filtering, washing (hexane), and drying weighed 7.0 g. This solid was found to be satisfactory without recrystallization, mp 116°. *Anal.* Calcd for $C_{41}H_{39}P_3CuO_2F_3$: C, 63.36; H, 5.06; P, 11.96; Cu, 8.17 (mol wt 777.24). Found: C, 63.20; H, 5.00; P, 11.69; Cu, 7.66 [mol wt 539 (dichlorobenzene)].

Bis[bis(diphenylphosphino)ethano]copper(I) Trifluoroacetate.—With stirring, a solution of 8.10 g of bis(diphenylphosphinoethane) in 50 ml of benzene was added to 1.8 g of copper(I) trifluoroacetate dissolved in 25 ml of benzene. After 30 sec a white solid appeared, but stirring was maintained 1 hr. Filtration gave 9.9 g of white powder. Recrystallization from ethanol–heptane yielded white crystals, mp 199–200° dec (effervescence). It appeared that a molecule of ethanol was included in the crystal from the analysis. *Anal.* Calcd for $C_{56}H_{54}P_4CuO_2F_3$: C, 65.97; H, 5.34; P, 12.15; Cu, 6.23 (mol wt 1018.51). Found: C, 65.81; H, 5.20; P, 12.04; Cu, 6.26 [mol wt 1440 ($CHCl_3$), 1045 (dichlorobenzene)].

Tetra-*n*-butylammonium Trifluoroacetate.—Two methanolic solutions were added together in a flask; the first contained 4.5 g (0.0204 mol) of silver trifluoroacetate in 5 ml; the second, 7.52 g (0.0204 mol) of tetra-*n*-butylammonium iodide also in 5 ml. After stirring several minutes, the yellow silver iodide was filtered (4.7 g). From the filtrate, 7.9 g of clear oil was obtained by flashing the alcohol. White crystals (6.2 g, mp 45°) were obtained from ethanol–ether–pentane. The solid was quite hygroscopic. After pumping at 55°, 0.01 Torr overnight, satisfactory analysis on the white crystals, mp 85.5–86°, was obtained. *Anal.* Calcd for $C_{18}H_{36}NO_2F_3$: C, 60.81; H, 10.21; N, 3.94 (mol wt 355.49). Found: C, 60.48; H, 9.85; N, 4.15.

(1,5-Cyclooctadiene)copper(I) Trifluoroacetate.—A solution composed of 2.16 g (0.02 mol) of 1,5-cyclooctadiene in 5 ml of pentane was added dropwise to a well-stirred slurry of 1.77 g (0.01 mol) of copper(I) trifluoroacetate in 50 ml of pentane. There was a slight temperature rise, and the solution took on a green cast. After stirring 2 hr the solid was filtered and washed with two 15-ml portions of pentane in a drybox, resulting in 1.7 g of a white solid, mp 188–190°. *Anal.* Calcd for $C_{10}H_{12}CuO_2F_3$: C, 42.18; H, 4.25; Cu, 22.31 (mol wt 284.75). Found: C, 41.64; H, 4.35; Cu, 22.75. Infrared spectrum (cm^{-1}): in KBr: ν_{CO_2} 1680 (vb); ν_{C-C} 1545 (w); ν_{CF_3} 1200, 1140 (vb); $\nu_{O_2C-CF_3}$ 840; in Nujol mull: ν_{CO_2} 1670 (b); ν_{CF_3} 1200, 1150 (b); $\nu_{O_2C-CF_3}$ 845.

(1,5-Cyclooctadiene)dicopper(I) Trifluoroacetate.—To a solution of 3.5 g of copper(I) trifluoroacetate (0.02 mol) in 25 ml of benzene, 1.0 g of 1,5-cyclooctadiene (0.0094 mol) was added dropwise, and the reaction was stirred at ambient temperature 3 days. A precipitate appeared which was then filtered, washed with pentane, and dried, resulting in the isolation of 2.75 g of light yellow solid, mp 192° dec. *Anal.* Calcd for $C_{12}H_{12}Cu_2O_4F_6$: Cu, 27.55 (mol wt 461.31). Found: Cu, 27.61. Infrared spectrum (cm^{-1}) (Nujol mull): ν_{CO_2} 1675; ν_{CF_3} 1145, 1205; $\nu_{O_2C-CF_3}$ 850.

(Cyclooctatetraene)dicopper(I) Trifluoroacetate.—Cyclooctatetraene (1.04 g; 0.01 mol) in 5 ml of benzene was mixed dropwise with a solution of 25 ml of benzene containing 3.54 g (0.02 mol) of copper(I) salt. A dark red solution resulted concommtant with a temperature rise of 10°. After overnight stirring, a light green solution over a precipitate was evident. Filtration, washing (pentane), and drying yielded 3.0 g of faintly yellow solid (mp *ca.* 235°, some dec 170°). *Anal.* Calcd for $C_{12}H_8Cu_2O_4F_6$: C, 31.52; H, 1.76; Cu, 27.79 (mol wt 457.28). Found: C, 31.85; H, 1.89; Cu, 27.67. Infrared spectrum (cm^{-1}) (Nujol mull): ν_{CO_2} 1670 (b); ν_{CF_3} 1140, 1200 (vb); $\nu_{O_2C-CF_3}$ 845. Addition of excess cyclooctatetraene to slurries of the above complex in benzene dissolves the solid; however, isolation of the product by precipitation with pentane led to the starting complex (27.67% Cu), with an identical ir spectrum.

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Amino Acid Catalysis of the Transfer of Copper(II) from Oligopeptide Complexes to Ethylenediaminetetraacetate

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The reactions of the copper(II)–oligopeptide complexes of triglycine ($Cu(H_{-2}GGG)^-$), tetraglycine ($Cu(H_{-3}GGGG)^{2-}$), and L-alanyl-L-alanyl-L-alanine ($Cu(H_{-2}AAA)^-$) with EDTA are catalyzed by amino acids. Under conditions of excess EDTA and low amino acid concentrations the experimentally observed rates are first order in the copper–oligopeptide and pseudo first order in the amino acid. The resolved second-order rate constants for the amino acid catalysis exhibit a direct dependence on the stability of their corresponding copper(II) complexes. Steric hindrance prevents EDTA from being an effective nucleophile with the copper(II)–oligopeptide complexes and the postulated role of the amino acid catalyst is to facilitate the formation of a species with only one Cu–N(peptide) bond. This complex, $Cu(H_{-1}L)(aa)^-$ (where L = oligopeptide and aa is the amino acid), allows nucleophilic attack by a tertiary nitrogen of EDTA on a planar copper site. A general mechanism for catalysis of the transfer of copper ion from oligopeptide complexes to EDTA is proposed.

Introduction

Recent work in this laboratory has shown that the transfer of copper(II) from its triglycine complex to ethylenediaminetetraacetate ion (EDTA) is catalyzed by the released triglycine above pH 8.[1] The exchange reaction is given in eq 1 where GGG^- is the glycylglycylglycinate ion and $Cu(H_{-2}GGG)^-$ is the complex in which two protons are ionized from the peptide nitrogens. Steric factors are important in controlling which ligands are able to react *via* a nucleophilic path with $Cu(H_{-2}GGG)^-$. EDTA and other ligands with only

$$Cu(H_{-2}GGG)^- + \begin{bmatrix} EDTA^{4-} + 2H_2O \\ HEDTA^{3-} + H_2O \end{bmatrix} \longrightarrow \begin{bmatrix} 2OH^- \\ OH^- \end{bmatrix} + CuEDTA^{2-} + GGG^- \quad (1)$$

tertiary nitrogens are sterically hindered in their reactions with this complex.[2] The proposed path of autocatalysis involves the formation of a bis(triglycine) complex which is more readily attacked by EDTA.

In the present work amino acids are shown to be remarkably effective catalysts for the transfer of copper

(1) G. R. Dukes, G. K. Pagenkopf, and D. W. Margerum, *Inorg. Chem.*, **10**, 2419 (1971).

(2) G. K. Pagenkopf and D. W. Margerum, *J. Amer. Chem. Soc.*, **92**, 2683 (1970).

ion, not only from $Cu(H_{-2}GGG)^-$, but from $Cu(H_{-3}GGGG)^{2-}$ and $Cu(H_{-2}AAA)^-$ as well.

Experimental Section

Triglycine was obtained (chromatographically homogeneous) from Schwarz–Mann, Orangeburg, N. Y.; tetraglycine was obtained from the International Chemical and Nuclear Corp., Irvine, Calif.; L-alanyl-L-alanyl-L-alanine and the amino acids used were obtained from the Cyclo Chemical Division of Travenol Laboratories, Los Angeles, Calif. A 9.85×10^{-2} *M* stock solution of $Cu(ClO_4)_2$ was prepared from the twice-recrystallized salt and standardized against EDTA. The copper–oligopeptide solutions were freshly prepared (<3 hr) before each series of kinetic measurements. In addition, the copper–tetraglycine solutions were purged with nitrogen immediately after their preparation to prevent reaction of the complex with molecular oxygen.[3] Ionic strength was maintained at 0.10 *M* with $NaClO_4$. Hydrogen ion concentrations were calculated from pH measurements using the relationship $-\log [H^+] = pH - 0.11$,[4] and hydroxide ion concentrations were calculated from $pK_w = 13.78$. Sodium tetraborate was used as the buffering agent (total borate $= 5.0 \times 10^{-3}$ *M*), unless otherwise noted.

The reactions were monitored by following the appearance of $CuEDTA^{2-}$ at 280 nm (molar absorptivities (M^{-1} cm^{-1}) are 930 for $Cu(H_{-2}GGG)^-$, 1120 for $Cu(H_{-3}GGGG)^{2-}$, 1200 for $Cu(H_{-2}AAA)^-$, and 2650 for $CuEDTA^{2-}$). Kinetic runs were performed an a Durrum-Gibson stopped-flow spectrophotometer with a 2.0-cm cell path. The photomultiplier output was interfaced to a Hewlett-Packard 2115A general-purpose digital computer as described elsewhere.[5] Using this system, up to 250 points may be taken at a rate as fast as 1 point/100 μsec. All reactions in this study were run under first-order or pseudo-first-order conditions and each rate constant is the average of at least four kinetic runs. The standard deviations were calculated from the deviation of the rate constants from the mean.

The oligopeptides, their amine group protonation constants and the log K_H values for the copper complexes of interest in this study (where K_H is a protonation constant of the peptide complex, *e.g.*, for $Cu(H_{-2}GGG)^-$, $K_H = [Cu(H_{-1}GGG)]/[Cu(H_{-2}GGG)^-][H^+]$) are listed in Table I. Also listed in Table I are the amino acids and other ligands used with their amine group protonation constants and the K_2 values for their copper(II) complexes where $K_2 = [Cu(aa)_2]/[Cu(aa)^+][aa^-]$.

TABLE I

A. Protonation Constants of the Oligopeptides, 25°

Oligopeptide	log $K_H(H_2NR)$	Ref
Glycylglycylglycine (GGG)	7.88	*a*
Glycylglycylglycylglycine (GGGG)	7.89	*b*
L-Alanyl-L-alanyl-L-alanine (AAA)	8.08	*c*

B. Protonation Constants of the Copper–Oligopeptide Complexes, 25°

Species	log K_H	μ, *M*	Ref
$Cu(H_{-1}GGG)$	5.4	0.1 (KNO_3)	*b*
$Cu(H_{-2}GGG)^-$	6.63	0.1 (KNO_3)	*b*
$Cu(H_{-2}GGGG)^-$	6.77	0.1 (KNO_3)	*b*
$Cu(H_{-3}GGGG)^{2-}$	9.00	0.1 (KNO_3)	*b*
$Cu(H_{-2}AAA)^-$	6.63	~0	*c*

C. Other Constants Used in This Study, 25°

Ligand	log $K_H(H_2NR)$	log K_2[d]
Glycine (gly)	9.59[e]	6.87[e]
Glycinamide (Ga)	8.06[f]	4.21[f]
Sarcosine (sar)	10.01[h]	6.65[h]
L-Threonine (thr)	9.07[g]	6.74[g]
L-Aspartic acid (asp)	9.62[h]	6.78[i]
L-Phenylalanine (phe)	9.13[j]	6.90[j]
L-Histidine (his)	9.17,[k] 6.08[k,n]	8.00[i]
Ethylenediamine (en)	9.83, 7.05[l]	9.31[m]
N,N-Dimethylglycine (*N,N*-dimegly)	9.88[o]	6.35[o]
N,N-Dimethylethylenediamine (*N,N*-dimeen)	9.60, 6.32[l]	6.70[p]

[a] H. Hauer, E. J. Billo, and D. W. Margerum, *J. Amer. Chem. Soc.*, **93,** 4173 (1971). [b] M. K. Kim and A. E. Martell, *ibid.*, **88,** 914 (1966). [c] G. F. Bryce and F. R. N. Gurd, *J. Biol. Chem.*, **241,** 1439 (1966). [d] $K_2 = [Cu(aa)_2]/[Cu(aa)^+][aa^-]$. [e] F. Basolo and Y. T. Chen, *J. Amer. Chem. Soc.*, **76,** 953 (1954). [f] N. C. Li, B. E. Doody, and J. M. White, *ibid.*, **79,** 5859 (1957). [g] J. E. Letter, Jr., and J. E. Bauman, Jr., *ibid.*, **92,** 437 (1970). [h] R. F. Lumb and A. E. Martell, *J. Phys. Chem.*, **57,** 690 (1953). [i] N. C. Li and B. E. Doody, *J. Amer. Chem. Soc.*, **72,** 1891 (1950). [j] N. C. Li and B. E. Doody, *ibid.*, **74,** 4184 (1952). [k] A. Chakravorty and F. A. Cotton, *J. Phys. Chem.*, **67,** 2878 (1963). [l] E. J. Billo, G. F. Smith, and D. W. Margerum, *J. Amer. Chem. Soc.*, **93,** 2635 (1971). [m] P. L. Peczok and J. Bjerrum, *Acta Chem. Scand.*, **11,** 1419 (1957). [n] Log K_H of imidazole group. [o] A. L. Remizov, *Zh. Obshch. Khim.*, **34,** 3192 (1964). [p] H. Irving and J. M. M. Griffiths, *J. Chem. Soc.*, 4370 (1954).

Results

General Mechanism.—In the amino acid catalysis of the transfer of copper ion from $Cu(H_{-2}GGG)^-$, $Cu(H_{-3}GGGG)^{2-}$, and $Cu(H_{-2}AAA)^-$ the anionic form of the amino acid is found to be the kinetically reactive species. The zwitterion form accounts for less than 1% of the reaction rate with the exception of the L-histidine reaction which will be discussed later. The inactivity of the zwitterion form of the amino acids is consistent with the results of several recent kinetic investigations of the reactions of amino acids with Co^{2+}, Ni^{2+}, and Cu^{2+}.[6-9]

The rate constants observed under conditions of excess EDTA and low amino acid concentrations exhibit a first-order dependence on both the copper–oligopeptide and the amino acid concentrations. It has been found that a rapid preequilibration between the triply deprotonated and doubly deprotonated copper–tetraglycine species takes place (eq 2). The three doubly deprotonated copper–oligopeptide species, $Cu(H_{-2}GGGG)^-$, $Cu(H_{-2}GGG)^-$, and $Cu(H_{-2}AAA)^-$, exhibit similar kinetic dependence with the various amino acids. Hydroxide ion also affects the reactions of these complexes in a similar manner. Under conditions where the rate is first order in amino acid concentration, no dependence was found in the EDTA concentration. However, the rate becomes responsive to EDTA when the amino acid concentration is increased to the point where the rate is no longer first order in amino acid. In addition, as the amino acid concentration is increased, the rate increases, reaches a maximum, and then decreases as the amino acid concentration is further increased.

The general mechanism, given in eq 2–5, is proposed

$$Cu(H_{-3}L)^{2-} + H_2O \overset{K_h}{\rightleftharpoons} Cu(H_{-2}L)^- + OH^- \quad (2)$$

$$H_2O + Cu(H_{-2}L)^- + X \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Cu(H_{-1}L)X + OH^- \quad (3)$$

$$Cu(H_{-1}L)X + EDTA^{4-} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} Cu(H_{-1}L)(EDTA)^{4-} + X \quad (4)$$

$$H_2O + Cu(H_{-1}L)(EDTA)^{4-} \xrightarrow{k_3} CuEDTA^{2-} + L^- + OH^- \quad (5)$$

(3) G. L. Burce, E. B. Paniago, and D. W. Margerum, unpublished data.
(4) R. G. Bates, "Determination of pH," Wiley, New York, N. Y., 1964, p 92.
(5) B. G. Willis, J. A. Bittikoffer, H. L. Pardue, and D. W. Margerum, *Anal. Chem.*, **42,** 1430 (1970).
(6) A. F. Pearlmutter and J. Stuehr, *J. Amer. Chem. Soc.*, **90,** 858 (1968).
(7) R. F. Pasternack, E. Gibbs, and J. C. Cassatt, *J. Phys. Chem.*, **73,** 3814 (1969).
(8) R. L. Karpel, K. Kustin, and R. F. Pasternack, *Biochim. Biophys. Acta*, **177,** 434 (1969).
(9) R. G. Wilkins, *Accounts Chem. Res.*, **3,** 408 (1970).

where L is the oligopeptide and X is the catalyst (amino acids, amides, or amines). The detailed kinetic behavior of each of the copper–oligopeptide complexes and the relationship of this behavior to the general mechanism are presented in the subsequent sections.

Glycylglycylglycine.—The experimentally observed rate expression is $d[CuEDTA^{2-}]/dt = k_{obsd}[Cu(H_{-2}GGG)^{-}]$, where $k_{obsd} = k_1[aa^-] + k_d + k_5[EDTA^{4-}]$ ($k_d = 0.12$ sec^{-1} [10] and $k_5 = 600\ M^{-1}$ sec^{-1} [1]). The observed rate constants as well as the resolved k_1 values are given in Table II. To be especially noted from

TABLE II

DEPENDENCE OF k_{obsd} ON AMINO ACID CONCENTRATION FOR THE CATALYZED TRANSFER OF COPPER ION FROM $Cu(H_{-2}GGG)^-$ TO EDTA ($[Cu]_T = 3.95 \times 10^{-5}\ M$, $[EDTA]_T = 2.00 \times 10^{-4}\ M$, EXCEPT AS OTHERWISE NOTED, $\mu = 0.10$ ($NaClO_4$), 25.0 ± 0.1°)

X[a]	−log [H⁺]	$10^5[X]$,[b] M	k_{obsd}, sec^{-1}	k_1, M^{-1} sec^{-1}
Glycine[c]	9.10	0.498	0.157 ± 0.003	1.42×10^4
	9.12	0.996	0.239 ± 0.001	
	9.11	1.49	0.304 ± 0.004	
	9.12	1.98	0.379 ± 0.008	
	9.11	2.49	0.440 ± 0.002	
L-Histidine	9.10	0.0918	0.290 ± 0.003	2.27×10^5
	9.10	0.184	0.485 ± 0.003	
	9.13	0.191	0.536 ± 0.005	
	9.13	0.238	0.655 ± 0.004	
	9.13	0.333	0.86 ± 0.02	
L-Threonine	9.12	5.42	0.465 ± 0.010	6.11×10^3
	9.12	10.6	0.757 ± 0.007	
	9.12	15.9	1.09 ± 0.01	
Glycinamide	9.11	18.4	0.15[d]	2.70×10^3
	9.11	27.6	0.178[e]	
	9.30	47.5	0.250 ± 0.001	
	9.30	95.5	0.360 ± 0.005	
L-Aspartic acid	9.13	2.40	0.313 ± 0.003	8.10×10^3
	9.12	4.80	0.509 ± 0.003	
	9.11	7.20	0.707 ± 0.006	
L-Phenylalanine	9.11	4.88	1.15 ± 0.01	2.0×10^4
	9.11	9.76	2.08 ± 0.02	
Sarcosine	9.89	4.42	0.194 ± 0.001	1.64×10^3
	9.91	8.85	0.298 ± 0.009	
	9.90	13.2	0.374 ± 0.002	
N,N-Dimethylglycine	9.90	12.4	0.171 ± 0.003	2.90×10^2
	9.93	49.7	0.277 ± 0.004	
	9.92	124	0.459 ± 0.003	
N,N-Dimethylethylenediamine	9.11	0.263	0.440 ± 0.003	1.30×10^5
	9.50	0.478	0.79 ± 0.02	
	9.11	0.524	0.788 ± 0.003	
	9.11	0.788	1.12 ± 0.01	

[a] X = catalytic species (amino acids, amides, or amines). [b] [X] = concentration of unprotonated catalytic species. [c] $[EDTA]_T = 5.00 \times 10^{-4}\ M$. [d] Single run. [e] Two runs.

Table II is the fact that there is a large variation in the k_1 values for the catalytic species, X. The k_1 values correlate with the stability of the copper complexes of the respective catalytic species. A good correlation is found with the stepwise equilibrium constant for the formation of the $Cu(X)_2$ species ($K_2 = [Cu(X)_2]/[CuX][X]$) as might be expected from the postulated formation of the mixed complex, $Cu(H_{-1}L)X$, in eq 3. The proportionality of k_1 with K_2 is shown in Figure 1, where the solid line in this log–log plot is constrained to a slope of unity. If the catalytic species were bound in a monodentate fashion in the $Cu(H_{-1}L)X$ mixed complex, the observed correlation of k_1 with K_2 would not be expected. Thus, the catalytic species must be

(10) G. K. Pagenkopf and D. W. Margerum, *J. Amer. Chem. Soc.*, **90**, 6963 (1968).

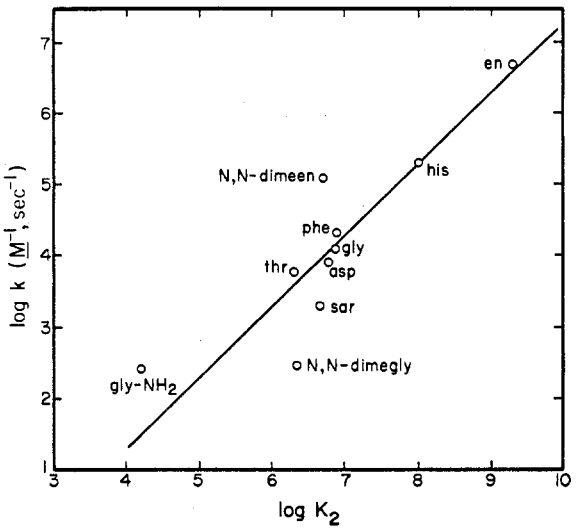


Figure 1.—Dependence of second-order catalytic rate constants on K_2 for catalysis of copper ion transfer from $Cu(H_{-2}GGG)^-$ to EDTA, $K_2 = [Cu(aa)_2]/[Cu(aa)^+][aa^-]$. The solid line is constrained to a slope of unity.

behaving as a bidentate chelate either prior to or during the rate-determining step.

The gly^- and his^- catalyzed reactions were intensively studied and exhibited the following characteristics in addition to their first-order dependence when present at low concentrations. (1) The rate is depressed at higher pH (Table III, Section A). (2) Under conditions where the rate is first order in the amino acid concentration, there is no EDTA dependence (Table III, Section B). (3) At pH 9, where the rate is first order in L-histidine at low concentration, large excesses of L-histidine inhibit the reaction rate (Table III, Section C). (4) The rates exhibit a dependence on EDTA when the reactions are run under conditions where there is no longer a first-order dependence in the amino acid concentration (Table III, Section D).

The general mechanism proposed in eq 3–5 accounts for these observations. Equation 2 is omitted because there is no $Cu(H_{-3}L)^{2-}$ species for L = GGG. By assuming steady-state concentrations of $Cu(H_{-1}GGG)(aa)^-$ (where X = aa^-) and $Cu(H_{-1}GGG)(EDTA)^{4-}$, eq 6 may be derived where $k'_{obsd} = k_{obsd} - (k_d + k_5[EDTA^{4-}])$. At constant pH and $EDTA^{4-}$ concentration, eq 6 may be rearranged to yield eq 7, where $A =$

$$k'_{obsd} = \frac{k_1k_2k_3[EDTA^{4-}][aa^-]}{k_{-1}k_{-2}[aa^-][OH^-] + k_{-1}k_3[OH^-] + k_2k_3[EDTA^{4-}]} \quad (6)$$

$$\frac{1}{k'_{obsd}} = \frac{C}{A[aa^-]} + \frac{B}{A} \quad (7)$$

$k_1k_2k_3[EDTA^{4-}]$, $B = k_{-1}k_{-2}[OH^-]$, and $C = k_{-1}k_3 \cdot [OH^-] + k_2k_3[EDTA^{4-}]$. Plots of eq 7 for the L-histidine catalyzed reaction at three different values of pH are shown in Figure 2. From the slopes and intercepts of these plots for L-histidine and glycine (data in Table III, Section A) the ratios of k_2/k_{-1} and k_{-2}/k_3, respectively, were calculated. Good agreement was found for the ratios calculated at different pH values. The averages for k_2/k_{-1} are 21 ± 7 and 6.6 ± 0.7 for

TABLE III
EFFECT OF pH AND AMINO ACID AND EDTA CONCENTRATIONS ON THE RATE OF COPPER TRANSFER FROM $Cu(H_{-2}GGG)^-$ TO EDTA ($[Cu(H_{-2}GGG)^-] = 3.95 \times 10^{-5}$ *M*, $\mu = 0.10$ ($NaClO_4$), 25.0 ± 0.1°)

A. Effect of pH on Glycine and L-Histidine Catalysis[a]

Glycine			L-Histidine		
−log [H⁺]	$10^5 \times$ [gly⁻], *M*	k_{obsd}, sec^{-1}	−log [H⁺]	$10^5 \times$ [his⁻], *M*	k_{obsd}, sec^{-1}
10.27	3.29	0.550 ± 0.004	10.25	2.79	0.552 ± 0.004
10.28	4.10	0.619 ± 0.006	10.29	3.74	0.625 ± 0.003
10.27	4.31	0.631 ± 0.003	10.27	4.63	0.701 ± 0.009
10.27	4.93	0.71 ± 0.01	10.26	4.65	0.734 ± 0.003
10.29	5.75	0.766 ± 0.005	10.26	5.62	0.806 ± 0.007
10.28	6.03	0.789 ± 0.008	10.25	6.51	0.949 ± 0.004
10.27	6.57	0.843 ± 0.003	10.28	8.34	0.98 ± 0.01
10.54	1.74	0.386 ± 0.005	10.54	1.92	0.421 ± 0.002
10.57	2.61	0.445 ± 0.004	10.53	2.89	0.518 ± 0.007
10.55	3.48	0.554 ± 0.003	10.57	3.88	0.554 ± 0.002
10.56	4.35	0.564 ± 0.003	10.53	4.80	0.625 ± 0.005
10.57	4.57	0.618 ± 0.003	10.52	4.81	0.632 ± 0.006
10.53	5.22	0.667 ± 0.005	10.57	5.82	0.686 ± 0.003
10.56	6.40	0.714 ± 0.006	10.52	6.74	0.760 ± 0.006
10.53	6.96	0.778 ± 0.004	10.54	8.64	0.809 ± 0.005
10.55	7.83	0.780 ± 0.012	10.86	2.93	0.405 ± 0.005
10.87	3.81	0.436 ± 0.001	10.85	4.89	0.485 ± 0.004
10.88	5.71	0.481 ± 0.003	10.85	6.85	0.511 ± 0.001
10.87	7.61	0.537 ± 0.004			

B. Independence of k_{obsd} on EDTA Concentration at pH 9.24[b]

10^4[EDTA]$_T$, *M*	k_{obsd}, sec^{-1}	10^4[EDTA]$_T$, *M*	k_{obsd}, sec^{-1}
2.00	0.536 ± 0.005	8.00	0.56 ± 0.02
4.00	0.528 ± 0.003	20.6	0.548 ± 0.009

C. Dependence of k_{obsd} on L-Histidine Concentration at Higher Levels of L-Histidine[c]

−log [H⁺]	$10^5 \times$ [his⁻], *M*	k_{obsd}, sec^{-1}	−log [H⁺]	$10^5 \times$ [his⁻], *M*	k_{obsd}, sec^{-1}
9.11	0.0918	0.290 ± 0.003	9.31	2.32	4.16 ± 0.05
9.11	0.184	0.485 ± 0.003	9.10	48.7	1.85 ± 0.01
9.31	0.578	1.28 ± 0.02	9.11	97.5	1.24 ± 0.02
9.31	1.16	2.34 ± 0.03	9.10	146	1.04 ± 0.01
9.30	1.73	3.30 ± 0.02			

D. EDTA Dependence in Region Where the Reaction Rate Does Not Exhibit a First-Order Dependence on L-Histidine[d]

−log [H⁺]	10^4-[EDTA]$_T$, *M*	k_{obsd}, sec^{-1}	−log [H⁺]	10^4-[EDTA]$_T$, *M*	k_{obsd}, sec^{-1}
10.39	2.01	2.07 ± 0.04	10.37	7.03	4.28 ± 0.06
10.40	5.02	3.52 ± 0.04	10.36	9.03	4.69 ± 0.03

[a] $[EDTA]_T = 2.00 \times 10^{-4}$ *M*. [b] $[his]_T = 4.00 \times 10^{-6}$ *M*. [c] $[EDTA]_T = 2.00 \times 10^{-4}$ *M*. [d] $[his]_T = 6.06 \times 10^{-5}$ *M*.

glycine and L-histidine, respectively. The respective values of k_{-2}/k_3 are $(4.3 \pm 0.4) \times 10^4$ M^{-1} and $(2.8 \pm 0.5) \times 10^5$ M^{-1}.

For glycine catalysis the resolution of the rate constants can be carried one step further since the stability constant for the reaction shown as eq 8 has been re-

$$H_2O + Cu(H_{-2}GGG)^- + gly^- \overset{K_1}{\rightleftharpoons} Cu(H_{-1}GGG)(gly)^- + OH^- \quad (8)$$

ported recently ($\log K_1 = -0.84$).[11] From this stability constant, the individual rate constants, k_2 and k_{-1}, may be calculated ($k_2 = 2.1 \times 10^6$ M^{-1} sec^{-1} and $k_{-1} = 9.8 \times 10^4$ M^{-1} sec^{-1}).

The catalysis of the transfer of copper ion from $Cu(H_{-2}GGG)^-$ to EDTA by L-histidine was investigated from pH 7 to 7.3 ($-\log [H^+] = 6.9$–7.2). At this acidity the observed rate constants, given in Table IV, contain, in addition to k_d and the rates due to cataly-

(11) R. P. Martin, L. Mosoni, and B. Sarkar, *J. Biol. Chem.*, **246**, 4944 (1971).

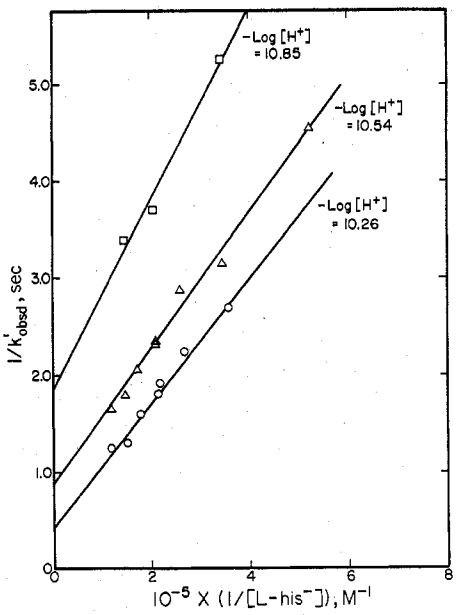


Figure 2.—Determinations of k_2/k_{-1} and k_{-2}/k_3 for his^- catalyzed transfer of copper ion from $Cu(H_{-2}GGG)^-$ to EDTA. The points are experimental and the solid lines are linear least-squares fits to the data.

TABLE IV
EFFECT OF pH AND L-HISTIDINE CONCENTRATION ON THE RATE OF COPPER ION TRANSFER FROM $Cu(H_{-2}GGG)^-$ AT LOWER pH ($[Cu(GGG)]_T = 3.95 \times 10^{-5}$ *M*, $[EDTA]_T = 2.00 \times 10^{-4}$ *M*, $[Lutidine]_T = 4.9 \times 10^{-3}$ *M*,[a] $\mu = 0.10$ ($NaClO_4$), 25.0 ± 0.1°)

−log [H⁺]	10^4[his]$_T$, *M*	k_{obsd}, sec^{-1}
6.90	0.505	1.52 ± 0.04
6.91	1.01	1.58 ± 0.01
6.91	3.03	2.12 ± 0.05
6.89	6.06	2.84 ± 0.08
7.17	0.0909	0.75 ± 0.03
7.22	0.505	0.864 ± 0.007
7.19	1.01	1.10 ± 0.03
7.22	5.05	2.73 ± 0.08
7.18	10.1	4.66 ± 0.05

[a] 2,6-Lutidine and its perchlorate salt were used as a buffer.

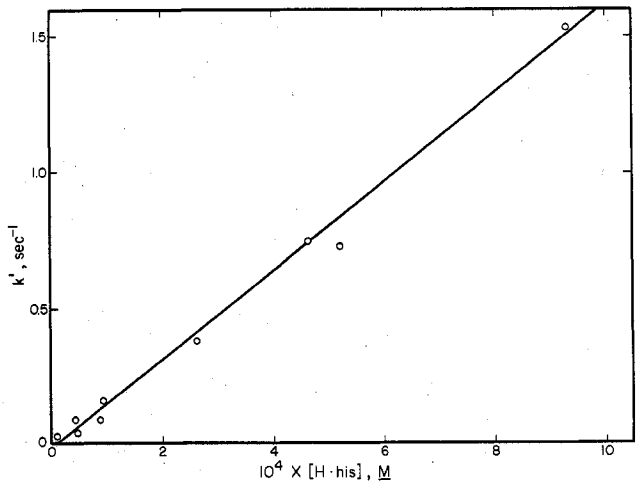


Figure 3.—Determination of k_1 for H·his catalyzed copper ion transfer from $Cu(H_{-2}GGG)^-$ to EDTA at physiological pH. The points are experimental and the solid line is a linear least-squares fit to the data: $[Cu]_T = 3.95 \times 10^{-5}$ *M*, $[EDTA]_T = 2.00 \times 10^{-4}$ *M*, $\mu = 0.10$ ($NaClO_4$), 25.0 ± 0.1°.

sis by H·his and his$^-$, contributions from the general acid species in solution (H_3O^+, H_2EDTA^{2-}, and H·lutidine$^+$; $k_{HX} = 4.9 \times 10^6\ M^{-1}\ sec^{-1}$, $3.1 \times 10^3\ M^{-1}\ sec^{-1}$, and $3.9 \times 10^2\ M^{-1}\ sec^{-1}$, respectively).[10] The first-order dependence of k' (k_{obsd} corrected for k_d, general acid catalysis and catalysis by his$^-$) on H·his concentration is shown in Figure 3. The value of k_1 for catalysis by H·his, obtained from the slope of Figure 3, is $(1.63 \pm 0.06) \times 10^3\ M^{-1}\ sec^{-1}$.

Tetraglycine.—The transfer of copper ion from $Cu(H_{-3}GGGG)^{2-}$ to EDTA also is catalyzed by amino acids and exhibits a kinetic dependence on the copper–oligopeptide and the amino acid concentration similar to that shown by the $Cu(H_{-2}GGG)^-$ reaction. The dependence of k_{obsd} at pH 10 on the amino acids and their concentration is given in Table V. The resolved

TABLE V

DEPENDENCE OF k_{obsd} ON THE AMINO ACID CONCENTRATION AND THE RESOLUTION OF THE SECOND-ORDER RATE CONSTANTS FOR CATALYSIS OF COPPER ION TRANSFER FROM $Cu(H_{-3}GGGG)^{2-}$ TO EDTA ($[Cu]_T = 3.95 \times 10^{-5}\ M$, $[EDTA]_T = 2.00 \times 10^{-4}\ M$, $\mu = 0.10$ ($NaClO_4$), $25.0 \pm 0.1°$)

X	−log [H$^+$]	10^5[X],[a] M	k_{obsd}, sec^{-1}	k, M^{-1} sec^{-1}
L-Histidine	9.92	0.103	0.503 ± 0.011	1.6×10^5
	9.91	0.205	0.665 ± 0.010	
	9.90	0.308	0.83 ± 0.02	
Glycine	9.91	0.364	0.604 ± 0.004	5.4×10^4
	9.93	0.757	0.779 ± 0.006	
	9.91	1.09	0.977 ± 0.005	
L-Threonine	9.89	1.83	0.70 ± 0.03	1.5×10^4
	9.90	3.66	0.966 ± 0.011	
	9.89	5.50	1.24 ± 0.02	
L-Aspartic acid	9.90	1.33	0.723 ± 0.010	2.7×10^4
	9.89	2.65	1.06 ± 0.01	
	9.90	3.98	1.28 ± 0.01	
L-Phenylalanine	9.89	0.848	0.620 ± 0.005	2.8×10^4
	9.90	1.70	0.89 ± 0.03	
	9.91	2.54	1.10 ± 0.04	
N,*N*-Dimethyl-glycine	9.87	1.00	0.48 ± 0.02	4.2×10^3
	9.88	1.99	0.496 ± 0.006	
	9.88	4.97	0.626 ± 0.002	
	9.89	12.4	0.90 ± 0.01	
Glycinamide	9.93	19.7	0.575 ± 0.007	9.2×10^2
	9.93	59.1	0.95 ± 0.02	
	9.93	98.4	1.24 ± 0.04	
N,*N*-dimethyl-ethylene-diamine	9.90	0.144	0.412 ± 0.003	6.1×10^4
	9.88	0.288	0.539 ± 0.004	
	9.87	0.362	0.590 ± 0.009	
	9.89	0.434	0.586 ± 0.007	
	9.87	0.725	0.798 ± 0.007	

[a] [X] = concentration of unprotonated catalytic species.

second-order rate constants, $k = k_{obsd}/[X]$, also are given in Table V for each amino acid, amide, or amine species. The correlation of k with K_2 (defined previously) is seen in Figure 4 and indicates that chelation of the catalytic species is as important in the rate-determining step of the $Cu(H_{-3}GGGG)^{2-}$ reactions as it is for those of $Cu(H_{-2}GGG)^-$.

Glycine catalysis was intensively studied for this case and a first-order dependence on gly$^-$ concentration was found from pH 8.4 to 10.4. The resulting second-order rate constants (k_{gly}), given in Table VI, Section A, exhibit a decreasing trend with increasing pH. In addition, under conditions where the reaction is first order in [gly$^-$], the reaction rate shows little or no dependence on the EDTA concentration (Table VI, Section B). Finally, when the glycine concentration is

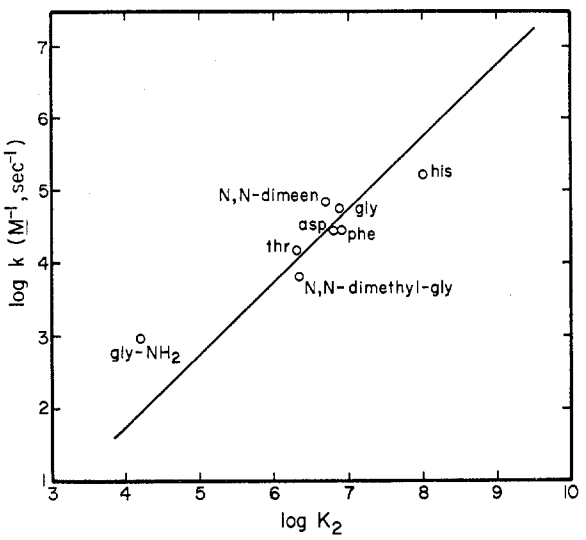


Figure 4.—Dependence of second-order catalytic rate constants on K_2 for catalysis of copper ion transfer from $Cu(H_{-3}GGGG)^{2-}$ to EDTA at pH 10. The solid line is constrained to a slope of unity.

TABLE VI

THE EFFECT OF GLYCINE AND EDTA CONCENTRATIONS AND pH ON THE RATE OF COPPER TRANSFER FROM COPPER–TETRAGLYCINE TO EDTA ($[Cu]_T = 3.95 \times 10^{-5}\ M$, $\mu = 0.10$ ($NaClO_4$), $25.0 \pm 0.1°$)

A. Effect of pH on the Second-Order Rate Constant for the Glycine-Catalyzed Exchange Reaction[a]

−log [H$^+$]	$10^{-4}k_{gly}$, M^{-1} sec^{-1}	−log [H$^+$]	$10^{-4}k_{gly}$, M^{-1} sec^{-1}
8.29	56.7	9.78	5.3
8.71	55	9.91	5.45
9.29	24.6	10.25	0.488

B. Independence of k_{obsd} on the EDTA Concentration at pH 8.82[b]

10^4[EDTA]$_T$	k_{obsd}, sec^{-1}	10^4[EDTA]$_T$	k_{obsd}, sec^{-1}
2.01	1.32 ± 0.02	7.03	1.56 ± 0.03
5.02	1.24 ± 0.02	9.03	1.48 ± 0.03

C. Effect of Excess Glycine on k_{obsd} at pH 10.57[c]

10^4[gly$^-$], M	k_{obsd}, sec^{-1}	10^4[gly$^-$], M	k_{obsd}, sec^{-1}
0.280	0.132 ± 0.002	1.05	0.162 ± 0.002
0.490	0.143 ± 0.003	3.50	0.190 ± 0.004
0.699	0.149 ± 0.001	6.99	0.263 ± 0.015

[a] $[EDTA]_T = 2.00 \times 10^{-4}\ M$. [b] $[gly]_T = 7.94 \times 10^{-6}\ M$. [c] $[EDTA]_T = 2.00 \times 10^{-4}\ M$.

increased, the reaction rate no longer exhibits a first-order dependence on the gly$^-$ concentration (Table VI, Section C).

The decreasing trend of the k_{gly} values with increasing pH indicates that the reactive species is $Cu(H_{-2}GGGG)^-$. Thus, the k_1 step must be preceded by a rapid preequilibrium between $Cu(H_{-3}GGGG)^{2-}$ and $Cu(H_{-2}GGGG)^-$ (eq 2).

For the data given in Table VI, Section A, k_1 may be calculated from the relationship given in eq 9. A plot

$$k_1 = k_{gly}\left(1 + \frac{K_h}{[H^+]}\right) \qquad (9)$$

of k_{gly} against the reciprocal of $(1 + (K_h/[H^+]))$ is shown as Figure 5 giving a value of k_1 equal to $(7.8 \pm 0.6) \times 10^5\ M^{-1}\ sec^{-1}$.

By assuming an equilibrium concentration of Cu-

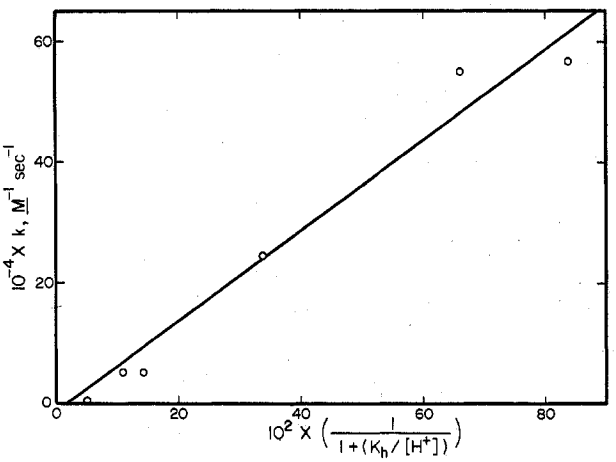


Figure 5.—Determination of k_1 for gly$^-$ catalyzed copper ion transfer from Cu(H$_{-2}$GGGG)$^-$ to EDTA. The points are experimental and the solid line is a linear least-squares fit to the data.

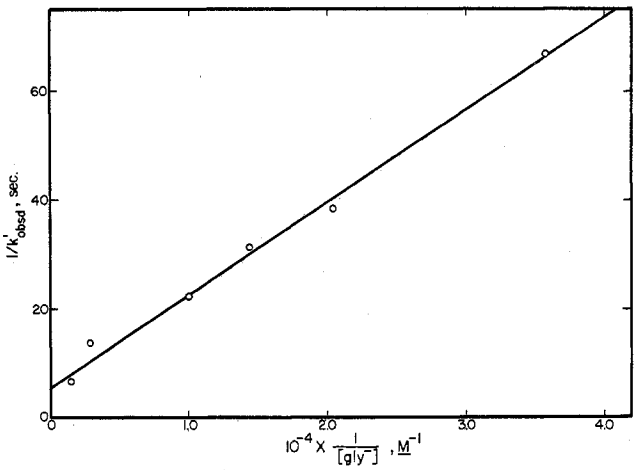


Figure 6.—Determination of k_2/k_{-1} and k_{-2}/k_3 for gly$^-$ catalyzed transfer of copper ion from Cu(H$_{-3}$GGGG)$^{2-}$ to EDTA. The points are experimental and the solid line is a linear least-squares fit to the data.

(H$_{-2}$GGGG)$^-$ and steady-state concentrations of Cu(H$_{-1}$GGGG)(aa)$^-$ and Cu(H$_{-1}$GGGG)(EDTA)$^{4-}$, eq 10 may be derived, where k'_{obsd} is obtained by subtracting all noncatalytic contributions from k_{obsd}. At constant pH and EDTA^{4-} concentration eq 10 may be rearranged to give eq 11, where $D = k_1k_2k_3[EDTA^{4-}]\cdot[1 + (K_h/[H^+])]^{-1}$, $E = k_{-1}k_{-2}[OH^-]$, and $F = k_{-1}k_3[OH^-] + k_2k_3[EDTA^{4-}]$. The double-reciprocal dependence of k'_{obsd} on [gly$^-$] is shown as Figure 6 (see Table VI, Section C, for data). From the slope (1.7×10^{-3} M sec) and intercept (5.5 sec) of Figure 6 the values of k_2/k_{-1} and k_{-2}/k_3 are 11 and 3.3×10^3 M^{-1}, respectively.

$$k'_{obsd} = \frac{k_1k_2k_3[EDTA^{4-}][gly^-][1 + (K_h/[H^+])]^{-1}}{k_{-1}k_{-2}[gly^-][OH^-] + k_{-1}k_3[OH^-] + k_2k_3[EDTA^{4-}]} \quad (10)$$

$$\frac{1}{k'_{obsd}} = \frac{F}{D[gly^-]} + \frac{E}{D} \quad (11)$$

L-Alanyl-L-alanyl-L-alanine.—The transfer of copper ion from Cu(H$_{-2}$AAA)$^-$ also is catalyzed by glycine and L-histidine. At pH 9 and under conditions of excess EDTA and low amino acid concentration the observed rate constants exhibit a first-order dependence on the amino acid concentration as shown in Table VII.

TABLE VII

EFFECT OF AMINO ACID CONCENTRATION ON THE RATE OF COPPER ION TRANSFER FROM Cu(H$_{-2}$AAA)$^-$ TO EDTA ([Cu(H$_{-2}$AAA)$^-$]$_T$ = 3.95×10^{-5} M, [EDTA]$_T$ = 2.00×10^{-4} M, pH = 9.09, μ = 0.10 M (NaClO$_4$), 25.0 ± 0.1°)

10^4[gly]$_T$, M	10^2k_{obsd}, sec^{-1}	10^4[his]$_T$, M	10^2k_{obsd}, sec^{-1}
0.794	2.08 ± 0.05	1.01	4.84 ± 0.10
1.59	2.27 ± 0.02	2.02	7.84 ± 0.07
2.38	2.22 ± 0.06	4.04	13.9 ± 0.6
3.18	2.28 ± 0.03	9.09	27.1 ± 0.7
11.9	3.20 ± 0.08		

From these data, the k_1 values are 51 M^{-1} sec^{-1} for glycine and 6.30×10^2 M^{-1} sec^{-1} for L-histidine. The ratio of the k_1 values for the his$^-$ to gly$^-$ reactions is 12 for Cu(H$_{-2}$AAA)$^-$ which is similar to that found for Cu(H$_{-2}$GGG)$^-$ (ratio = 16) and indicates that the k_1 values for the exchange reaction of Cu(H$_{-2}$AAA)$^-$ with EDTA are also a function of K_2 consistent with the general mechanism given in eq 3–5.

Discussion

The proposed role of triglycine in the mechanism of triglycine autocatalysis of the copper ion transfer from Cu(H$_{-2}$GGG)$^-$ to EDTA is to facilitate the formation of a complex with only one Cu–N(peptide) bond.[1] (The importance of this step is that the complex with only one Cu–N(peptide) bond allows EDTA to react *via* nitrogen coordination to a planar coordination site which is the most effective path for nucleophilic attack.[1]) The role of the amino acids in the proposed general mechanism is the same.

The rate constants evaluated in the determination of the proposed general mechanism are given in Table VIII.

TABLE VIII

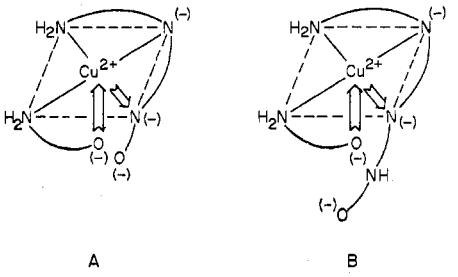
A SUMMARY OF RATE CONSTANTS EVALUATED FOR THE PROPOSED GENERAL MECHANISM μ = 0.10 (NaClO$_4$), 25.0°

Catalytic species	Oligopeptide	k_1, M^{-1} sec^{-1}	k_2/k_{-1}	k_{-2}/k_3, M^{-1}
gly$^-$	GGG	1.42×10^4	21[a]	4.3×10^4
	GGGG	7.8×10^5	11	3.3×10^3
	AAA	51		
his$^-$	GGG	2.27×10^5	6.6	2.8×10^5
	AAA	6.3×10^2		
H·his	GGG	1.63×10^3		
GGG$^-$	GGG		4.76	1.5×10^3

[a] $k_2 = 2.0 \times 10^6$ M^{-1} sec^{-1} and $k_{-1} = 9.8 \times 10^4$ M^{-1} sec^{-1}.

Figures 1 and 4 illustrate one of the most important aspects of this study, the fact that the k_1 value is dependent on the stability of the catalytic species as a chelate as opposed to its stability as a monodentate amine, carboxylate, or imidazole group. (The proposed bonding of histidine is *via* the amino and imidazole groups.)[12] Thus, the reaction path must involve chelate formation by the catalytic species either before or during cleavage of the terminal Cu–N(peptide) bond.

(12) J. L. Meyer, Jr., and J. E. Bauman, Jr., *J. Amer. Chem. Soc.*, **92**, 4210 (1970).



A B

Figure 7.—Proposed rate step for reaction of glycine with Cu(H$_{-2}$GGG)$^-$ and Cu(H$_{-2}$GGGG)$^-$.

An interesting feature of this investigation is that the general mechanism explains the kinetics of copper transfer from both tri- and tetrapeptide complexes. Based on the results of the protonation reactions of Cu(H$_{-2}$GGG)$^-$,[10] Ni(H$_{-2}$GGG)$^-$,[13] Ni(H$_{-3}$GGGG)$^{2-}$, and Ni(H$_{-3}$GGGa)$^-$[14] (GGGa is triglycinamide) the rate of proton transfer to Cu(H$_{-3}$GGGG)$^{2-}$ to form Cu(H$_{-2}$GGGG)$^-$ might be expected to be rather sluggish, especially above pH 8. However, the kinetic data in this study indicate a rapid preequilibrium between Cu(H$_{-3}$GGGG)$^{2-}$ and Cu(H$_{-2}$GGGG)$^-$. Preliminary results of an independent study of the protonation reaction of Cu(H$_{-3}$GGGG)$^{2-}$ have experimentally verified the facile nature of this process.[15] Further studies on the mechanism of this reaction are in progress.

A comparison of the k_1 values for gly$^-$ catalysis (Table VIII) reveals that the rate constant with Cu(H$_{-2}$GGGG)$^-$ is 55 times greater than with Cu(H$_{-2}$GGG)$^-$. From a thermodynamic standpoint this is a quite unexpected result for there is not a great deal of difference in stability between Cu(H$_{-2}$GGGG)$^-$ and Cu(H$_{-2}$GGG)$^-$ ($\Delta \log K < 0.2$ from Table I, Section B). The slight difference in stability presumably arises from the presence of a Cu–O(peptide) bond in Cu(H$_{-2}$GGGG)$^-$ as compared to a Cu–O(carboxylate) bond in Cu(H$_{-2}$GGG)$^-$. Since chelation is important in the k_1 step, the reaction may proceed by an associative type mechanism as shown in Figure 7A for Cu(H$_{-2}$GGG)$^-$ and 7B for Cu(H$_{-2}$GGGG)$^-$. The rate step in both cases is the cleavage of the terminal Cu–N(peptide) bond. As shown in Figure 7A and 7B, the incoming amino acid carboxylate group would be closer to the free carboxylate group in the triglycine complex than in the tetraglycine complex. The diminished electrostatic repulsion in the reaction with Cu(H$_{-2}$GGGG)$^-$ can account for the increased rate of catalysis. That electrostatic effects may be significant is supported by the similarity of the k_1 values for the reaction of a neutral catalyst with Cu(H$_{-2}$GGG)$^-$ and Cu(H$_{-2}$GGGG)$^-$. Thus, the k_1 value for *N,N*-dimeen with Cu(H$_{-2}$GGG)$^-$ is 1.30×10^5 M^{-1} sec^{-1} and the k_1 value with Cu(H$_{-2}$GGGG)$^-$ is 5.2×10^5 M^{-1} sec^{-1}. The ratio of these k_1 values is reasonably close to the ratio of the stability constants of the two oligopeptide complexes.

The k values in Table V are not corrected to the specific k_1 values for reaction with Cu(H$_{-2}$GGGG)$^-$. When this correction is made, all of the amino acids are found to react faster with Cu(H$_{-2}$GGGG)$^-$ than with Cu(H$_{-2}$GGG)$^-$. An accurate k_1 value for Cu(H$_{-2}$GGGG)$^-$ was determined only for glycine. However, the ratios of the k values in Table V to the corresponding k_1 values in Table II can be used to compare the reactivities of the amino acids with the tetraglycine and triglycine complexes. This ratio is more than five times larger for glycine than it is for histidine which corresponds to a reduced electrostatic factor for histidine bound by the amino and imidazole groups.

A comparison also may be made between the k_1 values of Cu(H$_{-2}$GGG)$^-$ and Cu(H$_{-2}$AAA)$^-$ (Table VIII) for both gly$^-$ and his$^-$ catalysis. This comparison reveals that the Cu(H$_{-2}$AAA)$^-$ rate constants are about 400 times smaller than those for the Cu(H$_{-2}$GGG)$^-$ case. Examination of the CPK atomic models shows that the presence of the methyl groups in trialanine presents a considerable hindrance to rotation of an alanine residue away from the copper coordination plane during bond cleavage. In addition, a kinetic study of the reactions of polyamines with the copper(II) complexes of L-leucyl-substituted tripeptides has shown that the presence of an L-leucine residue in the middle position reduces the rate of trien attack by a factor of 200 compared to that of Cu(H$_{-2}$GGG)$^-$. This reduction in rate is ascribed to steric difficulties in breaking the chelate ring terminated by the Cu–N(peptide) bond adjacent to the carboxylate group.[16] Apparently, a similar effect leads to the observed reduction of the k_1 step for Cu(H$_{-2}$AAA)$^-$ compared to Cu(H$_{-2}$GGG)$^-$.

A check on the consistency of the rate constants for the gly$^-$ and his$^-$ catalyzed reactions of Cu(H$_{-2}$GGG)$^-$ is possible. Although the rate constants and rate constant ratios were independently determined for each catalyst, the mechanism predicts that the product of k_1 and k_2/k_{-1} divided by k_{-2}/k_3 should be identical. The calculated $k_1k_2k_3/k_{-1}k_{-2}$ values agree within experimental error (7.0 for gly$^-$ and 5.4 for his$^-$) and thus provide an additional check on the mechanism.

Based on the k values, the correlation of k_1 with K_2, and the rate constant ratios for gly$^-$ and his$^-$ catalysis of the Cu(H$_{-2}$GGG)$^-$ reaction, relative values may be estimated for the stability constants of the Cu(H$_{-1}$GGG)X mixed complexes (where X = gly$^-$ or his$^-$) and of the k_2 values for gly$^-$ and his$^-$ catalysis. From the K_2 values of the amino acid complexes of copper(II), the stability constant, K_1^H (where $K_1^H = k_1^H/k_{-1}^H$, H being used to denote constants for histidine and G denoting glycine constants), for the Cu(H$_{-1}$GGG)(his)$^-$ complex may be estimated to be 13.5 times greater than that for the Cu(H$_{-1}$GGG)(gly)$^-$ complex. As mentioned earlier, the ratio $k_1^Gk_2^Gk_3^G/k_{-1}^Gk_{-2}^G$ is equal to $k_1^Hk_2^Hk_3^H/k_{-1}^Hk_{-2}^H$. Rearrangement of this identity gives the expression in eq 12. Substituting

$$\frac{k_2^H}{k_2^G} = \frac{K_1^Gk_{-2}^H/k_3^H}{K_1^Hk_{-2}^G/k_3^G} \qquad (12)$$

the known values of k_{-2}/k_3 (2.8×10^5 M^{-1} for histidine and 4.3×10^4 M^{-1} for glycine) and the estimated value of K_1^G/K_1^H (13.5), the ratio of k_2^H to k_2^G is estimated to be 0.5. From this estimate it can be predicted that if an exchanging ligand should cause the k_2 step to become rate determining, histidine could ac-

(13) E. J. Billo and D. W. Margerum, *J. Amer. Chem. Soc.*, **92**, 6811 (1970).

(14) E. B. Paniago and D. W. Margerum, *ibid.*, **94**, 6704 (1972).

(15) D. C. Young and D. W. Margerum, unpublished results.

(16) H. Hauer, G. R. Dukes, and D. W. Margerum, to be published.

tually be a slightly poorer catalyst than glycine. However, for EDTA under all the conditions used in this study, histidine is the better catalyst.

Steric factors involving the catalytic species also can be important as evidenced by the rate constant (k_1) for *N*,*N*-dimethylglycine catalysis with $Cu(H_{-2}GGG)^-$ in Figure 2. This rate constant is smaller than would be predicted by the K_2 value. The reduced value is attributed to steric hindrance due to the difficulty of coordinating a tertiary nitrogen in the planar position vacated by the triglycine carboxylate group.[2] A similar, but somewhat smaller, reduction in the k_1 value for *N*,*N*-dimegly$^-$ catalysis compared to that for gly$^-$ catalysis (a factor of about 13) is seen for the reaction of $Cu(H_{-2}GGGG)^-$ with EDTA. The reaction path for this catalyst may proceed by initial coordination of either the carboxylate or the amino terminal followed by an associative displacement of the peptide nitrogen by the other end. The reduced rate could be due to either the lower stability of Cu(II) with oxygen donors as opposed to nitrogen donors or the steric difficulty encountered by the amine group in coordinating to the vacated planar position.

The apparent unreactivity of the zwitterion form of the amino acids raises an interesting question. In the study of the general acid catalyzed protonation reactions of $Cu(H_{-2}GGG)^-$ [9] and $Ni(H_{-2}GGG)^-$,[11] acids which could coordinate to the metal (*i.e.*, coordinating acids) are as much as 100 times more effective as general acid catalysts than would be indicated by their pK_a values. Thus, it is reasonable to ask if the zwitterion could act as a general acid catalyst. Initial coordination of the carboxylate end of the zwitterion would put the protonated amine end in close proximity to the deprotonated peptide nitrogen, facilitating proton transfer. Based on its pK_a of 9.59, the expected rate constant (k_{HX}) for H·gly acting as a noncoordinated general acid with $Cu(H_{-2}GGG)^-$ is about 1 M^{-1} sec^{-1}. If H·gly were to act as a coordinating acid, k_{HX} could increase to a value as large as 100 M^{-1} sec^{-1}. However, this is still too small to be observed in this study. Thus, the zwitterion is a relatively unreactive species.

L-Histidine, however, is a different case. The pK_a of the imidazole group is 6.08 and, on the basis of this value, it would be expected to have a value of $k_{HX} \approx 10^3$ M^{-1} sec^{-1} for general acid catalysis of the protonation reaction of $Cu(H_{-2}GGG)^-$. However, the data taken at pH 7.0 and 7.3 do not exhibit a dependence on H_2·his$^+$ as a general acid. It does, however, exhibit a first-order dependence on H·his (see Figure 3) which, with a pK_a of 9.17, would be expected to be a weak noncoordinating general acid ($k_{HX} \approx 2$ M^{-1} sec^{-1}). The k_1 value for H·his (1.63×10^3 M^{-1} sec^{-1}) may be due to initial coordination by the imidazole nitrogen followed either by nucleophilic displacement of the terminal peptide nitrogen by the carboxylate group or by proton transfer from the protonated amine terminal of H·his acting as a coordinated acid. The initial coordination of the imidazole nitrogen as opposed to the carboxylate oxygen may be explained by the well-known preference of Cu(II) for nitrogen donors over oxygen donors as demonstrated by the increased stability of the copper(II) imidazole complex ($\log K_2 = 4.2$) compared to that of copper(II) acetate ($\log K_1 = 2.0$).[17]

Amino acids are remarkably effective catalysts for the transfer of copper ion from an oligopeptide environment to EDTA, even at physiological pH. In addition, the general mechanism (eq 2–5) proposed for this process is analogous to that which has been suggested for the amino acid catalysis of the transfer of copper ion from serum albumin in the blood.[18]

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