Kinetics and mechanism of the reactions of Ni(II) oligopeptideamide-cyanide and Ni(II) oligopeptide-cyanide complexes with triethylene-tetramine
by William Anthony Marchese

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Chemistry
Montana State University

Abstract:
The kinetics and mechanism of the reaction between - TRIEN and certain Nickel(II) oligopeptideamide cyanide and Nickel(II) oligopeptide cyanide complexes have been investigated. The individual rate constants for each reaction of the different substrates were evaluated. The contribution of the TRIEN's subspecies to the overall rate constant were, also, evaluated. The effects of steric hindrances in the substrate ring and alteration of the solvent's pH on the rate constants were investigated.

The rate constants for the Nickel(II) oligopeptideamide cyanide and Nickel(II) oligopeptide cyanide complexes are approximately 10,000 times slower than for the Nickel(II) triglycinate ion. Both the steric hindrances and pH effects have a direct effect on the rate constants;

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ABSTRACT

The kinetics and mechanism of the reaction between TRIEN and certain Nickel(II) oligopeptideamide cyanide and Nickel(II) oligopeptide cyanide complexes have been investigated. The individual rate constants for each reaction of the different substrates were evaluated. The contribution of the TRIEN's subspecies to the overall rate constant were, also, evaluated. The effects of steric hindrances in the substrate ring and alteration of the solvent's pH on the rate constants were investigated.

The rate constants for the Nickel(II) oligopeptideamide cyanide and Nickel(II) oligopeptide cyanide complexes are approximately 10,000 times slower than for the Nickel(II) triglycinate ion. Both the steric hindrances and pH effects have a direct effect on the rate constants.

STATEMENT OF THE PROBLEM

The investigation and elucidation of the kinetics and mechanism for the reactions of some Ni(II) Oligopeptide cyanide complexes with triethylenetetramine are the major problems presented in this work.

The studies involve the determination of the general rate expression, pH effects and steric effects introduced by variation in substrate complex.

INTRODUCTION

Oligopeptide-metal complexes have generated much interest because of their possible biologically enzymatic properties (1).

Two major areas that have received much attention are the structural and kinetic aspects of these complexes.

Originally, the question of how Ni(II) and Cu(II) oligopeptide complexes formed was pursued hotly. After some initial confusion, it became evident that the formation of these complexes is assisted by the ionization of the peptide's imide protons and the peptide's nitrogen atoms are coordinated to the metal (2,3)

The kinetics of both Cu(II) and Ni(II) complexes of oligopeptides have been investigated quite thoroughly. The kinetics of these complexes involving proton transfer, ligand exchange, steric and pH effects have been studied.

The proton transfer reactions of these complexes are of both the general acid catalysis (4,5,6) and specific hydrogen ion catalysis (7,8).

The ligand exchange reactions of these Cu(II) and Ni(II) oligopeptide complexes most often involve the substitution of a multidenate ligand for the oligopeptide.

Triethylenetetramine and ethylenediamine tetraacetate ion are used most frequently (4,5,9).

The Cu(II)-Triglycine, $Cu(H_{-2}GGG)^{-}$, reaction with multidentate ligands proceeds by two general mechanisms (9). In a general acid catalysis mechanism, a proton is transferred to a peptide nitrogen to assist the dissociation of the complex. The nucleophilic mechanism involves coordination of the displacing ligand to the metal, and this speeds the breaking of the metal-peptide nitrogen bonds. Steric effects are very important and steric hindrance in the substituting ligand can block the reaction.

Ni(II)-Triglycinate, $Ni(H_{-2}GGG)^{1-}$, can react by a nucleophilic mechanism similar to that proposed for the analogous copper complex, $Cu(H_{-2}GGG)^{1-}$ (4). Ethylenediamine and polyamines have second-order rate constants of $(1.2 - 1.7)\times10^{4}\underline{M}^{-1}$ sec^{-1} with these complexes (4). Steric effects, chelation and donor ability are important factors in the substituting ability of the nucleophile (4).

In this work, both oligopeptideamides and Oligopeptides were complexed with Nickel(II). Oligopeptideamides are small peptides composed of two amino acids, and the small peptide's carboxylic acid end, its C end, has been converted into an amide. Glycyl-glycine amide is an example of an oligopeptideamide. Oligopeptide refers to a small peptide composed of three amino acids, and the carboxylic acid of this small peptide is untouched. Glycyl-glycyl-glycine is an example of an oligopeptide.

In this paper, a cyanide ion, CN^-, is added to enhance the stabilization of the complex. The CN^- occupies the fourth position of the square planar coordination sphere of Ni(II). Such complexes of Ni(II) Oligopeptide or oligopeptideamide and cyanide shall be referred to in this work as "mixed complexes".

The mixed complexes are square planar, yellow and diamagnetic. $Ni(H_{-2}GGa)CN^{1-}$ has λmax = 405 nm, $\varepsilon = 170\ \underline{M}^{-1}Cm^{-1}$, Cf. λmax = 452 nm, $\varepsilon = 136\underline{M}^{-1}Cm^{-1}$, for the aquo-complex $Ni(gga)H_2O$ (7). The $Ni(H_{-2}GGG)CN^{2-}$ absorption maxima and molar absorptivities are λmax = 410 nm and $\varepsilon = 174\underline{M}^{-1}Cm^{-1}$ (7). The mixed complexes behave as weak acids, with pKa's Ca 9. Previous attempts at determining the stability constants of these complexes have been made. Because of the complete formation of these complexes, the stability constants must be greater than 10^7 (7).

Previous studies with the Ni(II) mixed complexes have been limited to proton transfer studies (7,8). It was found with these proton transfer studies that the system responded to specific hydrogen catalysis only, and the cyanide ion had a stabilization effect on the substrate that reduced the rate of proton transfer 38,000 times (7).

EXPERIMENTAL SECTION

Apparatus

For the gathering of all spectral data, either absorption spectra or absorbance measurements at a fixed wavelength, a Cary Model 14 spectrophotometer was used. The instrument is equipped with thermostated cell compartments capable of maintaining a constant temperature to within ±0.1°C.

A Radiometer Model PHM26c pH meter was used to measure pH. The instrument was standardized using standard buffer solutions.

To record the passage of time, a A.R. & J.E. Meylan type 208A stopwatch was used.

Reagents

Nickel (II) perchlorate stock solution, 0.00916M, was prepared by diluting to volume a pre-determined volume of 0.0916M $Ni(ClO_4)_2$. The 0.0916M $Ni(ClO_4)_2$ was prepared from twice recrystallized $Ni(ClO_4)_2$ and standardized by EDTA titration (10).

Sodium cyanide solution (0.095M) was standardized by the argentimetric method (11), then diluted to 0.0095M.

Anhydrous sodium perchlorate was prepared by dissolving a weight of hydrated sodium perchlorate in boiling, double distilled water, filtering the solution using a millipore filter, evaporating to dryness and heating in an oven overnight. To prepare a 2.00<u>M</u> solution of sodium perchlorate, the required weight of sodium perchlorate was dissolved in the required volume of double distilled water.

A stock solution of borate buffer was prepared by dissolving enough sodium borate in double distilled water to provide total boron concentration equal to 0.2973<u>M</u>.

Triethylenetetramine was converted to its disulfate salt by adding dropwise 2.0 moles of concentrated sulfuric acid to 1.0 mole of triethylenetetramine dissolved in ice cold toluene. The temperature of the triethylenetetramine-toluene solution was maintained at $0^{o}C$ by using an ice bath. After the addition of the sulfuric acid, the resulting mixture was stirred for one hour. The precipitated disulfate salt was twice recrystallized from double distilled water.

To dissolve the disulfate salt in double distilled water for the preparation of the standard solution, a small amount of dilute NaOH solution was required.

Standardization was achieved by mole ratio at 550 nm using 0.077M $Cu(ClO_4)_2$ and acetate buffer.

Prior to each kinetic run, the required volume of the standard triethylenetetramine was adjusted to the desired pH using strong NaOH solution and then diluted to the desired volume.

The mixed complexes were prepared by mixing aliquots of 0.00916M $Ni(ClO_4)_2$ and the particular oligopeptide, 0.001M, to be studied so that a 100% molar excess of the oligopeptide was present. The ionic strength was adjusted to 0.10M $NaClO_4$. The pH of the resulting solution was raised to 9.5 with dilute NaOH, borate buffer and a stoichiometric amount of NaCN, equivalent to total Nickel(II), added and the pH adjusted to the desired value with either dilute NaOH or $HClO_4$.

Solutions of the complexes were always prepared, within three hours, prior to any kinetic run.

After preparation, the Ni(II) oligopeptide cyanide solution was placed in a water bath at 25°C, for two hours, to insure temperature homogenitity during the reaction.

Names and their abbreviations of all the chemicals used in this study are summarized in Table I. Also, the suppliers and compounds' purities are supplied.

Table I. Names of Compounds Used in This Study and Their Abbreviations.

Name	Abbreviation
Glycyl-Glycine amide	GGa [a,g]
Alanyl-Glycine amide	AGa [a,g]
Glycyl-Alanine amide	GAa [b,g]
Alanyl-Alanine amide	AAa [a,g]
Glycyl-Glycyl-Glycine	GGG [a,g]
Glycyl-Alanyl-Glycine	GAG [a,g]
Alanyl-Alanyl-Glycine	AAG [a,g]
Glycyl-Glycyl-Glycine-Benzyl/Ester	GGG-BnzE [a,g]
Sarcosyl-Glycyl-Glycine	SGG [c,g]
Nickel(II) perchlorate	$Ni(ClO_4)_2$ [d,h]
Sodium cyanide	NaCN [e,h]
Sodium perchlorate	$NaClO_4$ [e,h]
Triethylenetetramine	TRIEN [f,h]
Sodium borate	$Na_2B_4O_7 \cdot 10H_2O$ [e,h]

a) Fox Chemical Company
b) Cyclo Chemical Company
c) Nutritional Biochemicals Corporation
d) G. Frederick Smith Chemical Company
e) J. T. Baker Chemical Company
f) E. H. Sargent & Company
g) Chromatagraphically Pure
h) Reagent Grade

Kinetic Measurements

The substrate molecule exhibits a characteristic maximum absorption peak at 245 nm. The course of each reaction was followed by monitoring the disappearance of this peak, using the previously described Cary 14 spectrophotometer, with time after mixing of the reactants.

The reaction was initiated by mixing 50 ml of substrate solution and from 0.5 ml to 4.0 ml of the TRIEN solution. After the addition of the TRIEN, the reactants were stirred for an unspecified amount of time to insure mixing.

Ionic strength was maintained at 0.10M $NaClO_4$. The temperature throughout the reaction time was maintained at 25^oC.

The reaction rates were measured over a range of TRIEN concentrations and except where specified constant substrate concentration.

A_t will be defined as a substrate absorption at anytime, t, during the reaction. And A_∞ is the absorption that does not decrease with further time, i.e., the end of the reaction. $A_t - A_\infty$ is a function of the substrate concentration. Therefore, by calculating the slope of $-\ln(A_t - A_\infty)$ plotted versus time, one obtains the individual rate constants for each reaction, designated as k_{obs}.

Experimentally determined pH values were converted into $[H^{+}]$ values using the relationship

(1) $$-\log[H^{+}] = (pH - 0.10)$$ [12]

Table II lists equilibria and their corresponding constants used in this work.

Table II. Equilibria and Equilibrium Constants

Equilibrium	Equilibrium Constant
$htrien^{\oplus} \overset{\beta_1}{\rightleftharpoons} H^{\oplus} + trien$	$\beta_1 = 10^{-9.81}$ [13]
$h_2trien^{+2} \overset{\beta_2}{\rightleftharpoons} 2H^{t} + trien$	$\beta_2 = 10^{-18.90}$ [13]
$h_2trien^{+2} \overset{K_2}{\rightleftharpoons} H^{\oplus} + htrien^{\oplus}$	$K_2 = 10^{-9.09}$ [13]
$Ni^{+2} + 4CN^{-} \overset{\beta_4}{\rightleftharpoons} Ni(CN)_4^{-2}$	$\beta_4 = 10^{30.4}$ [14]
$Ni^{+2} + TRIEN \overset{\beta_1'}{\rightleftharpoons} NiTrien^{+2}$	$\beta_1' = 10^{14.1}$ [15]

13 - G. Schwarzenbach, Helv. Chim. Acta, 33,974(1950)
14 - F. A. Long, J. Am. Chem. Soc., 73,537(1951)
15 - C. N. Reilley and R. W. Schmid, J. Elisha Mitchell Sci. Soc., 73,279(1957)

RESULTS

Determination of the General Rate Expression for the Reaction of TRIEN with $Ni(H_{-2}GGa)CN^{-1}$

The general rate expression for the TRIEN reaction with $Ni(H_{-2}GGa)CN^{1-}$ was determined by observing the rate as a function of the concentration of TRIEN while maintaining a constant pH. A 20 to 100 fold excess of $TRIEN_t$ to substrate was present at the beginning of each reaction.

The general rate expression has the form

$$(2) \qquad \frac{-d[Ni(H_{-2}GGa)CN^{-}]}{dt} = k_{t_t}[TRIEN]_t[Ni(H_{-2}GGa)CN^{1-}]$$

Under pseudo first-order conditions, the observed rate constant is shown in equation (3).

$$(3) \qquad k_{obs} = k_{t_t}[TRIEN]_t$$

Figure 1 shows the relationship between k_{obs} and $[TRIEN]_t$.

Table III lists the individual $k_{obs}\{sec^{-1}\}$ for each $[TRIEN]_t$.

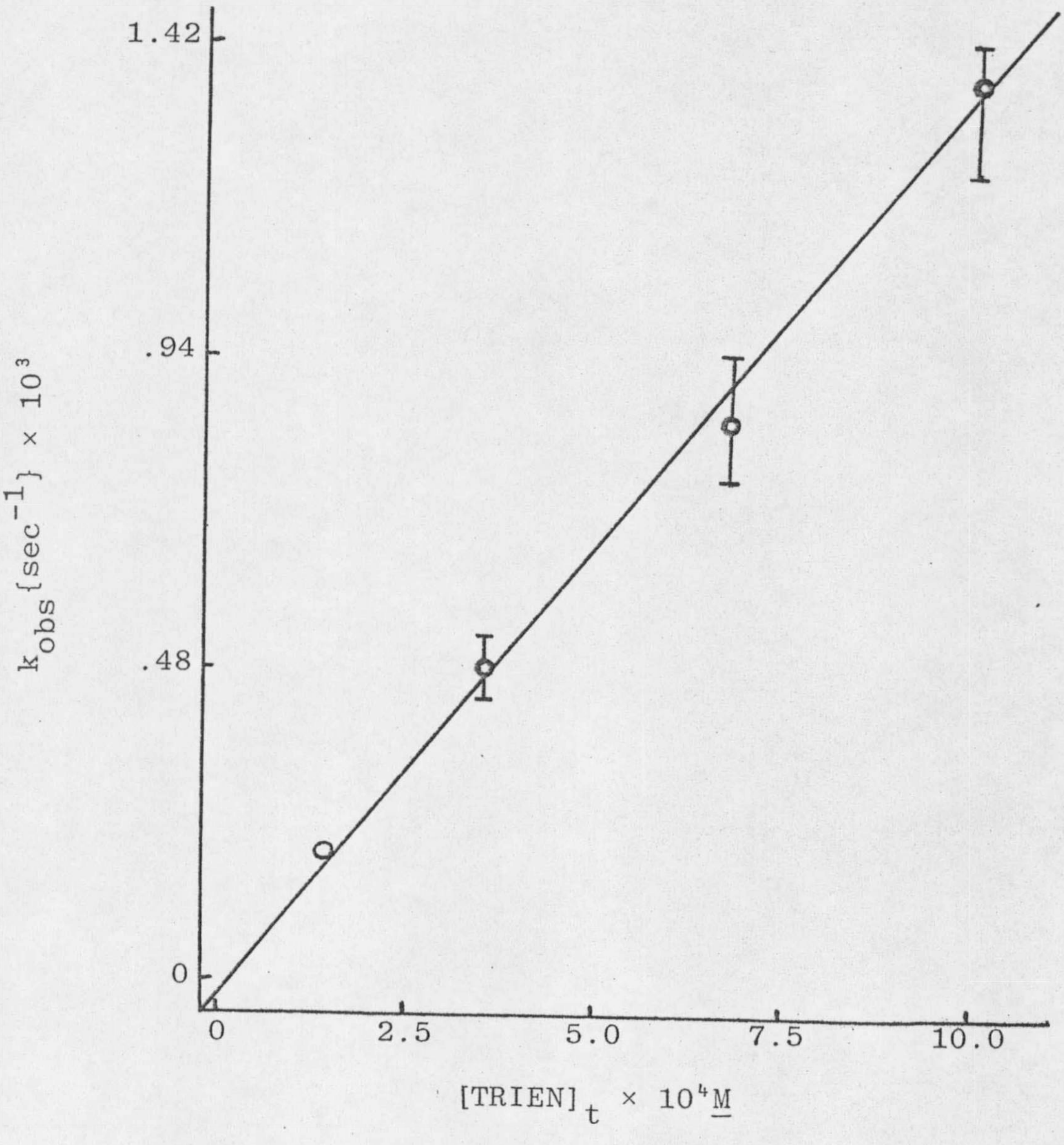

Figure 1. Pseudo First-Order Kinetics for Reaction of TRIEN with $Ni(H_{-2}GGa)CN^{1-}$. $-\log[H^+]$ + 10.10.

Table III. Concentrations of Species and k_{obs} for $Ni(H_{-2}GGa)CN^{1-}$ Reaction with TRIEN

$[Ni(H_{-2}GGa)CN^{-}] \times 10^{5}$ <u>M</u>	$-\log[H^{+}]$	$[TRIEN]_{t} \times 10^{4}$ M	$k_{obs}\{sec^{-1}\} \times 10^{3}$
1.0	10.00	1.85	2.42
1.0	10.10	3.5	4.75
0.5	10.15	3.5	4.70
1.0	10.00	6.85	8.60
1.0	10.00	10.00	13.70

k_{t_t} is defined as the rate constant for TRIEN total. It is the slope of Figure 1. k_{t_t} has a value of $13.72\underline{M}^{-1}$ sec^{-1} at $-\log[H^+] = 10.1$.

The linearity of the plot and its zero intercept, along with the fact that the rate constant, k_{obs}, does not change with halving the initial concentration of substrate demonstrate that the conditions are pseudo first order.

pH Dependence of the $Ni(H_{-2}GGa)CN^{1-}$ System

The pH of the reaction medium was lowered to 9.00 and raised to 10.60 to elucidate the relative contributions of TRIEN species. For each run, the $[TRIEN]_t$ was equal to $0.00035\underline{M}$.

TRIEN is composed of several species. An unprotonated species, trien and two protonated species; (1) monoprotonated, $htrien^{\oplus}$, and (2) diprotonated, h_2trien^{2+}. The equilibrium constants for these species were listed earlier in Table II. After reviewing the literature, the contributions of $h_2trien^{2\oplus}$ species was neglected (4,6). $h_2trien^{2\oplus}$ had a relative strength of 1/100 of the other species in these studies.

The correspondence of k_{obs} with pH for the GGa system is given in Table IV.

Table IV. Correspondence of k_{obs} with pH for $Ni(H_{-2}GGa)CN^{1-}$ System.

$-\log[H^+]$	$k_{obs}\{sec^{-1}\} \times 10^{+3}$, experimental*
8.97	1.92
9.26	2.81
9.75	4.22
10.08	4.99
10.50	5.03

* $[TRIEN]_t$ = 0.00035M; Temperature = 25°C; μ = 0.10M $NaClO_4$; $[Ni(H_{-2}GGa)CN^{1-}] = 1 \times 10^{-5}$M.

The relationship of $[TRIEN]_t$ to its species is given by equation (4).

$$(4) \qquad [TRIEN]_t = [trien] + [htrien^{\oplus}] + h_2trien^{2\oplus}$$

To resolve the relative contributions of trien and $htrien^{\oplus}$, it was necessary to derive an equation that related k_{obs} and $[H^+]$ to the various species.

From the equilibrium relationships listed in Table II for the TRIEN system, defining such terms as