Rate Controlling Chelate Ring Closing in the Complexing of Nickel(II) by Picolinic and Fusaric Acids

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Abstract: The kinetics of complexing of nickel(II) by picolinic acid (pyridine-2-carboxylic acid) and fusaric acid (5-butylpicolinic acid) have been studied in the pH range 2.6–6.7 at 25° in 0.5 M $NaClO_4$. The pH dependence of the rates is not a linear function of $(H^+)^{-1}$, but can be accounted for by a reaction scheme in which initial complexing occurs at the carboxylate group and chelate ring closing is an important kinetic factor. Extension of the same reaction scheme to simple α-amino acids reveals that, if the correct microacid dissociation constant is used, rate differences can be rationalized on the basis of a steric effect of α-substituents on the dissociation of the initially formed carboxylate complex.

The complexing of a metal ion (M) by a simple amino acid ($^-$O–N, where $^-$O and N represent the carboxylate and amino portions, respectively) may be described by the reaction scheme (1) where electrostatic attraction is assumed to make the carboxylate group complex first in the anion. Previous analyses[1,2] of this scheme have shown that, if

$$\begin{array}{ccccc} M + {}^-O\text{–}\overset{+}{N}H & \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} & M\text{–}O\text{–}\overset{+}{N}H & & \\ \quad K_1 \Big\Vert H^+ & & \quad K_1' \Big\Vert H^+ & & \\ M + {}^-O\text{–}N & \underset{k_{34}}{\overset{k_{43}}{\rightleftharpoons}} & M\text{–}O\text{–}N & \xrightarrow{k_{35}} & M\!<\!\begin{matrix}O\\ \\ N\end{matrix}\!> \end{array} \qquad (1)$$

$$-\frac{d[M]}{dt} = k_{obsd}[M][\text{total amino acid}] \qquad (2)$$

then

$$\frac{k_{obsd}(K_1 + (H^+))}{(H^+)} = \frac{(k_{12} + k_{43}K_1(H^+)^{-1})k_{35}K_1'}{k_{21}(H^+) + K_1'(k_{34} + k_{35})} \qquad (3)$$

For most simple amino acids (for which $pK_1 \gtrsim 8$) reacting with nickel(II) under the usual experimental conditions of pH $\leq$7, $Ni^{2+} \leq 0.3$ M, it was found[2] to be most probable that $k_{21}(H^+) \gg K_1'(k_{34} + k_{35})$ and consequently $k_{21}(H^+) \gg K_1'k_{34}$. Since $(k_{43}/k_{34}) = (k_{12}K_1'/k_{21}K_1)$ it is readily shown that

$$k_{21}(H^+)\left(\frac{k_{43}K_1}{(H^+)}\right) = k_{12}(K_1'k_{34}) \qquad (4)$$

This equation and the previous inequality can be satisfied only if $k_{12} \gg k_{43}K_1/(H^+)$. Then eq 3 simplifies to

$$\frac{k_{obsd}(K_1 + (H^+))}{(H^+)} = \frac{k_{12}K_1'}{k_{21}(H^+)}k_{35} \qquad (5)$$

This is consistent with the experimental rate law generally found for these systems. The implication of eq 5 is that chelate ring closure (k_{35}) is rate controlling in these reactions under the conditions specified and often used. This conclusion rests on the inequalities used to obtain eq 5, which in turn depend on values of k_{34}, k_{35}, k_{21}, and K_1' for which only reasonable guesses can be made.

Previously,[3] comparisons of the rate constants for reaction of a α- and β-alanine have indicated rate controlling ring closure for the latter with cobalt(II) and manganese(II). Analysis in terms of eq 5 confirms these conclusions in that, other factors being equal, k_{35} may be smaller when the larger chelate ring is formed with β-alanine. It should be emphasized that eq 5 implies that ring closure will be important for any simple amino acid, with $pK_1 > 8$, even in the much less labile nickel(II) system.

In order to better test the scheme outlined in eq 1 and to evaluate some of the assumptions used to obtain eq 5, it would be desirable to study systems in which eq 3 does not simplify to eq 5. Systems must be used for which (under normal experimental conditions of pH $\lesssim$7) $k_{21}(H^+) \approx K_1'(k_{34} + k_{35})$. A consideration of the magnitudes of the rate and equilibrium constants indicates that this approximate equality might be attained if $pK_1 \approx 6$. No simple amino acids have such a low pK, therefore two pyridine carboxylic acids, picolinic (pyridine 2-carboxylate) and fusaric (5-butylpicolinic) acids have been chosen for study. The picolinic acid system was studied earlier by Cassatt and Wilkins,[4] and their results were reanalyzed recently in terms of eq 3.[2] Unfortunately the previous study,[4] although over a wide pH range, was done in too large steps of pH to clearly show if the full form of eq 3 had to be used. In the present work the complexing of nickel(II) by picolinic and fusaric acids has been studied more intensively and it is shown that the complete form of eq 3 must be applied. From the results values of k_{34}/k_{35}, k_{43}, and k_{12} have been determined.

Experimental Section

Materials. The picolinic acid (Eastman Organic Chemicals) was recrystallized twice from 95% ethanol. Fusaric (5-butylpicolinic) acid was used as supplied (Sigma Chemical Co.).

Stock buffer solutions (0.125 M) were prepared from the following chemicals as supplied: sodium formate (Fisher), chloroacetic acid (Fisher), sodium acetate (Fisher), 2-(*N*-morpholino)ethanesulfonic acid (MES) (Pierce). Because pivalic acid (Eastman) is sparingly soluble in water the sodium salt was prepared by adding a stoichiometric amount of NaOH to a suspension of the acid in water and the product was precipitated by the addition of acetone. An integrated NMR spectrum of the sodium salt in anhydrous Me_2SO-d_6 indicated 4.5 mol of water per mole of sodium pivalate. This product was used to prepare the stock buffer solution.

Aqueous nickel(II) perchlorate was prepared by mixing reagent grade nickel carbonate (Baker and Adamson) with perchloric acid. The nickel(II) concentration was determined by EDTA titration with murexide indicator.[5]

Kinetic Measurements. The transmittance change during reaction was monitored on a standard Aminco-Morrow stopped flow system at 265 nm for picolinic acid and at 240 nm (pH $\geq$5.53) or 274 nm (pH $\leq$5.39) for fusaric acid. All reactions were carried out in 0.50 M sodium perchlorate and the effects of buffer ($\sim 10^{-2}$ M) and metal ion ($< 5 \times 10^{-2}$ M) on ionic strength were neglected. All runs were done with a tenfold molar excess of Ni^{2+} over ligand to maintain pseudo-first-order conditions. The logarithm of the absorbance change vs. time data for each run was analyzed by a simple least-squares program to give the apparent pseudo-first-order rate constant (k_O), from which the second-order rate constant $k_{obsd} = k_O[Ni^{2+}]^{-1}$ was evaluated. Each k_{obsd} value in Table I is the average of eight–ten runs for each set of concentrations.

The pH values reported in Table I are the average of values before and after mixing as measured on a Metrohm E 300-B pH

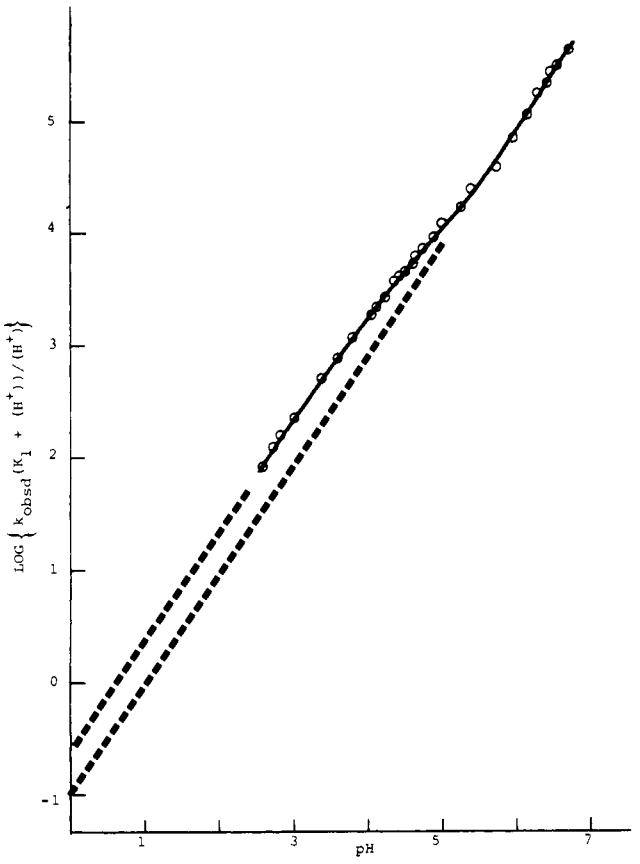


Figure 1. Variation of log $\{k_{obsd}(K_1 + (H^+))/(H^+)\}$ with pH for the complexing of nickel(II) by picolinic acid.

meter with a 2 pH unit full scale expansion. The pH difference before and after mixing was generally <0.05 units.

The temperature of reactants in the drive syringes of the stopped-flow system was controlled by a standard water circulating system. The temperature was constantly monitored with a digital thermocouple thermometer and found to have a long term value of 25 ± 0.2°.

Determination of Acid Dissociation Constants. The values of the pK_a for picolinic and fusaric acid in 0.50 M $NaClO_4$ were determined by pH titration at 25 ± 0.2°. A Beckman Expandomatic pH meter with a 2 pH unit full scale expansion was used. The pK_a values were measured from the half-neutralization points and found to the 5.03 ± 0.04 for picolinic acid and 5.64 ± 0.04 for fusaric acid.

Results

The experimental values of k_{obsd} for picolinic and fusaric acids are given in Table I. In the case of picolinic acid the results of this study are in reasonable agreement with those of Cassatt and Wilkins,[4] with differences being <20%.

The purpose of this study is to determine if it is necessary to use eq 3 to describe the pH dependence of k_{obsd}, or if some simplified form of eq 3 will suffice. One such form is given by eq 5 which predicts a simple $(H^+)^{-1}$ dependence of $k_{obsd}(K_1 + (H^+))(H^+)^{-1}$. Another form which has been used previously is based on the assumption that $k_{21}(H^+) < K_1'(k_{34} + k_{35})$, and $k_{35} > k_{34}$, in which case eq 3 reduces to

$$\frac{k_{obsd}(K_1 + (H^+))}{(H^+)} = k_{12} + \frac{k_{43}K_1}{(H^+)} \quad (6)$$

As already discussed[2] the approximations leading to eq 6 do not appear to be realistic, but this equation has been widely used, and was used by Cassatt and Wilkins[4] to analyze their results for picolinic acid.

From the point of view of presenting the results it is convenient to discuss the expected behavior of a plot of log $\{k_{obsd}(K_1 + (H^+))(H^+)^{-1}\}$ vs. pH (Figure 1). Clearly eq 5 predicts that such a plot should be linear with unit slope. The results in Figure 1 do not conform to this prediction in that the plot is not linear and the best straight line would not have unit slope.

On the other hand eq 6 predicts that the plot in Figure 1 should be linear and of unit slope at high pH and level off to zero slope and a constant value of log k_{12} at low pH. There is no sign of such leveling in Figure 1.

The behavior expected from eq 3 can be described best in terms of the limiting forms of this equation. At low pH the following limiting conditions can be expected, $k_{12} \gg k_{43}K_1(H^+)^{-1}$; $k_{21}(H^+) \gg K_1'(k_{34} + k_{35})$ so that eq 3 reduces to

$$\frac{k_{obsd}(K_1 + (H^+))}{(H^+)} = \frac{k_{12}k_{35}K_1'}{k_{21}(H^+)} = \frac{k_{43}k_{35}K_1}{k_{34}(H^+)} \quad (7)$$

At high pH the opposite limiting conditions could be revealed and eq 3 reduces to

$$\frac{k_{obsd}(K_1 + (H^+))}{(H^+)} = \frac{k_{43}k_{35}K_1}{(k_{34} + k_{35})(H^+)} \quad (8)$$

Table I. Kinetic Results for Complexing of Nickel(II) by Picolinic and Fusaric Acid (25°, 0.5 M $NaClO_4$)

Ligand	$10^2[Ni^{2+}]$, M	pH	$10^{-3}k_{obsd}$, M^{-1} s^{-1} Exptl	Calcd
	5.00	2.58	0.0858	0.0868
Pico-	5.00	2.71	0.121	0.116
linic	5.00	2.83	0.151	0.151
acid	5.00	3.01	0.222	0.225
	1.00	3.37	0.479	0.484
	1.00	3.58	0.727	0.739
	1.00	3.77	1.11	1.07
	0.500	4.06	1.71	1.82
	0.500	4.12	1.97	2.02
	0.500	4.23	2.38	2.45
	0.500	4.35	3.30	2.99
	0.500	4.41	3.50	3.31
	0.500	4.49	3.88	3.76
	0.500	4.60	4.30	4.44
	0.500	4.63	4.80	4.65
	0.500	4.73	5.44	5.36
	0.500	4.87	6.48	6.44
	0.500	5.02	7.68	7.66
	0.500	5.24	8.40	9.43
	0.500	5.39	10.3	10.5
	0.100	5.96	11.8	13.3
	0.100	6.15	13.2	13.8
	0.100	6.29	15.0	14.1
	0.100	6.37	16.4	14.2
	0.100	6.41	14.3	14.3
	0.200	6.46	14.3	14.3
	0.100	6.54	15.9	14.4
	0.100	6.71	14.8	14.5
Fusaric	0.500	3.27	0.144	0.145
acid	0.500	3.42	0.208	0.203
	0.500	3.64	0.332	0.331
	0.500	3.85	0.532	0.522
	0.500	4.09	0.830	0.862
	0.500	4.23	1.10	1.14
	0.500	4.40	1.58	1.59
	0.500	4.57	2.22	2.17
	0.250	4.86	3.77	3.55
	0.250	5.03	4.84	4.63
	0.250	5.19	5.96	5.84
	0.250	5.39	7.32	7.60
	0.200	5.53	8.10	8.95
	0.200	5.74	10.8	11.0
	0.200	5.94	12.8	12.9
	0.200	6.13	14.5	14.4
	0.200	6.35	16.3	15.7
	0.200	6.57	17.6	16.7

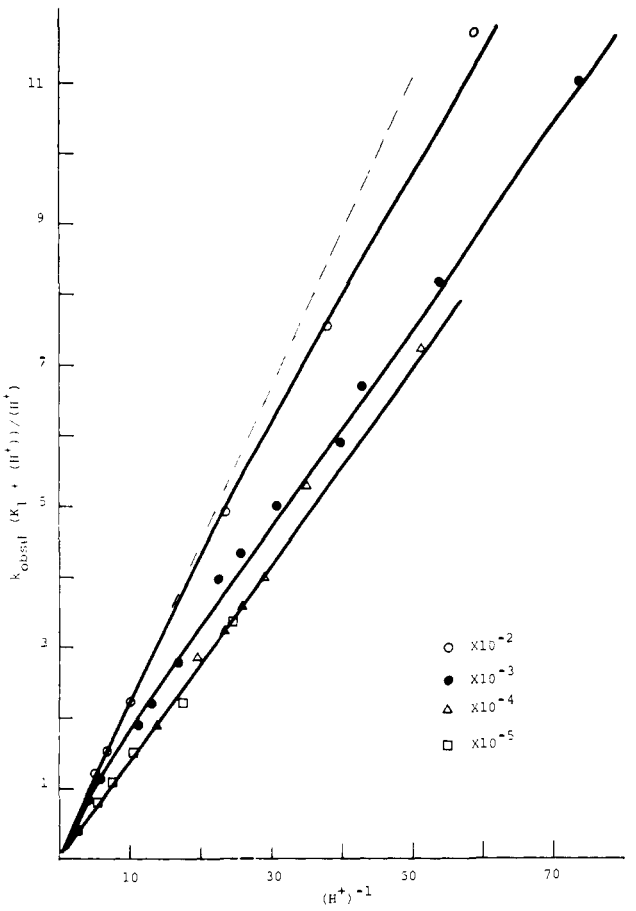


Figure 2. Variation of $k_{obsd}(K_1 + (H^+))/(H^+)$ with $(H^+)^{-1}$ for the complexing of nickel(II) by picolinic acid. Both ordinate and abscissa have been multiplied by the factor indicated for each set of points. The dashed line represents the calculated low pH limit.

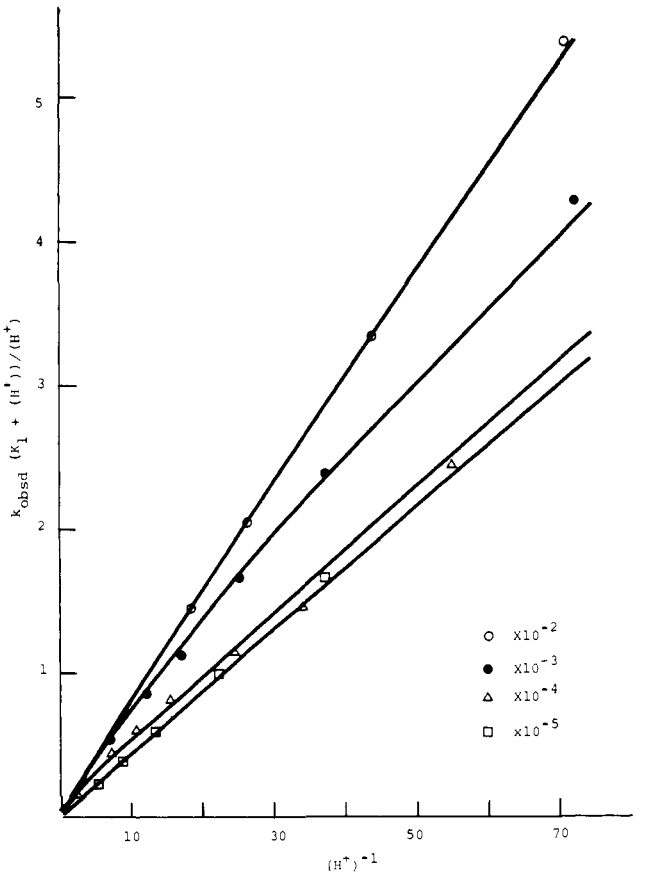


Figure 3. Variation of $k_{obsd}(K_1 + (H^+))/(H^+)$ with $(H^+)^{-1}$ for the complexing of nickel(II) by fusaric acid. Both ordinate and abscissa have been multiplied by the factor indicated for each set of points.

Both eq 7 and eq 8 predict a linear plot of unit slope at each pH limit in Figure 1. However, eq 7 predicts an intercept of log $(k_{43}k_{35}K_1/k_{34})$ from the low pH data, while eq 8 predicts an intercept of log $(k_{43}k_{35}K_1/(k_{34} + k_{35}))$ from the high pH data. All of these predictions are consistent with the results in Figure 1. The data are described by two straight lines of unit slope (extrapolated dotted lines on Figure 1) with a transition between the two limiting regions between pH 4 and 5.

The fact that the slope of a simple $(H^+)^{-1}$ plot is not constant is more easily seen from Figures 2 and 3. This type of presentation gives a plot in terms of the usual variables, rather than their logarithms, but also allows data over a wide pH range to be presented in one figure. The ordinate and abscissa are simultaneously increased by a constant factor, ten in this case. Therefore, if a line of constant slope represented all the results, then only one line with that slope should be needed on Figures 2 and 3.

It is clear that the slope of any line describing the data in Figures 2 and 3 is not constant but decreases as the pH increases. This behavior is not consistent with either eq 5 or 6 but is predicted by eq 3. The low pH limiting slope is attained at higher pH by fusaric acid as expected because of its larger pK_1.

This qualitative analysis shows that the complete form of eq 3, or its mathematical equivalent, must be used to explain the pH dependence of k_{obsd} for picolinic and fusaric acids. In order to extract specific rate constants it is convenient to rearrange eq 3 by dividing numerator and denominator by $k_{35}K_1'$, and to note that $(k_{21}/K_1') = (k_{34}k_{12}/k_{43}K_1)$, to obtain

Table II. Summary of Results (25°, 0.50 M $NaClO_4$)

	Picolinic acid	Fusaric acid
10^6K_1, M	9.33 ± 0.01	2.29 ± 0.1
$10^{-3}k_{12}$, M^{-1} s^{-1}	4.19 ± 2	8.06 ± 3
$10^{-4}k_{43}$, M^{-1} s^{-1}	3.63 ± 0.8	3.93 ± 0.8
k_{34}/k_{35}	1.45 ± 0.3	1.13 ± 0.1
10^2k_{53}, s^{-1}	1.14[a]	

[a] Rate constant for chelate ring opening; calculated from the formation constant of 2.19×10^6 (25, 0.5 M $NaClO_4$) measured by R. Faure, H. Loiseleur, and G. Thomas, *Bull. Soc. Chim. Fr.*, 2343 (1971).

$$\frac{k_{obsd}(K_1 + (H^+))}{(H^+)} = \frac{k_{12} + k_{43}K_1(H^+)^{-1}}{\left(\frac{k_{34}}{k_{35}}\right)\left(\frac{k_{12}}{k_{43}K_1}\right)(H^+) + \frac{k_{34}}{k_{35}} + 1} \quad (9)$$

A nonlinear least-squares analysis of the data in Table I was used to evaluate k_{12}, k_{43}, and (k_{34}/k_{35}) in eq 9. The resulting values are given in Table II.

It seems reasonable to expect the specific rate constants for the picolinic and fusaric acid systems to be similar since the butyl group in fusaric acid is far removed from the reaction site. The results in Table II confirm this expectation in that k_{12} and k_{43} are the same, within their estimated uncertainties, for the two systems. In addition, if the ion-pair dissociative mechanism[6,7] is operative, then k_{12} and k_{43} should be comparable to the rate constants for other neutral and uninegative ligands, respectively.[6,7] Rate constants of about 5×10^3 M^{-1} s^{-1} seem to be typical for neutral ligands reacting with nickel(II) and the magnitude of k_{12} is therefore normal. Similarly k_{43} is comparable to the rate constant of

Table III. Kinetic Results for Some Amino Acids Reacting with Nickel(II) (25°)

Amino acid	α substituent	pK_a[a]	pK_x	pK_N[b]	$10^4(k_{43}k_{35}/k_{34})$,[c] M^{-1} s^{-1}
Glycine	H	9.75			2.2, 4.1
α-Alanine	$-CH_3$	9.87			2 (20°)
Serine	$-CH_2OH$	9.34			2.9
Aspartic acid amide	$-CH_2CONH_2$	8.8			0.87
Methionine	$-(CH_2)_2SCH_3$	9.12			0.72[d]
Tyrosine	$-CH_2(C_6H_4OH)$	9.12[e]	9.63[e]	9.28[e]	0.54[d] (1.4)[f]
L-Dopa	$-CH_2(C_6H_3(OH)_2)$	8.76[g], 9.84[g]	8.97[g]	9.17[g]	0.57[h]
Diaminobutyric acid	$-(CH_2)_2NH_3^+$	8.1, 10.4	~8.4[i]	~8.4[i]	0.56
Ornithine	$-(CH_2)_3NH_3^+$	8.75, 10.50	9.55[i]	8.83[i]	0.43
Lysine	$-(CH_2)_4NH_3^+$	9.17, 10.71	10.09[i]	9.22[i]	0.58
Cysteine	$-CH_2SH$	8.22	8.55	8.88	(11)[d,j]
Penicillamine	$C(CH_3)_2SH$	7.96	8.05	~8.7	(11)[d,j]

[a] Values given in original kinetic study unless otherwise indicated. [b] Microacid dissociation constant for the α-amino group. [c] Calculated from results compiled in ref 7 unless otherwise indicated. [d] Reference 2. [e] R. B. Martin et al., *J. Biol. Chem.*, **233**, 1429 (1958). [f] M. L. Barr et al., *J. Coord. Chem.*, **2**, 263 (1973). [g] R. B. Martin, *J. Phys. Chem.*, **75**, 2657 (1971). [h] Reference 10. [i] D. L. Rabenstein, private communication, determined by ^1H NMR. [j] See text for discussion of these values.

3.4×10^4 M^{-1} s^{-1} measured for the monohydrogen phthalate ion,[8] and $\sim 1 \times 10^4$ M^{-1} s^{-1} for the acetate ion.[9]

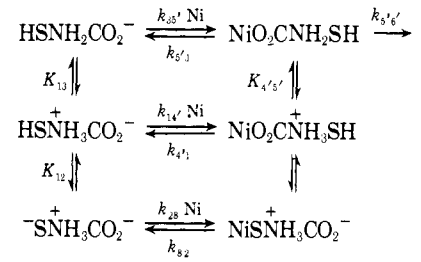
If picolinic and fusaric acids follow the reaction scheme in eq 1 it seems reasonable to conclude that simple α-amino acids will do the same. The consequences of this conclusion with respect to previous work can then be explored. Since the amino group of an α-amino acid generally has $pK_a > 8$, and the experimental conditions are normally pH $\lesssim (pK_a - 2)$, then kinetic studies yield values for $(k_{12}k_{35}K_1'/k_{21}) = (k_{43}k_{35}K_1/k_{34})$ (see eq 7). It is then possible to obtain $k_{43}k_{35}/k_{34}$ if K_1 is known. It is important to note that for amino acids with several ionizable groups K_1 is the micro-, or molecular acid dissociation constant for the $-NH_3^+$ group, and not just the macroconstant determined from a simple pH titration. Values of the macro- and microconstants, and of $k_{43}k_{35}/k_{34}$ are collected in Table III.

If the dissociative ion-pair mechanism[6,7] is retained one would expect the magnitude of k_{43} and k_{35} to be similar for similarly charged amino acid ligands, and variations in the experimental $k_{43}k_{35}/k_{34}$ may be largely due to changes in k_{34}. For glycine, α-alanine, and serine $k_{43}k_{35}/k_{34}$ is fairly constant at $3 \pm 1 \times 10^4$ M^{-1} s^{-1}. However, with more extensive α-substituents $k_{43}k_{35}/k_{34}$ seems to decrease, for example, to $\sim 0.8 \times 10^4$ M^{-1} s^{-1} for methionine and aspartic acid amide. It seems most straightforward to attribute this decrease to a steric effect causing an increase in k_{34}.

The effect of α-substituents seems to extend to tyrosine and L-Dopa, which appear very similar if the most recent result[2] for tyrosine is used. The earlier conclusion[10] that L-Dopa reacted unusually slowly resulted from the incorrect use of the macroacid dissociation constant instead of the microconstant. Recently Martin[11] has commented on this problem in connection with copper(II) systems.

Diaminobutyric acid, ornithine, and lysine, each with a nonreacting $-NH_3^+$ substituent, have $k_{43}k_{35}/k_{34}$ values similar to tyrosine but smaller than methionine. The $-NH_3^+$ substituent would be expected to lower k_{43} through a charge effect on ion-pair formation. However, the effect does not seem to be a major one and the expected attenuation with increasing separation of $-CO_2^-$ and $-NH_3^+$ could be concealed by uncertainties in the microacid dissociation constants.

The systems cysteine and penicillamine require special consideration because the sulfur atom is a possible site for initial metal ion attack. Earlier work[2] indicates that the term in the rate law with essentially an inverse hydrogen ion dependence (k_2' of ref 2) can be attributed to the reactions shown in eq 10 (with the numbering scheme of ref 2). The



(10)

appropriate experimental rate constant could be $k_{14'}k_{5'6'}K_{4'5'}/k_{5'3'}$, as expected by analogy to eq 1 and eq 7. However, $k_{28}K_{12}$ might be contributing (see eq 24 and the following discussion in ref 2). Thus for cysteine, for example, the experimental result is expected to be given by

$$14.5 \times 10^{-5} = \left(\frac{k_{35}k_{5'6'}}{k_{5'3}}\right) K_{13} + k_{28}K_{12} \qquad (11)$$

If the $k_{28}K_{12}$ term is neglected, then, with $pK_{13} = 8.88$, one obtains $(k_{35}k_{5'6'}/k_{5'3}) = 11 \times 10^4$, and the same value is obtained for penicillamine. It is difficult to understand why this value for cysteine is ca. four times greater than the analogous $k_{43}k_{35}/k_{34}$ for serine when the two ligands should be sterically quite similar. It appears that the reaction rates for cysteine and penicillamine are much larger than expected if the $k_{28}K_{12}$ term is ignored. If the latter term is retained in eq 11, and if $(k_{25}k_{56'}/k_{5'3'}) \approx 3 \times 10^4$ M^{-1} s^{-1} (by analogy to serine) then it is possible to calculate that k_{28} is 3.8×10^4 M^{-1} s^{-1} for cysteine and 1.8×10^4 M^{-1} s^{-1} for penicillamine. As already noted a rate constant of $\sim 3 \times 10^4$ M^{-1} s^{-1} seems normal for reaction of an anion with nickel(II), so that the magnitude of k_{28} seems reasonable. Therefore a consistent interpretation requires that nickel(II) reacts at the deprotonated sulfhydryl group as well as the carboxylate group of cysteine and penicillamine.

In summary, several points should be emphasized. The results for picolinic and fusaric acids show that the reaction scheme in eq 1 can adequately describe the kinetic behavior, and that chelate ring closing (k_{35}) is an important factor. Extension of these results to simple amino acids reveals that experimental results yield $(k_{43}k_{35}/k_{34})$ if the microacid dissociation constant of the α-amino group is used. Variations in $(k_{43}k_{35}/k_{34})$ for similarly charged amino acids seem to be qualitatively consistent with expected steric effects on k_{34}.

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Kinetic Studies of the Oxidation and Reduction of *Chromatium* High Potential Iron–Sulfur Protein (HiPIP) by Inorganic Complexes. Comparison of the Electron Transfer Reactivities of HiPIP and Horse Heart Cytochrome *c*

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Abstract: Kinetic measurements of the oxidation of reduced *Chromatium* high potential iron-sulfur protein (HiPIP) by $Fe(CN)_6^{3-}$ and $Co(phen)_3^{3+}$ have been made. The rate of reduction of oxidized HiPIP by $Fe(EDTA)^{2-}$ has also been studied. The second-order rate constants are $(2.0 \pm 0.1) \times 10^3$ M^{-1} s^{-1} (25°, μ 0.1 M, pH 7.0 (phosphate)) for HiPIP-$Fe(CN)_6^{3-}$, $(2.8 \pm 0.1) \times 10^3$ M^{-1} s^{-1} (26°, μ 0.1 M, pH 7.0 (phosphate)) for HiPIP-$Co(phen)_3^{3+}$, and $(1.6 \pm 0.1) \times 10^3$ M^{-1} s^{-1} (25°, μ 0.1 M, pH 7.0 (phosphate)) for oxidized HiPIP-$Fe(EDTA)^{2-}$. Activation parameters are: $\Delta H^{\ddagger} = -0.4 \pm 0.1$ kcal/mol, $\Delta S^{\ddagger} = -45 \pm 1$ eu (HiPIP-$Fe(CN)_6^{3-}$); $\Delta H^{\ddagger} = 14.9 \pm 0.7$ kcal/mol, $\Delta S^{\ddagger} = 7 \pm 2$ eu (HiPIP-$Co(phen)_3^{3+}$); $\Delta H^{\ddagger} = 0.8 \pm 0.3$ kcal/mol, $\Delta S^{\ddagger} = -41 \pm 1$ eu (HiPIP-$Fe(EDTA)^{2-}$). The differences between electron transfer kinetic parameters of HiPIP and horse heart cytochrome *c* have been analyzed in terms of relative Marcus theory. The analysis indicates that in both the cytochrome *c* self-exchange and the $Co(phen)_3^{3+}$ cross reaction, electron transfer takes place at the partially exposed heme edge of the protein. The cytochrome *c* k_{11} value based on the $Fe(EDTA)^{2-}$ cross reaction is somewhat smaller than the experimental self-exchange rate constant, which suggests that this redox agent has difficulty in approaching the partially exposed heme edge. The difference in self-exchange rate constants calculated for HiPIP from cross reactions with $Co(phen)_3^{3+}$ and $Fe(EDTA)^{2-}$ is much greater than that for cytochrome *c*. The k_{11} value for HiPIP obtained from the $Co(phen)_3^{3+}$ oxidation is 1×10^4 M^{-1} s^{-1}, whereas that for $Fe(EDTA)^{2-}$ reduction is 1×10^{-2} M^{-1} s^{-1}. It is proposed that HiPIP-$Co(phen)_3^{3+}$ and HiPIP-$Fe(EDTA)^{2-}$ employ different mechanisms of electron transfer. The low reactivity in the latter case suggests that $Fe(EDTA)^{2-}$ is not able to approach the buried [Fe_4S_4*] cluster in HiPIP very closely, and is thus forced to transfer an electron over a relatively large distance ($\geq$3.5 Å).

The kinetics and mechanisms of the electron transfer reactions involving iron-sulfur proteins and simple inorganic complexes have been little studied. In an important recent paper, Bennett and co-workers have reported[1] second-order rate constants and activation parameters for the outer sphere reduction of clostridial rubredoxin, a mononuclear iron-sulfur protein, by $Ru(NH_3)_6^{2+}$. The reduction of rubredoxin by $V(H_2O)_6^{2+}$ and $Cr(H_2O)_6^{2+}$ was also investigated.

We have initiated kinetic studies of the oxidation and reduction of polynuclear iron-sulfur proteins by inorganic complexes that normally employ outer sphere electron transfer pathways. This paper reports rate constants and activation parameters for the oxidation of reduced *Chromatium* high potential iron-sulfur protein (HiPIP) by ferricyanide and by tris(1,10-phenanthroline)cobalt(III), as well as for the reduction of oxidized HiPIP by $Fe(EDTA)^{2-}$. An analysis of the electron transfer reactivities of HiPIP and horse heart cytochrome *c* has been performed based on the relative Marcus theory.[2]

Experimental Section

Reagent grade chemicals were used without further purification. Distilled deionized water was used in making solutions. $K_3Fe(CN)_6$ solutions were prepared from weighed samples; concentrations were checked by absorbance measurements at 420 nm (ϵ 1.0×10^3).[3] $[Co(phen)_3]Cl_3 \cdot 7H_2O$ was prepared from $[Co(NH_3)_5Cl]Cl_2$ (Alfa Inorganics) and 1,10-phenanthroline (phen) by a standard method.[4] The isolated crystals were characterized by spectroscopic measurements in the region 380–220 nm. Concentrations of solutions used for kinetic studies were determined by absorbances at 350 (ϵ 3.7×10^3) and 330 nm (ϵ 4.7×10^3).[5] Solutions of $Fe(EDTA)^{2-}$ were prepared by standard procedures.[6]

HiPIP was extracted from cells of *Chromatium*, strain D (ATCC no. 17899), as described by Bose.[7] Cells were harvested by continuous centrifugation after 4 days of growth, and the protein was purified by variations on published methods.[8,9] The cells were first disrupted by freeze–thaw lysing, with 1% Triton-X added, then centrifuged for 1 h at 10 000 rpm, and cell fragments and mitochondrial particles, which were at the top, were removed. To the resulting yellowish solution, ammonium sulfate was added to 90%