NO_2^-, and HOH) with pK' values from 5.0 to 7.3[59] have also been assigned to the axial water ligand, in this case on the basis of indirect spectral evidence.

The alkaline proton dissociation has been assigned to the axial water ligand in the complexes $XCo(D_2H_2)HOH$[24] where X = NO_2^- (pK' = 7.28),[59] SO_2^{-2} (pK' = 10.23),[26] and CH_3 (pK' = 12.68) on the basis of the following approximate ratio of the rates of axial water replacement by SCN^-, N_3^-, and py: $NO_2^-/SO_3^{2-}/CH_3$ = $1/10^4/10^5$.[24-26] The kinetic data, it is argued, reflect the strength of water bonding to cobalt and the acidity of the axial water ligand both of which decrease through the series NO_2^-, SO_3^{2-}, CH_3. However, the similarly alkaline proton dissociation constants for the complexes $Me(D_2H_2)L$ (Tables I and II) which, as noted above, must be assigned to the equatorial ligand system indicate that a similar assignment merits serious consideration for $Me(D_2H_2)HOH$ as well. Indeed, if methylcobalt complexes are predominately pentacoordinate in aqueous solution at room temperature,[50] then the proton dissociation from $Me(D_2H_2)HOH$ *must* be assigned to the equatorial ligand system. For example, methylcobinamide, which has been shown to be predominately pentacoordinate in aqueous solution,[50] shows no evidence of proton dissociation up to pH 14.0.[58]

(59) A. V. Ablov, B. A. Borykin, and N. M. Samus, *Russ. J. Inorg. Chem.*, 11, 978 (1966).

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Kinetics of Protonation Reactions of Nickel(II) Peptide Complexes

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Abstract: Nickel tetraglycine, $Ni(H_{-3}GGGG)^{2-}$, and nickel triglycinamide, $Ni(H_{-2}GGGa)^-$, react with H_3O^+ with second-order rate constants (M^{-1} sec^{-1}) of 7.1×10^4 and 3.3×10^4, respectively. The $Ni(H_{-3}GGGG)^{2-}$ and $[NiH_{-2}GGGa]^-$ reactions are not general-acid catalyzed while the reactions of nickel triglycine, $Ni(H_{-2}GGG)^-$, and the corresponding copper complex are subject to general-acid catalysis. Hydrogen carbonate ion is an effective general acid for the protonation of $Ni(H_{-2}GGG)^-$ and carbonate ion is an inhibitor. A general mechanism is proposed to account for the kinetics of the various protonation reactions of metal peptide complexes.

Tetraglycine, triglycine, and triglycinamide are known to ionize peptide and amide protons in the formation of yellow, diamagnetic, square-planar nickel(II) complexes.[1-6] The deprotonated nitrogen atoms become coordinated to nickel.[7] In this manner the triglycine anion ionizes two protons to give $Ni(H_{-2}GGG)^-$, while triglycinamide and the tetraglycine anion lose three protons to give $Ni(H_{-3}GGGa)^-$ and $Ni(H_{-3}GGGG)^{2-}$, respectively.[1,5,6,8]

The rates of the H_3O^+ reactions with the triglycine complexes, $Ni(H_{-2}GGG)^-$ and $Cu(H_{-2}GGG)^-$, are much slower than normal diffusion-controlled reactions.[5,6,9,10] A general-acid catalysis mechanism is observed in the reaction of other acids (HX) with these complexes.[5,10] The reactions are first order in the concentrations of the acids and first order in the concentration of the complex. The rate expression (eq 1) includes a solvent dissociation rate constant (k_d).

$$\text{rate} = (k_d + k_H[H_3O^+] + k_{HX}[HX])[M(H_{-2}GGG)^-] \quad (1)$$

Recently Wilkins and coworkers[6] have reported that the protonation rate of $Ni(\text{glycinamide-H})_2$ was dependent on the concentration of buffer, excess ligand, and hydrogen ion as is the case with the triglycine complex, $Ni(H_{-2}GGG)^-$. However, they also reported that the tetraglycine complex, $Ni(H_{-3}GGGG)^{2-}$, undergoes a two-stage protonation at pH ~7 and that the rate constants are pH independent over some 0.5-unit range of pH. We disagree with these results for tetraglycine.

Ligand exchange reactions of $Ni(H_{-3}GGGG)^{2-}$ with polydentate amines have been reported[11] to take place in two measurable steps from pH 10.5 to 12. The first reaction supposedly produced yellow mixed complexes of unusual stoichiometries, such as (nickel tetraglycine)$_3$trien. Some of the peculiar reactions attributed to the tetraglycine complex of nickel may be due to other species produced in its spontaneous reaction with molecular oxygen.[12] We have found that under anaerobic conditions $Ni(H_{-3}GGGG)^{2-}$ reacts with trien (triethylenetetramine) without forming yellow mixed complexes and without undergoing the initial

(1) R. B. Martin, M. Chamberlin, and J. T. Edsall, *J. Amer. Chem. Soc.*, 82, 495 (1960).
(2) M. K. Kim and A. E. Martell, *ibid.*, 89, 5138 (1967).
(3) R. Mathur and R. B. Martin, *J. Phys. Chem.*, 69, 668 (1965).
(4) M. K. Kim and A. E. Martell, *J. Amer. Chem. Soc.*, 91, 872 (1969).
(5) E. J. Billo and D. W. Margerum, *ibid.*, 92, 6811 (1970).
(6) C. F. V. Mason, P. I. Chamberlain, and R. G. Wilkins, *Inorg. Chem.*, 10, 2345 (1971).
(7) H. C. Freeman, J. M. Guss, and R. L. Sinclair, *Chem. Commun.*, 485 (1968).
(8) J. W. Chang and R. B. Martin, *J. Phys. Chem.*, 73, 4277 (1969).
(9) G. K. Pagenkopf and D. W. Margerum, *J. Amer. Chem. Soc.*, 90, 501 (1968).
(10) G. K. Pagenkopf and D. W. Margerum, *ibid.*, 90, 6963 (1968).
(11) N. W. H. Ma, D. A. White, and R. B. Martin, *Inorg. Chem.*, 6, 1632 (1967).
(12) E. B. Paniago, D. C. Weatherburn, and D. W. Margerum, *Chem. Commun.*, 1427 (1971).

fast reaction previously reported.[11] On the other hand, solutions of nickel tetraglycine not protected from air appear to have more than one reaction with trien and similarly the disappearance of the yellow color takes place in several stages in the reaction of such solutions with acids.

The protonation reactions of $Ni(H_{-3}GGGG)^{2-}$ and of $Ni(H_{-3}GGGa)^{-}$, unlike reactions of $Ni(H_{-2}GGG)^{-}$, are not general-acid catalyzed. A general mechanism is proposed to explain the behavior of the nickel(II) oligopeptide complexes in protonation reactions.

Experimental Section

Reagents. Nickel(II) perchlorate was prepared from nickel carbonate and perchloric acid and was recrystallized from water. A stock solution of the nickel salt was standardized by EDTA titration using murexide indicator.

Nickel(II) tetraglycine solutions were freshly prepared for each set of experiments. The chromatographically pure tetraglycine (Schwarz/Mann, Orangeburg, N. Y.) was added in a 1:1 mole ratio to a deaerated nickel perchlorate solution. Sodium hydroxide, CO_2-free and stored under N_2, was added slowly to bring the solution to pH 10–11. Recrystallized sodium perchlorate was added to adjust the ionic strength to 0.10 *M* and the solution was passed through a Millipore filter. All steps were carried out in a N_2 atmosphere and the solutions were stored under N_2 while being used. The O_2 uptake reactions are fastest in neutral solutions, so special care must be taken to avoid dissolved oxygen when studying reactions between pH 6 and 9.

Nickel(II) triglycinamide solutions were prepared similarly using chromatographically pure triglycinamide hydrochloride (Fox Chemical Co., Los Angeles, Calif.).

Triglycine solutions were prepared from the chromatographically pure solid (Mann Research Laboratories, New York, N. Y.). Nickel triglycine solutions were prepared by the addition of CO_2-free NaOH solution to a mixture containing triglycine and nickel ion in a 2:1 ratio. The ionic strength was adjusted to 0.16 *M* with $NaClO_4$ and the solutions were passed through a Millipore filter and kept under nitrogen. Oxygen uptake is not a significant problem with nickel triglycine solutions, but CO_2 uptake can cause catalysis of the dissociations reactions by HCO_3^-.

Measurements. The kinetics of protonation and subsequent dissociation reactions were measured spectrophotometrically and in some instances by means of a pH-Stat. To avoid reversibility the reactions above pH 7.5 were run in the presence of EDTA as a scavenger. The EDTA concentration has no effect on the rate of the reactions of the tetraglycine and triglycinamide complexes. Only above pH 10 does EDTA contribute slightly to the rate of the nickel triglycine reaction as reported earlier.[5] Ionic strength was adjusted to 0.10 *M* ($NaClO_4$) except for the nickel triglycine reactions which were at 0.16 *M* ($NaClO_4$) for comparison with earlier studies. All rates were measured at 25.0°.

The rates of disappearance of the yellow complexes were followed at their visible absorption maxima: $Ni(H_{-3}GGGG)^{2-}$, 412 nm, ϵ 200 M^{-1} cm^{-1}; $Ni(H_{-3}GGGa)^{-}$, 410 nm, ϵ 140 M^{-1} cm^{-1}; $Ni(H_{-2}GGG)^{-}$, 430 nm, ϵ 260 M^{-1} cm^{-1}. The complexes were mixed with an excess of various buffers to give constant pH during the run. The slower reactions were followed using a Cary 14 or a Cary 16 spectrophotometer. Faster reactions were measured using a Durrum stopped-flow spectrophotometer. Data were reduced using an on-line digital computer (Hewlett-Packard 2115A) interfaced to the stopped-flow instrument as described elsewhere.[13] A plot and least-squares analysis of the conformance of the data to a first-order rate law were obtained after each run. Data from all runs were recorded on punched paper tape for later off-line calculations, when desired. The reactions were first order in the nickel complex and gave excellent plots.

Kinetic data from the pH-Stat also were reduced using the digital computer on-line interfaced to a Radiometer automatic titration assembly (titrator TTT11, autoburet ABU 13, pH meter PHM 26, equipped with a G202C glass electrode and a K401 calomel electrode with NaCl electrolyte).

Measurements of pH were made with the Radiometer pH meter (PHM 26) and pH readings were converted to $-\log[H^+]$ by subtracting 0.11.[14]

Results and Discussion

Nickel(II) Tetraglycinate Anion. The reaction of $Ni(H_{-3}GGGG)^{2-}$ with buffers or acid, observed at 412 nm, was first order in the concentration of the complex. There was no evidence of two protonation rates and above pH 5 the absorbance extrapolated to the time of mixing was that expected from $Ni(H_{-3}GGGG)^{2-}$. The observed first-order rate constants (Table I) are dependent on the hydrogen ion concen-

Table I. $Ni(H_{-3}GGGG)^{2-}$ Protonation Rate Constants from Spectrophotometric Studies[a]

Buffer	*M*	pH	k_{obsd}, sec^{-1} [b]
NaOH	0.050[c]	12.48[i]	4.3×10^{-6}
EDTA	0.001	10.90	1.8×10^{-5}
	0.002	10.50	2.1×10^{-5}
	0.005	11.50	1.6×10^{-5}
	0.005	10.30	1.7×10^{-5}
Borate	0.018[d]	8.78	2.1×10^{-4}
	0.018[e]	8.48	3.4×10^{-4}
	0.040[f]	9.48	4.1×10^{-5}
	0.050[f]	9.40	4.6×10^{-5}
	0.050[g]	9.00	$(9.6 \pm 0.4) \times 10^{-5}$
	0.050[e]	9.00	$(9.8 \pm 0.2) \times 10^{-5}$
	0.050[h]	9.00	$(1.1 \pm 0.2) \times 10^{-4}$
	0.100[f]	8.41	3.5×10^{-4}
Phosphate	0.025	6.90	1.9×10^{-2}
Lutidine	0.003	6.62	2.0×10^{-2}
	0.005	6.73	1.7×10^{-2}
	0.008	7.30	4.3×10^{-3}
	0.008	7.23	4.7×10^{-3}
	0.010	7.37	4.3×10^{-3}
	0.010	6.84	1.4×10^{-2}
	0.050	7.07	6.1×10^{-3}
	0.050	6.96	$(5.6 \pm 0.2) \times 10^{-3}$
	0.050	6.70	1.3×10^{-3}
	0.050	6.62	1.4×10^{-2}
	0.050	6.20	$(4.1 \pm 0.1) \times 10^{-2}$
	0.050	6.00	1.4×10^{-1}
	0.100	6.20	4.3×10^{-2}
Malonate	0.025	5.50	$(3.9 \pm 0.2) \times 10^{-1}$
	0.025	4.90	1.02 ± 0.04
Acetate	0.075	4.89	1.00 ± 0.02
	0.075	4.60	1.60 ± 0.02
	0.075	4.28	2.34 ± 0.04
	0.100	4.60	1.59 ± 0.03
Formate	0.075	3.95	3.63 ± 0.04
	0.075	3.67	4.4 ± 0.1
	0.075	3.37	5.2 ± 0.1
Chloroacetate	0.050	2.80	7.55 ± 0.04
	0.075	3.11	6.18 ± 0.08
	0.075	2.57	10.5 ± 0.1
	0.100	2.77	7.8 ± 0.1
Glycine	0.050	3.04	6.7 ± 0.1
	0.050	2.59	10.3 ± 0.2
	0.050	2.28	16.2 ± 0.7
Maleate	0.075	2.37	14.4 ± 0.2
	0.075	2.12	23.8 ± 0.6
	0.075	1.95	$36. \pm 1$
$HClO_4$	0.050	1.41[i]	$156. \pm 4$

[a] $Ni(H_{-3}GGGG)^{2-}$: 10^{-4}–10^{-3} *M*, 25°, 0.10 *M* $NaClO_4$. [b] Rate constants with error ranges are for standard deviations of three to seven runs. Other rate constants are from individual runs or from the average of two runs. [c] With EDTA added: 5×10^{-4} *M*, [d] 10^{-3} *M*, [e] 6×10^{-4} *M*, [f] 5×10^{-4} *M*, [g] 3×10^{-4} *M*, [h] 1.3×10^{-3} *M*. [i] Calculated from concentrations.

(13) B. G. Willis, J. A. Bittikoffer, H. L. Pardue, and D. W. Margerum, *Anal. Chem.*, 42, 1340 (1970).

(14) R. G. Bates, "Determination of pH," Wiley, New York, N. Y., 1964, p 92.

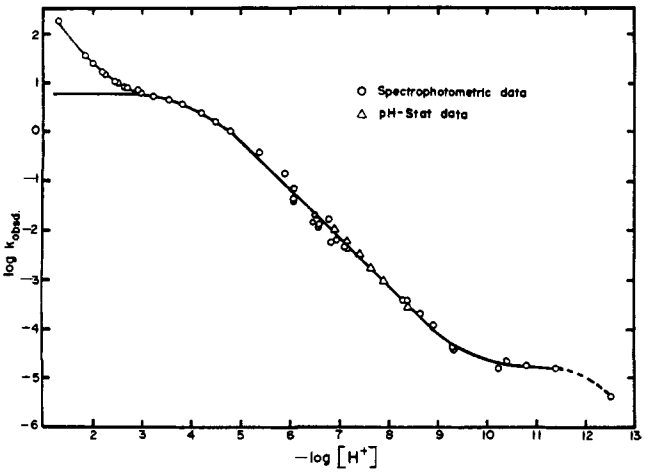


Figure 1. Hydrogen-ion dependence of the $Ni(H_{-3}GGGG)^{2-}$ protonation reaction (solid line is calculated from eq 5 where $k_{-1} = 1.6 \times 10^{-5}$ sec^{-1}, $K = 10^{4.1}$ M^{-1}, $k_1 = 5.6$ sec^{-1}).

tration but show no dependence on the concentration of buffers or other acids. Earlier work[6] had reported a biphasic protonation at pH ~7 with a small (~10%) change in absorbance at 410 nm for a fast reaction (k = 0.22 sec^{-1}) followed by a slower reaction with complete loss of absorbance (k = 0.028 sec^{-1}); neither reaction depended on the pH. We found no evidence for the faster reaction and although the slower reaction corresponds to the rate we observed at pH ~6.5, it is very pH dependent.

When $Ni(H_{-3}GGGG)^{2-}$ (initially pH 10–11) is mixed with excess buffer at pH <4 there is an immediate, but small (~4%), absorbance decrease which occurs within stopped-flow mixing times (~1 msec). The pH dependence of the first-order rate constant also tends to level off at pH 3–4 as seen in Figure 1.

Rates were measured also by a pH-Stat method. Solutions with a 2:1 ligand to nickel ion ratio were used to provide some buffering capacity and prevent temporary pH fluctuations as acid is added. First-order rate plots were obtained by plotting ln ($V - V_f$) against time, where V_f is the total volume of acid solution consumed and V is the volume at specified times. At pH 7.5 and above EDTA was added to the solution to avoid reversibility. The rates measured from pH 7 to 8.5 (Table II) show a first-order hydrogen-ion dependence and coincide with the rate constants determined by following the absorbance decrease of the yellow complex (Figure 1). Our initial experiments appeared to show a fast hydrogen ion uptake (other than that for excess ligand or EDTA) as the reaction pH was lowered. The amount of acid consumed in this initial step varied from 0 at pH 8 to about 1 equiv at pH 7. However, tests without $Ni(H_{-3}GGGG)^{2-}$ present showed that the apparent fast initial step could be due to the response characteristics of the electrode system.

Table II. Rate Constants for $Ni(H_{-3}GGGG)^{2-}$ Protonation from pH-Stat Studies[a]

pH	$10^3 k_{obsd}$, sec^{-1}
7.00	10.1, 10.1
7.25	5.5
7.50	3.5, 3.2
7.50[b]	3.0
7.75[b]	1.74
8.00[b]	0.94
8.50[b]	0.31

[a] Solution: $[Ni^{2+}] = 5 \times 10^{-3}$ M, [tetraglycine] = 1×10^{-2} M, pH 10.0, 25°, 0.10 M $NaClO_4$, titrant, 0.96 M $HClO_4$. [b] 0.014–0.020 M EDTA added as a scavenger.

In order to test further for any evidence of a faster reaction with uptake of hydrogen ion by $Ni(H_{-3}GGGG)^{2-}$, experiments were performed on the stopped-flow with acid–base indicators and partially buffered solutions. The results at pH 5.5 (chlorophenol red followed at 580 nm, after a 0.05 M malonate buffer containing 5×10^{-4} M indicator was mixed with a 2×10^{-3} M nickel tetraglycine solution at pH 10) and at pH 6.9 (2×10^{-5} M Bromthymol Blue in 0.05 M phosphate buffer, followed at 620 nm under similar conditions) failed to show the fast reaction and gave reaction rates consistent with that observed by following the loss of the yellow color. We conclude therefore that above pH 5 there is no rapid uptake of protons by $Ni(H_{-3}GGGG)^{2-}$.

The k_{obsd} dependence on $[H^+]$ shown in Figure 1 tends to diminish above pH 9 which is explicable in terms of the water dissociation path for $Ni(H_{-3}GGGG)^{2-}$. The value for k_d, the first-order aqueous dissociation rate constant for $Ni(H_{-3}GGGG)^{2-}$, is 1.6×10^{-5} sec^{-1}. This is smaller by a factor of 3000 than the k_d value (~0.05 sec^{-1}) for $Ni(H_{-2}GGG)^{-}$. The k_{obsd} value starts to decrease again at $-\log [H^+] = 12.5$. Similar effects at high pH were observed in the dissociation reactions of nickel triglycine which had EDTA as a scavenger.

Another plateau is seen in Figure 1 in the vicinity of pH 3. As was mentioned there is small absorbance change at 412 nm upon mixing the nickel tetraglycine with acids below pH 4. In this region reactions in the presence of indicators apparently show a rapid consumption (too fast to measure by stopped-flow) of at least one proton. This agrees with our interpretation of the mechanism, but the indicator experiments are difficult at lower pH and can only be considered as tentative supporting evidence. The reactions proposed to explain the pH dependence from 3 to 12 are given in eq 2–4. The products of the reaction depend upon the pH

$$Ni(H_{-3}GGGG)^{2-} + H_2O \xrightarrow{k_d} \text{products} \quad (2)$$

$$Ni(H_{-3}GGGG)^{2-} + H_3O^+ \underset{}{\overset{K_H}{\rightleftharpoons}} Ni(H_{-3}GGGG)H^- + H_2O \quad (3)$$

$$Ni(H_{-3}GGGG)H^- \xrightarrow{k_1} \text{products} \quad (4)$$

but the protonation of the other peptide nitrogens, such as $Ni(H_{-2}GGGG)^-$ and $Ni(H_{-1}GGGG)$, are rapid compared to the rate of protonation of $Ni(H_{-3}GGGG)^{2-}$. Thus, the pH-Stat reactions without EDTA present indicate a first-order dependence in $[H^+]$ but with the release of approximately three protons per nickel tetraglycine. The solid curve in Figure 1 is calculated from eq 5, where $K_H = 10^{4.1}$ M^{-1}, $k_d = 1.6 \times 10^{-5}$ sec^{-1},

$$k_{obsd} = k_d + \left(\frac{K_H[H^+]}{1 + K_H[H^+]}\right)k_1 \quad (5)$$

and $k_1 = 5.6$ sec^{-1}. The curve does not fit above pH 12 (possibly due to hydroxide inhibition) and it does not fit below pH 3 due to additional protonation reactions.

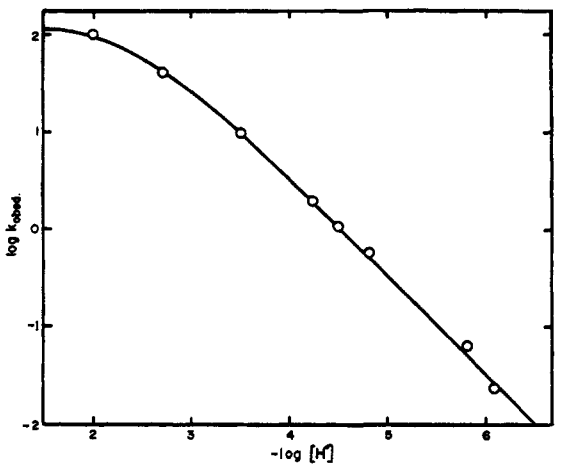


Figure 2. Hydrogen-ion dependence of the $Ni(H_{-3}GGGa)^-$ protonation reaction (solid line is calculated from eq 6 where $K_H' = 263\ M^{-1}$ and $k_1' = 126\ sec^{-1}$).

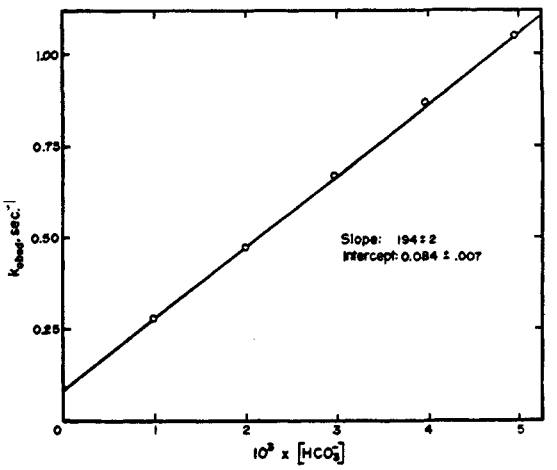


Figure 3. Hydrogen carbonate catalysis of the $Ni(H_{-2}GGG)^-$ protonation reaction (data from buffers at pH 8.0 ± 0.1, Table IV).

The additional increase in k_{obsd} below pH 3 did not give a simple dependence in $[H^+]$ but can be understood after consideration of the behavior of the triglycinamide complex.

Nickel(II) Triglycinamide Anion. The rates of acid dissociation of $Ni(H_{-3}GGGa)^-$ were measured with the stopped flow, monitoring the loss of the yellow color at 410 nm. The reactions are first order in the concentration of the nickel complex. Variation of acetate buffer concentrations at constant pH showed no effect of the acetic acid concentration on the rate (Table III). The

Table III. Rate Constants for the $Ni(H_{-3}GGGa)^-$ Protonation[a]

Buffer	M	pH	k_{obsd}, sec^{-1} [b]
EDTA	0.010	6.20	$(2.40 \pm 0.05) \times 10^{-2}$
Malonate	0.025	5.90	$(6.3 \pm 0.3) \times 10^{-2}$
Acetate	0.050	4.60	1.09 ± 0.02
Acetate	0.075	4.90	0.56 ± 0.01
Acetate	0.075	4.60	1.07 ± 0.02
Acetate	0.075	4.30	2.04 ± 0.08
Acetate	0.100	4.60	1.08 ± 0.03
Formate	0.075	3.60	9.8 ± 0.2
Chloroacetate	0.100	2.80	$41. \pm 3$
Maleate	0.075	2.10	$96. \pm 7$

[a] Nickel triglycinamide: $2 \times 10^{-4}\ M$, 25°, 0.10 M $NaClO_4$. [b] Standard deviation from average of five runs.

dependence of k_{obsd} with hydrogen ion is shown in Figure 2. The reactions were not studied above pH 6.2 so the k_d value was not obtained. Omitting the k_d term, an expression for k_{obsd} can be written which is similar to that for nickel tetraglycine and is given in eq 6 where $K_H' = 263\ M^{-1}$ and $k_1' = 126\ sec^{-1}$.

$$k_{obsd} = \left(\frac{K_H'[H^+]}{1 + K_H'[H^+]}\right)k_1' \qquad (6)$$

Although the k_1, k_1', and K_H, K_H' terms are significantly different for the reactions of $Ni(H_{-3}GGGG)^{2-}$ and $Ni(H_{-3}GGGa)^-$, their products are similar with $K_H k_1 = 7.1 \times 10^4\ M^{-1}\ sec^{-1}$ for tetraglycine and $K_H' k_1' = 3.3 \times 10^4\ M^{-1}\ sec^{-1}$ for triglycinamide.

Nickel(II) Triglycinate Anion. Proton-transfer reactions of $Ni(H_{-2}GGG)^-$ were examined in detail in an earlier study.[5] Exposure of solutions of the nickel triglycinate complex to the atmosphere increased its dissociation rate when measured at pH 8–9. This effect was not due to reactions with O_2 but was a result of CO_2 uptake and catalysis of the dissociation reaction by HCO_3^-.

Hydrogen carbonate catalysis of the dissociation reaction was measured in a borate buffer (total borate = 0.16 M) at pH 8.0 ± 0.1. The results in Figure 3 show a first-order dependence in HCO_3^- concentration, where k_{obsd} (sec^{-1}) = 0.084 + 194$[HCO_3^-]$. These reactions are run in the presence of EDTA and the intercept agrees with earlier studies under similar conditions.

The rate constant for HCO_3^- is very much larger than would be expected simply from its acid strength (pK_a = 10.1). In fact, it is about four orders of magnitude more effective than expected from its acidity. A similar effect was found for $H_2PO_4^-$ which was about 50 times more effective than predicted from its pK_a value. Thus, acids which can simultaneously coordinate and donate a proton are very good catalysts for the nickel triglycinate dissociation reaction.

Reactions run at higher pH, when both HCO_3^- and CO_3^{2-} are present, show a CO_3^{2-} inhibition of the catalysis by HCO_3^-. The inhibiting effect could be attributed in part to hydroxide ion but fits best with changes in the CO_3^{2-} concentrations. The kinetic data are given in Table IV. It is proposed that the HCO_3^- and CO_3^{2-} effect takes place by the steps given in eq 7 and 8.

$$Ni(H_{-2}GGG)^- + HCO_3^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} Ni(H_{-2}GGG)H + CO_3^{2-} \qquad (7)$$

$$Ni(H_{-2}GGG)H \xrightarrow{k_2} Ni(H_{-1}GGG) \xrightarrow{EDTA} \text{products} \qquad (8)$$

(Another probable intermediate, $Ni(H_{-2}GGG)\cdot HCO_3^{2-}$, is omitted for simplicity.) Assuming a steady-state condition for $Ni(H_{-2}GGG)H$ and rapid reactions with EDTA gives eq 9. Since the reactions

$$\frac{-d[Ni(H_{-2}GGG)^-]}{dt} = \frac{k_1 k_2 [HCO_3^-][Ni(H_{-2}GGG)^-]}{k_{-1}[CO_3^{2-}] + k_2} \qquad (9)$$

were run under pseudo-first-order conditions k_{obsd} is given by eq 10. Therefore, plotting $[HCO_3^-]/k_{obsd}$

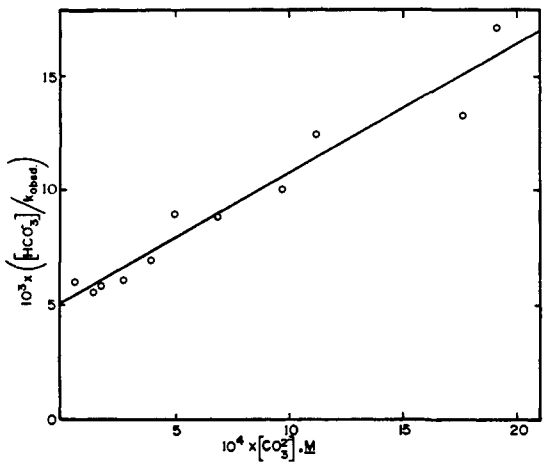


Figure 4. Carbonate inhibition of the $Ni(H_{-2}GGG)^-$ protonation reaction.

Table IV. Rate Constants for the HCO_3^- Catalyzed Protonation of Nickel Triglycine[a]

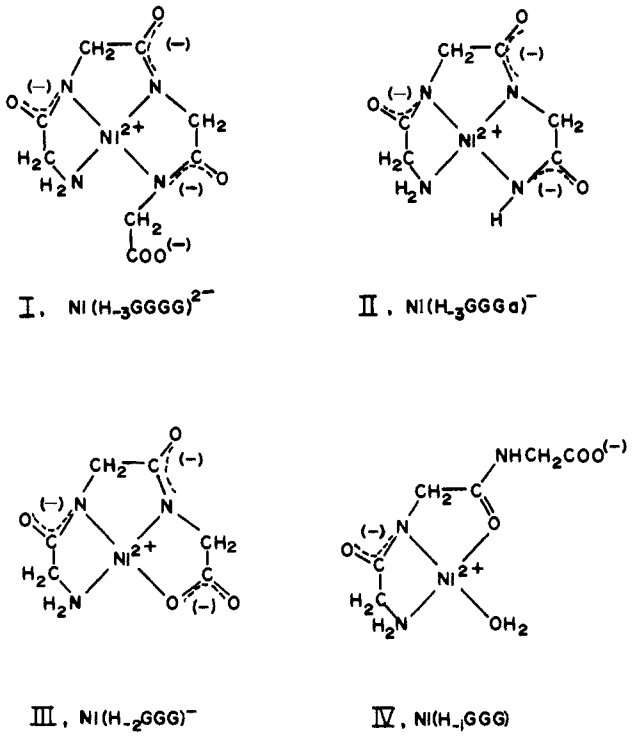
10^3-$[CO_3^{2-}]_T$	pH	$10^3 \cdot [HCO_3^-]$	$10^4 \cdot [CO_3^{2-}]$	k_{obsd}, sec^{-1} [b]
1.0	7.95[c]	0.99	0.05	0.278 ± 0.004
2.0	8.01[c]	1.99	0.10	0.47 ± 0.01
2.0	8.72[d]	1.94	0.60	0.322 ± 0.002
2.0	9.21[d]	1.83	1.74	0.313 ± 0.003
2.0	9.75[d]	1.50	4.98	0.168 ± 0.002
3.0	8.07[c]	2.98	0.20	0.67 ± 0.03
4.0	8.05[c]	3.97	0.30	0.87 ± 0.02
4.0	8.80[d]	3.86	1.43	0.69 ± 0.01
4.0	9.27[d]	3.60	3.95	0.516 ± 0.006
4.0	9.82[d]	2.88	11.20	0.232 ± 0.001
5.0	8.11[c]	4.96	0.40	1.05 ± 0.02
6.0	8.91[d]	5.73	2.74	0.94 ± 0.02
6.0	9.34[d]	5.32	6.85	0.602 ± 0.008
6.0	9.90[d]	4.09	19.12	0.238 ± 0.006
8.0	9.36[d]	7.03	9.70	0.70 ± 0.02
10.0	9.56[d]	8.24	17.61	0.62 ± 0.01

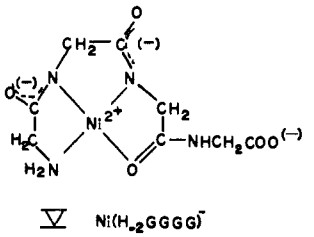
[a] $[Ni^{2+}] = 1.25 \times 10^{-4}$ *M*, [triglycine] = 2.5×10^{-4} *M*, initial pH 10, 25°, 0.16 *M* $NaClO_4$. [b] Standard deviation from average of at least five runs. [c] 0.16 *M* borate buffer (5×10^{-4} *M* EDTA). [d] 0.04 *M* borate buffer (5×10^{-4} *M* EDTA).

$$k_{obsd} = k_1k_2[HCO_3^-]/(k_{-1}[CO_3^{2-}] + k_2) \quad (10)$$

against $[CO_3^{2-}]$ gives $1/k_1$ as the intercept and k_{-1}/k_1k_2 as the slope. This plot, Figure 4, gives $k_1 = 196\ M^{-1}\ sec^{-1}$ and $k_{-1}/k_2 = 110\ M$.

General Mechanism for the Protonation of Metal Peptide Complexes. The preceding data and the results of earlier studies of the kinetics of proton transfer to nickel and copper triglycine complexes[5,10] pose several questions. Why should the triglycine complexes be subject to general-acid catalysis while $Ni(H_{-3}GGGG)^{2-}$ and $Ni(H_{-3}GGGa)^-$ are not? Why should CO_3^{2-} inhibit the $Ni(H_{-2}GGG)^-$ protonation reactions while the conjugate bases of other general acids (such as acetate ion) have no effect? What are the reasons for the different kinetic–pH profiles for $Ni(H_{-3}GGGG)^{2-}$ and $Ni(H_{-3}GGGa)^-$? Why should the "terminal" peptide nitrogen in nickel tetraglycine (structure I) and the amide nitrogen in nickel triglycinamide (structure II) be as slow or slower to react with acids than the peptide nitrogen in nickel triglycine (structure III)? In the latter case the carboxylate coordination must be broken before the necessary rearrangement can take place to give the protonation product, $NiH_{-1}GGG$ (structure IV). Thus, the coordinated carboxylate group might be expected to protect the triglycine peptide from rapid protonation while this is not the case for $Ni(H_{-3}GGGG)^{2-}$ and $Ni(H_{-3}GGGa)^-$. This was the basis for the not unreasonable mechanism proposed by Wilkins[6] in which $Ni(H_{-2}GGGG)^-$ (structure V) formed rapidly. However, as the data show, $Ni(H_{-2}GGGG)^-$ does not form rapidly.



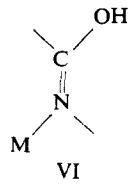


A general mechanism for the protonation of all the metal peptide and amide complexes is proposed and is given in eq 11 and 12 (charges are omitted, L is the peptide, H_{-n} corresponds to *n* metal–N(peptide or amide) bonds, HX is H_3O^+ or some weaker acid). The species written as $M(H_{-n}L)H$ indicates an intermediate protonated complex without cleavage of any metal–N (peptide or amide) bonds which we will term "outside" protonation. Crystal structure determination[15] of the protonated bis(glycylglycinato)cobaltate(III) complexes indicates that the proton adds to the O(peptide) (VI) rather than to the N(peptide). The proton addition increases the double bond character of the C–N bond and decreases that of the C–O bond; it lengthens the Co–N

$$MH_{-n}L + HX \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} M(H_{-n}L)H + X \quad (11)$$

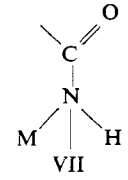
$$M(H_{-n}L)H \xrightarrow{k_2} M(H_{-n+1}L) \quad (12)$$

(15) M. T. Barnet, H. C. Freeman, D. A. Buckingham, I. Hsu, and D. van der Helm, *Chem. Commun.*, 367 (1970).



VI

bond and would make it easier to break. The alternate structure (VII) would be less stable but should



VII

weaken the M–N bond to a greater extent than VI and therefore may be important kinetically. The actual location of the proton in the metal–N(peptide) bond cleavage step is uncertain but it is presumed that any observable concentration of $M(H_{-n}L)H$ corresponds to VI.

Four kinetic situations observed in the present study can be shown to fit particular conditions of the general mechanism.

Case I. The concentration of $M(H_{-n}L)H$ is negligible and the steady-state approximation gives eq 13. If

$$\frac{-d[MH_{-n}L]}{dt} = \frac{k_1k_2[MH_{-n}L][HX]}{k_{-1}[X] + k_2} \qquad (13)$$

$k_{-1}[X]$ and k_2 are of the same order of magnitude, then catalysis by HX and inhibition by X would result as is observed with HCO_3^- and CO_3^{2-} in the protonation of $Ni(H_{-2}GGG)^-$ given in eq 9. In this case X must be a moderately strong base to make $k_{-1}[X]$ appreciable compared to k_2. Thus, CO_3^{2-} and OH^- have this effect with $Ni(H_{-2}GGG)^-$ but acetate ion does not.

Case II. $[M(H_{-n}L)H]$ is negligible and $k_2 \gg k_{-1}[X]$. This is the condition which fits the general-acid catalysis of $Ni(H_{-2}GGG)^-$ and $Cu(H_{-2}GGG)^-$ given in eq 1. The rate-determining step involves the proton transfer from HX to give the reactive $M(H_{-n}L)H$ species. The rearrangement of this species to $M(H_{-n+1}L)$ is fast by comparison to the proton-transfer step. Because we are accustomed to thinking of proton-transfer reactions to nitrogen or oxygen as very fast reactions, this situation will be discussed in detail after the other general cases are considered. An essential requirement in addition to slower proton transfer is that the protonated intermediate, in this case $M(H_{-2}GGG)H$, be relatively fast to break the M–N(peptide) bond.

Case III. $[M(H_{-n}L)H]$ is negligible and $k_2 \ll k_{-1}[X]$. This condition is equivalent to specific hydrogen-ion catalysis because eq 11 may now be treated as a rapid preequilibrium for the reaction in eq 12 and $k_1[HX]/k_{-1}[X] = K_1[H^+]$, where K_1 is the protonation constant for $M(H_{-n}L)H$. The reactions of $Ni(H_{-3}GGGG)^{2-}$ and of $Ni(H_{-3}GGGa)^-$ fit this condition over wide pH ranges as seen in Figures 1 and 2.

Case IV. $[M(H_{-n}L)H]$ is appreciable and $k_2 \ll k_{-1}[X]$. This is the case below pH 4 for nickel tetraglycine and below pH 2 for nickel triglycinamide and gives the kinetics in eq 5 and 6. If only one proton added rapidly, the k_{obsd} value would become constant at lower pH as all the initial complex would convert rapidly to $M(H_{-n}L)H$ before the rate-determining step in eq 12. The reoccurrence of a hydrogen-ion dependence at low pH with nickel tetraglycine can be attributed to the addition of more than one proton.

Another general case could occur but was not encountered in the present studies. If the concentration of $M(H_{-n}L)H$ was appreciable and if $k_2 \cong k_{-1}[X]$ then the reaction order would not be simple.[16]

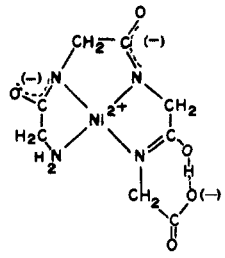
Ligand-Field Stabilization. The general mechanism accounts for most of the kinetic behavior observed provided that the changes in the relative values of k_2 and k_{-1} can be understood. As the metal–N(peptide or amide) bonds become more stable, the k_2 values become smaller and the acid reaction shifts from general-acid catalyzed to a specific hydrogen-ion dependence. The $N^{(-)}$–peptide group is a much stronger ligand-field donor than is the $-COO^{(-)}$ group and, in fact, is higher in the spectrochemical series than is the amine group.[17] This is reflected in the spectral properties of the nickel complexes: λ_{max} 412 nm (Δ' 69.3 kcal mol^{-1}) for $Ni(H_{-3}GGGG)^{2-}$, λ_{max} 410 nm (Δ' 69.7 kcal mol^{-1}) for $Ni(H_{-3}GGGa)^-$, and λ_{max} 430 (Δ' 66.4 kcal mol^{-1}) for $Ni(H_{-2}GGG)^-$. The crystal-field stabilization energy for a d^8 square-planar complex in a strong field is 24.6 Dq,[18] while the spectral transitions (Δ') are about half this value. Therefore the difference in Δ' of 3 kcal mol^{-1} between the tetraglycine and triglycine complexes would correspond to about 6-kcal difference in stability. Our postulate is that the $Ni(H_{-3}L)$ complexes are sufficiently stabilized by the fourth nickel–nitrogen bond that their "outside" protonated forms, $Ni(H_{-3}L)H$, are substantially slower to break the nickel–nitrogen bond. The ligand-field stabilization effect is great enough with nickel to overcome other factors, such as the absence of protective carboxylate coordination found with triglycine, or possible influence of $N^{(-)}$–peptide groups trans to one another. This is not the case with copper where the d^9 system gives only half the crystal-field stabilization energy. A comparison of metal–nitrogen bond lengths[7,19] for nickel(II) and copper(II) illustrates the effect; in general, Ni–NH_2 (2.11 Å) > Cu–NH_2 (2.00 Å), but in the $M(H_{-3}GGGG)^{2-}$ complex Ni–NH_2 (1.93 Å) < Cu–NH_2 (2.03 Å) and Ni–N(peptide) distances average 1.85 Å while Cu–N(peptide) distances average 1.93 Å.

The ligand-field stabilization effect with nickel also accounts for the fact that the conversion of $Ni(H_{-3}GGGG)^{2-}$ to $Ni(H_{-2}GGGG)^-$ is slower than the subsequent protonations to $Ni(H_{-1}GGGG)$ and to $Ni(GGGG)^+$. The same is true with $Ni(H_{-3}GGGa)^-$. The same type of effect is seen with $Ni(CN)_4^{2-}$ where the loss of the first CN^- is the rate-determining step.[20] The removal of the strong ligand-field donors is accompanied by a change from square-planar to octahedral coordination.

Differences in the "Outside" Protonation Constants. The K_H value is $10^{4.1}$ for $Ni(H_{-3}GGGG)H^-$ but K_H' is only $10^{2.4}$ for $Ni(H_{-3}GGGa)H$. This strongly suggests that the free carboxylate group helps to stabilize the

(16) R. P. Bell, "The Proton in Chemistry," Cornell University Press, Ithaca, N. Y., 1959, p 141.
(17) H. C. Freeman, personal communication.
(18) F. Basolo and R. G. Pearson, "Mechanisms of Inorganic Reactions," 2nd ed, Wiley, New York, N. Y., 1967, p 70.
(19) H. C. Freeman, *Advan. Protein Chem.*, 22, 354 (1966).
(20) G. B. Kolski and D. W. Margerum, *Inorg. Chem.*, 7, 2239 (1968).

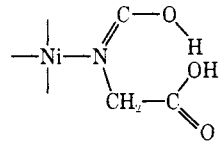
"outside" protonation of nickel tetraglycine as shown in structure VIII. Such a hydrogen-bonded structure



VIII $Ni(H_3GGGG)H^-$

accounts for the larger value of K_H and also accounts for the fact that k_1 (eq 5) is less than k_1' (eq 6), because the greater the H bonding to the COO^- group the less effective the proton is at causing the Ni–N(peptide) bond to weaken.

The "outside" protonation could take place at the other peptide sites as well although it would be more difficult to lengthen the metal–N(peptide) bond distance when the group is chelated on both sides. The kinetically important protonation would be only that which affects the reactive sites (the terminal peptide or amide in the tetraglycine or triglycinamide complexes). However, a second protonation to give the $Ni(H_{-3}GGGG)H_2$ would affect this site as shown in structure IX because with protons on both the peptide oxygen



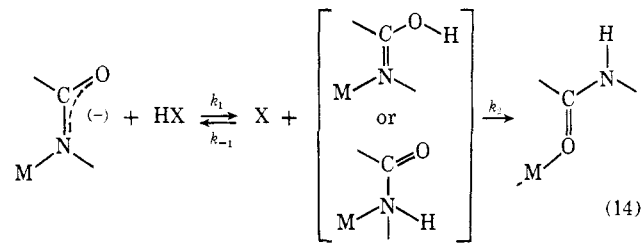
IX

and the carboxylate oxygen the nickel–N(peptide) bond would be expected to cleave about 20 times faster (ratio k_1'/k_1 in eq 5 and 6). This accounts for the reoccurrence of the hydrogen-ion dependence at low pH with the nickel tetraglycine complex.

Mechanism of Proton Transfer with Nickel Triglycine. An earlier paper[5] discussed alternate mechanisms (A and B) for the reaction of acids with $Ni(H_{-2}GGG)^-$. The general mechanism proposed in the present work encompasses both of the earlier ones. As has been indicated $[M(H_{-n}L)HX]$ can be considered as a reaction intermediate as well as $[M(H_{-n}L)H]$ and this is consistent with mechanism A. On the other hand with $Ni(H_{-2}GGG)^-$ the rate-determining step is postulated to be the proton-transfer step and not the nickel–N(peptide) bond cleavage and this is consistent with mechanism B (however, mechanism A proved to be valid for tetraglycine and triglycinamide). A question which remains to be discussed is how could the proton-transfer step be as slow as it is in the reactions of HX with $NiH_{-2}GGG^-$? For example, with acetic acid $k_{HX} = 9.7 \times 10^2\ M^{-1}\ sec^{-1}$ at 25°. There are several factors which could explain this. (1) The pK_a value for $Ni(H_{-2}GGG)H$ is small and can be estimated to be ~2.4 from the triglycinamide constant. Therefore, the ΔpK_a for transfer of a proton from HOAc ($pK_a = 4.5$) is approximately −2 units. This will reduce the proton-transfer rate by at least a factor of 10^2 below that of diffusion-controlled reactions with HOAc.[21] (2) The proton transfer to the peptide group (structure III) will not be effective in causing dissociation unless the carboxylate group is not bonded to the metal. Such a preequilibrium would introduce an unfavorable factor (estimated to be about 10^2) for the proton-transfer reaction. (3) To accomplish the proton transfer some electronic and structural rearrangement is necessary. This is the case whether the proton goes to the peptide-oxygen or to the peptide-nitrogen (both prior to Ni–N (peptide) bond cleavage). As is the case with carbon acids[22] this will lead to slower reactions. (4) It could be argued that the $Ni(H_{-2}GGG)H$ structure might parallel the hydrogen-bonding arrangement in structure VIII. If this were so the ΔpK_a differences would be less negative, but slow proton transfer now would be anticipated due to the hydrogen bonding itself.[21] It would appear that more than one of the above factors are coming into play in controlling the proton-transfer rates of HX with $Ni(H_{-2}GGG)^-$ and with $Cu(H_{-2}GGG)^-$.

Conclusion

The general mechanism of protonation of nickel peptide complexes accounts for four types of observed kinetic behavior (cases I–IV). Changes in the relative values of k_2 and k_{-1} for these cases are consistent with changes in the ligand-field stabilization of the nickel complex and the basicity of X. Other metal peptide complexes are predicted to follow the same general mechanism but may not have as much variation in the ratios of k_2 and k_{-1} as is found for the nickel complexes. The more labile metal ions such as copper(II) and cobalt(II) will tend toward larger k_2 values and the general-acid catalysis behavior as demonstrated by $Cu(H_{-2}GGG)^-$. The more sluggish palladium(II) peptide complexes will tend toward smaller k_2 values and specific hydrogen-ion catalysis. Observation of the "outside" protonated species will depend largely on the k_2 values and will be easier with the more sluggish complexes. The rate of formation of the kinetically reactive "outside" protonated metal peptide species is much less than the diffusion-controlled rate. The kinetically reactive protonated species is the one which leads to metal–N(peptide) bond cleavage. It is not known if this species is protonated at the peptide–nitrogen or at the peptide–oxygen before the metal–N(peptide) bond breaks (eq 14). The fact that the proton-transfer step



(14)

itself is sluggish suggests that the proton may go to the nitrogen. After breaking the metal bond the proton is found on the nitrogen.

Acknowledgment. This investigation was supported by Public Health Service Research Grant No. GM 12152 from the National Institute of General Medical Sciences, National Institutes of Health, and by a Rockefeller Foundation Grant to E. B. P.

(21) M. Eigen, *Angew. Chem., Int. Ed. Engl.*, 3, 1 (1964).

(22) Reference 16, p 103.