D. The species with composition C is similar to the Cannon and Gardiner ion in which $Fe(H_2O)_6^{2+}$ is coordinated to the iminodiacetate fragment of carboxylate bound $Co(NH_3)_5NTA$.[23] The ESR spectrum of the reacting mixture (Figure 4) reveals the overlapping spectra of the VO^{2+} resonance of C with the Mn(II) resonance of D. As in the case of the MnNTA system alone, some competitive ligand oxidation or solvent oxidation also takes place. The final spectrum reveals a small amount of unconsumed V(IV) due to the thermal side reactions of the Mn(III) center in the binuclear complex. In the presence of O_2 there is a catalytic production of a peroxo species which is coordinated to the V(V) center. The induced formation of peroxo complexes is not observed for the oxidation of VO^{2+} amino carboxylate–Mn(III) aminocarboxylate monomeric complexes. Free radicals produced by competitive ligand oxidation at the Mn(III) center may be involved and the system is under investigation.[24] A peroxo complex is readily formed directly from $VO(DTPA)^{3-}$ and H_2O_2. It has a spectrum similar to the one reported by Tanaka et al. for $VO(O_2)(NTA)$.[25] No long-lived intermediates which might be attributed to a hydroxy or oxo bridged Mn(III)–V(IV) complex on the same $DTPA^{5-}$ ligand could be determined from the visible spectrum. Since the proximity effect should favor a higher concentration of a bridged species, these results suggest an outer-sphere mechanism for Mn(III)–V(IV) systems in aminocarboxylate environments.

Acknowledgment. We wish to thank the Research Corporation for funds which allowed us to purchanse the stopped-flow unit.

Registry No. $Mn(EDTA)(H_2O)^-$, 12318-32-0; $VO(EDTA)^{2-}$, 12276-03-8; $VO(HEDTA)^-$, 20648-99-1; $VO(NTA)(H_2O)^-$, 12347-63-6; $Mn(CH_3CO_2)_3$, 993-02-2; $VO(DTPA)^{3-}$, 65605-14-3; $Mn(HEDTA)(H_2O)^-$, 65545-48-4; $VO(H_2O)_5^{2+}$, 15391-95-4; chloroacetate, 14526-03-5.

References and Notes

(1) L. E. Bennet, *Prog. Inorg. Chem.*, **18**, 1 (1973); M. Goldberg and I. Pecht, *Biochemistry*, **15**, 4197 (1976).
(2) J. H. Swinehart, W. R. Biggs, D. J. Halko, and N. C. Schroeder, *Biol. Bull. (Woods Hole, Mass.)*, **146**, 302 (1974).
(3) K. Kustin, D. S. Levine, G. C. McLeod, and W. A. Curby, *Biol. Bull. (Woods Hole, Mass.)*, **150**, 426 (1976).
(4) G. M. Woltermann, R. A. Scott, and G. P. Haight, Jr., *J. Am. Chem. Soc.*, **96**, 7569 (1974).
(5) F. J. Kristine, D. R. Gard, and R. E. Shepherd, *J. Chem. Soc., Chem. Commun.*, 994 (1976).
(6) F. J. Kristine and R. E. Shepherd, submitted for publication.
(7) D. J. Boone, R. E. Hamm, and J. P. Hunt, *Inorg. Chem.*, **11**, 1060 (1972).
(8) R. G. Wilkins, "The Study of Kinetics and Mechanism of Reactions of Transition Metal Complexes", Allyn and Bacon, Boston, Mass., 1974, pp 262–263.
(9) A. Zwickel and H. Taube, *J. Am. Chem. Soc.*, **81**, 1288 (1959); J. H. Espenson and O. J. Parker, *ibid.*, **90**, 3689 (1968); A. Adin and A. G. Sykes, *J. Chem. Soc. A.*, 351 (1968).
(10) G. Davies, *Coord. Chem. Rev.*, **4**, 199 (1969).
(11) W. P. Griffith and T. D. Wickens, *J. Chem. Soc. A*, 400 (1968); F. A. Cotton and G. Wilkinson, "Advanced Inorganic Chemistry", Interscience Publishers, New York, N.Y., 1972.
(12) W. R. Scheidt, R. Countryman, and J. L. Hoard, *J. Am. Chem. Soc.*, **93**, 3878 (1971).
(13) W. R. Scheidt, C. Tsai, and J. L. Hoard, *J. Am. Chem. Soc.*, **93**, 3867 (1971).
(14) G. Brauer, "Handbook of Preparative Inorganic Chemistry", Vol. II, Academic Press, New York, N.Y., 1965, p 1470.
(15) R. E. Hamm and M. A. Suwyn, *Inorg. Chem.*, **6**, 139 (1967).
(16) Y. Yoshino, A. Ouchi, Y. Tsunoda, and M. Kojima, *Can. J. Chem.*, **40**, 775 (1962).
(17) W. M. Latimer, "Oxidation Potentials", Prentice-Hall, Englewood Cliffs, N.J., 1952.
(18) A. E. Martell, Ed., *Chem. Soc., Spec. Publ.*, **No. 17** (1964).
(19) R. E. Hamm and J. C. Templeton, *Inorg. Chem.*, **12**, 755 (1973); ref 10.
(20) T. E. Jones and R. E. Hamm, *Inorg. Chem.*, **13**, 1940 (1974).
(21) H. Fischer, G. Tom, and H. Taube, *J. Am. Chem. Soc.*, **98**, 5512 (1976).
(22) C. R. Johnson and R. E. Shepherd, *Bioinorg. Chem.*, in press; K. Wuthrich and R. E. Connick, *Inorg. Chem.*, **7**, 1377 (1968); K. Wuthrich, *Helv. Chim. Acta*, **48**, 779 (1965).
(23) R. D. Cannon and J. Gardiner, *Inorg. Chem.*, **13**, 390 (1974); *J. Am. Chem. Soc.*, **92**, 3800 (1970).
(24) J. Nelson, work in progress.
(25) S. Funahashi, K. Haraguchi, and M. Tanaka, *Inorg. Chem.*, **16**, 1349 (1977).
(26) M. M. Taqui Khan and A. E. Martell, *J. Am. Chem. Soc.*, **90**, 6011 (1968).
(27) This assumes that $Mn(HEDTA)A^-$ is unreactive toward $VO(EDTA)^{2-}$.
(28) L. W. Amos and D. T. Sawyer, *Inorg. Chem.*, **11**, 2692 (1972).
(29) M. Suwyn and R. E. Hamm, *Inorg. Chem.*, **6**, 2150 (1967).

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Trivalent Nickel Catalysis of the Autoxidation of Nickel(II) Tetraglycine

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Received July 6, 1977

Molecular oxygen reacts with nickel(II)–peptide complexes in aqueous solution by a facile autocatalytic process in which nickel(III) intermediates play a major role. With tetraglycine the formation of $Ni^{III}(H_{-3}G_4)^-$ initiates the reaction which results in the formation of carbon dioxide, triglycyl-*N*-(hydroxymethyl)amide, and glycinamide as the main products. The *N*-(hydroxymethyl)amide can further hydrolyze to produce triglycinamide and formaldehyde. When a free carboxylate group is adjacent to a deprotonated-peptide nitrogen (as in the tetraglycine complex), decarboxylation occurs and the autoxidation reactions are more rapid than for other peptide complexes. Initiation of the reaction by electrolytically prepared nickel(III)–peptide complexes is used to resolve the reaction order, which at low concentrations is first order in $[Ni^{III}G_4]_{total}$, in $[Ni^{III}(H_{-3}G_4)^-]$, and in $[O_2]$ but approaches zero order for each at higher concentrations.

Introduction

Autoxidation of *N*-alkylamides and *N*,*N*-dialkylamides is induced by either thermal[1-3] or photochemical[4] means to produce hydroperoxide intermediates by radical chain mechanisms. Transition-metal salts (e.g., Co(II), Fe(II), and Cu(I)) are often used to catalyze such O_2 reactions.[5] Ionizing radiation induces similar oxidation of oligopeptides.[6] These processes are slow, and the oxidation of oligopeptides is not self-sustaining in the absence of radiation.

Our laboratory has found that some copper(II) peptides[7,8] and nickel(II) peptides[9,10] react with oxygen in an autocatalytic manner with oxidation of the ligand. We have found recently that the trivalent oxidation states of copper[11,12] and nickel[13,14] are stabilized by deprotonated-peptide ligands. In the present

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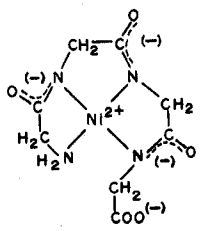
work an extensive study of the reaction of nickel(II) tetraglycine (I) with oxygen identifies the products and shows that



I $Ni^{II}(H_{-3}G_4)^{2-}$

a trivalent nickel peptide complex is the reactive intermediate. The accessibility and stability of the trivalent nickel peptide complexes can account for the autocatalytic nature and the initiation of the reaction.

Experimental Section

The peptides and peptide amides were obtained from Biosynthetika and Vega-Fox Chemical Co. Solutions of the nickel–peptide complexes were prepared by the reaction of 5–10% excess ligand with $Ni(ClO_4)_2$ solutions, except for the initial rate studies where no excess ligand was used. A stock solution of $Ni(ClO_4)_2$ was prepared with nickelous carbonate and perchloric acid and standardized by EDTA titrations. The pH was adjusted slowly with NaOH in order to avoid formation of $Ni(OH)_2$. The ionic strength was adjusted with $NaClO_4$ (prepared from Na_2CO_3 and $HClO_4$). Di-*tert*-butyl nitroxide was obtained in purified form from N. Kornblum, Purdue University. Solutions of reagent grade benzoyl peroxide were prepared with ethanol.

The dissolved oxygen concentration of aqueous solutions was monitored with a Yellow Springs Instrument Co. Model 53 biological oxygen monitor thermostated at 25.0 °C. The pH of nickel(II)–tetraglycine solutions was adjusted under nitrogen. For the initial rate studies these solutions were mixed with an equal volume of borate buffer solution at the same pH in the O_2 monitor, and the dissolved oxygen concentration was then adjusted. The reaction was started by injection of 0.10 mL of initiator. Visible absorption measurements were obtained using Cary 14 and Cary 16 spectrometers.

Electrochemical oxidation to form the trivalent nickel–peptide complexes was performed with a flow system in which the electrode arrangement included a graphite powder working electrode packed in a porous glass column, wrapped externally with a Pt wire electrode.[15] In general the fully deprotonated form of the divalent metal complex was oxidized at a potential 200 mV above its value of $E°$ [13,14] and at a flow rate of 0.4 mL min^{-1}.

Product Analysis. Peptides and modified peptide products were separated (eluting with 0.2 M sodium citrate at pH 3.2) and analyzed on a 15-cm column packed with Beckman PA35 cation-exchange resin using a Beckman Model 120B-121 amino acid analyzer. The concentration of peptide samples was between 10^{-4} and 10^{-5} M. Preparative scale liquid chromatography was performed on a 24-cm stainless steel column (0.25 in. o.d.) packed with Bio-Rad Laboratories Aminex-A7 cation-exchange resin. A Varian Model 4100 liquid chromatograph was used with a Waters Model R401 differential refractometer detector in combination with a Varian 635 UV–visible variable wavelength spectrophotometer. A 0.1- or a 1.0-mL loop injector was used. Peptides were eluted at 80 mL h^{-1} with a 0.2 M ammonium formate buffer (pH 2.4), which subsequently was removed by freeze-drying using a VirTis manifold in conjunction with a Neslab Model CC60 vapor freezing trap. The main peptide fragment and peptide standards were analyzed by ^{13}C NMR using a Varian CFT-20 or a Varian XL-100 instrument.

Neutral alumina sheets (Eastman Kodak) were used in the TLC separation and identification of the 2,4-DNP derivative of CH_2O, which was eluted with a hexane–ethyl acetate (9:1) mixture.[16] Quantitative colorimetric analysis of CH_2O also was performed[17] before and after mild hydrolysis (2 M HCl, 110 °C, 12 h) of the reaction solution. No interference was observed due to excess peptide or metal ion. Carbon dioxide was detected using an Aerograph-202 gas chromatograph, equipped with a thermal conductivity detector and a 10 ft × $^1/_8$ in. o.d. stainless steel column packed with Waters Poropak Q resin. Quantitative analysis was obtained using a Radiometer E5037 P_{CO_2} electrode, calibrated between 2 × 10^{-3} and 5 × 10^{-5} M CO_2 using Na_2CO_3 and $HClO_4$.

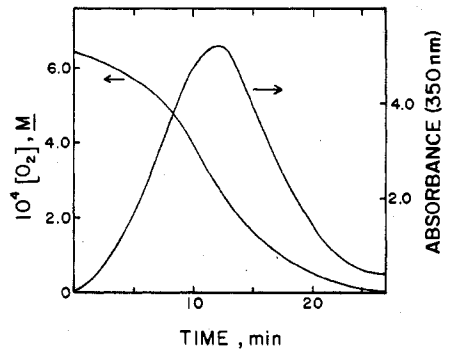


Figure 1. Molecular oxygen reaction with nickel(II) tetraglycine in aqueous solution. $[Ni^{II}G_4]_T$ = 2 × 10^{-3} M, pH 8.2, 25 °C. The right-hand ordinate is the absorbance in a 1-cm cell at 350 nm of the nickel(III)–peptide intermediate.

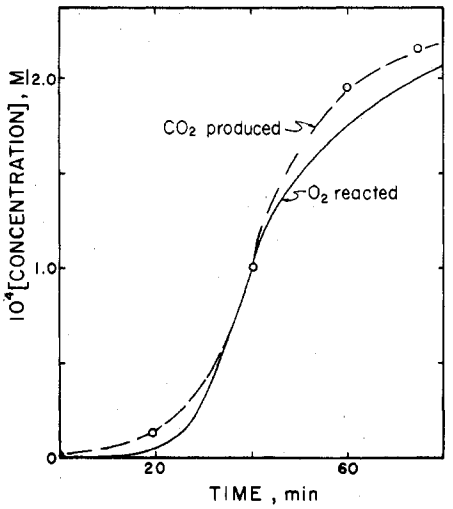


Figure 2. O_2 reacted (—) and CO_2 produced (O) for the autoxidation of nickel(II) tetraglycine. $[Ni^{II}G_4]_T$ = 1.5 × 10^{-3} M, pH 8.5.

The oxidizing intermediate was monitored by the quantitative reaction with acidic iodide solutions or by spectral means at 350 nm. The molar absorptivity was determined at 327 nm by monitoring changes of the absorbance in a 1-cm cell upon additions of aliquots of standardized hydroquinone solutions. One equivalent of hydroquinone reacts with 2 equiv of the intermediate which was identified as trivalent nickel tetraglycine.

Results and Discussion

Nickel(II) tetraglycine reacts with molecular oxygen to produce small amounts of nickel(III) peptide with eventual oxidation of the ligand. Rapid oxygen uptake occurs after a short induction period as shown in Figures 1 and 2. The S-shaped curves are indicative of an autocatalytic reaction. (The reaction in Figure 2 is slower because the H^+ and $Ni^{II}G_4$ concentrations are lower than the concentrations used in Figure 1.) Similar oxygen uptake is found for Ni(II) complexes of other peptide ligands containing four or more potential nitrogen donor sites. The tetrapeptides and higher order peptides which can bind the metal through an amine nitrogen and three deprotonated-peptide nitrogens bound to the metal are reactive, whereas the doubly deprotonated tripeptides with a bound carboxylate group in place of a deprotonated-peptide nitrogen appear to be unreactive. The tripeptide complex of glycylglycyl-L-histidine (GGhis) does react with oxygen, but the doubly deprotonated form of the complex has a bound imidazole group to provide the fourth bound nitrogen. Of the complexes that do react with oxygen, those with a free carboxylate group adjacent to a deprotonated-peptide nitrogen have more facile O_2 uptake. Nickel(II) complexes of G_4, tetra-L-alanine, and GGhis react rapidly with O_2 and produce

Table I. Maximum Rate of O_2 Uptake for the Autoxidation of Nickel(II) Tetraglycine as a Function of pH[a]

pH	$10^6 \times$ rate$_{max}$, M s^{-1}	pH	$10^6 \times$ rate$_{max}$, M s^{-1}
7.5	0.5	8.15	1.1
7.9	1.3	8.25	1.0
8.0	1.5	8.6	0.6

[a] $[Ni^{II}G_4]_T = 1.0 \times 10^{-2}$ M, $[O_2]_i = 1.2 \times 10^{-3}$ M, 25 °C.

Table II. Comparison of the Half-Lives of Acidity-Jump and Preequilibrated O_2 Uptake Reactions[a]

pH	$10^{-3}t_{1/2}$(equil), s	$10^{-3}t_{1/2}$(jump),[b] s
8.0	3.7	0.8
8.1	4.9	0.9
8.3	5.5	1.0
8.4	6.6	1.1
8.5	6.8	1.9

[a] $[Ni^{II}G_4]_T = 2.0 \times 10^{-2}$ M, $[O_2]_i = 6.3 \times 10^{-4}$ M, $[borate]_T = 4.0 \times 10^{-1}$ M, 25 °C, $\mu = 0.16$. $t_{1/2}$ is time at which $[O_2] = 0.5[O_2]_i$. [b] Initial pH 11.

CO_2. On the other hand nickel(II) pentaglycine (G_5) reacts approximately 4 times slower than nickel tetraglycine under similar conditions and nickel tetraglycinamide is about a factor of 10 slower even at 37 °C. Neither of these two reactions produces CO_2 during O_2 uptake.

The Reactive Nickel(II)–Tetraglycine Species. Nickel(II) tetraglycine can exist in solution in a number of forms, which depending upon the pH can include NiG_4^+, $Ni(H_{-2}G_4)^-$, and $Ni(H_{-3}G_4)^{2-}$, but $Ni(H_{-1}G_4)$ is not present in appreciable concentrations. The pK values for equilibrium constants[18] are 24.22 for $[Ni(H_{-3}G_4)^{2-}][H^+]^3/[NiG_4^+]$ and 8.1 for $[Ni(H_{-3}G_4)^{2-}][H^+]/[Ni(H_{-2}G_4)^-]$. The total concentration of the divalent complex is defined as $[Ni^{II}G_4]_T$. The reactivity of $[Ni^{II}G_4]_T$ with oxygen as a function of pH is given in Table I. The maximum rate refers to the steepest part of the curve for reactions where a limited amount of dissolved oxygen is consumed as shown in Figure 1. The reaction does not occur below pH 7; the rate increases up to a pH value of 8 and then decreases at high pH values. At pH 8 the peptide complex is present as a mixture of $Ni(H_{-3}G_4)^{2-}$, $Ni(H_{-2}G_4)^-$, and NiG_4^+. The pH dependence can be explained in terms of the triply deprotonated species in combination with a proton dependence or in terms of the doubly deprotonated complex. The two possibilities are differentiated by taking advantage of the novel feature that the rate of the O_2 uptake reaction can be made to be greater than the rate of H^+ reaction with $Ni(H_{-3}G_4)^{2-}$. The transition from $Ni(H_{-3}G_4)^{2-}$ to $Ni(H_{-2}G_4)^-$ is relatively slow due to the change in the geometry from square planar to octahedral. At pH 8 the half-life of the transition is 12.5 min.[19] If the $Ni(H_{-3}G_4)^{2-}$ complex is formed at a high pH and an acidity jump is used to lower the pH, then a substantially higher concentration of the triply deprotonated species is present compared to a Ni(II) solution which is preequilibrated at the same final pH. In the acidity-jump experiments, solutions containing $Ni(H_{-3}G_4)^{2-}$, O_2, and borate buffer initially at pH 11 are rapidly mixed with sufficient $HClO_4$ to give pH 8.0–8.5 (Table II). In the preequilibrated study O_2 is added after allowing the NiG_4 solutions to reach equilibrium (>1 h) at pH 8.0–8.5. Table II lists the final pH values and compares the half-lives of oxygen uptake for the preequilibrated and the acidity-jump experiment. The data indicate that the O_2 uptake occurs approximately 5 times faster under the acidity-jump conditions than under conditions of preequilibration. Both reactions are autocatalytic. Hence the rate appears to be dependent upon $[Ni^{II}(H_{-3}G_4)^{2-}]$ and upon $[H^+]$ rather than upon $[Ni^{II}(H_{-2}G_4)^-]$.

Table III. Methylene ^{13}C NMR Chemical Shifts (ppm vs. Me_4Si)[a]

Compound	pH	δ(amine)	δ(middle)	δ(carboxylate)	δ(other)
G_3a	1.6	42.2 (1)	44.1 (1)	43.7 (1)[b]	
G_4	1.8	42.4 (1)	44.1 (2)	42.8 (1)	
G_4	4.2	42.4 (1)	44.3 (2)	44.7 (1)	
G_3aCH_2OH[c]	4.4	42.7 (1)	44.5 (2)		64.9 (1)

[a] Aqueous solutions. Relative intensity is given in parentheses. [b] Methylene group on the amide end of the peptide amide. [c] G_3aCH_2OH fraction from preparative liquid chromatography.

Identification of Reaction Products. Analytical and preparative liquid chromatography have been used for the identification of the peptide fragments resulting from oxidative cleavage of tetraglycine. The cation-exchange separation of peptides after depletion of a millimolar solution of oxygen for the autoxidation of a 5×10^{-3} M solution of $[Ni^{II}G_4]_T$ at pH 8.0 shows three main peaks. The separation was performed on an amino acid analyzer modified for peptide analysis and only ninhydrin active products are detected. Standard elution times were measured for glycine and the glycyl (di through penta) peptides, along with the corresponding amides. The first and third eluting species were identified as G_4 and Ga, respectively. The second peak was the major peak. It did not correspond to the elution time of any of our standards, and the G_3a peak appeared if the oxidized solutions were allowed to stand for long periods of time at room temperature before analysis. After hydrolysis of these solutions (2 M HCl, 110 °C, 12 h) there was complete conversion to G_3a. The hydrolyzed solutions also were analyzed for formaldehyde and the concentration found corresponds to the amount of G_3a. Hence, peak 2 is triglycyl-*N*-(hydroxymethyl)amide (G_3aCH_2OH).

Continuous bubbling of O_2 through a concentrated solution of nickel tetraglycine was used to build up the concentration of products prior to isolation by preparative cation-exchange chromatography. In addition to the unoxidized G_4 (identified by its elution time and ^1H NMR) small amounts of G_3a, G_2a, Ga, and NH_3 are formed as well as G_3aCH_2OH. Isolation and concentration of the latter species permitted ^{13}C NMR analysis to be performed. Table III lists the chemical shifts of the methylene carbons for G_4, G_3a, and G_3aCH_2OH in acid solution. A pH variation allowed the assignment of peaks for G_4 and G_3a.[20] A comparison of the NMR data for G_4 and the G_3aCH_2OH fraction shows that the carboxylate end of the peptide has been altered. The signal of 64.9 ppm is in the region expected for the carbon of a primary alcohol[21] and corresponds to the chemical shift predicted on the basis of replacing the carboxylate group of G_4 with a hydroxy group.[22] The NMR identification of the product as triglycyl-*N*-(hydroxymethyl)amide (G_3aCH_2OH) is consistent with the production of G_3a and CH_2O upon hydrolysis. The larger molecule of G_3aCH_2OH is also expected to elute before G_3a.[23]

N-(Hydroxymethyl)amides have been prepared by the reaction of CH_2O with acylamides in aqueous solution and they hydrolyze in acid solution.[24,25] We have not found previous reports of *N*-(hydroxymethyl)amide derivatives of amino acids or peptides.

Carbon dioxide is also a major product of the O_2 uptake reaction for nickel(II) tetraglycine. It was detected by gas chromatography and quantitatively analyzed with a gas-sensing electrode. Since the CO_2 is produced simultaneously with the O_2 uptake as shown in Figure 2, the CO_2 is formed from an initial oxidation and is not the result of the oxidation of peptide fragments in a later stage of the reaction. The oxidative decarboxylation of tetraglycine forms triglycyl-*N*-(hydroxymethyl)amide.

Figure 3 shows the consumption of G_4 and the production of peptide products during continuous oxygenation of a 0.05

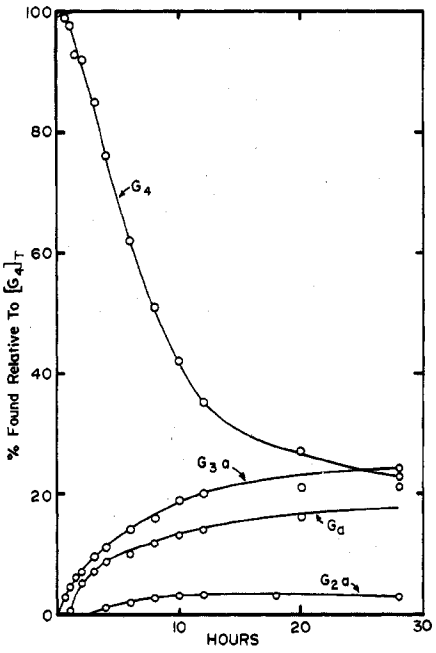


Figure 3. Peptide products formed and tetraglycine reacted during continuous O_2 saturation of a 0.05 M nickel(II) tetraglycine solution at pH 8.0.

M nickel–tetraglycine solution. Triglycyl-*N*-(hydroxymethyl)amide is hydrolyzed to G_3a. Even after 28 h G_4 is not completely depleted. There is an expected decrease in the rate of ligand oxidation with decreased G_4 and the production of CO_2 reduces the pH of the solution which will slow down the O_2 uptake reaction. However, even with pH adjustments depletion of G_4 does not occur after continuous oxygenation over long periods of time suggesting that some products inhibit the reaction.

Identification of the Oxidizing Intermediate. The intermediate species followed at 350 nm as shown in Figure 1 is an oxidizing agent. The maximum formation of the 350-nm intermediate coincides with the maximum rate of O_2 uptake. This corrects an earlier plot[1] in which the absorbance at 350 nm appeared to precede the oxidizing intermediate. We now know this intermediate is primarily a nickel(III) peptide rather than a peroxide. In the present work identical solutions were used to follow simultaneously the O_2 uptake, the absorbance, and the concentration of oxidizing agent formed. This avoids the irreproducibility of the induction period for these reactions.

Preparative cation-exchange separation of an oxidized solution of nickel tetraglycine elutes the oxidizing species in the solvent front. Chemical analysis of this fraction indicates that the metal ion is still present, although such a separation normally retards all of the divalent metal. Mild acid hydrolysis of this fraction followed by cation-exchange separation shows that both tetraglycine and triglycinamide are present.

The spectrum of the intermediate shows an absorption shoulder at 245 nm and a peak at 327 nm. The molar absorptivity at 327 nm was determined by quantitative reactions with hydroquinone and calculated to be 5600 ± 200 M^{-1} cm^{-1}. The rates of acid decomposition of the intermediate are much slower than those of nickel(II) tetraglycine or nickel(II) triglycinamide.[19] The spectral properties, the oxidizing power, the sluggish decomposition kinetics, and the decomposition products of this intermediate species indicate that it is the trivalent nickel complex of tetraglycine.

Properties of $Ni^{III}(H_{-3}G_4)^-$. Studies in our laboratory have shown that the fully deprotonated peptide complexes of trivalent nickel are attainable in aqueous solution by mild electrolytic or chemical oxidation.[14] The electrode potential of the $[Ni^{III,II}(H_{-3}G_4)]^{-,2-}$ couple has a value of 0.79 V (NHE). The Ni(III) complex has a band at 327 nm with a molar absorptivity of 5420 ± 200 M^{-1} cm^{-1} and a second, more intense shoulder near 250 nm. The complex is slightly more stable in acid than in base. Even in 1.0 M $HClO_4$ the observed first-order rate constant is only 7.1×10^{-3} s^{-1}. Unlike the trivalent copper tetraglycine complex,[7,8,26] the decomposition of the nickel complex is unaffected by light. In the decomposition half the peptide is oxidized and half the peptide is recovered intact. The products of the decomposition, formed in the presence or absence of oxygen, are the same as those found in the O_2 uptake reaction of nickel(II) tetraglycine and include CO_2, formaldehyde, triglycinamide, and glycinamide. However, in the $Ni^{III}(H_{-3}G_4)^-$ decomposition slightly more glycinamide than triglycinamide is formed in contrast to the autoxidation experiments. The trivalent complex decomposes in acid or neutral solution to give ninhydrin-analyzed peptide products which correspond to the amount of oxidized tetraglycine. However, about 25% of the ninhydrin activity is lost in the base decomposition.

Table IV. Stoichiometry for the Reaction of Nickel(II) Tetraglycine and Oxygen[a]

$10^4[Ni^{II}G_4]_i$, M	$10^4[O_2]$, M	$\Delta G_4/\Delta O_2$	$\Delta G_3a/\Delta G_4$	$\Delta Ga/\Delta G_4$
2.9	1.24	1.5	0.35	0.13
3.3	1.86	1.5	0.41	0.16
6.0	4.31	1.5	0.55	0.14
7.5	6.22	1.3	0.59	0.15

[a] pH 8–8.4, 37 °C. O_2 uptake allowed to run to completion before acid quench to inhibit further oxidation.

Reaction Stoichiometry for the Autoxidation of Nickel(II) Tetraglycine. The reaction of nickel(II) tetraglycine with oxygen indicates that one CO_2 is produced for each O_2 reacted. The ratio for formaldehyde formed to O_2 reacted is approximately 0.9. Table IV lists data compiled on the peptide products formed during O_2 uptake. The distribution of products and the formation of CO_2 indicate that the main peptide oxidation occurs at the carboxylate end of the peptide. The formation of glycinamide (Ga) must be due to further oxidation of triglycyl-*N*-(hydroxymethyl)amide because the total amount of G_3aCH_2OH and Ga corresponds to the total CO_2 formed. Separate experiments showed that in the decomposition of $Ni^{III}(H_{-3}G_3a)$ 50% of the peptide is oxidized to give glycinamide and 50% G_3a is recovered. On the basis of the formation of CO_2, CH_2O, and G_3a the expected stoichiometry is expressed by eq 1. However, the observed

$$2G_4 + O_2 \rightarrow 2G_3a + 2CH_2O + 2CO_2 \quad (1)$$

stoichiometry is given by eq 2, where nn stands for non-

$$3G_4 + 2O_2 \rightarrow 1.5G_3a + 0.5Ga + 2CH_2O + 2CO_2 + 1.0nn \quad (2)$$

ninhydrin-active products. It is apparent that some oxidation of the products and tetraglycine occurs which does not produce CO_2 and CH_2O. The non-ninhydrin-active product may be due to the reaction of formaldehyde with the amine function of the products or due to the oxidation of the amine group by reactive intermediates. Small amounts of other aldehydes, in addition to formaldehyde, have been detected after O_2 uptake.

Activation and Inhibition of the O_2 Uptake Reaction. The reaction is very sensitive to a number of activators and inhibitors. The addition of 1×10^{-2} M methyl methacrylate (99% pure), which is a radical scavenger,[27] lengthens the induction period by a factor of 4. Excess G_4 and high concentrations of borate buffer also inhibit the reaction. At 0.4 M borate the O_2 reaction with nickel tetraglycine slows down by a factor of 5. The addition of trace concentrations of a reducing agent (e.g., 10^{-5}–10^{-7} M di-*tert*-butyl nitroxide)

lengthens the induction period or, if added after the induction period, completely stops the O_2 uptake. The addition of H_2O_2 at 5 mol % or more of the total nickel complex also inhibits the reaction. The reducing agents react with the trivalent nickel peptide intermediate. Figure 3 indicates that products may inhibit the reaction. Nickel(III) peptides do not react with formaldehyde but do decompose faster in the presence of amino type buffers.

On the other hand some oxidizing agents are initiators and can shorten or eliminate the induction period. Initiators include benzoyl peroxide and trivalent nickel and trivalent copper peptide complexes.

Reaction Mechanism. The addition of low levels (10^{-5} M) of H_2O_2 does not catalyze the O_2 uptake and higher levels inhibit the reaction. Hydrogen peroxide has the potential to oxidize the G_4 complex of Ni(II) to Ni(III) and at the same time reduce Ni(III) to Ni(II). Both eq 3 and eq 4 are

$$H_2O_2 + 2Ni^{III}G_4 \rightleftarrows 2Ni^{II}G_4 + O_2 + 2H^+ \quad (3)$$

$$2H^+ + H_2O_2 + 2Ni^{II}G_4 \rightleftarrows 2Ni^{III}G_4 + 2H_2O \quad (4)$$

$$2H_2O_2 \rightleftarrows O_2 + 2H_2O \quad (5)$$

thermodynamically favorable at pH 8. The addition of traces of H_2O_2 to a $Ni^{II}G_4$ solution produces a small amount of Ni(III), but addition of H_2O_2 to a solution of $Ni^{III}G_4$ causes decomposition of the trivalent complex. The net results is that a Ni(III,II) couple catalyzes the disproportionation of H_2O_2 (eq 5) under the pH conditions of the O_2 uptake reaction. Copper complexes of *N,N'*-diglycylethylenediamine and homologues also are known to catalyze the disproportionation of H_2O_2.[28,29]

A mechanism for O_2 activation and consumption by nickel(II) is needed which avoids the formation of H_2O_2 and accounts for the main pathway for the oxidation of tetraglycine to form G_3aCH_2OH and CO_2. $Ni^{III}(H_{-3}G_4)^-$ also must build up in the early stages of the reaction. The proposed mechanism given by eq 6–12 provides an autocatalytic pathway for the consumption of oxygen and the oxidation of tetraglycine when $Ni^{III}(H_{-3}G_4)^-$ is present. The reactions expressed in eq 6–12 are based on studies of the initial rates of oxygen uptake as a function of $[Ni^{II}G_4]_T$, $[Ni^{III}(H_{-3}G_4)^-]$, and $[O_2]$. They are also consistent with the rate of formation of $Ni^{III}(H_{-3}G_4)^-$ at the initial stages of the reaction.

In the mechanism for O_2 uptake of nickel(II) tetraglycine as given by eq 6–12, L· is G_3aCH_2·, LO_2· is $G_3aCH_2O_2$·, P is

$$Ni^{III}G_4 \xrightarrow{k_1} Ni^{II}L\cdot + CO_2 \quad (6)$$

$$Ni^{II}L\cdot + O_2 \xrightarrow{k_2} Ni^{II}LO_2\cdot \quad (7)$$

$$Ni^{II}L\cdot + Ni^{III}G_4 + OH^- \xrightarrow{k_3} Ni^{II}G_4 + Ni^{II}P \quad (8)$$

$$Ni^{II}LO_2\cdot + Ni^{III}G_4 + OH^- \xrightarrow{k_4} Ni^{II}G_4 + Ni^{II}P + O_2 \quad (9)$$

$$Ni^{II}LO_2\cdot + Ni^{II}G_4 + H_2O \xrightarrow{k_5} Ni^{III}G_4 + Ni^{II}LOOH + OH^- \quad (10)$$

$$Ni^{II}LOOH + Ni^{II}G_4 + 2H_2O \xrightarrow{k_6} Ni^{III}G_4 + Ni^{III}P + 2OH^- \quad (11)$$

$$Ni^{III}P + Ni^{II}G_4 \xrightarrow{k_7} Ni^{III}G_4 + Ni^{II}P \quad (12)$$

the *N*-(hydroxymethyl)amide product and all complexes are assumed fully deprotonated. The formation of peptide amide products is consistent with the proposed ligand hydroperoxide intermediates. The intermediates can be further oxidized or reduced to give the peptide *N*-(hydroxymethyl)amide or dehydropeptide species that can hydrolyze to the peptide amide. The hydroperoxides can be formed via the reaction of an oxygen molecule with a carbon radical to give a peroxy radical.

The source of the ligand free radical can be from hydrogen atom abstraction or one-electron decomposition of a trivalent nickel peptide complex. Decarboxylation of tetraglycine during O_2 uptake suggests that ligand free radicals are a consequence of nickel(III) peptide decomposition rather than hydrogen atom abstraction. The reaction in eq 6 indicates that decarboxylation can occur by direct transfer of an electron from the methylene radical. Direct electron transfer from the carboxylate group is unlikely since decarboxylation does not occur for complexes where the carboxylate group is not adjacent to a deprotonated peptide nitrogen. The decomposition of nickel(III)–tetraaza(macrocyclic amine) complexes proceeds through a nitrogen free radical[30] and irradiation of peptides in aqueous solution results in the preferential formation of methylene radicals during oxidative cleavage.[31,32] In addition, the presence of metal ions with two accessible oxidation states, including Cu^I, Cu^{II} and Pb^{II}, Pb^{IV}, are known to catalyze decarboxylation of the type shown in eq 6.[33,34] Metal ion catalysts which act as oxidizing agents have been found to cause oxidative decarboxylation of citrate and a variety of α-hydroxypolycarboxylic acids.[35]

The reactions given in eq 7–12 show the fate of the ligand radical in a mixture of oxygen and nickel(III) peptide. The reactions are based on fast electron transfers via the nickel(III,II)–peptide complexes, which studies in our laboratory confirm.[36] Reactions 8 and 9 are termination reactions, since both pathways form product by destroying the reactive nickel(III)–peptide intermediate. A reaction similar to the one in eq 9 has been proposed for the reactions of lead tetraacetate with hydroperoxides.[37] If reactions 7 and 10 predominate, a source of nickel(III) peptide is available for the continued production of ligand radicals.

It was observed that when large amounts of nickel(III) tetraglycine (~10% $[Ni^{II}G_4]_T$) are used to initiate O_2 uptake, there is a decrease in Ni(III) absorbance until a lower level is reached. On the other hand it is possible to initiate the reaction with much smaller amounts of $Ni^{III}(H_{-3}G_4)^-$, resulting in an increase in Ni(III) absorbance during O_2 uptake to approximately the same level of Ni(III) as in the previous case. The uninitiated reaction has a similar buildup of nickel(III) peptide as shown in Figure 1. These observations are consistent with oxidation of nickel(II) peptide by the ligand hydroperoxide. This reaction is expressed by eq 11. Hydroperoxides can oxidize a variety of metals, including Fe(II) in acidic solution.[38] Although the potentials of the nickel peptides are higher than those of Fe(III,II), it appears that the hydroperoxides formed in this system are capable of oxidation of nickel(II) peptides. The reaction is expected to be fairly rapid since no buildup in hydroperoxides has been detected. As eq 12 indicates the trivalent complex of triglycyl-*N*-(hydroxymethyl)amide is expected to easily oxidize the tetraglycine complex of Ni(II) to Ni(III). The summation of eq 6, 7, 10, 11 and 12 gives

$$Ni^{III}G_4 + O_2 + 3Ni^{II}G_4 \rightarrow 3Ni^{III}G_4 + Ni^{II}P + CO_2$$

or a net reaction corresponding to a gain of two molecules of Ni(III) per molecule of O_2 reacted. As the concentration of Ni(III) increases, reactions 8 and 9 become more important. The summation of eq 6 and 8 or eq 6, 7, and 9 gives

$$2Ni^{III}G_4 \rightarrow Ni^{II}G_4 + Ni^{II}P + CO_2$$

and the net result is the decay of $Ni^{III}G_4$ without O_2 consumption. This accounts for the buildup and decay of Ni(III) as seen by the absorbance change in Figure 1.

Determination of Reaction Order in $[Ni^{II}G_4]_T$, $[Ni^{III}(H_{-3}G_4)^-]$, and $[O_2]$ by Initial Rate Studies. Oxidizing agents, such as $Ni^{III}(H_{-3}G_5)^-$ and benzoyl peroxide which are capable of forming initial amounts of $Ni^{III}(H_{-3}G_4)^-$, can be used to initiate the O_2 uptake reaction under controlled conditions. The

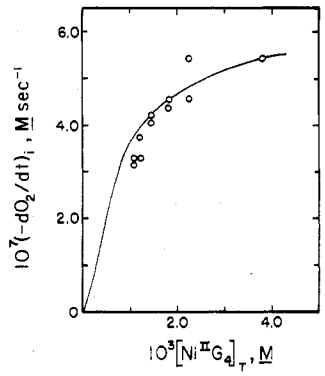


Figure 4. Dependence of initial rates of oxygen uptake on $[Ni^{II}G_4]_T$. $[O_2]_i = 6.1 \times 10^{-4}$ M, $[Ni^{III}(H_{-3}G_4)^-]_i = 1.2 \times 10^{-4}$ M, 2.5×10^{-2} M borate buffer, pH 8.6, $\mu = 0.10$ M $NaClO_4$, 25 °C. Solid line calculated on the basis of eq 13 and the rate constants in Table V.

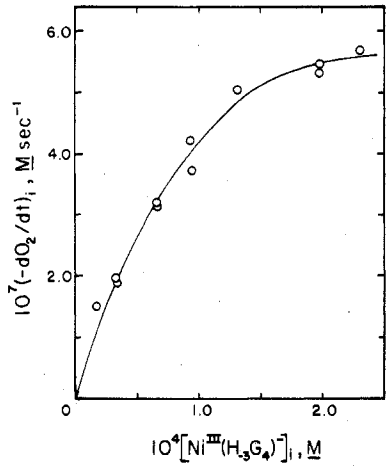


Figure 5. Dependence of the initial rates of oxygen uptake on $[Ni^{III}(H_{-3}G_4)^-]_i$. $[Ni^{II}G_4]_T = 1.95 \times 10^{-3}$ M, $[O_2]_i = 6.1 \times 10^{-3}$ M, 2.5×10^{-2} M borate buffer, pH 8.6, $\mu = 0.10$ M $NaClO_4$, 25 °C. Solid line calculated on the basis of eq 13 and the rate constants in Table V.

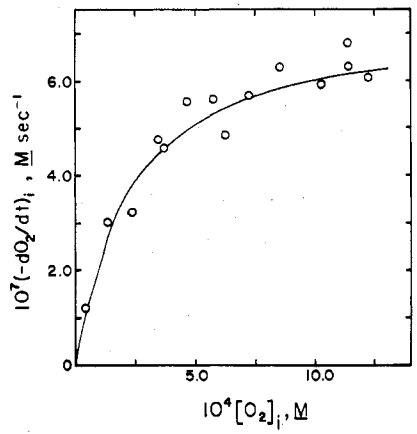


Figure 6. Dependence of initial rates of oxygen uptake on $[O_2]_i$. $[Ni^{II}G_4]_T = 1.95 \times 10^{-3}$ M, $[Ni^{III}(H_{-3}G_4)]_i = 1.8 \times 10^{-4}$ M, 2.5×10^{-2} M borate buffer, pH 8.6, $\mu = 0.10$ M $NaClO_4$, 25 °C. Solid line calculated on the basis of eq 13 and rate constants in Table V.

dependence of the initial rates of O_2 uptake with variations in the concentration of $[Ni^{II}G_4]_T$, initial $[Ni^{III}(H_{-3}G_4)]$, and initial $[O_2]$ can be obtained in such a manner and are given in Figures 4, 5, and 6, respectively, for pH 8.6 borate buffer solutions. In each case the dependence appears first order at low concentration of the reactant followed by zero order at higher concentrations.

The rate of oxygen consumption can be derived from eq

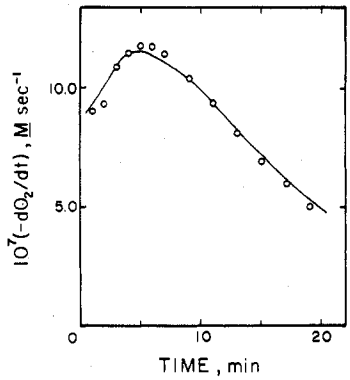


Figure 7. Change in $d[O_2]/dt$ during the autoxidation of nickel(II) tetraglycine. $[Ni^{II}G_4]_T = 5 \times 10^{-3}$ M, $[O_2]_i = 1.2 \times 10^{-3}$ M, $[Ni^{III}(H_{-3}G_4)^-]_i = 8 \times 10^{-5}$ M, pH 8.1, 25 °C. Solid line calculated on the basis of eq 13 and the rate constants in Table V.

Table V. Resolved Rate Constants

pH	k_1, s^{-1}	$k_3k_2^{-1}$	$k_4k_5^{-1}$	Conditions
8.6	7.0×10^{-3}	1.2	7	2.5×10^{-2} M borate buffer, pH 8.6, initial rate study
8.1	1.4×10^{-2}	1.7	0.1	Buffered with excess peptide ligand, pH 8.1, O_2 uptake curve

6–12 using steady-state assumptions for $Ni^{II}L\cdot$ and $Ni^{II}LO_2\cdot$ and the resulting expression is given in eq 13. The expression

$$-\frac{d[O_2]}{dt} = \frac{k_1[Ni^{III}G_4][O_2][Ni^{II}G_4]_T}{([O_2] + k_3k_2^{-1}[Ni^{III}G_4]) \times ([Ni^{II}G_4]_T + k_4k_5^{-1}[Ni^{III}G_4])} \quad (13)$$

is given in terms of the total concentration of nickel(II) tetraglycine. The solid lines in Figures 4–6 are calculated on the basis of a nonlinear least-squares analysis of all the data and eq 13. Although the observed dependences are very complex, eq 6–12 provide a good description of the O_2 uptake reaction and give an excellent fit to the experimental data. The values of k_1, $k_3k_2^{-1}$, and $k_4k_5^{-1}$ calculated under initial rate conditions are given in Table V. The values of k_2 and k_3 are very similar under these conditions and the rate of reaction of oxygen and a carbon radical is expected to be nearly diffusion controlled.[39] The value of 7 for $k_4k_5^{-1}$ suggests that the termination reaction of the peroxy radical predominates over propagation at this pH. A different analysis was performed at the lower pH of 8.1 and in the absence of borate buffer. An entire O_2 uptake curve for the depletion of a millimolar solution of oxygen by nickel(II) tetraglycine is fit using eq 13. Figure 7 shows a reasonable fit between the experimental and calculated spontaneous rates of O_2 uptake as a function of time. This is further evidence that eq 6–12 provide an adequate description of the autocatalytic reaction. At pH 8.1 the value of k_1 is higher than expected. However, the reaction was run in excess ligand as buffer and it has been observed that amine type buffers accelerate the rate of decomposition of the nickel(III) peptides.

Formation of $Ni^{III}(H_{-3}G_4)^-$ by the O_2 Reaction. At low $[Ni^{III}(H_{-3}G_4)^-]$ the stoichiometry of reactions 6–12 indicates that the rate of formation of $Ni^{III}(H_{-3}G_4)^-$ should be twice the rate of loss of O_2 and can be expressed by eq 14. Equation 14

$$d[Ni^{III}G_4]/dt = 2k_1[Ni^{III}G_4] \quad (14)$$

expresses the autocatalytic nature of the reaction and indicates that $Ni^{III}(H_{-3}G_4)^-$ initially forms by a first-order process. The formation of $Ni^{III}(H_{-3}G_4)^-$ was monitored at 350 nm for the

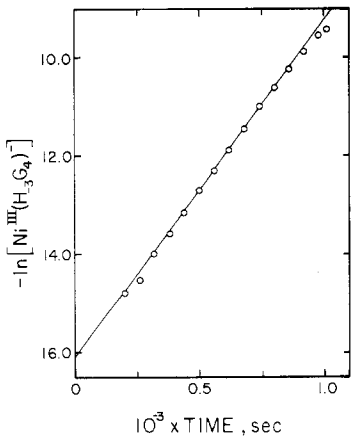


Figure 8. First-order formation of $Ni^{III}(H_{-3}G_4)^-$ during oxygen uptake reaction of nickel(II) tetraglycine: 2.5×10^{-2} M borate buffer, pH 8.6, $[Ni^{III}(H_{-3}G_4)]_i = 1.5 \times 10^{-7}$ M, slope = 6.9×10^{-3} s^{-1}.

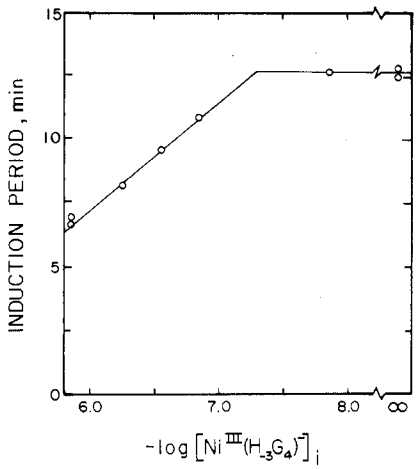


Figure 9. Induction period as a function of added $Ni^{III}(H_{-3}G_4)^-$. $[Ni^{II}G_4]_T = 2 \times 10^{-3}$ M, 2.5×10^{-2} M borate buffer, pH 8.6, μ = 0.10 M $NaClO_4$, 25 °C. Induction period is defined as time to build up to 8×10^{-6} M $[Ni^{III}(H_{-3}G_4)^-]$.

O_2 reaction with nickel(II) tetraglycine at pH 8.6 and 2.5×10^{-2} M borate buffer. An exponential increase in Ni(III) was observed. Figure 8 is a plot of ln $[Ni^{III}(H_{-3}G_4)^-]$ as a function of time which shows excellent first-order behavior from 3×10^{-7} to 4×10^{-5} M $Ni^{III}(H_{-3}G_4)^-$. Curvature at the higher concentration is expected due to contributions from the terminating reactions. The intercept is 1×10^{-7} M $[Ni^{III}(H_{-3}G_4)^-]$, which agrees with the amount of initiator added to the system.

A similar description of the autocatalytic increase in $Ni^{III}(H_{-3}G_4)^-$ is obtained by monitoring the induction period as a function of the concentration of initiator added to the system. Low concentrations of $Ni^{III}(H_{-3}G_5)^-$ (E° = 0.83 V) were used to provide the initial amount of trivalent nickel tetraglycine (E° = 0.79 V) for the O_2 reaction with nickel(II) tetraglycine. As Figure 9 indicates there is a linear relationship between the induction period and log $[Ni^{III}(H_{-3}G_4)^-]$ above 5×10^{-8} M Ni(III). Additions of Ni(III) at concentrations below this level do not affect the induction period. The induction period is defined as the time required to build up to 8×10^{-6} M $Ni^{III}(H_{-3}G_4)^-$, which is about 10% of the maximum amount of Ni(III) formed. The linear portion is evidence for an exponential increase in $Ni^{III}(H_{-3}G_4)^-$.

Determination of k_1 Values. Values of k_1 have been obtained under the same conditions by four different methods as listed in Table VI. From initial rate studies the best fit of all the data to eq 13 gave a value of 7×10^{-3} s^{-1} for k_1. The initial rate for the decomposition of electrolytically prepared $Ni^{III}(H_{-3}G_4)^-$ under the same conditions gives an observed rate constant of 1.3×10^{-2} s^{-1}. Equations 6–12 indicate that 2 mol of $Ni^{III}G_4$ is involved with the decomposition, which is consistent with recovery of 50% of the reactant after decomposition. Therefore, the value of k_1 is 6.5×10^{-3} s^{-1}, which agrees with the value obtained from the initial rate studies. Formation of $Ni^{III}(H_{-3}G_4)^-$ is expressed by eq 14. The value of k_1 is determined from the slope of the exponential increase in $Ni^{III}(H_{-3}G_4)^-$ shown in Figure 8 and calculated to be 3.5×10^{-3} s^{-1}. From the induction period study in Figure 9 a similar analysis gives a value of 3.8×10^{-3} s^{-1} for k_1.

Table VI. Values of k_1[a]

Method	k_1, s^{-1}	Comment
$Ni^{III}(H_{-3}G_4)^-$ base decomposition (under N_2)	6.5×10^{-3}	$k_{obsd} = 2k_1$
		$k_{obsd} = 1.3 \times 10^{-2}$ s^{-1}
Initial rates of O_2 uptake	7×10^{-3}	Eq 13
First-order formation of $Ni^{III}(H_{-3}G_4)^-$ by O_2 reaction	3.5×10^{-3}	Figure 8
		$k_{obsd} = 2k_1$
Induction period vs. initial $[Ni^{III}(H_{-3}G_4)^-]$	3.8×10^{-3}	Figure 9
		$k_{obsd} = 2k_1$

[a] pH 8.6, 2.5×10^{-2} M borate buffer.

This discrepancy in the values of k_1 suggests that some hydroperoxide decomposition may occur via a competing pathway that does not produce $Ni^{III}G_4$. This would tend to lower the stoichiometry in the formation of $Ni^{III}G_4$ in the presence of O_2 but would still give the autocatalytic buildup of $Ni^{III}G_4$. When present in high concentration borate buffer inhibits the reaction of nickel(II) tetraglycine with oxygen. Perborates are readily formed by H_2O_2 oxidation.[40] Another possible competitive reaction involves amine oxidation. Hydroperoxides oxidize amine to hydroxylamines,[41,42] which would be consistent with the observed loss of ninhydrin-active products. The reaction of hydroperoxides with amines is normally slow, and for such a process to be competitive with the reaction of Ni(II) to Ni(III), coordination by nickel would have to labilize the amine toward oxidation. Although other terminating steps of the peroxy radical or the ligand hydroperoxide may be involved, we believe that the basic nature of the autocatalytic reaction has been described.

pH Dependence for the Reaction of $Ni^{II}G_4$ and O_2. Equation 13 was derived for a single pH. The peroxy radical oxidation of Ni(II) to Ni(III) (eq 10) is dependent upon $[H^+]$. If this dependence is included in eq 13, the following relationship is obtained (eq 15). This relationship indicates that the rate

$$-\frac{d[O_2]}{dt} = \frac{k_1[Ni^{III}G_4][O_2][Ni^{II}G_4]_T[H^+]}{([O_2] + k_3k_2^{-1}[Ni^{III}G_4]) \times ([Ni^{II}G_4]_T[H^+] + k_4k_5^{-1}[Ni^{III}G_4])} \quad (15)$$

of O_2 uptake should become faster as the pH is lowered. However, an offsetting effect results because the reactive form of nickel(II) tetraglycine is the triply deprotonated complex whose concentration increases with increasing pH. The data in Table I indicate that the rate of O_2 uptake is most rapid near a pH value of 8 and the experimentally observed concentration of $Ni^{III}(H_{-3}G_4)^-$ formed during the autoxidation reaction as a function of pH is highest near the same pH value.

Initiation of the Autoxidation of Nickel(II) Tetraglycine. The autocatalytic reaction begins rapidly even in the absence of added initiators. The value of k_1 obtained from the first-order formation of Ni(III) (Table VI) corresponds to an increase in the Ni(III) concentration by an order of magnitude every 5.5 min. Figure 9 shows that an uninitiated reaction takes approximately 13 min to attain 8.0×10^{-6} M Ni(III) (this is the induction period as defined in Figure 9). This requires the presence of 3.5×10^{-8} M Ni(III) at time zero.

The possibility was entertained that this low initial Ni(III) concentration was formed from residual O_2 or from some trace of an oxidizing impurity. To eliminate this possibility the Ni(III) formation experiments were repeated with samples which had been sealed for 20 h to permit any adventitiously formed Ni(III) to decompose. These results essentially reproduced those shown in Figure 9 with the rate constant $2k_1 = 6.1 \times 10^{-3}$ s^{-1} and the same induction period as before.

In examining the system for another process which could produce the apparent initiating concentration of Ni(III), the direct oxidation of $Ni^{II}(H_{-3}G_4)^{2-}$ by O_2 was considered. Three different processes may be involved according to whether O_2 is reduced by 1, 2, or 4 equiv per mole:

$$O_2 + e^- \rightarrow O_2^- \quad E^\circ = -0.33 \text{ V}^{43} \tag{16}$$

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \quad E^\circ = +0.18 \text{ V at pH 8.6} \tag{17}$$

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \quad E^\circ = +0.72 \text{ V at pH 8.6} \tag{18}$$

None of these half-reactions is sufficiently positive to quantitatively oxidize $Ni^{II}(H_{-3}G_4)^{2-}$ to $Ni^{III}(H_{-3}G_4)^-$ (E° = +0.81 V at pH 8.6); however, all might produce small equilibrium amounts of Ni(III).

If we write an isolated equilibrium using the half-reaction in eq 16 to oxidize $Ni^{II}(H_{-3}G_4)^{2-}$ we obtain

$$Ni^{II}(H_{-3}G_4)^{2-} + O_2 \underset{k_{-8}}{\overset{k_8}{\rightleftharpoons}} Ni^{III}(H_{-3}G_4)^- + O_2^- \tag{19}$$

$E^\circ = -1.14$ V at pH 8.6

$$K_8 = \frac{[Ni^{III}(H_{-3}G_4)^-][O_2^-]}{[Ni^{II}(H_{-3}G_4)^{2-}][O_2]} = 10^{-19.3}$$

For $[Ni^{II}G_4]_T = 2.0 \times 10^{-3}$ M and $[O_2] = 1.0 \times 10^{-3}$ M we find an equilibrium concentration of $10^{-12.5}$ M for $Ni^{III}(H_{-3}G_4)^-$.

Similarly, the half-reaction of eq 17 gives

$$2Ni^{II}(H_{-3}G_4)^{2-} + O_2 + 2H^+ \rightleftarrows 2Ni^{III}(H_{-3}G_4)^- + H_2O \tag{20}$$

$E^\circ = -0.63$ V at pH 8.6

$$K_{eq} = \frac{[Ni^{III}(H_{-3}G_4)^-]^2[H_2O_2]}{[Ni^{II}(H_{-3}G_4)^-]^2[O_2]} = 10^{-21.3} \text{ (at pH 8.6)}$$

and an equilibrium concentration of $\sim 2 \times 10^{-10}$ M $Ni^{III}(H_{-3}G_4)^-$. Even this is much too low for the Ni(III) concentration needed to initiate the reaction as observed. However, this assumes that the H_2O_2 produced in eq 20 reacts no further. Thermodynamically H_2O_2 is capable of oxidizing $Ni^{II}(H_{-3}G_4)^{2-}$ quantitatively and if that process were rapid the equilibrium Ni(III) concentration could rise significantly higher than this estimate (as can be seen from the E° value in eq 18).

There also is a kinetic limitation with eq 19. If we let the extremely favorable reverse reaction have a rate constant $k_{-8} \approx 10^9$ M^{-1} s^{-1} (diffusion controlled), the forward rate constant k_8 ($=K_8k_{-8}$) cannot exceed 10^{-10} M^{-1} s^{-1}. Then the outer-sphere electron transfer cannot produce $Ni^{III}(H_{-3}G_4)^-$ faster than 10^{-16} M s^{-1} for millimolar $Ni^{II}(H_{-3}G_4)^{2-}$ and O_2. This is much too slow for the initiation process, barring the existence of a branching chain reaction in subsequent steps. However, if there existed a $Ni^{II}(H_{-3}G_4)^{2-}\cdot O_2$ complex of appreciable stability, this kinetic limitation would not apply. An internal electron transfer could produce a $Ni^{III}\cdot O_2^-$ species at a significantly greater rate. This superoxide species could abstract a hydrogen atom from tetraglycine to give hydroperoxide and a ligand-oxidized nickel(II)–peptide complex. There is no direct evidence for an O_2 complex, although it is not unreasonable for Ni^{II} to bind O_2. Metal–oxygen complexes involving cobalt amines[5] and iron(II) complexes[44] have been characterized, but oxygen complexes with nickel are known only for the zerovalent state.[45]

Michelson[46] has presented evidence for the formation of superoxide from the interaction of O_2 with a complex he believed to be nickel(II) tetraglycine. Under his conditions of a high concentration of ammonium buffer at pH 9–10.4 we observed no formation of the nickel(II) peptide complex. The color of the solutions was that of characteristic octahedral rather than square-planar nickel complexes. However, the formation of superoxide from interaction of O_2 with any Ni(II) complex is relevant. In addition, Michelson's suggestion that ligand hydroperoxides are the cause of a later signal is consistent with the autocatalytic mechanism proposed here.

Initiation of the autoxidation by a possible nickel(II) peptide oxygen complex need not be intramolecular. The reaction of the $Ni^{II}\cdot O_2$ complex with a second nickel(II) peptide would have the thermodynamic advantage of a more favorable two-electron process offsetting the unfavorable third-order reaction.

The Ni(III) formation experiments with samples which had been sealed overnight exclude initiation by a trace oxidant, since any $Ni^{III}G_4$ formed during sample preparation would decompose. There remains the possibility that these solutions contain a small amount of some species which is more reactive toward O_2 than is $Ni^{II}(H_{-3}G_4)^{2-}$ or some other long lasting nickel(III) complex. Recent work has shown that bis(peptide) complexes of Ni(III) can have much lower standard electrode potentials.[47]

In summary, there are several possibilities for the initiation process, but the data appear to eliminate outer-sphere electron transfer from O_2 or initiation by trace oxidizing impurities. Once $Ni^{III}(H_{-3}G_4)^-$ reaches a concentration level of $\sim 10^{-8}$ M, the autocatalytic pathway (described in eq 6–12) controls the rate of O_2 uptake.

Conclusions

Some of the novel features of the reaction of nickel tetraglycine with molecular oxygen include the autocatalytic nature of the reaction, the ease of initiation in neutral solution and room temperature, the peptide oxidation involving decarboxylation, the formation of triglycyl-*N*-(hydroxymethyl)amide, and the formation of trivalent nickel peptides as reactive intermediates in aqueous solution. We feel that this work proves that $Ni^{III}(H_{-3}G_4)^-$ is formed in appreciable amounts and catalyzes the reaction. The hydroperoxide formation is postulated and provides a good fit of the experimental data with the proposed mechanism. The reaction mechanism also requires rapid electron-transfer interactions with the nickel peptides. Studies in our laboratory indicate that rapid electron exchange does occur.[36] The nature of the metal ion is important in the specificity of the peptide oxidation. In similar studies of copper peptide autoxidations, oxidative cleavage of the peptide ligands occurs at the third bound amino acid residue and no CO_2 is produced.[7,8]

The accessibility of nickel(III) peptides in aqueous solution and their ability to catalyze metal peptide degradation may be of interest in biological systems. Very little is known about nickel in biological systems, although it appears to be an essential metal[48] and the enzyme jack bean urease has recently been shown to contain nickel.[49] Our previous study indicates that there is relatively little variation in Ni(III)–Ni(II) electrode potential with changes in the nature of the ligand for mono(peptide) complexes.[14] However, bis(peptide)-nickel(III) complexes may be attainable under even milder oxidizing conditions.

Acknowledgment. This investigation was supported by Public Health Service Grant No. GM 19775 from the National Institute of General Medical Sciences. In addition F. P. Bossu acknowledges support from the 1973 Fellowship of the Analytical Division of the American Chemical Society

sponsored by the Society of Analytical Chemists of Pittsburgh, E. B. Paniago from the Rockefeller Foundation, and S. T. Kirksey, Jr., from the Purdue Doctoral Fellowship Program for Black Students.

Registry No. $Ni(H_{-3}G_4)^{2-}$, 39016-92-7; $Ni^{III}(H_{-3}G_4)^{-}$, 60165-88-0; G_3aCH_2OH, 65621-94-5.

References and Notes

(1) M. V. Lock and B. F. Sargar, *Proc. Chem. Soc., London*, 358 (1960).
(2) M. V. Lock and B. F. Sargar, *J. Chem. Soc. B*, 690 (1965).
(3) B. F. Sargar, *J. Chem. Soc. B*, 428, 1047 (1967).
(4) W. H. Sharkey and W. E. Mochel, *J. Am. Chem. Soc.*, **81**, 3000 (1959).
(5) R. G. Wilkins, *Adv. Chem. Ser.*, **No. 100**, 111 (1971).
(6) O. H. Wheeler, "The Chemistry of Amides", J. Zabicky, Ed., Interscience, New York, N.Y., 1970, pp 320–329.
(7) G. L. Burce, E. B. Paniago, and D. W. Margerum, *J. Chem. Soc., Chem. Commun.*, 261 (1975).
(8) G. L. Burce, Ph.D. Thesis, Purdue University, 1975.
(9) E. B. Paniago, D. C. Weatherburn, and D. W. Margerum, *Chem. Commun.*, 1427 (1971).
(10) E. B. Paniago, Ph.D. Thesis, Purdue University, 1972.
(11) D. W. Margerum, K. L. Chellappa, F. P. Bossu, and G. L. Burce, *J. Am. Chem. Soc.*, **97**, 6894 (1975,
(12) F. P. Bossu, K. L. Chellappa, and D. W. Margerum, *J. Am. Chem. Soc.*, **99**, 2195 (1977).
(13) F. P. Bossu and D. W. Margerum, *J. Am. Chem. Soc.*, **98**, 4003 (1976).
(14) F. P. Bossu and D. W. Margerum, *Inorg. Chem.*, **16**, 1210 (1977).
(15) B. R. Clark and D. H. Evans, *J. Electoanal. Chem.*, **69**, 181 (1976).
(16) G. M. Nano in "Thin-Layer Chromatography", G. B. Marini-BeHôlo, Ed., Elsevier, Amsterdam, 1964, p 138.
(17) J. Christie and J. Wilson, *Anal. Biochem.*, **63**, 202 (1975).
(18) A. Kaneda and A. E. Martell, *J. Coord. Chem.*, **4**, 137 (1975).
(19) E. B. Paniago and D. W. Margerum, *J. Am. Chem. Soc.*, **94**, 6704 (1972).
(20) M. Christie and J. D. Roberts, *J. Am. Chem. Soc.*, **94**, 4565 (1972).
(21) G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists", Wiley-Interscience, New York, N.Y., 1972, p 49.
(22) W. Horsley, H. Sternlicht, and J. S. Cohen, *J. Am. Chem. Soc.*, **92**, 680 (1970).
(23) G. L. Burce and D. W. Margerum, *Anal. Chem.*, submitted for publication.
(24) B. C. Challis and J. A. Challis in "The Chemistry of Amides", J. Zabicky, Ed., Wiley, London, 1970, p 755.
(25) J. F. Walker, "Formaldehyde", Reinhold, New York, N.Y., 1953, pp 290–293.
(26) D. W. Margerum, L. F. Wong, F. P. Bossu, K. L. Chellappa, J. J. Czarnecki, Sanford T. Kirksey, Jr., and T. A. Neubecker, *Adv. Chem. Ser.*, **No. 162**, 281 (1977).
(27) K. Takemoto, K. Azuma, K. Nakamichi, and D. K. Hamann, *Makromol. Chem.*, **150**, 51 (1971).
(28) H. Sigel, *Helv. Chim. Acta*, **51**, 947 (1968).
(29) H. Sigel, *Angew. Chem., Int. Ed. Engl.*, **8**, 167 (1969).
(30) E. K. Barefield and M. T. Mocella, *J. Am. Chem. Soc.*, **97**, 4238 (1975).
(31) A. F. Usatyi and V. I. Panin, *Biofizika*, **18**, 439 (1973).
(32) M. Simic, P. Neta, and E. Haynon, *J. Am. Chem. Soc.*, **92**, 4763 (1970).
(33) J. D. Bacha and J. K. Kochi, *J. Org. Chem.*, **33**, 83 (1968).
(34) P. Poupko, J. Rosenthal, and D. Elad, *Photochem. Photobiol.*, **17**, 395 (1973).
(35) R. W. Hay in "Metal Ions in Biological Systems", Vol. 5, H. Sigel, Ed., Marcel Dekker, New York, N.Y., 1976, p 164.
(36) J. J. Czarnecki and D. W. Margerum, to be submitted for publication.
(37) M. S. Kharasch, A. Fono, W. Nadenberg, and B. Bischof, *J. Org. Chem.*, **17**, 207 (1952).
(38) J. K. Kochi, Ed., "Free Radicals", Vol. 1, Wiley-Interscience, New York, N.Y., 1973, p 635, and references therein.
(39) O. L. Magelli and C. S. Sheppard in "Organic Peroxides", Vol. 1, D. D. Swern, Ed., Wiley-Interscience, New York, N.Y., 1970, p 15.
(40) H. Menzel, *Z. Anorg. Alleg. Chem.*, **164**, 1 (1927).
(41) H. E. J. DeLaMare, *J. Org. Chem.*, **25**, 2114 (1960).
(42) B. C. Challis and A. R. Butler in "Chemistry of the Amino Group", S. Patai, Ed., Interscience, New York, N.Y., 1968, pp 321–333.
(43) P. M. Wood, *FEBS Lett.*, **44**, 22 (1974).
(44) J. P. Collman, R. R. Gagne, H. B. Gray, and J. W. Hare, *J. Am. Chem. Soc.*, **96**, 6522 (1974).
(45) E. W. Stern in "Transition Metals in Homogeneous Catalysis", G. N. Schrauzer, Ed., Marcel Dekker, New York, N.Y., 1971, p 126.
(46) A. M. Michelson, *Biochimie*, **55**, 925 (1973).
(47) A. G. Lappin, C. K. Murray, and D. W. Margerum, *Inorg. Chem.*, in press.
(48) F. W. Sunderman, Jr., in "Nickel", National Academy of Science, Washington D.C., 1975, pp 89–93.
(49) N. E. Dixon, C. Gazzola, R. L. Blakeley, and B. Zener, *J. Am. Chem. Soc.*, **97**, 4131 (1975).

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Microcalorimetric Determination of the Enthalpy of a Slow Reaction: Destruction with Cyanide of the Macrocyclic (1,4,8,11-Tetraazacyclotetradecane)nickel(II) Ion

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Received August 10, 1977

The calorimetric determination of the enthalpy of formation of (1,4,8,11-tetraazacyclotetradecane)nickel(II) has been made by displacing the macrocyclic ligand with cyanide and following the reaction to completion (5 h) in a batch microcalorimeter of the heat-flow type. The $\Delta H°$ value found (−20.3 kcal mol^{-1}) is 11 kcal mol^{-1} less than that previously reported. Thermodynamic parameters of both octahedral and square-planar forms of the complex have been calculated. The macrocyclic effect in Ni(II) complexes has been rationalized with that already established for Cu(II) complexes.

Introduction

The term "macrocyclic effect" was first introduced in 1969 by Cabbiness and Margerum[2] to highlight the large increase in stability constants which they had found for the macrocyclic complex $[Cu(tet\text{-}a)]^{2+}$ when compared to that of the equivalent complex with a noncyclic ligand in which the chelate rings were present in the same alternating ring sequence (5,6,5), namely, $[Cu(2,3,2\text{-}tet)]^{2+}$. In general terms, the "macrocyclic effect" refers to the decrease in Gibbs energy for the metathetic reaction

$$ML^{n+}_{noncyclic} + L_{cyclic} \rightarrow ML^{n+}_{cyclic} + L_{noncyclic}$$

and, as for the chelate effect before it, considerable attention has been directed toward separating $\Delta G°$ into its component enthalpic and entropic parts in order to understand the origins of the enhanced stability of macrocyclic complexes, which play an important role in many naturally occurring systems.

However, macrocyclic complexes also display an enhanced kinetic inertness, with respect to either their formation in aqueous solution or their degradation, and, until recently, it has not been possible to determine $\Delta H°$ directly by calorimetry. Thus, the first attempts to separate $\Delta G°$ involved $\Delta H°$ values which had been obtained empirically or indirectly, and these are summarized below together with more recent calorimetric determinations of $\Delta H°$.

(1) $\Delta H°$ for $[Cu(tet\text{-}a)]^{2+}$ was obtained[3] by extrapolating the empirical $\Delta H°$ vs. ν(d–d) relationship already established for Cu(II) polyamine complexes, and the authors concluded that the enhanced stability of the macrocyclic complex could be attributed equally to both enthalpy and entropy terms arising from a stronger Cu–N interaction and a smaller loss in configurational entropy, respectively.

(2) $\Delta H°$ for $[Ni[14]aneN_4]^{2+}$ and $[Ni(tet\text{-}a)]^{2+}$ were obtained[4] from temperature-dependent stability constant

0020-1669/78/1317-1042$01.00/0