Kinetic and Equilibrium Studies of the Reactions of Cysteine and Penicillamine with Aqueous Iron(III)

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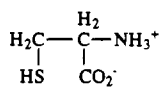
Received March 22, 1995[⊗]

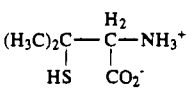
The reactions of cysteine (cys) and penicillamine (H_3L^+, pen) with aqueous iron(III) have been studied in dilute acid solutions (0.01−0.2 M H^+) with iron(III) concentrations (0.01−0.1 M) in excess over the amino acid ((~1.5−8.5) × 10^{-4} M) at 25 °C in 1.0 M $LiClO_4/HClO_4$. Stopped-flow and standard spectrophotometry were used to measure the kinetics of the complexation and oxidation−reduction reactions, respectively. Complexation equilibrium constants (K_f = $[MLH][H^+]^2/[M][LH_3]$) have been determined from the absorbance immediately after the complexation reaction. Both monomeric ($Fe(OH_2)_6^{3+}$) and dimeric ($Fe_2(OH_2)_8(\mu\text{-}OH)_2^{4+}$) iron(III) species form complexes with K_f values of 0.063 and 0.38 M with penicillamine and of 0.025 and 0.07 M with cysteine, respectively. The dependence of the complexation rate on iron(III) and H^+ concentrations indicates that the dominant reaction pathways and their rate constants (M^{-1} s^{-1}) are as follows: $(H_2O)_5Fe(OH)^{2+} + H_3L^+$, k = 1.4 × 10^3 (cys), 1.2 × 10^3 (pen); $(H_2O)_5Fe(OH)^{2+} + H_2L$, k = 7.4 × 10^3 (cys), 7.4 × 10^3 (pen); $Fe_2(OH_2)_8(\mu\text{-}OH)_2^{4+} + H_2L$, k = 4.3 × 10^3 (cys), 8.1 × 10^3 (pen). The oxidation of cysteine is second order in cysteine with terms in the rate law first, second, and third order in $Fe(OH_2)_6^{3+}$. From the $[H^+]$ dependence, these are assigned to reactions of $(H_2O)_4FeLH^{2+} + H_2L$, $(H_2O)_4FeLH^{2+} + (H_2O)_4FeLH^{2+}$ or $Fe_2(OH_2)_6(\mu\text{-}OH)_2(LH)^{3+} + H_2L$, and $Fe_2(OH_2)_6(\mu\text{-}OH)_2(LH)^{3+} + (H_2O)_4FeLH^{2+}$. Penicillamine reacts similarly but more slowly, and the first pathway is not observed, but one fourth order in iron(III) is found. Limited studies with excess penicillamine show that the reaction is second order in penicillamine and is inhibited by H^+ and iron(II), and the reactive species is suggested to be $Fe(LH)_2^+$. Spectrophotometric results under these conditions yield K_{f12} = $[M(LH)_2][H^+]^2/[MLH][LH_3]$ = 1.6 × 10^{-2} M.

Introduction

Mercaptocarboxylic acids of the general formula ($HSRCO_2H$) react with aqueous iron(III) to form blue complexes that subsequently undergo oxidation−reduction to the corresponding disulfide and iron(II). Equilibrium studies[1-4] have shown that two protons are released on complexation so that the $-CO_2^-$ and $-S^-$ groups are coordinated to iron(III) in the blue species. Cysteine (**I**) and penicillamine (**II**) also form blue complexes,



I



II

and the observations of Stadtherr and Martin[5] show that $-S^-$ and $-CO_2^-$ are coordinated because *S*-methyl-L-cysteine and cysteine methyl and ethyl esters give no blue color. Furthermore, the amino group is not coordinated in acidic solution because the *N*-acetyl derivatives of cysteine and penicillamine give the blue color, and penicillamine complexation[4] involves release of only two protons. Tomita et al.[6] characterized 1:1 complexes of cysteine and thioglycolic acid at −78 °C in 90% ethanol:water with absorption maxima at 620 nm ($\epsilon \approx$ 500−600 M^{-1} cm^{-1}). They also isolated violet tris complexes (λ_{max} ~ 590 nm, ϵ ~ 3 × 10^3 M^{-1} cm^{-1}, for cysteine) and red complexes (λ_{max} ~ 490 nm, ϵ ~ 1 × 10^3 M^{-1} cm^{-1}, for cysteine) which they assigned to $Fe(OH)(SRCO_2)_2^{2-}$.

The complexation kinetics of several $HSRCO_2H$ systems have been studied by McAuley and co-workers[1-3] and by Baiocchi et al.[4] These studies involved different conditions, with iron(III) in excess in the former and $HSRCO_2H$ in excess in the latter, and the kinetic results are in reasonable agreement and follow the usual pattern for substitution on aqueous iron(III). Cysteine was not studied, but Baiocchi et al. did study penicillamine.

More recently, Jameson and co-workers[7,8] studied the reaction of aqueous iron(III) with excess cysteine at pH 2.7−4.87 and 8.5−11.68. This work has introduced some confusion into the general picture, because these workers seem to have been unaware of the earlier studies of Stadtherr and Martin, Tomita et al., and Baiocchi et al. In the lower pH range,[7] Jameson et al. assigned a blue species (λ_{max} = 614 nm, ϵ = 1.03 × 10^3 M^{-1} cm^{-1}) to a mono complex of fully deprotonated cysteine with S- and N-coordination, while all previous work would indicate S- and O-coordination with an uncoordinated $-NH_3^+$. At pH > 8, the species assigned as $Fe(OH)(L)$ and $Fe(OH)(L)_2^{2-}$ by Jameson et al. appear to be $Fe(OH)(L)_2^{2-}$ and $Fe(L)_3^{3-}$, respectively, from the observations of Tomita et al.[6] Reliance on the conclusions of Jameson et al. led Ghosh and Gould[9] to suggest that the iron catalyzed oxidation of mercaptoacetate and cysteine by a peroxochromium(IV) complex involved an iron(III) complex, formulated as $Fe^{III}(SR^-)$. Ghosh and Gould reported the rates of formation and decay of this species by

[⊗] Abstract published in *Advance ACS Abstracts,* October 15, 1995.

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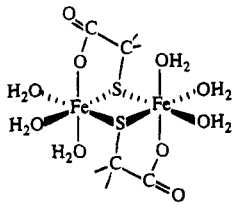
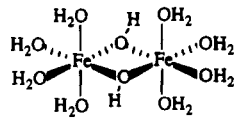
reacting aqueous iron(III) with mercaptoacetate (pH 4.4 acetate buffer, $[HSCH_2CO_2H] = (2-8) \times 10^{-3}$ M, $[Fe(III)] = (2-4) \times 10^{-4}$ M, $\lambda_{max} = 575$ nm). However the rate constants reported by Ghosh and Gould are $\sim 10^2$ times smaller than those predicted by extrapolation of the results of Baiocchi et al.[4] at higher acidity, and the latter also found a different λ_{max} of 640 nm. One can infer that Ghosh and Gould probably were dealing with a mixture of the red ($\lambda_{max} = 490$ nm) and blue ($\lambda_{max} = 590$ nm) species assigned by Tomita et al.[6] to $Fe(OH)(L)_2^{2-}$ and $Fe(L)_3^{3-}$, respectively, and were largely observing the kinetics of formation and decomposition of the tris complex.

McAuley and co-workers[3] and Baiocchi et al.[4] studied the oxidation step for several $HSRCO_2H$ systems, although neither studied cysteine, and McAuley noted only that penicillamine oxidation is much slower. With excess reductant ($[HSRCO_2H] > [iron(III)] = 1 \times 10^{-4}$ M), Baiocchi et al. proposed formation of a steady-state amount of bis complex ($Fe(SRCO_2)^{2-}$) as the reactive species to explain the second-order dependence on $[HSRCO_2H]$. Our analysis of the published observations for 2-mercaptosuccinic acid confirms the second-order dependence, but there is some problem with the quantitative fitting of the data. McAuley and co-workers found that the redox reactions were effectively second order in both iron(III) and $[HSRCO_2H]$ under conditions of $[iron(III)] > [HSRCO_2H]$ (0.2–0.4 M H^+). The detailed analysis indicated that the reaction was second order in the complex $(H_2O)_4Fe(SRCO_2)^+$. McAuley suggested that the oxidation might proceed through a sulfur bridged diiron(III) species (**III**). One purpose of the present study is to determine if the structurally analogous μ-dihydroxy dimer (**IV**) might show complexation and oxidation reactivity.



III



IV

The oxidation rate laws show the general feature that two mercaptocarboxylic acids are necessary to reach the transition state, and two iron(III) units are used if iron(III) is in excess. It is remarkable that these systems use this high-order reaction pathway rather than simple one electron oxidation by iron(III) to give an unstable organic radical which is rapidly oxidized further.

However, Jameson et al.[7] proposed that oxidation of cysteine at pH 2.7–4.8 does involve simple intramolecular electron transfer within the monocysteine–iron(III)) complex. But it is difficult to explain why such a pathway was not found for the mercaptocarboxylates[3,4] nor found in the present study on cysteine. It seems more probable that Jameson et al. were observing decomposition of the bis(cysteine–iron(III)) complex, since this makes their observations more compatible with those of Baiocchi et al., as discussed in more detail below. For the iron(III)–mercaptoacetate system, Ghosh and Gould[9] suggest that an iron(III) complex reacts with another "unit of reductant" in the oxidation step, and this also is inconsistent with Jameson's proposal for cysteine, although the studies are under very similar conditions. However, the rate constants of Ghosh and Gould are about 500 times smaller than those predicted by the rate law of Baiocchi et al. for mercaptoacetate, and this is a further indication that Ghosh and Gould were not dealing with the mono complex. This also would explain why the iron(III)–cysteine complexation constant determined from the peroxochromium(IV)–iron(III)–cysteine kinetics is ~50 times smaller than that found in the present work, if the kinetic value really refers to higher complex formation. For both the mercaptoacetate and cysteine systems,[9] the kinetics show that the iron(III) complex is oxidized by peroxochromium(IV) rather than undergoing intramolecular electron transfer, and this is consistent with the general observation made in the preceding paragraph. Except for the underestimation of the degree of iron(III) complexation, the mechanisms suggested by Gould et al. are qualitatively consistent with most earlier observations.

The energetics of the oxidation of thiol functions provides some indication of why these systems prefer routes more complex than simple intramolecular electron transfer. Armstrong and co-workers[10,11] have provided values for reduction potentials of a number of species of interest, including mercaptoacetate, cysteine, and penicillamine. The potentials are rather insensitive to the substituents on the HS– group and generally may be summarized as follows:

$$^{\bullet}S\sim CO_2^- + e^- = {}^-S\sim CO_2^- \qquad E^\circ = +0.77 \text{ V}$$

$$^-O_2C\sim SS^{\bullet -}\sim CO_2^- + e^- = 2\ {}^-S\sim CO_2^- \qquad E^\circ = +0.57 \text{ V}$$

$$^-O_2C\sim SS\sim CO_2^- + e^- = {}^-O_2C\sim SS^{\bullet -}\sim CO_2^- \qquad E^\circ = -1.57 \text{ V}$$

$$^{\bullet}S\sim CO_2^- + H^+ + e^- = HS\sim CO_2^- \quad E^\circ = +1.33 \text{ V}$$

$$^-O_2C\sim SS^{\bullet -}\sim CO_2^- + 2\ H^+ + e^- = 2\ HS\sim CO_2^- \qquad E^\circ = +1.72 \text{ V}$$

$$^-O_2C\sim SS\sim CO_2^- + 2\ H^+ + 2\ e^- = 2\ HS\sim CO_2^- \qquad E^\circ = -0.03 \text{ V}$$

The E° for the last reaction (at 1.0 M H^+) is consistent with measured values[12] of about −0.25 V at pH ~ 7 for various species. It is apparent from the first two equations that oxidation to form a radical in these systems is highly unfavorable, although overall two electron oxidation in the last reaction is possible with quite mild oxidizing agents. The E° for the first reaction can be combined with the known complexation constants[4] and $E^\circ(Fe^{III}/Fe^{II}) = 0.75$ V to estimate the driving force for the following intramolecular electron transfer reaction.

$$Fe^{III}(S\sim CO_2^-) \rightleftharpoons Fe^{II} + {}^{\bullet}S\sim CO_2^-$$

This reaction has $E^\circ = -0.54$ V ($K = 9 \times 10^{-10}$ M) for cysteine and penicillamine. Clearly the intramolecular electron transfer is quite unfavorable thermodynamically. If the reverse of this reaction is assumed to have a diffusion controlled rate constant of $\sim 10^9$ M^{-1} s^{-1}, then the upper limit for the forward rate constant is <0.9 s^{-1}.

The situation is even more unfavorable for the following reaction,

$$Fe^{III}(S\sim CO_2^-)_2 \rightleftharpoons Fe^{II} + {}^-O_2C\sim SS^{\bullet -}\sim CO_2^-$$

which has $E^\circ = -0.79$ V ($K = 4 \times 10^{-14}$ M) for cysteine and

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penicillamine. This calculation has assumed that the formation constant of the bis complex is one-fifth that of the known value of the mono complex, but changes of a factor of 10 in this ratio just change the $E°$ by ±0.06 V.

On the other hand, the following overal bimolecular reaction is quite favorable,

$$2Fe^{III}(S\sim CO_2^-) \rightleftharpoons 2Fe^{II} + {}^-O_2C\sim SS\sim CO_2^-$$

and has $E° = 0.24$ V ($K = 1 \times 10^8$ M) for cysteine and penicillamine.

The present work was undertaken to determine how the biologically important cysteine and penicillamine fit into the general picture from the mercaptocarboxylic acids. The conditions of [iron(III)] > [amino acid] and modest acidities (0.01–0.2 M H^+) were chosen to determine if the μ-dihydroxy dimer (**IV**) of iron(III) might show unusual reactivity. This species could bring two iron(III) and two −S− groups together, in a manner somewhat analogous to the intermediate **III** proposed by McAuley, and might impart unusual reactivity.

Results

When mixed with aqueous iron(III), both cysteine and penicillamine give a blue color, which fades with time. The fading is slower and the color appears more intense with penicillamine. The kinetics of both color formation and fading have been studied here. The rather slow loss of color with penicillamine has allowed the initial absorbance values to be used to determine the complex formation equilibrium constant for penicillamine with $Fe(OH_2)_6^{3+}$ and the dimer $(H_2O)_8Fe_2(\mu\text{-}OH)_2^{4+}$. The cysteine results are consistent with an analogous equilibrium system.

Complex Formation Kinetics. The absorbance−time traces for formation of the blue color with cysteine and penicillamine are well fitted to a first-order rate expression. The fading of the blue color is sufficiently slow so that it does not interfere with the analysis of the faster absorbance increase. The kinetic results are summarized in Tables 1 and 2. For most of the runs, before mixing the acid was in the iron(III) solution and the amino acid solutions contained $<1 \times 10^{-3}$ M H^+. In order to test for the reactivity contribution of the μ-dihydroxy dimer of iron(III) ($Fe(OH_2)_8(\mu\text{-}OH)_2^{4+}$), a few runs were done with less H^+ in the iron solution and more with the amino acid. These conditions favor formation of the dimer before mixing, and it does not undergo dissociation on the time scale of the complexation. Therefore, any change in rate constant from otherwise identical conditions after mixing may be attributed to a contribution of the dimer.

The kinetic results follow the usual pattern for complexation by aqueous iron(III) and have been analyzed in terms of the reactions in Scheme 1, where $Fe_2(OH)_2^{4+} = (H_2O)_8Fe_2(\mu\text{-}OH)_2^{4+}$. The fully protonated forms (H_3L^+) of both cysteine and penicillamine undergo acid dissociation at the carboxyl group with $pK_a \approx 1.9$, while ionization of the SH and NH_3^+ groups have $pK_a \geq 8$[13] and these species are not included. Although there is no kinetic evidence for the reaction of $Fe(OH_2)_6^{3+}$ with H_3L^+, the K_{f1} reaction is included in Scheme 1 to provide a definition for the equilibrium constant often used to characterize such systems.

Scheme 1 actually predicts that the reaction should be biphasic, because the dimer $Fe_2(OH)_2^{4+}$ is not in equilibrium with the monomeric species on the time scale of these reactions. However, the reactions appear monophasic, and this happens for two reasons: the dimer contribution is generally small, and the molar absorptivities of the monomer and dimer complex are not greatly different. Therefore the rate constants have been fitted to eq 1, in which the first term for the monomeric species

$$k_{obsd} = ((k_2K_m + k_3K_a)[H^+] + k_4K_mK_a)\left\{\frac{[Fe(III)]_m}{(K_a + [H^+])(K_m + [H^+])} + \frac{1}{K_{f1}}\right\} + k_dK_a\left\{\frac{[Fe_2(OH)_2]}{K_a + [H^+]}\right\} \quad (1)$$

is calculated from Scheme 1, but the dimer contribution is simply added to account for the fact that qualitative observations show that it is contributing. More quantitative analysis below shows that this procedure gives surprisingly good results. The experimental and least squares calculated values of k_{obsd} are compared in Tables 1 and 2. The results give values of ($k_2K_m + k_3K_a$), $k_4K_mK_a$, k_dK_a ($M\ s^{-1}$), and K_{f1} (M) of 2.29 ± 0.55, 0.15 ± 0.02, 54 ± 13, and 0.0248 ± 0.005 for cysteine and 1.90 ± 0.5, 0.15 ± 0.016, 103 ± 10, and 0.0634 ± 0.01 for penicillamine, as summarized in Table 6.

In order to test the justification for eq 1, several kinetic runs have been analyzed by numerical integration. This analysis requires numerical values for the molar absorptivities and the equilibrium constant for dimer complex formation (K_{f2}), which were obtained from the equilibrium data for penicillamine described in the next section. The calculated curves are in

Table 1. Kinetic Results for the Complexation of Aqueous Iron(III) by Cysteine at 25 °C in 1.00 M $LiClO_4/HClO_4$

$[H^+] \times 10^2$, M	$[Fe(III)]_m$[a] $\times 10^3$, M	[cysteine] $\times 10^4$, M	$[Fe_2(OH)_2^{4+}] \times 10^3$, M	k, s^{-1} obsd	k, s^{-1} calcd
1.00	1.80	1.63	0.0995	8.27	8.40
1.01	3.30	1.63	0.353	9.38	9.98
1.00	4.55	5.11	0.229	10.7	10.5
1.00	4.55	5.11	0.229	9.92	10.5
1.00	4.55	5.11	0.229	9.76	10.5
0.990	7.80	1.55	1.11	15.2	14.8
1.01	8.31	5.11	0.849	15.4	14.4
1.01	8.31	5.11	0.849	14.7	14.4
1.01	8.31	5.11	0.849	14.6	14.4
1.00	11.4	5.11	1.80	19.3	18.8
1.00	11.4	5.11	1.80	19.8	18.8
1.00	11.4	5.11	1.80	19.9	18.8
1.01	11.7	1.63	1.67	18.1	18.5
1.01	14.2	1.63	2.89	21.5	23.1
1.25	7.76	5.25	1.12	12.4	13.5
2.00	4.82	1.55	0.0920	9.31	9.38
2.00	9.29	1.55	0.358	11.3	11.1
2.00	9.54	1.55	0.235	11.8	10.9
2.00	9.54	5.11	0.235	11.5	10.9
2.00	9.54	5.11	0.235	11.1	10.9
2.00	13.4	5.25	0.806	12.6	12.9
2.01	13.5	1.55	0.778	14.2	12.9
2.01	17.4	1.55	1.33	15.5	14.9
2.00	18.2	5.11	0.919	14.1	14.5
2.00	18.2	5.11	0.919	14.1	14.5
2.00	18.2	5.11	0.919	14.2	14.5
2.00	20.9	1.55	2.06	16.2	17.1
5.01	9.74	1.55	0.137	11.8	11.5
4.92	11.5	4.69	1.75[b]	15.1	13.0
5.01	19.0	1.55	0.532	13.4	12.6
10.0	9.99	5.25	0.0104	14.8	15.5
10.0	19.9	5.25	0.0415	15.6	15.9
10.0	34.8	5.25	0.127	15.5	16.4

[a] Concentration of monomeric species, $Fe(OH_2)_6^{3+} + Fe(OH_2)_5(OH)^{2+}$. [b] Mixing a cysteine solution containing 8.30×10^{-2} M H^+ with a 3.00×10^{-2} M iron(III) solution containing 6.19×10^{-3} M H^+ to give a higher concentration of $Fe_2(OH)_2^{4+}$.

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Table 2. Kinetic Results for the Complexation of Aqueous Iron(III) by Penicillamine at 25 °C in 1.00 M $LiClO_4/HClO_4$

$[H^+] \times 10^2$, M	$[Fe(III)]_m$[a] $\times 10^3$, M	[penicillamine] $\times 10^4$, M	$[Fe_2(OH)_2^{4+}] \times 10^3$, M	k, s^{-1} obsd	k, s^{-1} calcd
0.998	1.93	1.47	0.0361	3.93	4.10
1.01	4.58	1.47	0.214	6.59	6.59
1.03	4.58	5.21	0.214	6.62	6.52
1.02	4.58	5.12	0.214	6.76	6.55[d]
0.991	8.35	1.47	0.831	12.8	12.0
0.979	8.35	1.52	0.831	13.2	12.1
0.980	10.4	1.65	2.29[b]	20.0	20.2[e]
0.984	11.5	1.52	1.74	19.5	18.3
0.989	11.5	1.54	1.75	20.3	18.3[d]
2.01	4.89	1.47	0.0574	4.62	4.47
1.99	9.55	1.47	0.232	6.12	6.30
2.00	9.55	5.21	0.232	5.99	6.27
1.98	14.0	5.32	0.525	7.68	8.45
1.98	14.0	1.52	0.508	8.63	8.40
1.98	18.2	1.52	0.895	10.8	10.8
1.98	18.2	5.32	0.922	9.44	10.9
1.97	18.2	5.12	0.923	10.7	10.9[d]
5.02	9.94	5.21	0.0376	5.02	4.70
4.97	15.0	4.99	1.75[c]	7.27	7.94[d]
4.99	19.7	5.13	0.152	5.84	5.64
4.98	19.7	5.12	0.152	5.99	5.64[d]
10.1	9.99	5.21	0.00938	5.63	5.71
10.0	19.9	5.13	0.0379	5.89	6.02
9.99	29.9	5.13	0.0858	6.62	6.35

[a] Concentration of monomeric species, $Fe(OH_2)_6^{3+} + Fe(OH_2)_5(OH)^{2+}$. [b] Mixing a penicillamine solution containing 1.30×10^{-3} M H^+ with a 3.00×10^{-2} M iron(III) solution containing 6.19×10^{-3} M H^+ to give a higher concentration of $Fe_2(OH)_2^{4+}$. [c] Mixing a penicillamine solution containing 8.30×10^{-2} M H^+ with a 3.00×10^{-2} M iron(III) solution containing 6.19×10^{-3} M H^+ to give a higher concentration of $Fe_2(OH)_2^{4+}$. [d] New supply of DL-penicillamine. [e] D-Penicillamine.

Scheme 1

$$Fe(OH_2)_6^{3+} \overset{K_m}{\rightleftharpoons} (H_2O)_5Fe(OH)^{2+} + H^+$$

$$H_3L^+ \overset{K_a}{\rightleftharpoons} H_2L + H^+$$

$$Fe(OH_2)_6^{3+} + H_3L^+ \overset{K_{f1}}{\rightleftharpoons} (H_2O)_4Fe(LH)^{2+} + 2H^+$$

$$(H_2O)_5Fe(OH)^{2+} + H_3L^+ \overset{k_2}{\rightleftharpoons} (H_2O)_4Fe(LH)^{2+} + H^+ + H_2O$$

$$Fe(OH_2)_6^{3+} + H_2L \overset{k_3}{\rightleftharpoons} (H_2O)_4Fe(LH)^{2+} + H^+$$

$$(H_2O)_5Fe(OH)^{2+} + H_2L \overset{k_4}{\rightleftharpoons} (H_2O)_4Fe(LH)^{2+} + H_2O$$

$$Fe_2(OH)_2^{4+} + H_2L \xrightarrow{k_d} Fe_2(OH)_2(LH)^{3+} + H^+$$

excellent agreement with the experimental single exponential curves for both penicillamine and cysteine. The agreement of these calculations provides justification for eq 1 and shows that the kinetic and equilibrium measurements are internally consistent.

Equilibrium Constant for Iron(III)−Penicillamine Complexation. In order to assess the equilibrium constant(s) for complexation, the initial absorbancies of iron(III)−penicillamine solutions were determined under various concentration conditions. Since the fading of the blue color is relatively slow, only a small linear extrapolation is necessary to obtain the initial absorbance. Since the fading is much faster with cysteine, analogous experiments are not possible. It should be noted that the time scale for these measurements (15−30 s) is such that the μ-dihydroxy−iron(III) dimer is in equilibrium with $Fe(OH_2)_6^{3+}$.

If the carboxylate and sulfhydryl groups are complexing, then monomeric complex formation can be described by the K_{f1} reaction in Scheme 1, in which two protons are released from the fully protonated penicillamine cation. Baiocchi et al. analyzed this system under conditions of [penicillamine] ≫ [iron(III)] = 1×10^{-4} M to obtain $K_{f1} = 0.0676$ M. This value is in excellent agreement with that of 0.064 M found in the kinetic study described above. Analysis of the present initial absorbance values to the single complex model gives a reasonable fit with $K_{f1} = 0.035$ M and a molar absorptivity coefficient (ϵ_1) of 1728 M^{-1} cm^{-1} for $Fe(LH)^{2+}$. Of course, this model must be unrealistic because the kinetic observations indicate that the μ-dihydroxy−iron(III) dimer is forming a complex with penicillamine under our higher iron(III) concentration conditions. Therefore the model was expanded to include $Fe_2(OH)_2(LH)^{3+}$ as described by eq 2. Then the initial absor-

Table 3. Stoichiometry Results for the Oxidation of Cysteine and Cystine by Aqueous Iron(III)

reductant	ratio of [Iron(II)] produced[a] to initial reductant for various reaction times (min): 1 min	1.5 min	3 min	10 min	20 min
cysteine[b]	1.0	1.5		2.0	2.0
cysteine[c]	0.85		1.0		1.2
cystine[d]			0.30	2.2	3.1

[a] Determined spectrophotometrically as the tris(1,10-phenanthroline) complex, with an estimated uncertainty of ±0.05, based on duplicate experiments. [b] Initial concentrations of iron(III), cysteine, and H^+ are 0.020, 3.0×10^{-4}, and 0.017 M, respectively. [c] Initial concentrations of iron(III), cysteine, and H^+ are 0.010, 3.0×10^{-4}, and 0.013 M, respectively. [d] Initial concentrations of iron(III), cystine, and H^+ are 0.020, 2.06×10^{-4}, and 0.015 M, respectively.

$$Fe_2(OH)_2^{4+} + H_3L^+ \overset{K_{f2}}{\rightleftharpoons} Fe_2(OH)_2(LH)^{3+} + 2H^+ \quad (2)$$

bance (A_0) is given by eq 3, where l is the path length, $[L]_{tot.}$ is the total amino acid concentration, and K_a is the acid dissociation constant of the amino acid.

$$A_0 = l[L]_{tot.}\frac{\epsilon_1 K_{f1}[FeOH_2] + \epsilon_2 K_{f2}[Fe_2(OH)_2]}{[H^+](K_a + [H^+]) + K_{f1}[FeOH_2] + K_{f2}[Fe_2(OH)_2]} \quad (3)$$

If the initial absorbancies are fitted to this model, the parameters are undefined because their magnitudes are comparable to their standard errors. Since the available conditions do not establish limiting values of K_{fn} or ϵ_n for either of the complexes, we have fixed K_{f1} at 0.064 M, a value consistent with the present kinetic and previous equilibrium measurements, to obtain $K_{f2} = 0.38 \pm 0.08$ M and ϵ_1 and ϵ_2 of 974 ± 23 and 1472 ± 98 M^{-1} cm^{-1}, respectively. These parameters provide self-consistent fits of the complexation absorbance time profiles for conditions where the dimer complex makes a significant contribution.

Oxidation−Reduction Kinetics for Cysteine. The fading of the blue color of the iron(III) complex of cysteine generally is associated with oxidation of the sulfhydryl group to the disulfide and reduction of iron(III) to iron(II).

$$2Fe^{III} + 2HSCH_2CH(NH_3^+)CO_2H \longrightarrow 2Fe^{II} + 2H^+ + \begin{matrix} SCH_2CH(NH_3^+)CO_2H \\ | \\ SCH_2CH(NH_3^+)CO_2H \end{matrix} \quad (4)$$

The stoichiometry, under the conditions of excess iron(III) in the present work, was investigated by determining the iron(II) produced, and the results are summarized in Table 3. On the time scale of disappearance of the blue color for cysteine (~1

min), the stoichiometry is one iron(II) produced per amino acid, but further oxidation does occur at longer times. It is also shown in Table 3 that the disulfide product, cystine, is oxidized by iron(III). However, the cystine solutions are colorless, and the spectrophotometric observations described below do not monitor the further oxidation of cystine.

The absorbance−time curves at 620 nm for the redox reaction are not fitted satisfactorily by a first-order rate law because the absorbance decreases too slowly during the later stages of the reaction. A first suspicion might be that the reaction is inhibited by the products. However, several experiments under anaerobic conditions with iron(II) added at the $(4-10) \times 10^{-3}$ M level show no kinetic effect of iron(II), and similar additions of cystine produced no effect. The half-time of the reaction also decreases with increases in the concentration of the deficient reagent, cysteine, under otherwise identical conditions. These observations indicate that the reaction is higher than first order in cysteine, and it is found that the absorbance−time profiles are well fitted by a second-order dependence on cysteine. Therefore, the rate is given by eq 5, where k_2 is a pseudo-second-order rate constant which will depend on the iron(III) and H^+ concentrations.

$$\text{rate} = k_2[\text{cysteine}]^2 \tag{5}$$

The various kinetic runs have been fitted graphically by numerical integration to obtain the values for k_2 that are given in Table 4. In the numerical analysis, the absorbance at the time of mixing ($t = 0$) was calculated from equilibrium constants and extinction coefficients that gave overall self-consistency for all the runs, with $K_{f1} = 0.0248$ M from the complexation kinetics. Details are given in the Experimental Section, and the results are as follows: $\epsilon_1 = 700\ M^{-1}\ cm^{-1}$; $\epsilon_2 = 1500\ M^{-1}\ cm^{-1}$; $K_{f2} = 0.07$ M.

The problem is then to determine the iron(III) and acidity dependence of k_2. The concentrations of the potential reactants are given in eq 6, where $DC = [H^+](K_a + [H^+]) + K_{f1}[FeOH_2] + K_{f2}[Fe_2(OH)_2]$.

$$[LH_3^+] = [L]_{tot.}\frac{[H^+]^2}{DC} \qquad [LH_2] = [L]_{tot.}\frac{K_a[H^+]}{DC}$$

$$[FeLH] = [L]_{tot.}\frac{K_{f1}[FeOH_2]}{DC}$$

$$[Fe_2(OH)_2LH] = [L]_{tot.}\frac{K_{f2}[Fe_2(OH)_2]}{DC} \tag{6}$$

The reaction rate is second order in the total cysteine concentration ($[L]_{tot.}$), and this might result from a number of reactant combinations. If termolecular terms, such as $[FeOH_2][H_3L^+]^2$ and $[FeOH_2][H_2L]^2$ are neglected, then the possible contributions to the rate are given by eq 7. If the concentrations

$$\text{rate} = k_2[L]_{tot.}^2 = k_1'[FeLH][H_3L^+] + k_1''[FeLH][H_2L] + k_2'[FeLH]^2 + k_2''[Fe_2(OH)_2LH][H_3L^+] + k_2'''[Fe_2(OH)_2LH][H_2L] + k_3'[Fe_2(OH)_2LH][FeLH] + k_4'[Fe_2(OH)_2LH][Fe_2(OH)_2LH] \tag{7}$$

are substituted from eq 6, then each term on the right hand side has a common factor of $[L]_{tot.}^2/(DC)^2$. Furthermore, the concentration of $Fe_2(OH)_2$ can be expressed as $[Fe_2(OH)_2] = K_D[FeOH_2]^2/[H^+]^2$. Then substitution and rearrangement give

Table 4. Kinetic Results for the Oxidation of Cysteine by Aqueous Iron(III) at 25 °C in 1.0 M $LiClO_4/HClO_4$

$[H^+] \times 10^2$, M	[cysteine] $\times 10^4$, M	$[Fe(III)]_m$[a] $\times 10^3$, M	$[Fe_2(OH)_2^{4+}] \times 10^4$, M	$10^{-2}k_2$, $M^{-1}\ s^{-1}$ obsd[b]	calcd
0.952	3.78	3.08	2.00	1.0	0.972
0.919	3.78	5.60	7.10	2.8	2.72
0.897	1.38	6.68	10.6	3.5	3.62
0.888	2.02	6.65	10.7	3.7_5[c]	3.67
0.888	2.03	6.65	10.7	3.6	3.67
0.872	3.25	6.60	10.9	3.7	3.77
0.879	3.78	6.61	10.8	3.8[d]	3.72
0.869	4.05	6.58	11.0	3.8_5[c]	3.78
0.873	4.14	6.59	10.9	3.7[d]	3.75
0.851	8.35	6.52	11.2	3.9[c]	3.89
0.770	1.94	7.93	20.3	5.8	5.59
0.770	1.94	7.93	20.3	5.8	5.59
0.770	1.94	7.93	20.3	5.8	5.59
0.843	2.01	8.98	21.7	5.4	5.51
0.843	2.01	8.98	21.7	5.2	5.51
0.843	2.01	8.98	21.7	5.1	5.51
0.830	1.94	10.3	29.4	6.3	6.27
0.830	1.94	10.3	29.4	6.4	6.27
0.830	1.94	10.3	29.4	6.2	6.27
0.912	2.01	11.9	32.3	6.4	6.24
0.912	2.01	11.9	32.3	6.4	6.24
0.912	2.01	11.9	32.3	6.4	6.24
1.96	3.78	3.58	0.638	0.17	0.165
1.94	3.78	6.94	2.45	0.57	0.527
1.89	3.67	8.50	3.87	0.80[c]	0.805
1.90	4.05	8.51	3.83	0.80	0.789
1.90	4.14	8.50	3.83	0.75[d]	0.791
1.85	8.35	8.46	4.00	0.80[c]	0.847
1.89	2.01	8.65	3.99	0.90	0.822
1.89	2.01	8.65	3.99	0.90	0.822
1.89	2.01	8.65	3.99	0.90	0.822
1.86	2.01	16.1	14.4	2.2_5	2.32
1.86	2.01	16.1	14.4	2.2_5	2.32
1.86	2.01	16.1	14.4	2.2_5	2.32
1.84	2.01	19.5	21.4	3.0	3.07
1.84	2.01	19.5	21.4	3.0	3.07
1.84	2.01	19.5	21.4	3.0	3.07
4.71	8.17	9.53	0.782	0.054	0.0577
4.87	8.24	9.54	0.732	0.052_5	0.0518
5.05	8.03	12.4	1.15	0.075	0.0719
5.06	8.03	18.9	2.66	0.15	0.149
10.0	5.10	19.6	0.731	0.015	0.0157
9.91	5.07	34.0	2.25	0.040	0.0425

[a] Concentration of monomeric species, $Fe(OH_2)_6^{3+} + Fe(OH_2)_5(OH)^{2+}$. [b] Runs are under aerobic conditions unless otherwise indicated. [c] Under anaerobic conditions. [d] Under anaerobic conditions in the presence of aqueous iron(II).

eq 8. This equation predicts that $k_2(DC)^2$ plotted versus $[FeOH_2]$

$$k_2(DC)^2 = k_1'K_{f1}[H^+]^2[FeOH_2] + k_1''K_{f1}K_a[H^+][FeOH_2] + k_2'K_{f2}[FeOH_2]^2 + k_2''K_{f2}K_D[FeOH_2]^2 + k_2'''K_{f2}K_DK_a[H^+]^{-1}[FeOH_2]^{2+} + k_3'K_{f1}K_{f2}K_D[H^+]^{-2}[FeOH_2]^3 + k_4'K_{f2}^2K_D^2[H^+]^{-4}[FeOH_2]^4 \tag{8}$$

should be a smooth function at a particular hydrogen ion concentration. The plot in Figure 1 clearly shows that $k_2(DC)^2$ has a greater than first-order dependence on $[FeOH_2]$. This plot also shows that $k_2(DC)^2$ decreases with increasing $[H^+]$ but seems to reach a limiting value for the higher hydrogen ion concentrations of ~0.05 and 0.1 M. This hydrogen ion dependence shows that the k_1' and k_1'' terms are not important contributors.

For qualitative analysis, the k_1' and k_1'' terms may be neglected, so that the above equation can be simplified and

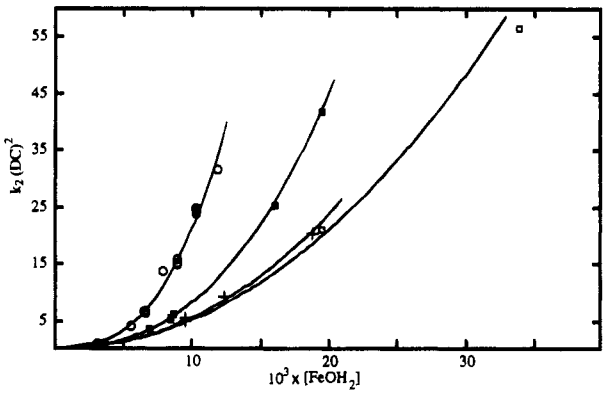

Figure 1. Variation of the second-order rate constant for oxidation of cysteine by aqueous iron(III), $k_2 \times DC$ (=$[H^+](K_a + [H^+]) + K_{f1}[FeOH_2^{3+}] + k_{f2}[Fe_2(OH)_2^{4+}]$) with the concentration of $FeOH_2^{3+}$. The points are grouped according to the approximate values of $[H^+]$ (M) as follows: 0.009 (○), 0.019 (■), 0.05 (+), and 0.1 (□).

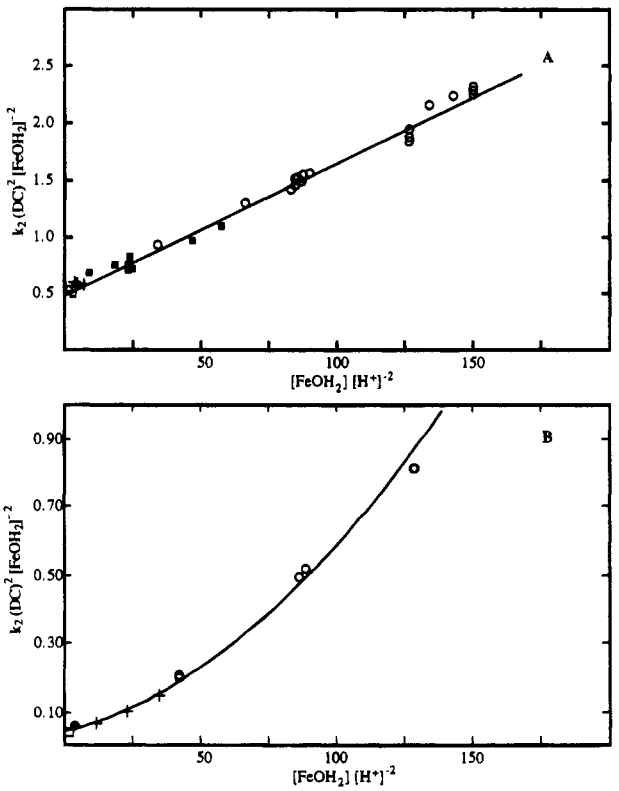

Figure 2. Plot predicted by eq 9 for the oxidation of cysteine (A) and penicillamine (B) by aqueous iron(III).

rearranged to give

$$k_2(DC)^2[FeOH_2]^{-2} = k_2'K_{f2} + k_2''K_{f2}K_D + k_2'''K_{f2}K_DK_a[H^+]^{-1} + k_3'K_{f1}K_{f2}K_D[H^+]^{-2}[FeOH_2] + k_4'K_{f2}^2K_D^2[H^+]^{-4}[FeOH_2]^2 \quad (9)$$

The linearity of the plot in Figure 2A shows that the k_2''' and k_4' terms are not major factors. Least-squares analysis gives values of $(k_2'K_{f2} + k_2''K_{f2}K_D) = 0.50 \pm 0.03$ and $k_3'K_{f1}K_{f2}K_D = (1.15 \pm 0.06) \times 10^{-2}$ (errors are 95% confidence limits). If the k_2''' and/or k_4' terms are included, there is no improvement in the quality of the fit and these parameters are undefined. The standard error of the fit is improved by 22% if the k_1'' term is included, and the best-fit values are as follows: $k_1''K_{f1}K_a = (2.9 \pm 1.7) \times 10^{-2}$, $(k_2'K_{f2} + k_2''K_{f2}K_D) = 0.394 \pm 0.07$, $k_3'K_{f1}K_{f2}K_D = (1.31 \pm 0.08) \times 10^{-2}$. From these values one obtains $k_1'' = 92$, $k_2' = 6.4 \times 10^2$ or $k_2'' = 3.0 \times 10^3$, and $k_3' = 4.0 \times 10^3\ M^{-1}\ s^{-1}$. The experimental and calculated values are compared in Table 4, and the results are summarized in Table 6.

Table 5. Kinetic Results for the Oxidation of Penicillamine by Aqueous Iron(III) at 25 °C in 1.0 M $LiClO_4/HClO_4$

$[H^+] \times 10^2$, M	[penicillamine][a] $\times 10^4$, M	$[Fe(III)]_m$[b] $\times 10^3$, M	$[Fe_2(OH)_2^{4+}] \times 10^4$, M	k_2, $M^{-1}\ s^{-1}$ obsd[c]	k_2, $M^{-1}\ s^{-1}$ calcd
0.861	2.62	6.56	1.11	22	20.7
0.880	2.68	6.63	1.08	21.5[d]	20.2
0.945	2.62	3.76	3.02	9.0	8.65
0.945	2.62	3.76	3.02	9.5[e]	8.65
0.955	2.62	11.7	31.4	25	26.3
0.956	2.68	11.7	31.4	25[d]	26.3
1.89	4.49	12.3	8.11	8.1	8.2
1.93	4.43	8.54	3.75	4.4	4.68
1.94	3.03	8.55	3.73	4.4	4.62
1.95	4.49	4.44	0.987	1.4_5	1.57
4.94	6.17	9.55	0.713	0.38	0.335
4.97	3.27	9.56	0.707	0.38	0.329
10.0	5.67	19.6	0.727	0.10	0.112

[a] All penicillamine stock solutions were prepared anaerobically and from penicillamine free base unless otherwise indicated. [b] Concentration of monomeric species, $Fe(OH_2)_6^{3+} + Fe(OH_2)_5(OH)^{2+}$. [c] Runs are under aerobic conditions unless otherwise indicated. [d] Penicillamine hydrochloride was used. [e] Under anaerobic conditions.

There is a kinetic ambiguity between the k_2' and k_2'' terms. The latter involves reduction of the iron(III) dimer complex by fully protonated cysteine (H_3L^+). We suspect the H_3L^+ is a poorer reducing agent than its conjugate base (H_2L) because the k_1' term makes no significant contribution compared to k_1'', although H_3L^+ is the major form of the ligand between 0.05 and 0.1 M H^+. Therefore assignment of this term largely to k_2' appears preferable.

Oxidation–Reduction Kinetics for Penicillamine. Qualitatively, this system is analogous to cysteine except that the reaction is about 10 times slower under similar conditions. In principle, the slower reaction should make the penicillamine reaction more tractable because the initial absorbance is well defined and normal mixing times are small compared to the reaction time. In fact, the initial absorbance values are quite reproducible, but the kinetics were reproducible only when freshly prepared, anaerobic solutions of penicillamine were used.

For freshly prepared anaerobic stock solutions of penicillamine, reproducible results were obtained and the absorbance–time profiles are satisfactorily fitted by the second-order rate law described for cysteine. As with cysteine, the addition of iron(II) under anaerobic conditions and penicillamine disulfide (or cystine) have no apparent effect on the absorbance–time profiles.

Graphical analysis (Figure 2B) shows that the data are consistent with eq 9, but the plot is not linear and indicates that the k_4' term is contributing. Least-squares analysis gives $(k_2'K_{f2} + k_2''K_{f2}K_D) = (4.15 \pm 0.3) \times 10^{-2}$, $k_3'K_{f1}K_{f2}K_D = (2.39 \pm 0.4) \times 10^{-3}$, and $k_4'K_{f2}^2K_D^2 = (3.54 \pm 0.5) \times 10^{-5}$. From these values one obtains $k_2' = 9.0$ or $k_2'' = 57$, $k_3' = 48.5$ and $k_4' = 25.5\ M^{-1}\ s^{-1}$. The experimental and calculated values are compared in Table 5, and the results are summarized in Table 6.

Oxidation Kinetics with Excess Penicillamine. This study was initiated to allow comparison with the similar study on cysteine of Jameson et al. Because of the low iron(III) concentrations, the second- and higher-order iron(III) contributions to the rate will be minimized and it may be possible to establish values or upper limits for k_1' and k_1''.

Reproducibility problems also were found under these conditions, even though fresh, anaerobic penicillamine solutions were

Table 6. Summary of Rate Constants for Complexation and Oxidation of Cysteine and Penicillamine by Aqueous Iron(III) at 25 °C in 1.0 M $LiClO_4/HClO_4$

reactants		rate constant, M^{-1} s^{-1} cysteine	penicillamine
	$k_2K_m + k_3K_a$	2.29	1.90
$(H_2O)_5Fe(OH)^{2+} + H_3L^+$	k_2[a]	$\leq 1.4 \times 10^3$	$\leq 1.2 \times 10^3$ (2.1×10^3)[b]
$Fe(OH_2)_6^{3+} + H_2L$	k_3[a]	$\leq 1.8 \times 10^2$	$\leq 1.5 \times 10^2$ (2.5×10^2)[b]
$(H_2O)_5Fe(OH)^{2+} + H_2L$	k_4	7.4×10^3	7.4×10^3 (5.2×10^3)[b]
$(H_2O)_8Fe_2(OH)_2^{4+} + H_2L$	k_d	4.3×10^3	8.2×10^3
$Fe(LH)^{2+} + H_3L^+$	k_1'	<6.0[c]	<0.08[c]
$Fe(LH)^{2+} + H_2L$	k_1''	9.2×10^1	<0.06[c]
	$k_2'K_{f2} + k_2''K_{f2}K_D$	0.394	0.415×10^{-1}
$Fe(LH)^{2+} + Fe(LH)^{2+}$	k_2'[d]	6.4×10^2	9.0
$Fe_2(OH)_2(LH)^{3+} + H_3L^+$	k_2''[d]	3.0×10^3	57
$Fe_2(OH)_2(LH)^{3+} + H_2L$	k_2'''	$<1.4 \times 10^3$ [c]	
$Fe_2(OH)_2(LH)^{3+} + Fe(LH)^{2+}$	k_3'	4.0×10^3	48.5
$Fe_2(OH)_2(LH)^{3+} + Fe_2(OH)_2(LH)^{3+}$	k_4'	$<1.2 \times 10^3$ [c]	25.5

[a] There is a proton ambiguity between k_2 and k_3 so that the value given is an upper limit assuming that the term is totally k_2 or k_3. [b] Values from ref 2 at 25 °C in 0.50 M $NaClO_4$. [c] Upper limits are determined on the basis that these terms are not detectable in the rate law and must be contributing less than 20% to the experimental rate constant under the conditions of their maximum possible contribution. [d] There is a kinetic ambiguity between k_2' and k_2''', and the former is suggested as the prefered assignment.

Scheme 2

$$Fe(OH_2)_6^{3+} + H_3L^+ \underset{}{\overset{K_{f1}}{\rightleftharpoons}} (H_2O)_4Fe(LH)^{2+} + 2\,H^+$$

$$(H_2O)_4Fe(LH)^{2+} + H_3L^+ \overset{K_{f12}}{\rightleftharpoons} (H_2O)_2Fe(LH)_2^+ + 2\,H^+$$

$$(H_2O)_2Fe(LH)_2^+ \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} Fe^{II}(LH)^+ + HL^\bullet$$

$$Fe^{II}(LH)^+ + H^+ \overset{K_f'}{\rightleftharpoons} Fe^{II}_{aq} + H_2L$$

$$H_2L + HL^\bullet \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} (HL^{\bullet}\!\!-\!\!LH)^\cdot + H^+$$

$$Fe^{III}_{aq} + (HL^{\bullet}\!\!-\!\!LH)^\cdot \xrightarrow{k_7} Fe^{II}_{aq} + HL\!-\!LH$$

$HL^\bullet \equiv {}^\bullet S\text{-}C(CH_3)_2CH(NH_3^+)CO_2^-$ $(HL^{\bullet}\!\!-\!\!LH)^\cdot \equiv$ $\begin{matrix} S\text{-}C(CH_3)_2CH(NH_3^+)CO_2^- \\ {}^\bullet| \\ S\text{-}C(CH_3)_2CH(NH_3^+)CO_2^- \end{matrix}$

used. After many trials, it was found that the mode of mixing was critical, so that solid penicillamine hydrochloride is added to the aqueous $HClO_4/LiClO_4$ solution, as described in the Experimental Section. Then the slowest rates are observed, and they are reproducible. On this basis we believe they represent the true behavior.

An analysis of the initial absorbance for these conditions indicated that there is an additional absorbing species. The dimer complex is not contributing significantly at the low iron(III) concentrations used. The new species was assigned to the bis complex $Fe(LH)_2^+$, with a molar absorptivity of 2.8×10^3 M^{-1} cm^{-1} and a formation constant (K_{f12} in Scheme 2) of 1.6×10^{-2} M to predict the initial absorbance.

The kinetics were studied with a pseudo-first-order excess of penicillamine ($(2.6-6.1) \times 10^{-3}$ M) over iron(III) ($(2-4) \times 10^{-4}$ M) in ~0.021 and ~0.05 M H^+, and iron(II) perchlorate was added at the $(1-6) \times 10^{-3}$ M level. The absorbance–time profiles appear to be normal first-order curves. However, there is some variation in the apparent pseudo-first-order rate constant with the concentration of the deficient reagent, iron(III), because of the contributions from the second-order terms discussed in the preceding section. The rate increases with increasing penicillamine concentration and decreases with increasing $[H^+]$ and iron(II) concentrations. The iron(II) inhibition is less at higher acidity. The $[H^+]$ dependence is consistent with the bis complex as the dominant reactive species. The observations can be most economically accounted for by the reactions in Scheme 2. If a steady state is assumed for the radical intermediates, and the complexation reactions for Fe(III) (K_{f1}, K_{f12}) and Fe(II) (K_f') are rapidly established equilibria, then Scheme 2 predicts that the rate is given by eq 10, where

$$\text{rate} = k_5k_6k_7[Fe^{III}][Fe(LH)_2]/\left[\frac{k_{-5}K_f'[Fe^{II}]_t}{[H^+] + K_f'[LH_2]}(k_{-6}[H^+] + k_7[Fe^{III}]) + k_6k_7[Fe^{III}]\right] \quad (10)$$

$[Fe^{II}]_t$ = total Fe(II) = $[Fe^{II}_{aq}] + [Fe(LH)^+]$. A consideration of the complex formation constants for thiocarboxylate ligands[13] indicates that $K_f' < 10^{-2}$, so that $[H^+] \gg K_f'[LH_2]$ for our conditions. This rate law becomes first order in $[Fe^{III}]$ if $k_7[Fe^{III}] \gg k_{-6}[H^+]$, and then eq 10 simplifies to eq 11, where

$$\text{rate} = k_5[Fe(LH)_2]/\left[\left(\frac{k_{-5}}{k_6}\right)\frac{K_f'[Fe^{II}]_t}{[H^+]} + 1\right] + \text{second-order terms} \quad (11)$$

the minor second-order terms described in the previous section have been added for completeness. Absorbance–time profiles calculated on the basis of this rate law are shown in Figure 3. In Figure 3A, no iron(II) has been added, and these runs show the penicillamine and $[H^+]$ dependence. In Figure 3B, the effect of iron(II) is shown; the dashed curves (calculated with added [iron(II)] = 0) show the magnitude of the inhibition and that the inhibition decreases at the higher acidity. All of the curves in Figure 3 have been calculated from eq 11 with $k_5 = 1.25 \times 10^{-2}$ and $k_{-5}K_f'/k_6 = 3.5$. These parameters provide a satisfactory description of all the data.

Models in which the dominant reaction is $Fe(LH)^{2+} + H_2L$ (k_1'') or $Fe(LH)^{2+} + H_3L^+$ (k_1') are also first order in iron(III) and second order in penicillamine but are not consistent with the $[H^+]$ dependence. A specific rate constant that predicts the rate at 0.02 M H^+ predicts a rate that is 2.5 and 5 times, respectively, too fast at 0.05 M H^+. This analysis leads to the upper limits given for k_1'' and k_1' in Table 6. It remains something of a mystery why these pathways, especially k_1'', do not seem to contribute significantly.

Conclusions

The equilibrium constants K_{f1} and K_{f2} are both smaller for cysteine than penicillamine. This difference is typical of other metal ion systems[13] in which formation constants have been determined for both ligands. Note that K_{f2} for complexation by the iron(III) dimer is larger than K_{f1} by 3 and 6 times for cysteine and penicillamine, respectively. For various α-mercaptocarboxylic acids, McAuley and co-workers and Baiocchi et al. are in reasonable agreement on the K_{f1} values in the general range of 0.5–3 M. For penicillamine, our value of $K_{f1} = 0.068$ M is in excellent agreement with that of Baiocchi et al. These authors suggested that K_{f1} is smaller for penicillamine because it forms a six membered chelate ring, while the α-mercaptocarboxylates form five membered rings.

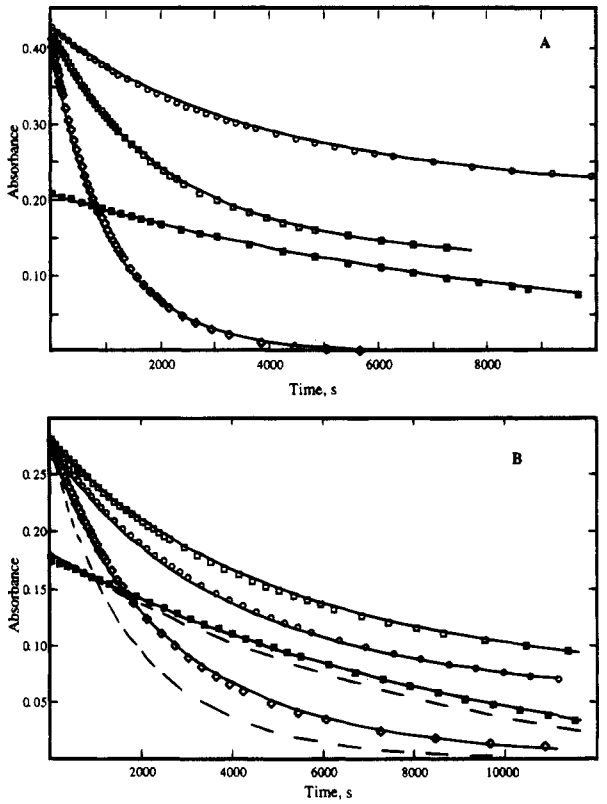

Figure 3. Absorbance–time profiles for the oxidation of excess penicillamine by aqueous iron(III). For A, the concentrations of penicillamine, total Fe(III) and H^+, respectively, are 2.60×10^{-3}, 2.01×10^{-4}, 2.06×10^{-2} (○); 3.80×10^{-3}, 2.01×10^{-4}, 2.11×10^{-2} (□); 6.00×10^{-3}, 2.01×10^{-4}, 2.18×10^{-2} (◇); and 6.15×10^{-3}, 4.00×10^{-4}, 5.08×10^{-2} (βsd). For clarity, the curves are offset by +0.20, +0.12, 0, and −0.04 absorbance units, respectively. For B, the concentrations of Fe(II), penicillamine, total Fe(III) and H^+, respectively, are 1.14×10^{-3}, 2.60×10^{-3}, 2.04×10^{-4}, 2.07×10^{-2} (○); 2.12×10^{-3}, 2.41×10^{-3}, 1.99×10^{-4}, 2.09×10^{-2} (□); 4.00×10^{-3}, 4.09×10^{-3}, 2.02×10^{-4}, 2.17×10^{-2} (◇); and 3.97×10^{-3}, 6.17×10^{-3}, 4.03×10^{-4}, 5.13×10^{-2} (■). For clarity, the curves are offset by +0.05, +0.06, 0, and −0.08 absorbance units, respectively. The curves are calculated from eq 11; dashed curves are calculated assuming [Fe(II)] = 0 for comparison to the ■ and ◇ data in B.

For the molar absorptivity coefficients of the iron(III)–α-mercaptocarboxylate complexes, McAuley and co-workers found values ranging from 140 to 1400 M^{-1} cm^{-1}, while Baiocchi et al. reported 700–1100 M^{-1} cm^{-1} for the same ligands. For penicillamine, our value of 9.7×10^2 is in only fair agreement with the 1200 M^{-1} cm^{-1} reported Baiocchi et al., and we find a somewhat lower value of 7×10^2 for cysteine. These values are somewhat difficult to determine because of the transient nature of the complexes.

As noted in the Introduction, previous work indicates that K_{f1} involves coordination of $-CO_2^-$ and $-S^-$ groups, and the same seems true for K_{f2} because the same number of protons are released, and the iron(III) monomer and dimer complexes have similar absorbance maxima. The dimer complex could involve chelation at just one iron(III) center, but then one might expect $K_{f1} > K_{f2}$ because of the higher charge per iron in the monomer. Alternatively, the amino acid could be bridging between the two iron centers in the dimer, in which case complexation of one end of the ligand would not adversely affect complexation of the other end at the other iron(III) center. This could explain the observation that $K_{f2} > K_{f1}$. The proton loss indicates that the μ-$(OH-)_2$ bridge is not lost, unless it is converted to a μ-$(O)_2$ bridge. The latter seems unlikely under the acidic conditions of this study.

For the complexation reaction, the specific rate constants can be calculated from the composite values determined from the least-squares analysis. These results are summarized in Table 6, and the values are quite typical for substitution on aqueous iron(III) and in reasonable agreement with the earlier work of Baiocchi et al. on penicillamine. There is the usual proton ambiguity between the paths involving $Fe(OH)^{2+} + H_3L^+$ (k_2) and $Fe(OH_2)^{3+} + H_2L$ (k_3). A choice between these terms is often based on the magnitude of the calculated rate constant compared to values for other ligands. In the present cases, this criterion does not eliminate either pathway as a reasonable contributor. For the first pathway, the rate constants of (1.2–1.4) $\times 10^3$ M^{-1} s^{-1} are high for reaction of a cation and are more typical of neutral ligand complexation, but the positive charge on the $-NH_3^+$ group is somewhat removed from the reaction center. Baiocchi et al. argued that assignment to k_3 leads to unreasonably high and variable values for several mercaptocarboxylic acids and penicillamine. On this basis assignment to k_2 seems more appropriate.

For the oxidation of cysteine, the dominant reaction pathways have a common feature in that they involve at least two iron(III) centers and two cysteines to reach the transition state. This appears to be a complicated way to proceed, but note that the product is the disulfide and that two electrons must be released. This feature was also observed by Ellis et al.[1,3] for the oxidation of several mercaptocarboxylic acids by aqueous iron(III). In the latter work, reactions of the iron(III) dimer were not observed because of the high acidity, and the only pathway identified corresponds to k_2' in the present terminology. The value of $k_2' = 6.4 \times 10^2$ M^{-1} s^{-1} for cysteine is somewhat larger than those of 48–167 M^{-1} s^{-1} (25 °C, 1.0 M $NaClO_4$) found by Ellis et al. for different reductants. However, it is still unclear how the nature of the reductant affects the reactivity, and the magnitude of k_2' seems reasonable. The alternative assignment of this kinetic term to k_2'' gives a five times larger rate constant and implies that $k_2'' > k_2'$. The latter order does not agree with the order $k_1' < k_1''$, nor with the expectation that H_2L should be a better reducing agent than H_3L^+.

From the earlier suggestion that the reaction of two $Fe(LH)^{2+}$ units might react through a sulfide bridged structure (**III**), one might expect that the dimer complex $Fe_2(OH)_2(LH)^{3+}$ would undergo facile reduction by cysteine as H_2L. In fact, the k_2''' contribution in eq 7 is too small to be detected, and only an upper limit can be given (Table 6). What is revealing is that k_2''' ($Fe_2(OH)_2(LH)^{3+} + H_2L$) is smaller than k_3' ($Fe_2(OH)_2(LH)^{3+} + Fe(LH)^{2+}$). This indicates that it is more favorable to have both cysteines coordinated to iron(III) centers for oxidation to occur.

The present results and the earlier work of McAuley and co-workers provide a general picture of the oxidation of mercaptocarboxylate systems under conditions of iron(III) in excess. The situation with a deficiency of iron(III) is not so clear. Baiocchi et al. studied several systems under these conditions and published details for mercaptosuccinic acid ($MSAH_3$). These data indicate a first-order dependence on [$MSAH_3$], and the proposed mechanism involves the bis complex ($Fe(MSAH)_2^-$) as a steady-state intermediate. However, the analysis assumed that formation of the mono complex ($Fe(MSAH)^+$) is complete under all conditions, but this is certainly not the case from the formation constant also determined by Baiocchi et al.

Our study with excess penicillamine clearly shows that the reaction is second order in penicillamine, that the bis complex forms as an equilibrium species, and that the latter is the kinetically dominant reactant. The penicillamine reaction also shows iron(II) inhibition, and this and other kinetic features can

be accounted for by the mechanism in Scheme 2. It must be acknowledged that this mechanism has some puzzling features; why, for instance, does the bis complex undergo intramolecular electron transfer, whereas the mono complex seems to be unreactive? The driving force, although slight in either case, would seem to favor the mono complex. The thiyl radical, once formed, couples with free penicillamine, rather than intramolecularly with the other penicillamine ligand in the bis complex precursor. This could be rationalized if the S− atoms are trans to each other in the bis complex and therefore not situated to couple efficiently. Further studies on analogous systems may clarify these issues.

Experimental Section

Materials. The L-cysteine hydrochloride monohydrate, L-cystine, D,L-penicillamine, D-penicillamine, D-penicillaminedisulfide (Aldrich), and D-penicillamine hydrochloride (Sigma) were used as received. Stock solutions of iron(III) perchlorate were prepared by dissolving primary standard grade iron wire (Allied Chemical) in excess 3.5 M $HClO_4$ and oxidizing the iron(II) so produced with H_2O_2. The concentration of $HClO_4$ in the final solution was determined by titrating the H^+ released from Dowex 50W−X8(H^+) cation resin and correcting for the H^+ released by adsorption of iron(III). Solutions of iron(II) perchlorate were prepared by dissolving hydrated $Fe(ClO_4)_2$ (Alfa) in aqueous perchloric acid. The iron(II) content was determined spectrophotometrically as the 1,10-phenanthroline complex, and the solutions were found to contain <4% of the total iron as iron(III).

The lack of reproducibility of the kinetics with a deficiency of penicillamine system was a source of frustration for some time. During over 40 runs, a number of variables were tested such as those that follow: the source of the penicillamine; the source and age of the iron(III) solutions; the addition of iron(II), penicillamine disulfide, or copper(II), at the same level as the penicillamine. None of these produced a consistent kinetic pattern or reproducible results. A key observation to resolving the problem involved addition of more penicillamine to a solution after the reaction is complete. For such an experiment, a typical reaction solution was prepared by adding 2.0 mL of penicillamine solution to 50 mL of acidic aqueous iron(III) (0.0188 M H^+, 0.010 M total iron(III) after mixing). After 70 min, the blue color had faded and a 12.5-mL aliquot of this solution was taken and mixed with 0.50 mL of the original penicillamine stock solution, and the reaction was monitored spectrophotmetrically. This was repeated for aliquots taken after 120 and 240 min. Qualitatively, the time for the absorbance to reach half of its initial value was 250, 110, and 180 s for runs started 70, 120, and 240 min, respectively, after the initial addition of penicillamine. At first sight these observations seem to imply that some catalytically active species is forming in the original reaction solution between 70 and 120 min and has started to disappear after 240 min. However, another explanation is that something is happening in the penicillamine stock solution and that the age and integrity of these solutions is the source of the problem. It was finally found that reproducible results were obtained if the penicillamine stock solution was prepared and kept under anaerobic conditions. Then, for example, if the experiment described in the preceeding paragraph was repeated, the rate constants were 3 ± 0.2 M^{-1} s^{-1} for an anaerobic penicillamine solution 10, 70, 120, and 240 min after preparation. It is suspected that the problem may be trace metal ion catalysis of the oxidation of penicillamine by O_2. Although these reactions yield disulfide as the dominant product by thiyl radical combination, the thiyl radicals thought to be produced may be trapped competitively by O_2 under the low penicillamine concentrations of this study.

The kinetics with excess penicillamine were also fraught with reproducibility problems, even though we thought these had been settled by working with fresh anaerobic solutions. It was found that the mode of mixing for these more concentrated penicillamine solutions was critical. If the stock solution of penicillamine is prepared by adding water or aqueous $HClO_4/LiClO_4$ to solid penicillamine, then highly variable results are obtained. However, if solid penicillamine hydrochloride is added to the aqueous solution then slower and reproducible rates are observed. The only obvious difference is that the former method may yield quite concentrated penicillamine solutions during the initial stages of mixing, but it remains unclear to us why this should be a critical feature.

Kinetic Measurements. A Tritech stopped-flow system was used for the complexation kinetics, the details of the equipment have been described previously,[14] and rate constants are the average of 5−8 runs. The reactions were followed at the absorbance maxima of 620 and 605 nm for cysteine and penicillamine, respectively, with iron(III) in large excess, except where noted. The redox reactions were mainly studied on a Cary 219 spectrophotometer in 50-mm-path length cells, but some runs were done on a Hewlett-Packard 8451 diode array spectrophotometer and some were done on the stopped-flow system. The absorbance scale of the latter was calibrated between 0.07 and 1.40 absorbance units by comparing the absorbance of bromothymol blue solutions at 615 nm on the stopped-flow and Cary 219 systems.

For the determination of k_2, the time between mixing and start of observation (dead time) must be taken into account for a second-order analysis. For our techniques, the dead time is 5−10 s and can be quite significant, because the reaction half-times are often in the range of 20−50 s, especially for cysteine. This problem can be alleviated if one can calculate the initial absorbance on mixing. This determination can be done easily with penicillamine because the reactions are often slow enough so that the dead time is not a significant factor. For cysteine, we have used the complexation model deduced for penicillamine. The value of K_{f1} was taken from the complexation kinetics and used to determine ϵ_1 from slow kinetic runs at the higher acidities, where the dimer and dead time are both insignificant. Then values of K_{f2} and ϵ_2 were determined from a number of other runs, with the initial assumption that ϵ_2 will be similar in magnitude to that for penicillamine and that the dead time must have a reasonable value. Overall good self-consistency is obtained with $\epsilon_1 = 700$, $\epsilon_2 = 1500$, and $K_{f2} = 0.07$.

Equilibrium Species Calculations. These calculations used previously determined equilibrium constants and take into account the hydrogen ion introduced with cysteine hydrochloride and that from the hydrolytic reactions of aqueous iron(III). The acid dissociation constants of cysteine and penicillamine were taken as 0.0126 M ($pK_a = 1.9$).[13] The hydrolysis (K_m) and dimerization constants (K_D) used were 1.62×10^{-3} M and 1.9×10^{-3} M, respectively.[15]

Acknowledgment. This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada.

IC950326M

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