed by stepwise dissociation of protons from the peptide linkages, according to the equilibria



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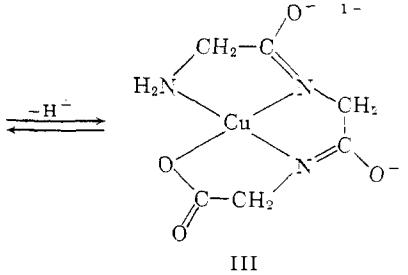

III

All of the compounds depicted by structures I–III would be expected to be extremely poor catalysts because there is no site on the copper atom available for strong coördination with the oxygen atom of DFP.

Assuming a value of $k_M = 8.0 \times 10^6$ to hold for the Cu(II)–GG system, it was possible to determine rate constants for $CuHGG^+$ and CuGG, using the slope intercept equation

$$\frac{k_{obs}^{**}}{[CuGG][OH^-]} = k_{MHGG}\frac{[CuHGG^+]}{[CuGG]} + k_{MGG} \qquad (5)$$

In the above expression k_{obs}^{**} is equal to $k_{obs} - k_{OH}[OH^-] - k_M[Cu^{2+}]$. Although only two experimental points are available, the values of $k_{MHGG} = 1.6 \times 10^6$ and $k_{MGG} = 6.5 \times 10^3$ indicate that the $CuHGG^+$ species possesses a catalytic activity in excess of one hundred times that of the neutral chelate, CuGG, even though both species probably possess one available coördination site. In both cases the rate constants were calculated on the basis of a third-order reaction involving DFP, the chelate species and hydroxyl ion. The greater catalytic activity of the Cu(II)–GGG system, relative to that of the Cu(II)–GG system at − log $[H^+]$ 6.90 may be explained by the fact that the highly active unbound cupric ion is present to the extent of 2.4% in the former case but only 0.5% in the latter.

TABLE VI

CATALYTIC HYDROLYSIS OF DFP IN THE PRESENCE OF Cu(II)-GG AND Cu(II)-GGG

Ligand	$T_M = T_L$ × 10^3 M	−log $[H^+]$	$t_{1/2}$, sec.	k_{obs}, sec.$^{-1}$
GG	1.46	6.90	38,500	1.80×10^{-5}
GG	1.45	8.90	4,500	1.54×10^{-4}
GGG	1.39	6.90	20,100	3.45×10^{-5}
GGG	2.69	6.90	14,300	4.85×10^{-5}
GGG	1.38	8.90	33,600	2.06×10^{-5}
GGG	2.66	8.90	22,800	3.04×10^{-5}

TABLE VII

DATA FOR Cu(II)-GGG CATALYZED HYDROLYSIS OF DFP DEMONSTRATING EFFECT OF UNCHELATED CUPRIC ION CONCENTRATION ON RATE OF REACTION

T_M × 10^3	k^*_{obs}[a]	[Cu]	$[OH^-]$	$\frac{k^*_{obs}}{[Cu][OH^-]}$ × 10^{-6}
1.39	3.45×10^{-5}	3.36×10^{-5}	1.28×10^{-7}	8.0
2.69	4.85	4.69×10^{-5}	1.28×10^{-7}	8.1
1.38	1.68	1.69×10^{-7}	1.28×10^{-5}	7.8
2.66	2.66×10^{-5}	2.34×10^{-7}	1.28×10^{-5}	8.9
			Average	$8.2 \pm 0.3 \times 10^6$

[a] $k^*_{obs} = k_{obs} - k_{OH}[OH^-]$.