A Kinetic Study of Nickel(II) Complexes of Sulfur-containing Amino Acids*

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ABSTRACT: The rate expression for the ethylenediaminetetraacetic acid (EDTA) ligand exchange reaction with nickel(cysteine)$_2^{2-}$ is: rate = k[complex] + k_{EDTA}·[complex][EDTA]. This two-term rate expression is characteristic of square planar complexes and agrees with the square planar configuration of Ni(cysteine)$_2^{2-}$ reported on the basis of spectral studies. The EDTA-independent term of the rate expression is an acid-catalyzed term and can be written k_H[H$^+$][complex]. The value of k_H is 2.5×10^5 M^{-1} sec^{-1} while the value of k_{EDTA} is only on the order of 10^{-3}–10^{-2} M^{-1} sec^{-1}. The EDTA reaction with Ni(cysteine methyl ester)$_2$ is very similar to the reaction with Ni(cysteine)$_2^{2-}$. When the sulfur-containing amino acid ligand contains a thiol ether, the kinetic differences are striking.

Ligand exchange reactions of EDTA, triethylenetetramine, and diethylenetriamine with nickel-methionine complexes are several orders of magnitude faster and give products that are mixed-ligand complexes. The results of this kinetic study of amino acid complexes containing sulfhydryl and thiol ether sulfur groups support other studies at equilibrium which suggest sulfur coordination and square planar complexes for the sulfhydryl groups and octahedral complexes with no sulfur coordination for thiol ethers.

In this study, the EDTA reactions with the nickel(II) complexes of cysteine ($HSCH_2CHNH_2COOH$), cysteine methyl ester ($HSCH_2CHNH_2COOCH_3$), and methionine ($CH_3SCH_2CH_2CHNH_2COOH$) are compared. This permits an evaluation of the presence or absence of sulfhydryl groups, thiol ethers, and carboxylate groups for possible coordination to nickel on the kinetics of the exchange reaction.

Equilibrium studies of some transition metal complexes of sulfur-containing amino acid ligands have shown that the stability of complexes formed when the sulfur is present as a sulfhydryl group is higher than when the sulfur is present as a thiol ether (White *et al.*, 1956; Albert, 1952; Lenz and Martell, 1964). The stability constants and absorption spectra of some metal complexes of methionine, where the sulfur is a thiol ether, are quite similar to metal complexes of glycine, valine, and serine (White *et al.*, 1956; Pelletier, 1960). On this basis methionine has been described as a bidentate ligand with nitrogen and carboxylate coordination. The nickel(II) complexes of methionine appear to be of the octahedral type.

On the other hand, the structure of a platinum chloro complex of methionine is reported to be square planar and to contain a sulfur bond from the thiol ether to Pt(II) in addition to a nitrogen bond (Volshtein and Mogilevkina, 1963). These authors point out that the bonding in this complex is different from that in zinc methionine where nitrogen and oxygen bonding are reported (Li and Manning, 1955). According to Hawkins and Perrin (1963), the copper(II) complexes of methionine also have metal–thiol ether bonding. However, Lenz and Martell (1964) use similar stability arguments to show that the thiol ether is not coordinated to any of nine transition metals including copper(II) and nickel(II).

The complexes of some of these ligands have been used to characterize the nature of the binding site in some metalloenzymes. The active site of zinc carboxypeptidase A is composed of zinc bonded to the sulfhydryl group of cysteine and to the α-amino group of the N-terminal asparagine residue of δ-carboxypeptidase (Coombs *et al.*, 1964). The apparent stability constants of carboxypeptidase A with seven transition metal ions correlate closely, both in sequence and magnitude, to the stability constants of model bidentate ligands containing nitrogen and sulfur groups (viz., cysteine and 2-mercaptoethylamine) (Vallee *et al.*, 1961; Coleman and Vallee, 1961).

The main reaction studied in the present work is

$$Ni(cysteine)_2^{2-} + EDTA \rightleftharpoons NiEDTA^{2-} + 2\ cysteine \quad (1)$$

as well as the similar reaction with the methyl ester of cysteine. The substitution reactions of the nickel methionine complexes with EDTA and with diethylenetriamine and triethylenetetramine are several orders of magnitude faster than the cysteine reactions and give products that are mixed-ligand complexes. In the case of the Ni(cysteine)$_2^{2-}$ reaction with EDTA the release of cysteine from nickel(II) is essentially complete.

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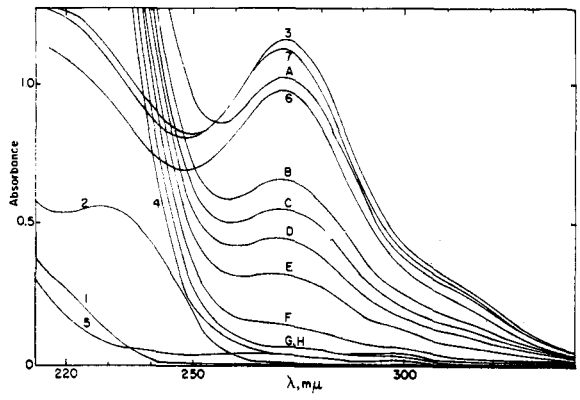

FIGURE 1: Spectral scan of the $Ni(cysteine)_2^{2-}$ and EDTA reaction. Borate buffer (*p*H 9.0) and ionic strength control in both the sample and reference cell. Cell path, 1 cm. (1) 1.0×10^{-4} M $NiEDTA^{2-}$; (2) 2.0×10^{-4} M cysteine; (3) 1.0×10^{-4} M $Ni(cysteine)_2^{2-}$; (4) 2.8×10^{-3} M EDTA; (5) 2.0×10^{-4} M cystine (prepared by air bubbling of (2) for 1 hour); (6) 1.0×10^{-4} M $Ni(cysteine)_2^{2-}$ (air bubbling for 1 hour); (7) 1.0×10^{-4} M $Ni(cysteine)_2^{2-}$ (aliquot of (3) after standing for 2 hours). Scans (A) through (H) taken at time intervals listed below for reaction of 9.3×10^{-5} M $Ni(cys)_2^{-2}$ and 2.8×10^{-3} M EDTA.

Scan	Time (sec) at 350 mμ	Time (sec) at 270 mμ
A	13	270
B	911	1069
C	1211	1370
D	1526	1685
E	2037	2195
F	3353	3512
G	5160	5318
H	7654	7812

Experimental

Reagents. L-Cysteine, L-cysteine methyl ester hydrochloride, and L-methionine were obtained in the highest purity available from Mann Research Laboratories and used without further purification. Solutions of these three amino acids were prepared daily in deaerated water by N_2 bubbling. The cysteine solutions were stored under N_2 in low actinic flasks at *p*H 5.5. Cysteine does not oxidize to cystine under these conditions (Elson and Edsall, 1962; McCormick and Gorin, 1962). $Ni(NO_3)_2$ was prepared from the reagent grade salt and standardized by EDTA titration (Welcher, 1958). Reagent grade triethylenetetramine disulfate was recrystallized twice by precipitating a hot solution of triethylenetetramine disulfate with ethanol. Triethylenetetramine was standardized by a mole-ratio plot against standard Cu(II) at 550 mμ at *p*H 5. Diethylenetriamine was purified by precipitating the hydrochloride salt by addition of an ethanol-HCl mixture (Jonassen *et al.*, 1952) and standardized by a mole-ratio plot against Cu(II) at 600 mμ. Reagent grade EDTA was standardized by titration with Cu(II) using Murexide indicator (Welcher, 1958). $Cu(NO_3)_2$ was prepared by dissolving pure copper wire in a minimum amount of hot nitric acid and standardized by electrolytic deposition (Kolthoff and Sandell, 1952). Potassium chloride, boric acid, sodium borate ($Na_2B_4O_7 \cdot 10H_2O$), and potassium hydroxide were of reagent grade quality and were used without further purification. High-purity nitrogen was passed through acidic vanadous sulfate to remove traces of oxygen. In all cases, solutions were prepared using distilled water which had been passed through a deionizing column containing Amberlite MB3 mixed-bed resin.

Apparatus. Spectral scans were taken on a Cary Model 10–11 recording spectrophotometer. Kinetic absorbance measurements were taken on a Beckman DU spectrophotometer equipped with a thermostated cell compartment. All *p*H measurements were made with a Beckman Model 1019 Research *p*H meter (±0.002 *p*H unit). A mixing system capable of mixing 15 ml of solution in less than 10 seconds was fabricated from two 10-ml syringes and a "T" junction. The reaction mixture entered a 5-cm cylindrical silica spectrophotometric cell through a hole in the cell cover. A rubber septum prevents light leakage. The per cent transmittance was recorded on a Sargent MR recorder connected to the Beckman DU spectrophotometer by an energy-recording adapter. Time for complete mixing was tested with indicator systems and was shown to be about 7 seconds.

Reaction Solutions. $Ni(cysteine)_2^{2-}$ was freshly prepared for each reaction by adding stoichiometric quantities of cysteine to nickel nitrate. The mixture was at *p*H 5 where $Ni(cysteine)_2^{2-}$ is not fully formed. The complex was then formed by the addition of borate buffer to raise the *p*H to about 9.0. The ionic strength was maintained at 0.3 M with KCl. Before being mixed, the solutions had been deaerated by nitrogen bubbling for 20 minutes. All subsequent operations except the absorbance measurements were carried out under a nitrogen atmosphere. $Ni(cysteine)_2^{2-}$ prepared in this manner gave constant and reproducible absorbances at 310 mμ for solutions prepared during a period of about 10 hours. After EDTA addition, aliquots were transferred to a covered 1-cm spectrophotometric cell. An identical procedure was employed to prepare Ni(cysteine methyl ester)$_2$. All reactions were studied at 25.0 ± 0.1°.

Nickel methionine solutions were prepared under nitrogen at *p*H 9 (borate buffer) by mixing nickel nitrate and excess methionine in order to give the 1:2 or 1:3 complexes. For the reactions with EDTA (5×10^{-5} M) or triethylenetetramine (5×10^{-5} M), the initial $[Ni^{2+}]$ was 5×10^{-5} M and [methionine] was 5×10^{-4}. At *p*H 9, the Ni(methionine)$_2$ complex was the major reactant species according to the constants given in Table I. These reactions required less than 1 minute to reach equilibrium. For the reaction of diethylenetriamine (5×10^{-5} M) in the *p*H range 8–10, the methionine concentration was 5×10^{-3} M to give th Ni-

TABLE I: Acid Dissociation Constants and Stability Constants.[a]

	Glycine[b]	L-Methionine[c]	L-Cysteine[d]	EDTA[b]	Diethylene-triamine[b]	Triethylene-tetramine[b]
			Acid Dissociation Constants			
pK_{H_4L}				1.99		3.25
pK_{H_3L}			1.96	2.69	4.34	6.55
pK_{H_2L}	2.43	2.12	8.13	6.10	9.13	9.08
pK_{HL}	9.62	9.28	10.11	10.19	9.94	9.80
			Stability Constants for Ni(II) Complexes			
$\log K_{Ni}^{NiL}$	5.86	5.67	9.64	18.56	10.7	14.0
$\log K_{Ni}^{NiL_2}$	10.64	10.26	19.04		18.9	
$\log K_{Ni}^{NiL_3}$	14.18	12.82				

[a] The constants for glycine are listed for comparison. [b] Bjerrum *et al.* (1957). [c] Pelletier (1960). [d] Lenz and Martell (1964).

(methionine)$_3^-$ complex. In these cases equilibrium was reached in 5–20 minutes but the initial reactions were much faster. All reactions of the nickel methionine complexes were studied at 255 mμ.

Product Study of the Ni(*Cysteine*)$_2^{2-}$ *and EDTA Reaction.* Cystine, an oxidation product of cysteine, has a less intense ultraviolet spectrum than cysteine (Gorin and Clary, 1960). The spectra of the reactants [Ni(cysteine)$_2^{2-}$ and EDTA] and products (NiEDTA^{2-} and cysteine) and cystine are shown in Figure 1. Curves 6 and 7 show the effect of exposure to air and air bubbling of Ni(cysteine)$_2^{2-}$, respectively, while the difference in intensity of the cysteine and cystine spectra is shown by curves 2 and 5. The change in the reaction spectrum with time is indicated by curves A through H. The molar absorptivities listed in Table II were used to characterize the chemical nature of the sulfur-containing ligand released from Ni(cysteine)$_2^{2-}$. At the concentrations used in Figure 1, the absorbance of NO_3^- at 240 mμ is negligible. If 2 moles of cysteine were released by complete reaction of Ni(cysteine)$_2^{2-}$, the calculated absorbance at 240 mμ is 1.223. If the released cysteine is oxidized to form cystine, the calculated absorbance at 240 mμ is 0.836. The final measured absorbance at 240 mμ is 0.91, indicating some oxidation of cysteine after it is released from nickel.

TABLE II: Molar Absorptivities, *p*H 9.0.

Compound	λ (mμ)	ε
Ni(methionine)$_2$	255	1020
Ni(methionine)$_3^-$	255	1200
Ni(cysteine)$_2^{2-}$	240, 310	9200, 3080[a]
Ni(cysteine methyl ester)$_2$	310	2980[a]
Ni-EDTA^{2-}	240, 255	400, 278
Ni(triethylenetetramine)$^{2+}$	255	14
Ni(diethylenetriamine)$_2^{2+}$	255	41
EDTA	240, 255	280, 98
Cysteine	240	2250
Cystine	240	300

[a] Molar absorptivity obtained for each reaction mixture (*p*H range 7.85–10.5) by measuring the absorbance of reaction mixture prior to the addition of EDTA.

An automatic potentiometric rate method for cystine using the azide-iodine reaction was used to test the reaction products for cystine content (Pardue and Shepherd, 1963). This method employs the catalytic effect of cystine on the rate of reduction of iodine to iodide by excess sodium azide. The presence of cysteine gives a much more rapid rate of reduction and the potentiometric response is very great. The system was found to respond to Ni(cysteine)$_2^{2-}$ in a manner very similar to cystine, but in the presence of free cysteine the typical intense response predominates (H. L. Pardue, W. E. Dahl, and R. A. Libby, unpublished results). Other studies indicated that the cystine and cysteine response is not altered by the presence of NiEDTA^{2-} or EDTA in the presence of KCl and borate buffer. The response of the reaction product indicated that it is a mixture of cysteine and cystine. This agrees with the results of the spectrophotometric study, which show a mixture of cysteine and cystine, and the report that the ultraviolet fading of 10^{-4} M cysteine in deaerated alkaline medium is significant after 2 minutes (Gorin and Clary, 1960). Thus the oxidation of cysteine released from Ni(cysteine)$_2^{2-}$ is expected, but at a slower rate because the EDTA removes trace metal impurities known to catalyze the cysteine oxidation (Tarbell, 1961).

The effect of aeration of Ni(cysteine)$_2^{2-}$ is shown in Figure 1. Solutions of Ni(cysteine)$_2^{2-}$ are much more stable in the presence of air and light than is cysteine itself, with only a few per cent decomposition of the complex in 30 minutes. Thus Ni(II) stabilizes cysteine with respect to oxidation while the Fe(III) and Co(III) cysteine complexes are reported to catalyze the oxidation (Tarbell, 1961).

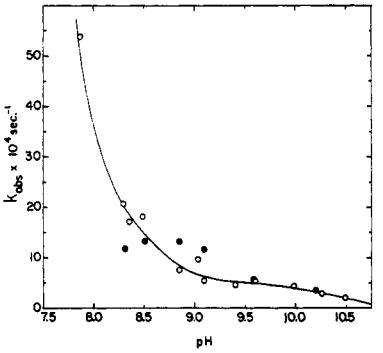

FIGURE 2: *p*H profile of observed first-order rate constants for the Ni(cysteine)$_2^{2-}$ and Ni(cysteine methyl ester)$_2$ reaction with EDTA. [EDTA] = 0.02 M. ○ = Ni(cysteine)$_2^{2-}$, ● = Ni(cysteine methyl ester)$_2$. T = 25°, μ = 0.3. Curve drawn to fit only Ni(cysteine)$_2^{2-}$ data.

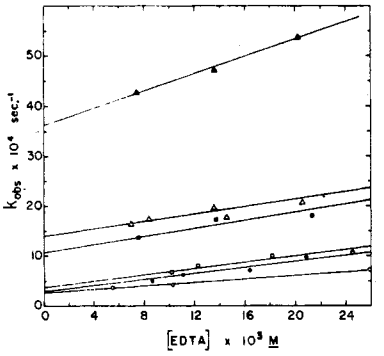

FIGURE 3: Plot of the observed first-order rate constant as a function of [EDTA]. T = 25°, μ = 0.3. ▲ = *p*H 7.85, △ = *p*H 8.26–8.31, ■ = *p*H 8.50, □ = *p*H 8.78–8.89, ● = *p*H 9.04–9.10, and ○ = *p*H 9.33–9.44.

Calculations. The concentration of Ni(cysteine)$_2^{2-}$ as a function of time was calculated from the molar absorptivity (see Table II) and the measured absorbance. Under conditions of 50-fold excess EDTA, linear plots were obtained by using the pseudo-first-order rate expression

$$\frac{-d[\text{Ni(cysteine)}_2^{2-}]}{dt} = k_{obs}[\text{Ni(cysteine)}_2^{2-}] \quad (2)$$

Results

Order Determination. The reaction of Ni(cysteine)$_2^{2-}$ was studied at 310 mμ in the presence of excess EDTA in the *p*H range 7.85–9.44 at 25°, μ = 0.30. The *p*H profile of the observed first-order rate constant for reactions of Ni(cysteine)$_2^{2-}$ and Ni(cysteine methyl ester)$_2$ with 0.02 M EDTA is shown in Figure 2. The graph of the observed first-order rate constant of Ni(cysteine)$_2^{2-}$ reactions as a function of EDTA is shown in Figure 3, where a first-order EDTA dependence is shown by the linear plots. The nonzero intercept of Figure 3 shows that the rate expression must include an EDTA-independent term. The slopes of Figure 3, which give the second-order rate constant for the EDTA-dependent term, and the intercepts are both *p*H dependent.

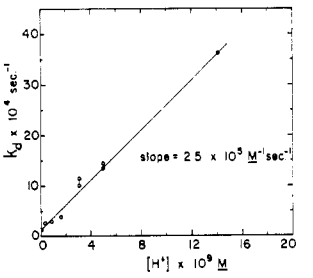

FIGURE 4: First-order [H$^+$] dependence of the EDTA-independent term for Ni(cysteine)$_2^{2-}$. The intercepts of Figure 3 give the k_d values which are shown here as a function of [H$^+$]. T = 25°, μ = 0.3.

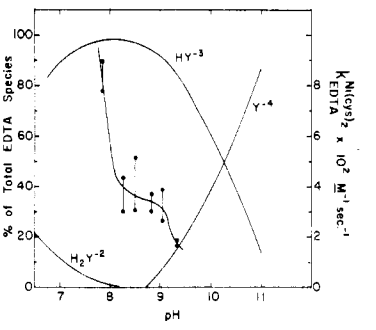

FIGURE 5: *p*H profile of $k_{EDTA}^{Ni(Cys)_2}$. T = 25°, μ = 0.3. The values of $k_{EDTA}^{Ni(Cys)_2}$ are taken from Figure 3 where the uncertainties shown here indicate the possible values of the slopes from Figure 3. The *p*H distribution of the EDTA species is also shown.

Thus the observed rate constant can be expressed in terms of equation (3):

$$k_{obs} = k_d + k_{EDTA}^{Ni(Cys)_2^{2-}}[\text{EDTA}] \quad (3)$$

where k_d is the first-order dissociation rate constant of the Ni(cysteine)$_2^{2-}$ complex and $k_{EDTA}^{Ni(Cys)_2^{2-}}$ is the second-order EDTA-dependent rate constant. A plot of k_d against [H$^+$] in Figure 4 shows that the EDTA-independent rate constant has a first-order dependence on [H$^+$]

$$k_d = k' + k_H^{Ni(Cys)_2}[H^+] \quad (4)$$

where $k_H^{Ni(Cys)_2}$ is the rate constant for H$^+$ reaction with the complex and has a value of 2.5 × 10^5 M^{-1} sec^{-1}. The *p*H profile of $k_{EDTA}^{Ni(Cys)_2}$ is shown in Figure 5, which also includes the *p*H distribution of the protonated EDTA species.

Reactions of nickel methionine with diethylenetri-

amine, triethylenetetramine, and EDTA were very fast. Low concentrations (5×10^{-5} M) of the complex and displacing ligand were used with the special mixing apparatus. In all cases, the absorbance changes at 255 mμ were rapid and did not show complete methionine displacement.

The molar absorptivities of nickel-methionine and NiEDTA, Ni-triethylenetetramine, and Ni(diethylenetriamine)$_2$ in Table II show that a large absorbance change is expected for the exchange reactions. The stability constants of these compounds in Table I predict an essentially complete reaction if no mixed complexes are formed. However, the observed absorbance changes were only about 50% of that expected. Although the products were not identified, a preliminary study indicated more than one mixed-ligand complex. It is believed that one or more of the methionine ligands are displaced in the reactions. The EDTA reaction must be at least 100 times faster with Ni(methionine)$_2$ than is the case with Ni(cysteine)$_2^{2-}$.

Discussion

The similarity in the acid-dissociation constants and the stability constants with Ni(II) of glycine and methionine shown in Table I is one indication that methionine functions as a bidentate ligand having nitrogen and oxygen coordination. Conductance studies by Pelletier (1960) have shown that methionine forms three complexes with Ni(II) corresponding to Ni(methionine), Ni(methionine)$_2$, and Ni(methionine)$_3$. Spectrophotometric studies have shown that the near-ultraviolet and visible spectra of nickel-methionine complexes are identical with the spectra of nickel-valine and nickel-serine complexes (Pelletier, 1960). These facts also suggest that methionine acts as a bidentate ligand and forms octahedral complexes with Ni(II).

The reactions observed for nickel-methionine complexes with EDTA, triethylenetetramine, and diethylenetriamine indicate the formation of stable mixed-ligand intermediates. Similar mixed-ligand complexes are known to form with other octahedral nickel species (Israeli, 1963a,b, 1964; Watters and DeWitt, 1960).

The relatively fast reactions of nickel methionine are similar to those of Ni-triethylenetetramine^{2+} and EDTA (Rorabacher and Margerum, 1964) and other nickel complexes under investigation in these laboratories. These reactions are typical of octahedral substitution. Thus there is no indication that the thiol ether group present in methionine alters the usual kinetic behavior expected of amine and carboxylate chelation with nickel.

The kinetic behavior reported for the Pt(II) complexes of methionine (Volshtein and Mogilevkina, 1963) is quite different from that observed with Ni(II). This appears to demonstrate that the thiol ether does bond to Pt(II) and gives a square planar complex, while it does not bond to Ni(II).

Nickel-Cysteine Reactions. The absorption maximum near 270 mμ for Ni(cysteine)$_2^{2-}$ (see Figure 1) corresponds to that reported for Co(II)-cysteine complexes (McCormick and Gorin, 1962) and Pb(II)-aldose-cysteinate complexes (Takahashi and Mizuguchi, 1962). The absorption maxima in the region near 280 mμ is reported as a spectrophotometric method for determining disulfides, sulfinic acid, thio ethers, and thiols (Åkerfeldt and Lövgren, 1964). This absorbance peak near 275 mμ for metal-sulfur complexes is thought to be characteristic of metal-sulfur bonds (Takahashi and Mizuguchi, 1962).

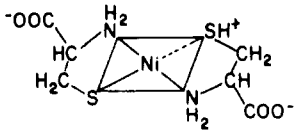

FIGURE 6: H_2O is believed coordinated above and below the planar structure.

When cysteine is coordinately bonded to Ni(II), the oxidative effect of aeration is greatly diminished (see Figure 1). This is supporting evidence of a Ni-sulfur coordinate bond which stabilizes the sulfhydryl group with respect to the oxidation of free RS^- ions to form cystine.

The rate of cysteine oxidation is reported to be quite fast (Gorin and Clary, 1960) and to increase as the *p*H is increased (Mathews and Walker, 1909; Fruton and Clarke, 1934; Garfinkel and Edsall, 1958; Benesch and Benesch, 1955). Since cystine was found as a product of the reaction in the study and the *p*H effect on the observed rate constant is opposite to that which would be observed if cysteine oxidation were rate limiting, it appears that the oxidation of cysteine and the oxidation of Ni(cysteine)$_2^{2-}$ are not important in the kinetics of the Ni(cysteine)$_2^{2-}$ reaction with EDTA.

The kinetics of the EDTA reaction with Ni(cysteine)$_2^{2-}$ gives a two-term rate expression characteristic of square planar complexes (Basolo and Pearson, 1958; Murmann, 1963). One term is the dissociation rate constant of the complex which itself is *p*H dependent, but independent of EDTA. The other term is first order in EDTA (see equation 3 and Figure 3) and also is *p*H dependent. The dissociation rate constant of Ni(cysteine)$_2^{2-}$ is shown in Figure 4 to have a $[H^+]$ dependence as given in equation (4). Two mechanisms can be proposed to account for the $k_H^{Ni(Cys)_2}$ term.

The nickel (cysteine)$_2^{2-}$ complex might react directly with H^+ through one electron pair of the coordinated sulfur (Figure 6). This could weaken the sulfur bond to nickel and could lead to the acid-catalyzed dissociation. Once the first sulfur-to-nickel bond is broken it can be assumed that the sluggish nature of the square planar complex is destroyed and that subsequent dissociation reactions or reactions with EDTA are rapid. The main drawback to this mechanism is that the basicity of the coordinated sulfur group is expected to be weak.

(2) The nickel(cysteine)$_2^{2-}$ complex might first break one of the chelate rings to give a reaction intermediate that has a basic group. The addition of a proton to this

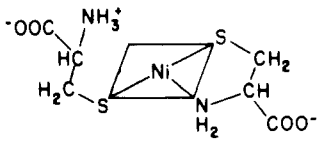

FIGURE 7: Intermediate AH^-. H_2O is believed coordinated above and below the planar structure.

intermediate can block the re-formation of the complex and thus accelerates the subsequent rate-determining dissociation steps. The reaction can be described as

$$Ni(cysteine)_2^{2-} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} (A^{2-}) \quad (5)$$

$$(A^{2-}) + H^+ \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} (AH^-) \quad (6)$$

$$(AH^-) \xrightarrow{k_3} \text{products} \quad (7)$$

A steady-state treatment of this mechanism with A^{2-} and AH^- as unstable reaction intermediates gives

$$\text{rate} = \frac{k_1k_2k_3[H^+][Ni(cysteine)_2^{2-}]}{k_{-1}k_3 + k_2k_3[H^+] + k_{-1}k_{-2}} \quad (8)$$

The proton-transfer rate constant, k_{-2}, can be expected to be much greater than the Ni-dissociation step in k_3. Furthermore, the re-formation rate constant k_{-1} can be expected to be much greater than the dissociation step in k_3. Thus, $k_{-1}k_{-2} >> k_{-1}k_3 + k_2k_3[H^+]$ and

$$\text{rate} = \frac{k_1k_2k_3[H^+][Ni(cysteine)_2^{2-}]}{k_{-1}k_{-2}} \quad (9)$$

The intermediate, AH^-, is shown in Figure 7 with a Ni-nitrogen bond broken.

The contribution of sulfur bonding has been shown to increase the stability constant more than 4 log units based on the difference in stabilities of the Ni(II) complexes with glycine and cysteine (White *et al.*, 1956). The stability constant for a Ni-nitrogen bond is about 3 log units. On the basis that the dissociation rate constant is inversely proportional to the stability (Wilkins, 1964), it would be expected that the less stable Ni-nitrogen bond will dissociate more rapidly than the Ni-sulfur bond.

EDTA-dependent Term. EDTA does appear to react directly with $Ni(cysteine)_2^{2-}$ but it is not very effective kinetically as a displacing ligand; the value of $k_{EDTA}^{Ni(Cys)_2}$ is quite small compared to $k_H^{Ni(Cys)_2}$. The fact that $k_{EDTA}^{Ni(Cys)_2}$ is itself quite acid dependent suggests the possibility that H_2Y^{2-} and HY^{3-} might be serving as acid catalysts. Unprotonated EDTA is abbreviated Y^{4-}. However, this seems unlikely because the rate of proton transfer from both water and from the boric acid–borate buffer can be shown to be sufficiently large (Eigen, 1963, 1964) to rule out specific H^+ transfer from the EDTA species. Furthermore, a change in the borate buffer concentration from 0.02 M to 0.05 M at *p*H 8.8 had no effect on the observed rate of reaction of $Ni(cysteine)_2^{2-}$ with EDTA. Therefore, it is postulated that the EDTA ion is involved in coordination with nickel prior to the rate-determining step.

The rate constant curve for $k_{EDTA}^{Ni(Cys)_2}$ is compared with the EDTA species as a function of *p*H in Figure 5. The rate constant curve can be fitted qualitatively to the pH decrease first to the appearance of HY^{3-} and then to the appearance of H_2Y^{2-}. Higher electrostatic repulsion of Y^{4-} compared to HY^{3-} and to H_2Y^{2-} may account for the decrease in rate constant with increasing *p*H. However, an intermediate involving both nickel coordination to EDTA and a proton transfer from EDTA also is possible.

Similar EDTA reactions with other square planar nickel complexes have been found (Murmann, 1963). The rate expressions were the same as in equation (3) and the EDTA-dependent term also decreased with increasing *p*H. In these amine oxime complexes, EDTA bonding to the fifth coordination position above the plane of the original complex was proposed. This is also possible with $Ni(cysteine)_2^{2-}$. It is interesting to note the similar *p*H dependence of the EDTA reaction in both cases. However, the reasons given by Murmann (1963) for the H^+ dependence are based on a hydrogen bridge structure of the amine oxime which is not present in the cysteine complex.

Reactions of Ni(*Cysteine Methyl Ester*)$_2$. Ni(cysteine methyl ester)$_2$ was studied at conditions identical with those of the EDTA reactions with $Ni(cysteine)_2^{2-}$ in order to examine the kinetic effect of eliminating the carboxylate portion of the cysteine ligand. The general kinetic behavior was very similar to that of cysteine.

The reported stability constants for Ni(II) complexes of cysteine methyl ester are log K_1 = 8.95 for Ni(cysteine methyl ester) and log K_2 = 8.45 for Ni(cysteine methyl ester)$_2$ (White *et al.*, 1956). These stability constants are about 1 log unit lower than the stability constants for comparable cysteine complexes with Ni(II). An estimate of the stability constant of a Ni—OOC bond (and chelate ring formation) based on the difference in stability of Ni-iminodiacetic acid (log K_1 = 8.26) and Ni-glycine (log K_1 = 5.86) (Bjerrum *et al.*, 1957) shows that when a Ni—OOC bond is formed the stability is increased by about 2.4 log units. Thus it appears that although the carboxylate group of cysteine does contribute slightly to the stability of $Ni(cysteine)_2^{2-}$ it is not as much as is predicted from the stability of forming a Ni—OOC bond. The small stability difference could be due to electrostatic activity.

It has been shown that $Ni(cysteine)_2^{2-}$ and Ni(cysteine methyl ester)$_2$ have similar visible absorption spectra at slightly different *p*H values (White *et al.*, 1956). Thus it appears that the contribution of carboxylate bonding in $Ni(cysteine)_2^{2-}$ is small, based on both equilibrium and spectrophotometric studies. If the Ni—OOC bond in $Ni(cysteine)_2^{2-}$ were of higher stability, it is possible that the Ni(II) complex of cysteine would have an octahedral structure to give different stability and spectra.

The kinetic study of Ni(cysteine methyl ester)$_2$ also suggests that any contribution from Ni—OOC bonding in Ni(cysteine)$_2^{2-}$ is quite weak. It is proposed that the slight differences in the observed rate constant shown in Figure 2 can be attributed to the different electrostatic attraction of H^+ and EDTA to Ni(cysteine methyl ester)$_2$ and Ni(cysteine)$_2^{2-}$.

If the proposed mechanism is correct, it is thought that substitutions on the ethylenic linkage separating the nitrogen and sulfur atoms in cysteine could modify the rate by blocking the axial positions of Ni(cysteine)$_2^{2-}$ or by changing the basicities of the nitrogen and sulfur atoms. Other ligands that can add to the axial positions of Ni(cysteine)$_2^{2-}$ and give thermodynamically favored products without forming mixed complexes should also displace cysteine from Ni(cysteine)$_2^{2-}$. Murmann (1963) has shown that other chelating ligands can catalyze the reaction rate of Ni-α-amine oxime complexes.

Conclusions

The kinetic behavior of the EDTA reactions with the nickel complexes of cysteine, cysteine methyl ester, and methionine are consistent with structures which propose sulfur and nitrogen bonding for cysteine and oxygen, and nitrogen bonding for methionine. Cysteine and its methyl ester form nickel complexes which have square planar characteristics both spectrally and kinetically. Thus the characteristic rate expression is found: rate = k[complex] + k_{ligand}[complex][ligand]. The presence or absence of a free carboxylate group makes little difference in the reaction of these complexes with EDTA. On the other hand, when sulfur is present as a thiol ether in methionine, the reaction of the nickel complex with EDTA is much faster and has the kinetic characteristics of octahedral nickel complexes containing only carboxylate and nitrogen coordination.

The hydrogen ion rate constant, $k_{\text{H}}^{\text{Ni(Cys)}_2}$, is about 3×10^5 M^{-1} sec^{-1} while the values for $k_{\text{ETDA}}^{\text{Ni(Cys)}_2}$ are only in the range of 2×10^{-3} M^{-1} sec^{-1} (pH 9.5) to 8×10^{-3} M^{-1} sec^{-1} (pH 8). This very high ratio of $k_{\text{H}}/k_{\text{EDTA}}$ is in marked contrast to the corresponding reactions with nickel triethylenetetramine (Rorabacher and Margerum, 1964).

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