Complexing of Nickel(II) by Cysteine, Tyrosine, and Related Ligands and Evidence for Zwitterion Reactivity

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Abstract: The kinetics of complexing of nickel(II) by methionine (I), cysteine (II), penicillamine (III), cysteine ethyl ester (IV), glycylmethionine (V), glutathione (VI), tyrosine (VII), *m*-tyrosine (VIII), and *o*-tyrosine (IX) have been measured by stopped-flow methods. The study generally covered the range of pH 6–7 with large excess of nickel(II), $(1\text{–}6) \times 10^{-2}$ *M*, compared to amino acid, $\sim 1 \times 10^{-3}$ *M*. A detailed analysis of the pH dependence of the rates indicates that initial complexing is at the carboxylate group of the zwitterion for I, II, III, VI, VII, VIII, and IX. Ligands II, III, and IV show a reaction path involving initial complexing at the sulfhydryl group. Previously used reasons for neglecting reaction of the simple amino acid zwitterion are reexamined.

The results of a number of kinetic studies of ligand complexing of nickel(II), summarized in two recent reviews,[1,2] have indicated that the rates depend largely on the charge of the reacting ligand. As has been shown by Wilkins and coworkers[1,3] these observations can be used to indicate the reactive form of a ligand when several tautomeric forms or basic sites are present. In the work reported here several ligands with at least two basic groups have been studied in order to determine the effect of the bifunctional nature of the ligand on the rates of complexing. Several ligands related to cysteine have been studied because the microacid dissociation constants for each group are more generally known for the sulfur containing amino acids. The microdissociation constants for tyrosine are also known, and it was of interest to study this system because it is fairly certain that the phenolic group is not involved in the complexing of nickel(II) by tyrosine.

A detailed analysis of various reaction pathways for metal ion complexing of an amino acid indicates that initial complexing probably occurs at the carboxylate group of the amino acid zwitterion. This is followed by proton loss from the amino group and chelate ring closure. This seems to be the dominant pathway as long as the hydrogen ion concentration is $>10^2$ times the acid dissociation constant of the amino group. Since the amino acid zwitterion previously has been assumed not to react, the reasons and experimental evidence for this assumption are reexamined.

Experimental Section

Materials. All amino acids were used as obtained from Aldrich Chemical Co. unless otherwise indicated. The glycylmethionine and glutathione were purchased from Calbiochem Co., and the L-tyrosine and 2,6-lutidine were from Eastman Organic Chemicals. The Bromothymol Blue was from British Drug Houses and the Chlorophenol Red from Fisher Scientific Co. The solutions of nickel(II) were prepared and standardized as described previously.[4]

Kinetic Measurements. A standard Aminco-Morrow stopped-flow system, described previously,[4] was used. The transmittance change was monitored at 405 nm when Chlorophenol Red ($pK_a \sim 6$) was the indicator and at 620 nm when either Bromothymol Blue ($pK_a \sim 7.3$) or no indicator was used. Absorbance values were calculated from the transmittance and the usual semilogarithmic plot of absorbance change vs. time was used to determine the reaction half-time ($t_{1/2}$). For each set of concentration conditions four–ten runs were made, and the average half-time was used to obtain the rate constants given here. The pH was measured as described previously.[4] The ionic strength was maintained at 0.15 *M* by addition of KNO_3.

Both indicators mentioned above were used in the study of cysteine ethyl ester. Reaction of the latter ligand and also penicillamine and cysteine was studied in the absence of indicator. Blank experiments in which indicator and nickel(II) solutions or indicator and ligand solutions were mixed showed no absorbance change of a magnitude or time scale such as to interfere with the results reported. Freshly prepared solutions of the amino acids were used in all cases, and no aging effects of these solutions were observed except for glutathione as noted in the following section. In several systems when no indicator and/or two different indicators were used, no significant difference in rate constants could be detected. Therefore, no correction has been applied for the acid–base properties of the indicator.

Results

Since the rate constants calculated from the kinetic data will often depend directly on the value of the ligand acid dissociation constant used, it seems appropriate to give some consideration to the latter values. Results from the literature at 25° are collected in Table I.

It is most common in the literature to give the apparent acid dissociation constant which is the hydrogen ion activity times the ratio of concentrations of the basic to acidic forms of the ligand. These values can be determined directly from standard potentiometric titrations. The apparent dissociation constant, when used in conjunction with the measured hydrogen ion activity (H^+) in the present type of study, yields rate constants in the usual terms of the concentrations of the reactants.

In two studies on cysteine type ligands[5,6] the acid dissociation constants reported are concentration constants in which an activity coefficient has been used to calculate the hydrogen ion concentration. The apparent dissociation constant at ~ 0.1 *M* ionic strength can be obtained from the concentration constant by multiplying by 0.83[7] or adding 0.08 to the pK_a.

A consideration of the results in Table I shows that the pK_a values are generally in agreement. Even uncertainties of 0.15 in the pK_a generally would not affect any qualitative kinetic arguments. It should be noted, however, that the microconstants K_{2XH} and K_{2NH} are obtained by using a spectrophotometric method to determine K_{2XH}, and then K_{2NH} is calculated from the relationship $K_{2NH} = K_{2a} - K_{2XH}$. Therefore errors in K_{2a} and K_{2XH} also appear in K_{2NH}, and the uncertainty in K_{2NH} may be large if K_{2XH} is similar to K_{2a}.

Cysteine and Related Ligands. The complex formation constants for nickel(II) complexes of methionine,[5] penicillamine,[5,8] and cysteine[5] indicate that the product will be >90% in the form of the monocomplex (Table II). Forma-

Table I. Summary of Ligand Acid Dissociation Constants (25°)

Ligand	pK_{2a}	pK_2XH[a]	pK_2NH[a]	Ref
Methionine	9.12[b]			*c*
	9.10			*d*
	9.15			*e*
Penicillamine	7.96[b]			*c*
	7.97			*f*
	7.95	8.05	~8.7	*g*
Cysteine[l]	8.21[b]			*c*
	8.22[b]			*h*
	8.38	8.55	8.88	*i*
	8.37	8.53	8.86	*j*
	8.27			*k*
Cysteine ethyl ester	6.69	7.45	6.77	*j*
	6.77			*i*
Glycylmethionine	8.51			*m*
Glutathione	9.20	9.20	9.20	*j*
	8.74	8.92	9.20	*n*
	8.75			*d*
		8.93	9.13	*o*
Tyrosine	9.21			*p*
	9.12	9.63	9.28	*q*
m-Tyrosine	9.09			*p*
o-Tyrosine	8.60			*p*

[a] pK_2NH and pK_2XH are microdissociation constants for the amino and either –SH or –OH groups on the amino acids. [b] Recalculated as an apparent pK as described in the text. [c] Reference 5, μ = 0.10 *M*. [d] N. C. Li and R. A. Manning, *J. Am. Chem. Soc.*, 77, 5225 (1955), μ = 0.15 *M*. [e] Ya. M. Azizov, A. Kh. Miftakhova, and V. F. Toropova, *Russ. J. Inorg. Chem.*, 12, 345 (1967), μ = 0.16 *M*. [f] E. J. Kuchinskas and Y. Rosen, *Arch. Biochem. Biophys.*, 97, 370 (1962), μ = 0.15 *M*. [g] E. W. Wilson and R. B. Martin, *ibid.*, 142, 445 (1971), μ = 0.16 *M*. [h] Reference 6, μ = 0.1 *M*. [i] R. G. Kallen, *J. Am. Chem. Soc.*, 93, 6227 (1971), μ = 1.0 *M*. [j] R. E. Benesch and R. Benesch, *ibid.*, 77, 5877 (1955), $\mu \approx 0.02$ *M*. [k] G. Gorrin, *ibid.*, 78, 767 (1956), μ = 0.10 *M*. [l] The activity constants determined by Coates et al, *Trans. Faraday Soc.*, 3032 (1969), have not been included because parameters were not given to calculate the apparent constants given here. [m] Reference 10, μ = 0.16 *M*. [n] Reference 11, μ = 0.16 *M*. [o] D. L. Rabenstein, *J. Am. Chem. Soc.*, 95, 2797 (1973), μ = 0.20–0.55 *M*. [p] Reference 17, μ = 0.16 *M*. [q] R. B. Martin et al, *J. Biol. Chem.*, 233, 1429 (1958), μ = 0.16 *M*.

tion constants have not been measured for cysteine ethyl ester and nickel(II), but values for the methyl ester[9] predict that the ligand is present >94% as the monocomplex. For glycylmethionine,[10] and glutathione[11] the ligand is >90% in the monocomplex form in the product. As a result only the kinetics of the formation of the monocomplex have been observed in this work.

In all cases, the reaction was monitored by observing the color change of an indicator. The magnitude of the absorbance change was twice as large with penicillamine, cysteine, and cysteine ethyl ester as it was with methionine, under identical conditions of pH, indicator, and ligand and metal ion concentration. This shows that twice as many protons are released when the former three ligands complex to nickel(II) and indicates that all three coordinate both the amino and sulfhydryl groups.

The above conclusion also is consistent with the fact that an absorbance change at 620 nm, in the absence of indicator, could only be observed for penicillamine, cysteine, and cysteine ethyl ester. The direct observation of an absorbance change under these concentration conditions is not typical for $-NH_2$ and CO_2^- coordination and implies that sulfur is bonding to nickel(II) in these systems. Neither glycylmethionine nor glutathione complexing showed any direct absorbance change implying that sulfur does not coordinate. This same conclusion was reached for glutathione on the basis of formation constant comparisons[11] and more recently on the basis of circular dichroism studies.[12]

Table II. Kinetic Data for Formation of Monocomplexes of Nickel(II) (0.15 *M* KNO_3, 23.7°)[a]

	10^3 [Ligand], *M*	10^2 [Nickel], *M*	pH	$10^{-2}k_{obsd}$, M^{-1} sec^{-1}
Methionine	0.96	3.45	6.36	0.13
	1.07	1.73	6.58	0.20
	1.07	1.73	6.79	0.34
	0.96	3.45	6.83	0.36
	1.07	1.73	6.87	0.43
Penicillamine	1.24	1.04	5.83	6.6[b]
	1.37	2.76	5.85	6.0
	1.24	1.04	6.19	8.3[b]
	1.08	1.04	6.20	8.4
	1.37	2.76	6.32	8.0
	1.08	1.04	6.43	10.4
	0.94	1.04	6.59	12.8
	1.37	2.76	6.62	12.5
	0.94	1.04	6.74	14.9
	1.24	1.04	6.96	21.9[b]
	0.94	1.04	7.06	26.9
	1.37	2.76	7.15	30.5
Cysteine	1.38	1.04	6.00	9.13
	1.14	1.38	6.11	10.5
	1.28	1.73	6.46	12.2
	0.97	3.45	6.48	12.3[b]
	1.28	1.73	6.49	13.6
	1.38	1.04	6.50	14.3
	1.38	1.04	6.62	15.5
	1.28	1.73	6.69	15.8
	1.28	1.73	6.80	16.8
	0.97	3.45	6.83	16.6[b]
	1.28	1.73	6.91	18.5
	1.38	1.04	7.04	23.3
Cysteine ethyl ester	1.10	1.73	5.93	13.6[b]
	1.08	1.73	6.08	16.7
	1.09	1.04	6.13	20.5[c]
	1.12	1.73	6.20	22.3[c]
	1.07	1.04	6.28	24.7[b]
	1.12	1.73	6.31	24.6[c]
	1.07	1.04	6.41	26.5[b]
	1.08	1.73	6.41	24.5
	1.12	1.73	6.59	34.6[c]
	1.07	1.04	6.64	35.4[b]
	1.12	1.73	6.85	40.7[c]
	1.07	1.04	6.91	47.2[b]
	1.10	1.73	6.99	44.2[b]
	1.12	1.73	7.04	44.5[c]
Glycylmethionine	1.02	6.19	6.41	0.61
	1.02	6.19	6.48	0.65
	1.00	2.48	6.59	0.84
	1.02	6.19	6.68	1.13
	1.00	6.19	6.83	1.46
	1.00	2.48	6.92	1.72
	1.00	6.19	6.96	2.05
	1.00	2.48	6.99	2.32
Glutathione	1.14	6.19	5.85	0.097
	1.04	6.19	5.89	0.11
	0.99	6.19	5.97	0.12
	1.04	6.19	6.02	0.15
	0.99	6.19	6.03	0.17
	1.14	1.98	6.11	0.23
	1.00	1.98	6.19	0.28
	1.14	6.19	6.19	0.27
	0.97	1.98	6.28	0.30
	0.86	1.98	6.35	0.37

[a] All experiments were done in 0.015 *M* lutidine buffer and were observed by monitoring the color change of Bromothymol Blue at 620 nm unless otherwise indicated. [b] No indicator added; the color change at 620 nm was observed. [c] Chlorophenol Red indicator used and observed at 405 nm.

It should be noted that if cysteine ethyl ester solutions were not freshly prepared or protected from oxygen, a second slower reaction than that reported here could be observed. In the case of glutathione the system could only be studied up to about a pH of 6.4. The results became irrepro-

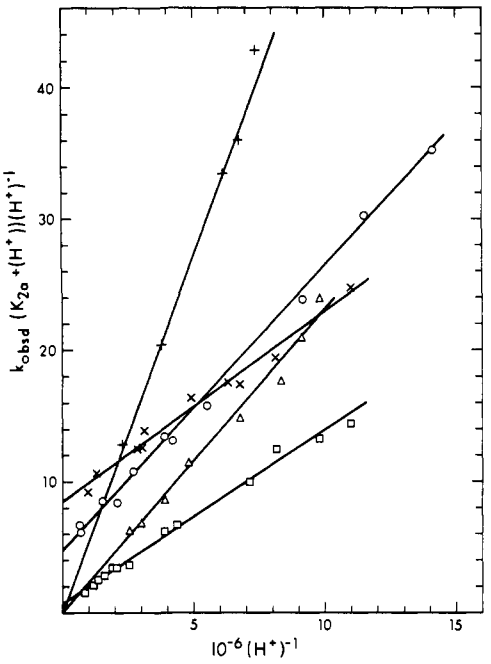


Figure 1. Variation of $k_{obsd}(K_{2a} + (H^+))(H^+)^{-1}$ with $(H^+)^{-1}$ at 23.7°: for cysteine (×), vertical scale $\times 10^{-2}$; for penicillamine (○), vertical scale $\times 10^{-2}$; for cysteine ethyl ester (□), vertical scale $\times 10^{-3}$; for methionine (+); for glycylmethionine (△), vertical scale $\times 10^{-1}$.

ducible at higher pH values and appeared to depend on the age of the glutathione solution.

The pH and nickel(II) dependence of the observed rate constants are consistent with the rate equation normally found for these reactions[1,3]

$$k_{obsd} = \frac{0.693}{t_{1/2}[Ni^{2+}]} = \frac{k_1'(H^+) + k_2'}{(K_a + (H^+))} \quad (1)$$

which can be rearranged to

$$k_{obsd}\frac{(K_a + (H^+))}{(H^+)} = k_1' + \frac{k_2'}{(H^+)} \quad (2)$$

The plot of the left-hand side of eq 2 vs. $(H^+)^{-1}$ is shown in Figure 1. Results for glutathione are not plotted because of the limited pH range studied. Values of k_1' and k_2' are given in Table IV.

Tyrosine and Related Ligands. The complex formation constants for nickel(II) with tyrosine have been determined by Albert,[13] Weber and Simeon,[14] and Barr, Baumgartner, and Kustin.[15] The results of the latter two studies are in moderate agreement and indicate that 70–85% of the tyrosine remains uncomplexed under the least favorable of our experimental conditions. Therefore, the system has been treated as one coming to equilibrium, and the results are found to be adequately represented by

$$k'_{obsd} = k_{obsd}[Ni^{2+}] = (k_3'/(K_a + (H^+))[Ni^{2+}] + k_{-3}' \quad (3)$$

No formation constants are available for *m*- and *o*-tyrosine with nickel(II). However, the results of Letter and Bauman[16] with copper(II) indicate that all these tyrosine systems may have similar formation constants. Therefore, the kinetic results for *m*- and *o*-tyrosine complexing have also been fitted to eq 3. The appropriate plots of the kinetic results are shown in Figure 2. In all three cases, a least-squares analysis of the data in Table III gives the values of k_3' and k_{-3}' in Table IV.

Discussion

The kinetic results are summarized in Table IV. The values of the intercept and slope of eq 2 or 3 are useful for comparison purposes since they are essentially independent of the K_{2a} value (since $K_{2a} \ll (H^+)$ in general) and of any mechanistic assumptions. Previous results of Wilkins et al.[3b] for cysteine ($k_1' = (3.5 \pm 1) \times 10^2\ M^{-1}\ sec^{-1}$, $k_2' = (20 \pm 2) \times 10^{-5}\ sec^{-1}$) and penicillamine ($k_1' = (3.5 \pm 1) \times 10^2\ M^{-1}\ sec^{-1}$, $k_2' = (22 \pm 3) \times 10^{-5}\ sec^{-1}$) are in substantial agreement with values found in the present work.

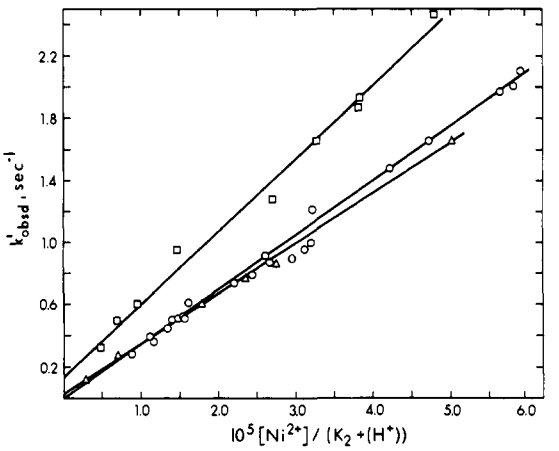


Figure 2. Variation of k'_{obsd} with $[Ni^{2+}](K_{2a} + (H^+))^{-1}$ for: tyrosine (○); *m*-tyrosine (□); and *o*-tyrosine (△), at 23.7°.

Table III. Kinetic Data for Formation of Monocomplexes of Nickel(II) (0.15 *M* KNO_3, 23.7°)[a]

	10^3 [Ligand], *M*	10^2 [Nickel], *M*	pH	k'_{obsd}, sec^{-1}
Tyrosine	0.96	1.73	6.89	0.45
	0.96	1.73	6.92	0.51
	0.96	1.73	6.97	0.61
	0.98	1.98	6.65	0.28
	0.98	1.98	6.77	0.36
	0.90	1.98	6.85	0.50
	0.90	1.98	7.12	0.91
	0.90	1.98	7.13	0.87
	0.73	3.45	6.85	0.79
	0.73	3.45	6.97	1.21
	0.52	4.95	6.35	0.39
	0.52	4.95	6.49	0.52
	1.00	4.95	6.50	0.51
	1.00	4.95	6.65	0.74
	0.52	4.95	6.80	0.96
	1.00	4.95	6.81	1.00
	0.52	4.95	6.93	1.38
	1.00	4.95	6.98	1.56
	1.00	4.95	7.07	1.91
	1.00	6.19	6.68	0.90
	1.00	6.19	6.96	1.87
	1.00	6.19	6.98	2.10
m-Tyrosine	0.97	1.70	6.44	0.33
	0.97	1.70	6.67	0.50
	0.98	1.70	6.75	0.61
	0.98	1.70	6.94	0.95
	0.48	3.45	6.98	1.67
	0.73	3.45	7.05	1.93
	0.88	4.50	6.78	1.28
	0.88	4.50	6.93	1.87
	0.88	4.50	7.03	2.47
o-Tyrosine	1.10	2.73	6.00	0.12
	1.10	2.73	6.41	0.34
	0.67	2.76	6.82	0.60
	0.67	2.76	7.01	0.86
	0.55	3.45	6.84	0.76
	0.55	3.45	7.18	1.66

[a] All experiments were done in 0.015 *M* lutidine buffer, and observed by monitoring the color change of Bromothymol Blue at 620 nm.

Table IV. Summary of Kinetic Results

Ligand	$10^{-2}k_1'$,[a] M^{-1} sec^{-1}	$10^5k_2'$,[a,c] sec^{-1}
Methionine		0.544
Penicillamine	4.8 ± 1	21.7
Cysteine	8.5 ± 1.5	14.5
Cysteine ethyl ester	~7.0	133
Glycylmethionine		2.30
Glutathione		1.63
Tyrosine	$(0.2 \pm 4.5) \times 10^{-4}$ [b]	0.332[b]
m-Tyrosine	$(0.12 \pm 0.07) \times 10^{-2}$ [b]	0.473[b]
o-Tyrosine	$(4.1 \pm 5.0) \times 10^{-4}$ [b]	0.316[b]

[a] k_1' and k_2' as defined by eq 2. [b] k_{-3}' and k_3' as defined by eq 3. [c] Estimated 95% confidence limits are generally ±10% of value given.

Our values of k_2' for cysteine seems consistent with that of Davies et al.[17] at 20°.

The results of Kustin et al.[15] predict $k_2' = (1.4 \times 10^4)(7.8 \times 10^{-10}) = 11 \times 10^{-6}$ sec^{-1}, compared to our value of 3.3×10^{-6} sec^{-1} for tyrosine. This discrepancy is larger than hoped for or found in other comparisons. The problem may result from the use by Kustin et al. of apparent acid dissociation constants measured by Martin et al.[18] in conjunction with hydrogen ion concentrations instead of activities. There may also be difficulties in separating the kinetics of formation of the first and second tyrosine complexes in the T-jump work.[15]

The complexing of a simple amino acid represented by $^-$O-N, where O$^-$ is the carboxylate end and N is the amino end of the ligand, is given by the reaction scheme

$$\begin{array}{ccccc} M + {}^-O\text{-}\overset{+}{N}H & \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} & MONH & & \\ \quad K_1\updownarrow +H^+ & & \quad K_1'\updownarrow +H^+ & & \\ M + {}^-O\text{-}N & \underset{k_{34}}{\overset{k_{43}}{\rightleftharpoons}} & MON & \underset{k_{53}}{\overset{k_{35}}{\rightleftharpoons}} & M\langle O \\ & & & & \quad\quad N \end{array} \qquad (4)$$

This scheme and numbering system is that used previously by Wilkins et al.,[3b] with the addition of the reverse step k_{53}.[19] With the assumption that the proton equilibria K_1 and K_1' are very rapidly attained compared to all other steps, it can be shown that the concentration changes will be exponential and characterized by two time constants

$$\gamma_\pm = -\{-(a_1 + a_2 + a_3 + k_{53}) \pm \sqrt{(a_1 + a_2 + a_3 + k_{53})^2 - 4(a_1a_3 + a_1k_{53} + a_2k_{53})}\}/2 \qquad (5)$$

where

$$a_1 = (k_{12}(H^+) + k_{43}K_1)\frac{[M]}{K_1 + (H^+)}$$

$$a_2 = \frac{k_{21}(H^+) + k_{34}K_1'}{K_1' + (H^+)}$$

$$a_3 = \frac{k_{35}K_1'}{K_1' + (H^+)} \qquad (6)$$

It may also be noted that the formation constant of the complex is related to the constants in the reaction scheme by

$$K_f = \left(\frac{k_{43}}{k_{34}}\right)\left(\frac{k_{35}}{k_{53}}\right) = \left(\frac{K_1'}{K_1}\right)\left(\frac{k_{12}}{k_{21}}\right)\left(\frac{k_{35}}{k_{53}}\right) \qquad (7)$$

If $(a_1 + a_2 + a_3 + k_{53})^2 \gg 4(a_1a_3 + a_1k_{53} + a_2k_{53})$, then eq 5 can be expanded to first order with the binomial expansion to give

$$\gamma_\pm = -\frac{(a_1 + a_2 + a_3 + k_{53})}{2}\left\{-1 \pm 1 \mp \left[\frac{2(a_1a_3 + a_1k_{53} + a_2k_{53})}{(a_1 + a_2 + a_3 + k_{53})^2}\right]\right\} \qquad (8)$$

or

$$\gamma_- = (a_1 + a_2 + a_3 + k_{53}) \qquad (9)$$

and

$$\gamma_+ = \frac{(a_1a_3 + a_1k_{53} + a_2k_{53})}{(a_1 + a_2 + a_3 + k_{53})} \qquad (10)$$

The observed reaction rate is presumed to be governed by the smaller time constant γ_+.[20] Therefore the expression for γ_+ will be simplified by a consideration of the experimental conditions ($[M] < 0.1$ *M*; $5 \lesssim pH \lesssim 7$) and the fact that for simple amino acids $pK_1 \gtrsim 9$. Recent work[21] has shown that for glycine coordinated to $(NH_3)_5Co^{3+}$ the K_a of the NH_3^+ group is about ten times greater than that for the free glycine zwitterion. This provides an upper limit for K_1' of an amino acid coordinated to a +2 metal ion, so that $K_1' \lesssim 10K_1$.

The values of a_1 and a_2 can be compared without making very restrictive assumptions. Hoffmann[22] has observed a correlation between rate of ligand dissociation and pK_a of carboxylic acid ligands. This correlation predicts $k_{21} \approx 10^4$ sec^{-1} and $k_{34} \approx 5 \times 10^3$ sec^{-1}. From numerous previous studies[1,2] the correlation of ligand charge and complexing rate predicts $k_{43} \approx 10^5$ M^{-1} sec^{-1} and $k_{12} \approx 5 \times 10^3$ M^{-1} sec^{-1}. Then with $(H^+) > K_1, K_1'$, and $[M] < 0.1$ it is easily shown that $a_2 \gg a_1$ and therefore

$$\gamma_+ = \frac{a_1a_3 + a_2k_{53}}{a_2 + a_3 + k_{53}} \qquad (11)$$

The substitution of expressions for a_1, a_2, and a_3 from eq 6, and multiplication of numerator and denominator by $(K_1' + (H^+))$ yields

$$\gamma_+ = \frac{\frac{(k_{12}(H^+) + k_{43}K_1)}{(K_1 + (H^+))}k_{35}K_1'[M] + k_{53}(k_{21}(H^+) + k_{34}K_1')}{k_{21}(H^+) + K_1'(k_{34} + k_{35}) + k_{53}(K_1' + (H^+))} \qquad (12)$$

If the reaction goes to completion then $k_{53} \approx 0$ and eq 12 can be simplified to the expression obtained by Wilkins et al.[3b]

$$\frac{\gamma_+}{[M]}\frac{(K_1 + (H^+))}{(H^+)} = k_{obsd}\left(\frac{K_1 + (H^+)}{(H^+)}\right) = \frac{(k_{12} + k_{43}K_1(H^+)^{-1})k_{35}K_1'}{k_{21}(H^+) + K_1'(k_{34} + k_{35})} \qquad (13)$$

In previous applications[3b] it was assumed that $k_{21}(H^+) < K_1'(k_{34} + k_{35})$ and that $k_{35} > k_{34}$ in which case eq 13 simplifies to

$$k_{obsd}\left(\frac{K_1 + (H^+)}{(H^+)}\right) = k_{12} + \frac{k_{43}K_1}{(H^+)} \qquad (14)$$

This rate law has been found to be consistent with experimental results for complexing of a wide range of ligands with basic groups. In the case of simple amino acids where there is only a carboxylate and an amino group capable of chelation, it has been found that $k_{12} = 0$. This is the origin of the oft quoted statement that an amino acid zwitterion, with the amino group protonated, does not react with a metal ion.

However, on close analysis the assumption that $k_{21}(H^+) < K_1'(k_{34} + k_{35})$, needed to obtain eq 14 from 13, seems questionable. The results of Taube et al. on $(NH_3)_5CoO_2CCH_2NH_3^{3+}$ give a value equivalent to K_1' of

3×10^{-9} M for coordinated glycine.[21] The K_1' for glycine coordinated to Ni^{2+} probably would be even smaller but 3×10^{-9} M will be taken as a reasonable upper limit. The work of Hoffmann[22] predicts that $k_{21} \approx 10^4$ sec^{-1} for the glycine zwitterion. Therefore the denominator of eq 13 is $10^4(H^+) + (3 \times 10^{-9})(k_{34} + k_{35})$, and $k_{21}(H^+) < K_1'(k_{34} + k_{35})$ only if $(k_{34} + k_{35}) \approx 10^7$ sec^{-1}. Hoffmann's results[22] show that k_{34} cannot be that large; therefore one must assume that $k_{35} \approx 10^7$ sec^{-1}. This is 10^3 times larger than the water exchange rate, and one is forced to the conclusion that either ring closing has considerable associative character or the presence of the coordinated amino acid has a large labilizing effect on the dissociation of a water molecule from nickel(II). There does not appear to be a precedent for such a large effect.

It seems therefore that one might better return to eq 13 with the assumption that $k_{21}(H^+) > K_1'(k_{34} + k_{35})$ rather than the reverse. It is convenient to rearrange eq 13, using the fact that $(k_{43}/k_{34}) = (K_1'k_{12}/K_1k_{21})$ to obtain

$$k_{obsd}\left(\frac{K_1 + (H^+)}{(H^+)}\right) = \frac{(k_{21}(H^+) + k_{34}K_1')\left(\frac{k_{43}K_1k_{35}}{k_{34}(H^+)}\right)}{k_{21}(H^+) + K_1'(k_{34} + k_{35})} \quad (15)$$

Clearly $k_{21}(H^+) > K_1'k_{34}$ if $k_{21}(H^+) > K_1'(k_{34} + k_{35})$ and eq 15 simplifies to

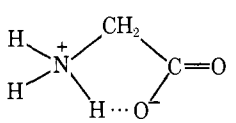
$$k_{obsd}\left(\frac{K_1 + (H^+)}{(H^+)}\right) = \frac{k_{43}K_1k_{35}}{k_{34}(H^+)} = \frac{k_{12}K_1'k_{35}}{k_{21}(H^+)} \quad (16)$$

This result gives the experimentally observed rate law for these reactions, and implies that *the reaction with nickel(II) proceeds by a rapid preequilibrium followed by rate controlling ring closure.*

In applying eq 16 to experimental results one might seek to determine if k_{35} is independent of the ligand as expected for a dissociative ring closure reaction. However, this is not truly valid because the ligand is in the first coordination sphere of the metal ion and might produce specific effects on the rate of water dissociation. A problem also arises in that neither K_1' nor (k_{12}/k_{21}) is known. If our experimental results for methionine are used in conjunction with previously used estimates of $K_1' \approx 3 \times 10^{-9}$ M and $(k_{12}/k_{21}) = (5 \times 10^3/10^4) = 0.5$ M, then a value for $k_{35} = 3.5 \times 10^3$ sec^{-1} is obtained. All that can be said is that this value is not unusual for substitution on nickel(II).

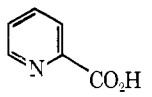
The important conclusion from this analysis is that the amino acid zwitterion may be kinetically active when it is the dominant species in solution. It has been assumed until now that the appearance of the $(H^+)^{-1}$ term in the experimental rate law meant that the zwitterion species was not reactive. However, such a term strictly means that a proton is lost before the transition state for the slowest step is attained. The above analysis shows that the highest energy transition state occurs in the ring closing step (k_{35}) after deprotonation of the coordinated ligand.

It is noteworthy that the conclusion, often drawn in earlier work, that $k_{12} = 0$ requires some explanation if one is to retain the dissociative ion pair mechanism. The problem is to explain why, for example, glycine zwitterion does not even react as a normal neutral ligand for which $k_{12} \approx 5 \times 10^3$ M^{-1} sec^{-1} might be expected. It has been proposed that hydrogen bonding and/or electrostatic interaction is responsible for blocking the reactivity of the carboxylate in a structure such as

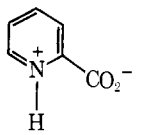


A similar effect would be expected for the ethylenediamine cation $H_3N^+CH_2CH_2NH_2$ where the greater basicity of the NH_2 group compared to CO_2^- might favor hydrogen bonding. However, this cation reacts only two times slower than nonhydrogen bonded cations.[23] In addition, pK_a values indicate hydrogen bonding in the phthalate cation, but it shows a normal reactivity ($k = 3.4 \times 10^4$ M^{-1} sec^{-1})[24] toward nickel(II). In another study Johnson and Wilkins[25] found that the neutral form of 8-hydroxyquinoline showed normal reactivity toward nickel(II). They concluded that hydrogen bonding must be weak or not effective in blocking the nitrogen atom. The normal reactivity of 8-hydroxyquinoline with OH^- [26] was noted as evidence for the kinetic ineffectiveness of hydrogen bonding in this system. By the same reasoning the fact that amino acid zwitterions also have nearly diffusion controlled rates of reaction with OH^- [27] may indicate that hydrogen bonding does not affect the reactivity of the amino acid zwitterion.

Other evidence for the lack of reactivity of carboxylate zwitterions comes from systems such as pyridine-2-carboxylate studied by Cassatt and Wilkins.[3a] The results, covering a range of pH 2–7, were analyzed according to eq 14, and it was concluded that $k_{12} = 30$ M^{-1} sec^{-1} at 25°. This rate constant was attributed to the presence of a small amount of the neutral species



and the zwitterion



was taken to be unreactive. However, if one returns to eq 13, if $k_{35} > k_{34}$, a simple rearrangement gives

$$k_{obsd} = \frac{k_{12}(H^+) + k_{43}K_1}{\left(\frac{k_{21}(H^+)}{K_1'k_{35}} + 1\right)(K_1 + (H^+))} \quad (17)$$

For pyridine-2-carboxylate $K_1 = 4 \times 10^{-6}$ M,[3a] and if values of k_{21}, k_{35}, and K_1' of 2.5×10^4 sec^{-1}, 2.5×10^4 sec^{-1}, and 4×10^{-5} M, respectively, are assumed, then $k_{21}(K_1'k_{35})^{-1} = 2.5 \times 10^4$ M^{-1}. The experimental results are very well fitted by eq 17 with $k_{12} = 8.5 \times 10^3$ M^{-1} sec^{-1} and $k_{43} = 4 \times 10^4$ M^{-1} sec^{-1}, as shown by the results in Table V. Cassatt and Wilkins fitted the results by the equation

$$k_{obsd} = \frac{30(H^+) + (1.04 \times 10^{-1})}{(4.0 \times 10^{-6}) + (H^+)} \quad (18)$$

As shown in Table V this equation does not predict the experimental results well in the pH range 3–4.6.

It cannot be concluded definitely that eq 17 is the correct representation of the results for pyridine-2-carboxylate because it was necessary to assume values of k_{21}, k_{35}, and K_1'. However, reasonable estimates of these parameters lead to a value of k_{12} which is typical for a neutral ligand. It seems probable that the zwitterion of pyridine-2-carboxylate does react with nickel(II).

The analysis of the results for the tyrosine systems requires that the reverse rate constant k_{53} be retained in eq 12. If other approximations already noted are made then it is readily shown that

$$\gamma_+ = \frac{\frac{k_{12}k_{35}K_1'(H^+)[M]}{K_1 + (H^+)} + k_{21}k_{53}(H^+)}{k_{21}(H^+) + k_{53}(K_1' + (H^+))} \quad (19)$$

Table V. Kinetic Data for Reaction of Nickel(II) with Pyridine-2-carboxylate

pH	$10^{-3}k_{obsd}$, M^{-1} sec^{-1} Exptl[a]	Calcd (eq 18[b])	Calcd (eq 17[c])
7	26	25.4	26.6
5	8.9	7.45	10.2
5	10	7.45	10.2
4.8	7.5	5.26	7.95
4.6	7.1	3.59	6.11
4.6	5.9	3.59	6.11
4.3	3.8	1.95	3.98
4.0	2.3	1.02	2.45
3.0	0.27	0.13	0.33
2.3	0.062	0.051	0.067

[a] Data from ref 3a at 25°, μ = 0.30 M. [b] R. G. Wilkins, private communication, has pointed out that the results are better represented by eq 18 if one assumes $k_{43} = 3.2 \times 10^4\ M^{-1}$ sec^{-1} and $pK_i = 5.3$, rather than $2.6 \times 10^4\ M^{-1}$ sec^{-1} and 5.4, respectively. A >20% deviation still exists for pH 3.0–4.3. [c] Calculated using values given in the text, and $k_{34} = 1.18 \times 10^4$ as required by microscopic reversibility.

The relative basicities of the leaving groups indicate that $k_{21} \gg k_{53}$ and since $(H^+) > K_1'$, then the denominator of eq 19 simplifies to $k_{21}(H^+)$ and

$$\gamma_+ = \frac{k_{12}k_{35}K_1'}{k_{21}}\frac{[M]}{(K_1 + H^+)} + k_{53} \qquad (20)$$

This equation has the same form as eq 3 with

$$k_3' = \frac{k_{12}k_{35}K_1'}{k_{21}} \qquad (21)$$

and

$$k_{-3}' = k_{53} \qquad (22)$$

The ratio of these experimental rate constants can be related to known constants through eq 7

$$\frac{k_3'}{k_{-3}'} = \frac{k_{12}k_{35}K_1'}{k_{21}k_{53}} = K_1K_f \qquad (23)$$

For tyrosine $pK_1 = 9.2$ and $\log K_f = 5.14$,[15] therefore $K_1K_f = 8.7 \times 10^{-5}$. This value can be combined with the experimental $k_3' = 3.3 \times 10^{-6}$ to calculate $k_{-3}' = 3.8 \times 10^{-2}$ sec^{-1}. The latter is less than the upper limit of 4.5×10^{-2} sec^{-1} obtained from the kinetic results so that at least the equilibrium constant and kinetic results are not inconsistent. However, the calculated value of k_{-3}' is about three times smaller than that calculated by Kustin et al.[15]

For *m*- and *o*-tyrosine K_f values for the nickel(II) complexes are not known. If it is assumed that the ratio of K_f values for nickel(II) is the same as that found for copper(II) with tyrosine, *m*-tyrosine, and *o*-tyrosine,[16] then k_{-3}' is calculated to be 5.5×10^{-2} and 9×10^{-2} sec^{-1} for *m*- and *o*-tyrosine, respectively. These calculations show that there are at least no apparent inconsistencies between the kinetic results and probable formation constant values for *m*- and *o*-tyrosine.

Again, as with methionine it is not possible to calculate k_{35} from k_3' because k_{12}, k_{21}, and K_1' are unknown. If $(k_{12}/k_{21}) = 0.5$ and $K_1' = 10K_1$, as assumed for methionine, then for tyrosine k_{35} is 1×10^3 sec^{-1}. This seems to be a reasonable value for substitution on nickel(II) but only indicates that the interpretation used does not lead to any obvious inconsistencies.

In order to analyze the results for systems with three coordinating groups, SH, NH_2, and CO_2^-, such as cysteine and penicillamine, the scheme shown in Scheme I will be used. In this scheme initial complexing by the NH_2 group has been neglected for reasons already discussed for methionine and tyrosine. In particular for cysteine and penicillamine the $^+NH_3$ group has a pK of ≈8.8 (see Table I) so that the approximation used to derive eq 16 will still be valid, and the term involving initial $-NH_2$ complexing ($k_{43}K_1$ in the previous scheme) should still be small relative to other terms. It has also been assumed in Scheme I that chelation will occur via the sequence of reactions which involve formation of the smallest chelate ring. Thus, if cysteine is represented as $HSNH_2CO_2^-$, then the monodendate complex MO_2CNH_2SH may react to bond the NH_2 group to the metal and not the SH group. Scheme I also applies only to systems in which complex formation goes to completion, because the last step has been written as irreversible.

The reaction scheme in Scheme I can be simplified as shown in Appendix A. It is also shown there that if various rate and equilibrium constants are estimated and the steady state approximation is used then from A25

$$\frac{k_{obsd}(K_a + (H^+))}{(H^+)} = k_{18'} + \frac{k_{14'}K_{4'5'}}{k_{4'1}(H^+)}k_{5'6'} \qquad (24)$$

Note that k_{obsd} here and in eq 1 and 2 equals $k'_{obsd}/[Ni^{2+}]$ in A25.

Equations 2 and 24 have the same form and the experimental results give $k_1' = k_{18'}$ values of 8.5×10^2 and $4.8 \times 10^2\ M^{-1}$ sec^{-1} for cysteine and penicillamine, respectively. The values of $k_{18'}$ are significantly lower than the estimate of $5 \times 10^3\ M^{-1}$ sec^{-1} used to obtain A23 and A25. This estimate was based on the rate constant for the neutral forms of tyrosine and methionine. However, the latter systems involved a neutral zwitterion reacting to complex its negative substituent whereas $k_{18'}$ involves a neutral zwitterion reacting to complex a third neutral substituent. This difference in the charge at the actual reacting site should influence the appropriate ion pair formation constant[28] and thereby lower the value of $k_{18'}$ as observed. The difference between penicillamine and cysteine could be rationalized as a steric effect.

It should be noted that if our estimate of $k_{18'}$ was too high then perhaps the neglect of the $k_{28}K_{12}$ term to get A23 is not justified. However, it is possible that the effects which make $k_{18'}$ smaller than estimated may also lower k_{28} and the approximation that $k_{18'}(H^+) > k_{28}K_{12}$ is still valid. If $k_{28}K_{12}$ cannot be neglected then it will appear as $k_{28}K_{12}/(H^+)$ in eq 24, and would be another contribution to the experimental k_2' value.

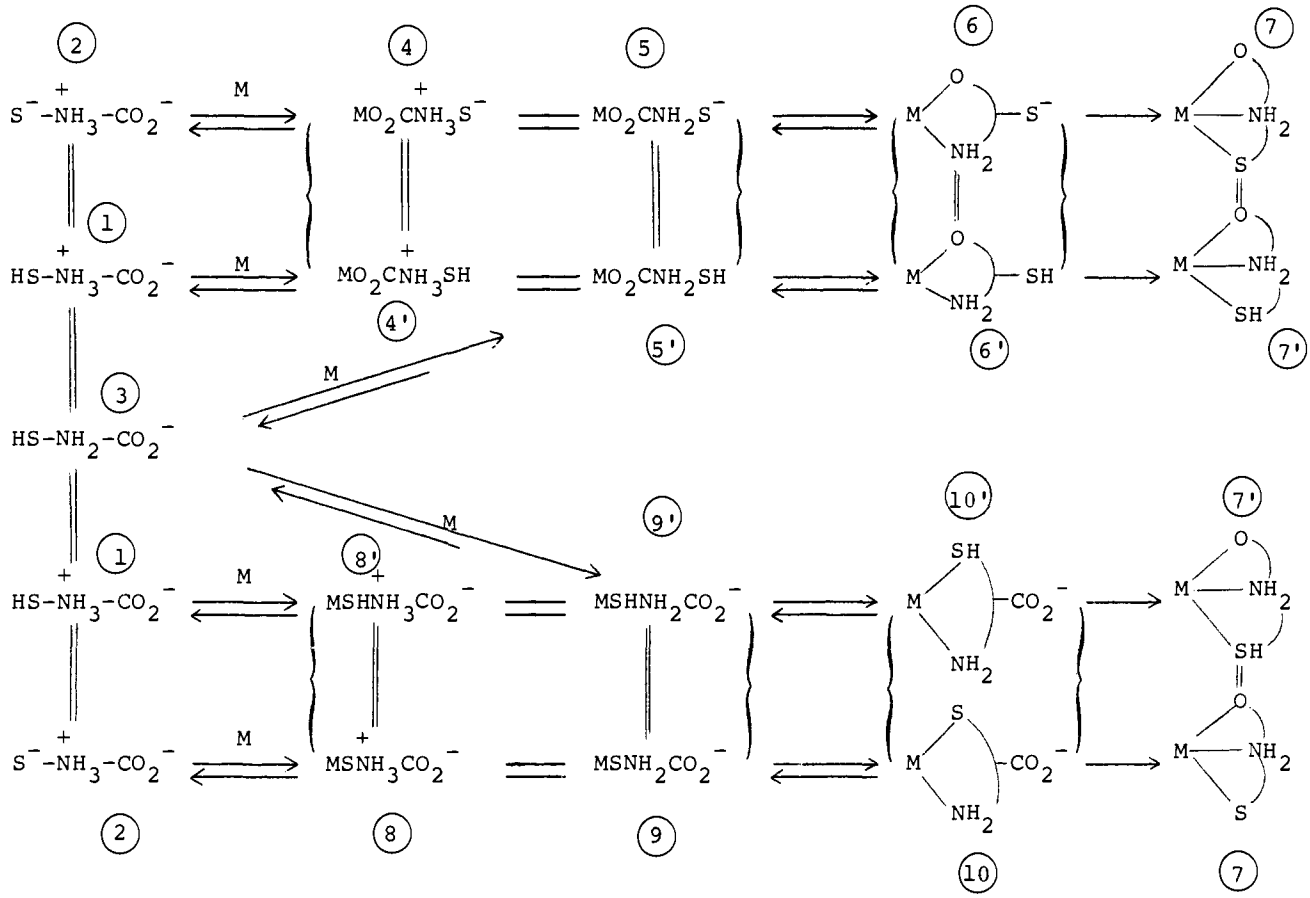
It is not possible to analyze the k_2' values of 1.45×10^{-4} and 2.2×10^{-4} sec^{-1} for cysteine and penicillamine because $k_{14'}/k_{4'1}$ and $K_{4'5'}$ are not known. If our previous estimate of the equivalent of $(k_{14'}/k_{4'1'}) \approx 0.5$ is used, and $K_{4'5'} \approx 10^{-8}\ M$, then for cysteine $k_{5'6'} = 4 \times 10^4$ sec^{-1}, not an unlikely value for chelate ring closing on nickel(II).

In summary the kinetic analysis indicates that complexing of nickel(II) by cysteine and penicillamine proceeds in one path through initial carboxylate binding via species 1, 4′, 5′, 6′, and 7′ successively in Scheme I. A competing path involves –SH complexing first by the 1 → 8′ reaction and possibly some contribution from reaction 2 → 8 in Scheme I.

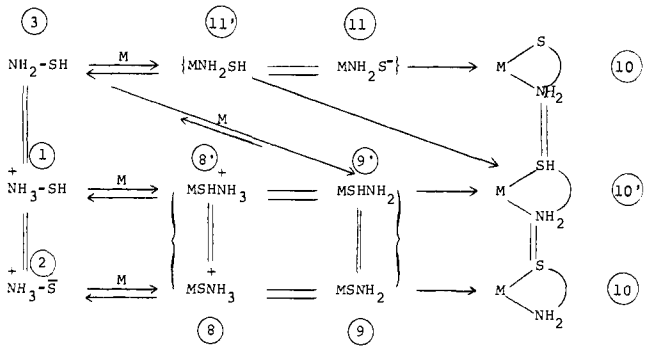
To analyze the results for cysteine ethyl ester another reaction scheme must be employed because the carboxylate group is no longer capable of reacting. Furthermore the amino group is sufficiently acidic (pK = 6.77) that the approximation used to derive eq 16 is no longer valid since $(H^+) \sim K_a$ now. The reaction scheme in Scheme II with a numbering system similar to Scheme I has been used.

The predicted rate law for this scheme has been simplified and analyzed in Appendix B. The resulting equation, B10, after substitution of $K_a = K_{12} + K_{13}$ and k_{obsd} =

Scheme I



Scheme II



$k'_{obsd}/[M]$, and rearrangement yields

$$k_{obsd}\frac{K_a + (H^+)}{(H^+)} = k_{18'} + \frac{(k_{3,11'} + k_{39'})K_{13} + k_{28}K_{12}}{(H^+)} \quad (25)$$

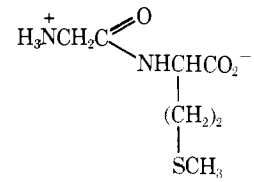
The experimental results are consistent with this rate law with $k_1' = k_{18'} = 7 \times 10^2\ M^{-1}\ sec^{-1}$ and $k_2' = (k_{3,11'} + k_{39'})K_{13} + k_{28}K_{12} = 1.33 \times 10^{-3}\ sec^{-1}$.

The value of $k_{18'}$ is similar to that obtained for cysteine. However, for the ester $k_{18'}$ is for complexing of a unipositive ion while for cysteine it is for the neutral zwitterion. This result indicates that the charge on the HSN^+H_3– reactive unit is more important than net charge in controlling the reactivity. This argument has been used here already to rationalize the low $k_{18'}$ values for the neutral zwitterions of cysteine and penicillamine.

In k_2', if it is assumed that $k_{28}K_{12}$ is the dominant term, then $k_{28} = 3.7 \times 10^4\ M^{-1}\ sec^{-1}$. This value seems too large for a neutral zwitterion. On the other hand if $(k_{3,11'} + k_{39'})K_{13}$ is the dominant term, then $(k_{3,11'} + k_{39'}) = 7.5 \times 10^3$. This is a reasonable value for reaction of a neutral ligand and indicates that this term dominates in k_2'.

The overall results for cysteine ethyl ester show that the rate controlling step in the complexing is initial binding of ligand to metal. The major reaction paths are through -SH complexing of the unipositive ion and NH_2 and SH complexing of the neutral molecule.

A discussion of the complexing of a peptide such as glycylmethionine



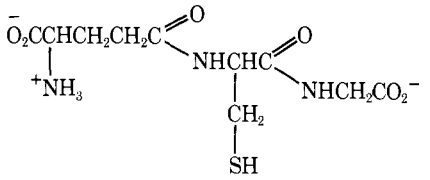
is complicated by uncertainty as to the nature of the final product. Recent evidence for polyglycine systems[29] indicates that the NH_2 and C=O groups are chelated in the final product. The results of the present study on methionine indicate that the SCH_3 group does not complex, probably for steric reasons.

The pK_a of glycylmethionine is similar to those of cysteine and penicillamine (Table I); however, the k_2' value for glycylmethionine is about ten times smaller than that for the other two ligands. A similar lower rate has been noted by Pasternack et al.[30] for polyglycine systems compared with glycine.

By analogy to the mechanism proposed here for cysteine and penicillamine one would predict that the nickel(II) would complex first at the CO_2^- of glycylmethionine. This would have to be followed by several chelate ring closing and opening steps if the final product has the NH_2 and C=O groups coordinated to the metal ion. These intermediate chelation steps may explain the lower reactivity of the polypeptide systems, as suggested by Pasternack et al.[30]

Alternatively the nickel(II) might complex at the C=O group first followed by proton loss and ring closure of the NH_2 group. This mechanism would lead to a rate law analogous to eq 16 where k_{12}/k_{21} would be the equilibrium constant for formation of the NiO=C- complex. If this equilibrium is less favorable than that with a carboxylate group, then this could explain the slower reaction of the peptide ligands.

In the case of glutathione



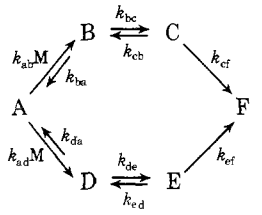
the available evidence[11,12] indicates that the SH group is not involved to any large extent in complexing to nickel(II). The kinetic results are consistent with this in that no k_1' could be detected. For penicillamine, cysteine, and cysteine ester, k_1' is associated with complexing at the SH group.

Although the k_2' value for glutathione is similar to that for glycylmethionine, this does not mean necessarily that the complexing mechanism is different from that of a simple amino acid. The reaction may occur at the glutamyl CO_2^- and NH_2 groups and the mechanism in eq 4 would apply. Then, from eq 16, assuming that K_1' parallels pK_{NH} (Table I), one would expect k_2' for glutathione to be similar to that for tyrosine and methionine as observed experimentally.

Acknowledgment. The authors wish to acknowledge the financial support of this research by the National Research Council of Canada.

Appendix A

If all the species related by proton transfer reactions are grouped together then Scheme I reduces to



(A1)

where the concentrations of A, B, C, etc. are related to those in Scheme I by

$$\begin{aligned}[A] &= [1] + [2] + [3]\\ [B] &= [4] + [4'] + [5] + [5']\\ [C] &= [6] + [6']\\ [D] &= [8] + [8'] + [9] + [9']\\ [E] &= [10] + [10']\\ [F] &= [7] + [7']\end{aligned} \quad \text{(A2)}$$

and total ligand concentration is

$$[T] = [A] + [B] + [C] + [D] + [E] + [F]$$

If a steady state is assumed for the mono- and bidendate complexes B, C, D, and E, and if the observed rate constant k'_{obsd} (as in eq 3) is defined by

$$\frac{d[F]}{dt} = k'_{obsd}([T] - [F]) \quad \text{(A3)}$$

then it can be shown that

$$k'_{obsd} = \frac{k_{ef}k_{de}k_{ad}[k_{ba}(k_{cf} + k_{cb}) + k_{bc}k_{cf}] + k_{cf}k_{bc}k_{ab}[k_{da}(k_{ef} + k_{ed}) + k_{ef}k_{de}]}{\{[k_{ba}(k_{cf} + k_{cb}) + k_{bc}k_{cf}][(k_{ef} + k_{ed})(k_{da} + k_{ad}) + k_{de}(k_{ef} + k_{ad})] + [k_{da}(k_{ef} + k_{ed}) + k_{ef}k_{de}][k_{cf} + k_{cb} + k_{bc}]k_{ab}\}} \quad \text{(A4)}$$

Fortunately this expression can be simplified by writing the composite rate constants in eq A4 in terms of specific values from Scheme I and then considering the magnitude of individual terms. A comparison of eq A1 and Scheme I shows that

$$k_{cf}[C] = k_{6'7'}[6'] + k_{67}[6] \quad \text{(A5)}$$

where $k_{6'7'}$ is the rate constant for conversion of species 6' to 7' in Scheme I, and other rate constants in the scheme are similarly defined.

Also, defining

$$K_6 = \frac{[6](H^+)}{[6']} \quad \text{(A6)}$$

and noting that [C] = [6'] + [6], it is easily shown that

$$[6] = \frac{K_6}{K_6 + (H^+)}[C] \text{ and } [6'] = \frac{(H^+)}{K_6 + (H^+)}[C] \quad \text{(A7)}$$

Substitution of eq A7 into eq A5 shows that

$$k_{cf} = \frac{k_{6'7'}(H^+) + k_{67}K_6}{K_6 + (H^+)} \quad \text{(A8)}$$

Similarly it can be shown that

$$k_{cb} = \frac{k_{6'5'}(H^+) + k_{65}K_6}{K_6 + (H^+)} \quad \text{(A9)}$$

It seems reasonable that the rate constants for chelate ring closing $k_{6'7'}$ and k_{67} will be much greater than those for ring opening $k_{6'5'}$ and k_{65}. Therefore

$$k_{cf} >> k_{cb} \quad \text{(A10)}$$

and similarly

$$k_{ef} >> k_{ed}$$

Furthermore defining

$$K_4 = \frac{[4](H^+)}{[4']};\ K_5 = \frac{[5](H^+)}{[5']};\ K_{45} = \frac{[5](H^+)}{[4]} \quad \text{(A11)}$$

it can be shown that

$$k_{ba} = \frac{k_{4'1}K_5(H^+)^2 + k_{42}K_5K_4(H^+)}{K_{45}K_4(K_5 + (H^+)) + K_5(K_4 + (H^+))(H^+)}$$

and (A12)

$$k_{bc} = \frac{k_{5'6'}K_4K_{45}(H^+) + k_{56}K_4K_5K_{45}}{K_{45}K_4(K_5 + (H^+)) + K_5(K_4 + (H^+))(H^+)}$$

with the approximation that, for pH <7, $(H^+) > K_4 > K_5 \approx K_{45}$,[31] then

$$k_{ba} = \frac{k_{4'1}K_5(H^+)}{K_{45}K_4 + K_5(H^+)};\ k_{bc} = \frac{k_{5'6'}K_4K_{45}}{K_{45}K_4 + K_5(H^+)} \quad \text{(A13)}$$

In addition it seems likely that $k_{4'1} > k_{5'6'}$, therefore

$$k_{ba} > k_{bc} \quad \text{(A14)}$$

With the added approximations that $(H^+) > K_6$ and $k_{6'7'} = k_{5'6'}$ then

$$k_{cf} > k_{bc} \quad \text{(A15)}$$

It can also be shown that for $(H^+) > K_{12'}$, $K_{13'}$, and $k_{10'7'} > k_{18'}[M]$, and $k_{10'7'} > k_{14'}[M]$, then

$$k_{ef} > k_{ad}, k_{ab} \quad \text{(A16)}$$

Conditions A10, A14, and A15 reduce A4 to

$$k'_{obsd} = \frac{k_{ef}[k_{de}k_{ad}k_{ba} + k_{bc}k_{ab}(k_{da} + k_{de})]}{k_{ba}[k_{ef}(k_{ad} + k_{da} + k_{de}) + k_{de}k_{ad}] + k_{ef}k_{ab}(k_{da} + k_{de})} \quad (A17)$$

and with A16 this reduces further to

$$k'_{obsd} = \frac{k_{de}k_{ad}k_{ba} + k_{bc}k_{ab}(k_{da} + k_{de})}{k_{ba}(k_{ad} + k_{da} + k_{de})} \quad (A18)$$

Further simplification in this way is not possible because there does not seem to be any way to reliably estimate $K_8(=[8](H^+)/[8'])$, K_9 $(=[9](H^+)/[9'])$, nor $k_{8'1}$. However, it is possible to resort to the experimental results and make one more simplification that

$$(k_{da} + k_{de}) > k_{ad} \quad (A19)$$

This must be so because k'_{obsd} is found to be first order in $[Ni^{2+}]$ in the present work, but only k_{ad} and k_{ab} contain the metal ion concentration. Therefore if the opposite of A19 were true, then $[Ni^{2+}]$ would cancel in numerator and denominator of A18 and k'_{obsd} would be independent of metal ion concentration. Therefore application of A19 to A18 gives

$$k'_{obsd} = \left(\frac{k_{de}}{k_{da} + k_{de}}\right)k_{ad} + \left(\frac{k_{bc}}{k_{ba}}\right)k_{ab} = \left(\frac{1}{1 + (k_{da}/k_{de})}\right)k_{ad} + \left(\frac{k_{bc}}{k_{ba}}\right)k_{ab} \quad (A20)$$

The ratio in the denominator of the first term is

$$\frac{k_{da}}{k_{de}} = \frac{K_9(H^+)(k_{82}K_8 + k_{8'1}(H^+))}{K_{89}(K_8 + (H^+))(k_{9,10}K_9 + k_{9',10'}(H^+))} \quad (A21)$$

Estimates of this ratio are difficult but some attempt can be made with the ultimate justification being consistency with the experimental rate law. The latter requires that (k_{da}/k_{de}) be either small relative to 1, or independent of (H^+). Based on the assumption that the pK_a of a coordinated HS-group will be increased about as much as that of a coordinated water molecule, then K_8 and K_9 will be $\sim 10^{-2}$ M and $K_8, K_9 \gg (H^+)$. It also seems probable that $k_{9,10} \approx k_{9',10'} \approx 10^4$ sec^{-1}. Then for $(H^+) \lesssim 10^{-6}$ the ratio $k_{da}/k_{de} < 1$ if $k_{82} < 10^{-1}$ and $k_{8'1} < 10^5$. These upper limits for the rate constants seem reasonable, and therefore A20 simplifies to

$$k'_{obsd} = k_{ad} + (k_{bc}/k_{ba})k_{ab} \quad (A22)$$

where

$$k_{ad} = \left(\frac{k_{18'}(H^+) + k_{28}K_{12} + k_{39'}K_{13}}{(H^+) + K_{12} + K_{13}}\right)[M] = \frac{k_{18'}(H^+)[M]}{(H^+) + K_{12} + K_{13}} \quad (A23)$$

if, on the basis of ligand charge $k_{18'} \approx 5 \times 10^3$ M^{-1} sec^{-1}, $k_{28} \approx k_{39'} \approx 2 \times 10^4$ M^{-1} sec^{-1}, and $K_{12}, K_{13} < 10^{-8}$ M (for cysteine and penicillamine).

Similarly

$$k_{ab} = \left(\frac{k_{14'}(H^+) + k_{24}K_{12} + k_{35'}K_{13}}{(H^+) + K_{12} + K_{13}}\right)[M] = \frac{k_{14'}(H^+)[M]}{(H^+) + K_{12} + K_{13}} \quad (A24)$$

Substitution of A13, A23, and A24 into A20 gives

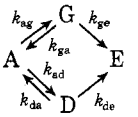
$$k'_{obsd} = \frac{k_{18'}(H^+)[M]}{(H^+) + K_{12} + K_{13}} + \left(\frac{k_{5'6'}K_4K_{45}}{k_{4'1}K_5(H^+)}\right)\left(\frac{k_{14'}(H^+)[M]}{(H^+) + K_{12} + K_{13}}\right)$$

With the substitution that $(K_4K_{45}/K_5) = K_{4'5'}$ and that the measured apparent acid dissociation constant $K_a = (K_{12} + K_{13})$, and after rearrangement, it is found that

$$\frac{k'_{obsd}}{[M]}\frac{(K_a + (H^+))}{(H^+)} = k_{18'} + \frac{k_{14'}K_{4'5'}}{k_{4'1}(H^+)}k_{5'6'} \quad (A25)$$

Appendix B

The reaction scheme in Scheme II may be simply represented as



(B1)

where letters represent the total concentration of species related by rapid proton transfer reactions in Scheme II.

$$\begin{aligned} [A] &= [1] + [2] + [3] \\ [D] &= [8] + [8'] + [9] + [9'] \\ [E] &= [10] + [10'] \\ [G] &= [11] + [11'] \\ [T] &= [A] + [D] + [E] + [G] \end{aligned} \quad (B2)$$

If a steady state is assumed for the nonchelated intermediates D and G, then it can be shown that the observed rate constant defined by

$$\frac{d[E]}{dt} = k'_{obsd}([T] - [E])$$

is given by

$$k'_{obsd} = \frac{k_{ge}k_{ag}(k_{da} + k_{de}) + k_{de}k_{ad}(k_{ga} + k_{ge})}{k_{ag}(k_{da} + k_{de}) + (k_{ga} + k_{ge})(k_{ad} + k_{da} + k_{de})} \quad (B3)$$

A comparison of B1 and Scheme II shows that

$$k_{ga}[G] = k_{11',3}[11'] = \frac{k_{11',3}(H^+)}{K_{11} + (H^+)}[G] \quad (B4)$$

where $K_{11} = [11](H^+)/[11']$. Therefore

$$k_{ga} = k_{11',3}(H^+)/(K_{11} + (H^+)) \quad (B5)$$

Similarly

$$k_{ge} = \frac{k_{11',10'}(H^+) + k_{11,10}K_{11}}{K_{11} + (H^+)} \quad (B6)$$

It seems very probable that the rate constant for ring closing $k_{11'10'}$ will be much greater than the rate constant for dissociation of the amine coordinated ligand $k_{11',3'}$ so that

$$k_{ge} \gg k_{ga} \quad (B7)$$

In addition, since only k_{ag} and k_{ad} contain the metal ion concentration, terms containing these constants in the denominator of B3 must be small with respect to the others. If this were not the case then k'_{obsd} would be independent of the $[Ni^{2+}]$ concentration, contrary to the experimental results.

Therefore B3 simplifies to

$$k'_{obsd} = \frac{(k_{da} + k_{de})k_{ag} + k_{de}k_{ad}}{(k_{da} + k_{de})} = k_{ag} + \frac{k_{ad}}{(1 + k_{da}/k_{de})} \quad (B8)$$

Further comparison of B1 and Scheme II leads to

$$k_{da} = \frac{k_{8'1}K_9(H^+)^2 + k_{82}K_8K_9(H^+) + k_{9',3}K_8K_{89}(H^+)}{K_8K_9K_{89} + K_8(K_9 + K_{89})(H^+) + K_9(H^+)^2}$$

and (B9)

$$k_{de} = \frac{k_{9',10'}K_8K_{89}(H^+) + k_{9,10}K_8K_9K_{89}}{K_8K_9K_{89} + K_8(K_9 + K_{89})(H^+) + K_9(H^+)^2}$$

where $K_8 = [8](H^+)/[8']$, $K_9 = [9](H^+)/[9']$, and $K_{89} = [9](H^+)/[8]$. With the reasonable assumption that $K_8, K_9 \gg (H^+)$, and since $K_{13} \approx (H^+)$, therefore $K_{89} > (H^+)$, and $k_{9'3} \approx k_{8'2} \gg k_{82}$ and $k_{910} \approx k_{9'10'}$. These conditions show that the last term in the numerator of the above two equations are the dominant ones. It seems likely, for reasons discussed in regard to cysteine, that $K_9 > 10^{-2}$, therefore $k_{de} > k_{da}$ as long as $k_{9'3} < 10^4 k_{9,10}$. If this condition is satisfied then

$$k'_{obsd} = k_{ag} + k_{ad} = \frac{k_{3,11'}K_{13}}{(H^+) + K_{12} + K_{13}}[M] + \frac{k_{18'}(H^+) + k_{28}K_{12} + k_{39'}K_{13}}{(H^+) + K_{12} + K_{13}}[M] \quad \text{(B10)}$$

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(19) In Eq 4 the rate constants k_{12} and k_{43} are really products of the specific rate constant and the ion pair formation constant if the ion pair mechanism is assumed.
(20) This assumption and those used to derive eq 8 and 9, can be justified by substitution of rate constant values derived later in this work. Calculations, using eq 5, show that $\gamma_- \gtrsim 10^2\gamma_+$, and γ_- is much larger than experimental values. Generally, γ_- is dominated by a_2, which is independent of [M], while experimental values are first order in [M]. The approximations will become less valid as pK_1 decreases, and pH and [M] increase; conservative limits seem to be pH <7, $pK_1 \approx 6$, and [M] < 0.1 *M* and pH <7, $pK_1 \approx 8$, and [M] < 0.3 *M* etc., for nickel(II) systems at least.
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Electronic Effects in Transition Metal Porphyrins. I. The Reaction of Piperidine with a Series of Para- and Meta-Substituted Nickel(II) and Vanadium(IV) Tetraphenylporphyrins[1]

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Abstract: Para- and meta-substituted tetraphenylporphyrin complexes of Ni(II), Ni(*p*-X)TPP and Ni(*m*-X)TPP (X = OCH_3, CH_3, H, F, Cl, $COOCH_3$, CN, and NO_2), and para-substituted V(IV) tetraphenylporphyrins, VO(*p*-X)TPP (X = OCH_3, CH_3, H, Cl, and CN), react in the presence of high concentrations of piperidine in toluene to form the bis- and monopiperidine adducts, respectively Ni(X)TPP + 2Pip $\rightleftharpoons$ Ni(X)TPP(Pip)$_2$ (β_2) and VO(X)TPP + Pip $\rightleftharpoons$ VO(X)TPP(Pip) (K_1^V). In most cases, equilibrium constants β_2 and K_1^V are less than unity. For reactions 1 and 2 a Hammett $\sigma\rho$ relationship is observed, with $\rho^{Ni(p)} = 0.331$, $\rho^{Ni(m)} = 0.413$, and $\rho^V = 0.113$. Thus substituents at such remote positions as the meta and para positions of the phenyl rings significantly affect the axial reactivity of the metal. The extent of this effect is greatly increased if the metal has a full compliment of dπ electrons (Ni(II), d^8, as compared to V(IV), d^1). Inductive and resonance contributions to the observed substituent effects are almost equal when X is in the para position, but inductive effects predominate when X is in the meta position. Either π induction or moderate π conjugation between phenyl and porphine rings (or a combination of both) may be the mode of transmission of resonance effects. In dilute piperidine solutions, NiTPP reacts to give the monopiperidine complex, whose electronic spectrum is almost indistinguishable from that of the reactant. ESR splitting constants and *g* values of the VO(*p*-X)TPP complexes and their piperidine adducts are independent of the substituent X.

The transmission of electronic effects from various points on the porphyrin ring through the four porphyrin nitrogens to the metal ion has long been an interest of those who have investigated the physical properties and chemical reactions of metalloporphyrins.[2-5] Because of the conjugated nature of the porphyrin ring system, electron donating or withdrawing substituents on the periphery of the molecule have been shown to affect the basicity of the porphyrin nitrogens.[2,3] This, in turn, often affects the visible absorption spectra, redox potentials, and axial ligation reactions of the free bases and their respective metalloporphyrin complexes.[2,4,5] Some of the most detailed investigations of the transmission of electronic effects in metalloporphyrins have been carried out on Ni(II) complexes of natural or modified