Inorg. Chem. **2005**, *44*, 3541–3550

Catalytic and Direct Oxidation of Cysteine by Octacyanomolybdate(V)

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Received March 22, 2005

The oxidation of cysteine by $[Mo(CN)_8]^{3-}$ in deoxygenated aqueous solution at a moderate pH is strongly catalyzed by Cu^{2+}, to the degree that impurity levels of Cu^{2+} are sufficient to dominate the reaction. Dipicolinic acid (dipic) is a very effective inhibitor of this catalysis, such that with 1 mM dipic, the direct oxidation can be studied. UV–vis spectra and electrochemistry show that $[Mo(CN)_8]^{4-}$ is the Mo-containing product. Cystine and cysteinesulfinate are the predominant cysteine oxidation products. The stoichiometric ratio ($\Delta n_{Mo(V)}/\Delta n_{cysteine}$) of 1.4 at pH 10.8 is consistent with this product distribution. At pH 1.5, the reaction is quite slow and yields intractable kinetics. At pH 4.5, the rates are much faster and deviate only slightly from pseudo-first-order behavior. With 2 mM PBN (N-phenyl-*tert*-butyl nitrone) present at pH 4.5, the reaction rate is about 20% less and shows excellent pseudo-first-order behavior, but the stoichiometric ratio is not significantly changed. The rates also display a significant specific cation effect. In the presence of spin-trap PBN, the kinetics were studied over the pH range 3.48–12.28, with $[Na^+]$ maintained at 0.09–0.10 M. The rate law is $-d[Mo(V)]/dt = k[\text{cysteine}]_{tot}[Mo(V)]$, with $k = \{2(k_bK_{a1}K_{a2}[H^+] + k_cK_{a1}K_{a2}K_{a3})\}/([H^+]^3 + K_{a1}[H^+]^2 + K_{a1}K_{a2}[H^+] + K_{a1}K_{a2}K_{a3})$, where K_{a1}, K_{a2}, and K_{a3} are the successive acid dissociation constants of $HSCH_2CH(NH_3^+)CO_2H$. Least-squares fitting yields $k_b = (7.1 \pm 0.4) \times 10^4\ M^{-1}\ s^{-1}$ and $k_c = (2.3 \pm 0.2) \times 10^4\ M^{-1}\ s^{-1}$ at $\mu = 0.1$ M ($NaCF_3SO_3$) and 25 °C. A mechanism is inferred in which k_b and k_c correspond to electron transfer to Mo(V) from the thiolate forms of anionic and dianionic cysteine.

Introduction

The oxidation of thiols is of widespread interest, and, in aqueous media, it is of special significance in biochemistry and environmental chemistry. Thiyl radicals are important intermediates in many of these reactions, and reactions that generate thiyl radicals in the rate-limiting step have the potential to be quite informative with respect to general questions about reactivity. Simple metal-ion oxidants such as Ce(IV) and Fe^{3+} have been demonstrated to produce thiyl radicals, but these reactions are complicated by the formation of various thiol/metal ion complexes. When the oxidants are of the outer-sphere type, one might anticipate significant gains in simplicity, but that has not often been the case; as a rule, aliphatic thiols react with outer-sphere oxidants through trace-copper-catalyzed mechanisms, such that the direct oxidation process is inaccessible. We have recently reported that this limitation can be overcome through the use of suitable chelating ligands.[1−3] In those studies, we used thioglycolate (TGA) as a representative thiol, and the oxidants were $[IrCl_6]^{2-}$, $[Mo(CN)_8]^{3-}$, and $[Os(phen)_3]^{3+}$; simple rate laws were obtained, indicating rate-limiting electron transfer from the thiolate forms of TGA.

The present study extends our work in this area to the oxidation of cysteine, the simplest biothiol. Prior reports on the oxidation of cysteine by typical outer-sphere oxidants include studies with $[Mo(CN)_8]^{3-}$,[4] $[Co^{III}W_{12}O_{40}]^{5-}$,[5] $[Fe(CN)_6]^{3-}$,[6] and $[Co_2(CN)_{10}(O_2)]^{5-}$.[7] The reactions of $[Fe(CN)_6]^{3-}$ and $[Co_2(CN)_{10}(O_2)]^{5-}$ were shown to be very sensitive to catalysis by copper ions, as is the rule. Although no such catalysis was reported for the reactions of $[Mo(CN)_8]^{3-}$ and $[Co^{III}W_{12}O_{40}]^{5-}$, the ubiquity of copper catalysis raises the possibility that it was simply overlooked. The present paper is a reinvestigation of the oxidation of cysteine by $[Mo(CN)_8]^{3-}$ with due attention paid to the possibility of copper catalysis. This oxidant is selected because of its relatively low charge and convenience.

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10.1021/ic050427c CCC: $30.25
Published on Web 04/21/2005

As is described below, we find that dipicolinic acid (dipic) is indeed effective in suppressing the copper catalysis, thus enabling an examination of the direct oxidation of cysteine by $[Mo(CN)_8]^{3-}$. In addition to revealing the anticipated rate-limiting electron transfer from the thiolate forms of cysteine, this reaction displays specific alkali-metal-ion catalysis and a mechanistic bifurcation arising after the rate-limiting step.

Experimental Section

Reagents and Solutions. Cysteine (Fluka), L-cystine, (Aldrich), cysteinesulfinic acid, disodium 2,6-pyridinedicarboxylate (sodium dipicolinate, abbreviated as dipic hereafter), and *N-tert*-butyl-α-phenyl-nitrone (abbreviated as PBN hereafter) were used as received from Aldrich Chemical Co. The high purity of the cysteine as used is demonstrated by ^{1}H NMR spectroscopy, as shown in Figure S-6 (Supporting Information). ^{1}H NMR spectroscopy also demonstrated the high purity of the PBN (data not shown). Sodium triflate ($NaCF_3SO_3$) was prepared by slowly adding sodium carbonate to triflic acid in an ice bath with vigorous stirring. The product was recrystallized from H_2O at 80−85 °C. $LiCF_3SO_3$ and KCF_3SO_3 were prepared by analogous methods. 3-(Trimethylsilyl)-1-propanesulfonic acid, sodium salt (abbreviated as DSS hereafter, Aldrich), was used as the NMR standard reference.

Solutions of $HClO_4$ (Fisher) and CF_3SO_3H were quantified by a standardized NaOH solution. $NaCF_3SO_3$ stock solution was standardized by passing an aliquot through a cation-exchange column packed with Dowex 50W-X8 resin and then titrating with standardized $NaOH_{(aq)}$. Selected buffers (acetate, citrate, monochloroacetate, cacodylate, and hydrogen phosphate buffers) were applied to maintain the pH. All solutions were purged with Ar or N_2 prior to reaction to prevent potential complications caused by O_2.[8−10] All solutions were prepared in deionized water obtained from a Barnstead NANO pure Infinity ultrapure water system.

The concentration of cysteine was determined spectrophotometrically with Ellman's reagent[11] at pH 7.5 in phosphate buffer. The concentrations of the oxidant $[Mo(CN)_8]^{3-}$ [abbreviated as Mo(V) hereafter], the limiting reagent, were determined optically. All cysteine and Mo(V) solutions were prepared freshly and purged with Ar or N_2 prior to reactions. For the kinetic study of fast reactions performed at high pHs, the ionic strength was approximately equal in both solutions to prevent Schlieren effects. All kinetic studies were conducted with the pH controlled by 2 mM selected buffers as described previously,[3] the ionic strength maintained at 0.1 M (sodium triflate), and the solutions thermostated at 25 °C.

$K_4[Mo(CN)_8]\cdot 2H_2O$. This compound was synthesized according to the literature.[12] The UV−vis spectrum shown in Figure S-1 (Supporting Information) has the same absorption pattern as that reported in the literature, but the molar absorption at a maximum wavelength of 240 nm is 9.14×10^3 M^{-1} cm^{-1}, which is about 30% less than the literature value [$(1.3 \pm 0.1) \times 10^4$ M^{-1} cm^{-1}].[13] With a glassy carbon disk as a working electrode, a $Ag/AgCl_{(s)}$ electrode as a reference, and a Pt wire as a counter electrode, the cyclic voltammogram (CV) (Figure S-2, Supporting Information) is quasi-reversible, with $\Delta E_p = 70$ mV and $E_{1/2} = 566$ mV versus $Ag/AgCl_{(s)}$ ($E^\circ_{Ag/AgCl(s)} = 0.205$ V vs normal hydrogen electrode),[14] that is, 0.771 V versus a normal hydrogen electrode. The E_p in the OSWV (Osteryoung Square-Wave Voltammogram) is similar.

$Cs_3[Mo(CN)_8]\cdot 2H_2O$. This compound was prepared according to the literature.[2] Its UV−vis spectrum displays peaks at 254, 268, and 388 nm (Figure S-1, Supporting Information) with $\epsilon = 2.76 \times 10^3$, 2.84×10^3, and 1.49×10^3 M^{-1} cm^{-1}, respectively. The CV (Figure S-3, Supporting Information) and OSWV properties (ΔE_p and $E_{1/2}$) are equivalent to those of $K_4[Mo(CN)_8]\cdot 2H_2O$. Both the optical properties and the electrochemistry of $Cs_3[Mo(CN)_8]\cdot 2H_2O$ are consistent with data reported earlier.[2]

Methods. Electrochemistry was performed on a BAS 100B electrochemical analyzer equipped with a BAS C3 cell stand with a purging and stirring system. A Corning 450 pH/ion meter with a Mettler Toledo InLab 421 pH electrode was used for pH measurements. ^{1}H NMR spectra of the reactants and products were obtained with Bruker AC 250 and AV 400 spectrometers. Determination of $[cysteine]_{tot}$ was conducted with constant stirring on a Hewlett-Packard 8453 diode-array spectrophotometer equipped with a 1-cm quartz cuvette and a Brinkmann BMS Lauda thermostat to maintain temperature at 25.0 ± 0.1 °C. A 375-nm optical cutoff filter was applied in recording the UV−vis spectra of Mo(V) to minimize the photochemical decomposition of $Cs_3[Mo(CN)_8]\cdot 2H_2O$[15] for all experiments run on the Hewlett-Packard 8453 diode-array spectrophotometer. The fast kinetic studies were performed on a Hi-Tech SF-51 stopped-flow spectrophotometer in the 1-cm optical path configuration with a tungsten lamp, using an OLIS data acquisition system. The stopped-flow optical slit width was limited to 1 mm as a precaution against the photolysis of Mo(V). All reactions were performed in a dark room and monitored at 388 nm [an absorption maximum for Mo(V)] at 25.0 ± 0.1 °C. Kinetic traces were fit to exponential functions by the use of OLIS-supplied software.

Results

Cysteine has three dissociable acidic protons with $pK_{a1} = 1.90$, $pK_{a2} = 8.18$, and $pK_{a3} = 10.30$ at ionic strength 0.1 M.[16] These pK_a values correspond to the dissociation of the cationic protonated form of cysteine, the neutral form, and the anionic form, largely as represented in eqs 1−3.

$$HSCH_2CH(NH_3^+)CO_2H \rightleftharpoons HSCH_2CH(NH_3^+)CO_2^- + H^+ \qquad pK_{a1} = 1.90 \quad (1)$$

$$HSCH_2CH(NH_3^+)CO_2^- \rightleftharpoons {}^-SCH_2CH(NH_3^+)CO_2^- + H^+ \qquad pK_{a2} = 8.18 \quad (2)$$

$${}^-SCH_2CH(NH_3^+)CO_2^- \rightleftharpoons {}^-SCH_2CH(NH_2)CO_2^- + H^+ \qquad pK_{a3} = 10.30 \quad (3)$$

Overall, cysteine can exist in four different degrees of ionization, and the neutral and anionic forms also have minor tautomer forms. All of these proton-transfer reactions should

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Table 1. Cu^{2+} Catalysis and Dipic Inhibition in the Oxidation of Cysteine by Mo(V)[a]

expt no.	$[Cu^{2+}]$, μM	[dipic], mM	$t_{1/2}$, s
1	0	0	0.21
2	5	0	<0.002
3	0	1	8.33
4	5	1	8.35
5	5	2	8.13

[a] Cu^{2+} added as $CuSO_4$. $[cysteine]_{tot} = 5$ mM and $[Mo(V)]_0 = 0.2$ mM at pH 4.2 (acetate buffer) and 25 °C.

be at equilibrium on the time scale of the reactions under study. The total concentration of all of the cysteine species present in the various experiments is represented by $[cysteine]_{tot}$.

Most of the reactions described below were performed with a large excess of cysteine. We designate the initial Mo(V) concentration in the reactions as $[Mo(V)]_0$.

Catalysis by Copper Ion. Studies of metal-ion catalysis were performed with 5 mM cysteine and 0.2 mM Mo(V) at pH 4.2, with various conditions described in Table 1. The half-life of the reaction in the absence of chelating reagent was 0.21 s. In the presence of 1 mM dipic (an excellent redox-inert chelating agent with large Cu^{2+}/dipic^{2-} formation constants: log $K_1 = 9.1$ and log $K_2 = 16.4$),[16] the half-life increased 40-fold, strongly suggesting trace metal ion catalysis. With the addition of 5 μM Cu^{2+} but no dipic, the half-life was immeasurably short ($t_{1/2} < 2$ ms). Thus, this reaction is strongly catalyzed by Cu^{2+}, to the degree that trace impurities of Cu^{2+} are sufficient to dominate the kinetics.

Table 1 also shows that with 1 or 2 mM dipic, the rates are unaffected by the addition of 5 μM Cu^{2+}. We infer that 1 mM dipic is sufficient to mask the catalytic effect of impurity levels of Cu^{2+} and to produce results characteristic of the direct oxidation of cysteine by Mo(V). All further experiments described below were performed with ~1 mM dipic unless specified otherwise.

Qualitative Kinetics Results. The oxidation of cysteine by $[Mo(CN)_8]^{3-}$ was described in a prior kinetics study, but there was no mention of copper catalysis in that study.[4] In view of the copper catalysis described above, we performed experiments to reexamine the reaction under conditions relevant to the prior study. Specifically, it was reported that with $[Mo(V)]_0 = 6 \times 10^{-5}$ M, $[cysteine]_{tot} = 0.01$ M, and pH 1.40, the reaction was pseudo-first-order for ~70% of the reaction with $t_{1/2} \sim 1.7$ s.[4] Under the same conditions (with no dipic), we observed a much slower reaction ($t_{1/2} =$ 39.1 s) that obeyed nearly zero-order kinetics. In the presence of 1 mM dipic, the half-life increased to 73.5 s with complex kinetic behavior: a slow reaction at first, which became faster, then with zero-order behavior at the end. These results imply that the prior study was heavily influenced by metal-ion catalysis. Moreover, the concentration of catalytic ion impurities is substantially less in our reactions.

As is described in detail below, the reaction rates are highly pH-dependent, increasing by orders of magnitude between pH 2 and 12. Below pH 3, the kinetic traces are intractably complex when the concentration of cysteine is in large excess over Mo(V). This effect may be due to the protonation of dipic (p$K_{a1} = 2.07$ and p$K_{a2} = 4.66$),[16] which would make dipic less effective as an inhibitor below pH 3. Alternatively, the complications in strongly acidic media might be due to acid-induced aquation of $[Mo(CN)_8]^{3-}$, which has been reported to be a significant process below pH 3.5.[17] Above pH 3, the traces approach pseudo-first-order behavior, except for an abrupt termination phase (as shown for pH 4.5 in Figure S-4 of the Supporting Information).

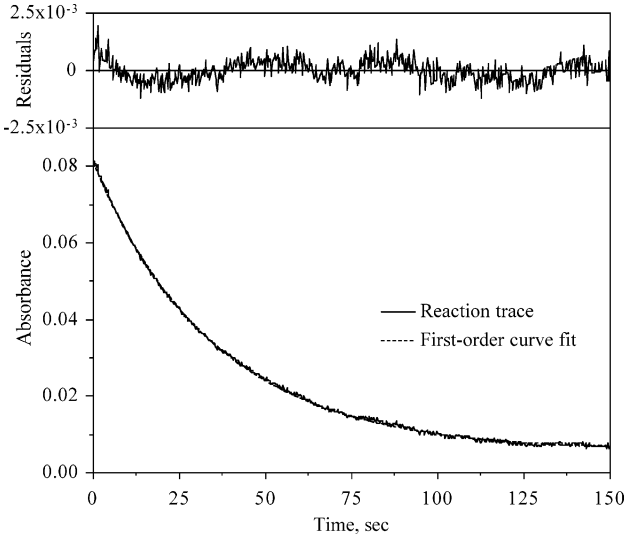

Figure 1. Kinetic trace of oxidation of 5.9×10^{-4} M cysteine by 5.9×10^{-4} M Mo(V) with 1 mM dipic present at pH 4.54 (acetate buffer), $\mu =$ 0.1 M ($NaCF_3SO_3$), and 25 °C, monitored at 388 nm in the presence of 2 mM PBN. A first-order curve fit ($k_{obs} = 2.75 \times 10^{-2}$ s^{-1}) is superimposed, and residuals are presented above.

A series of experiments at pH 4.5 showed that the spin-trap PBN is effective in producing good pseudo-first-order kinetics without grossly perturbing the reaction rates. Figure 1 demonstrates the result when the reaction is conducted with 2 mM PBN: the loss of Mo(V) obeys excellent first-order kinetics, and the half-life is only 20% greater than that in the absence of PBN. As is summarized in Table S-1 (Supporting Information), the effective amount of PBN was determined by varying its concentration from 1 to 5 mM. These results show that 2 mM PBN is sufficient for complete inhibition and that PBN does not react directly with Mo(V). Consequently, all of the kinetic experiments described below pertain to solutions containing 2 mM PBN.

Reactivity of RSSR and RSO_2^- and Alkaline Decomposition of Mo(V). Cystine, a common impurity in cysteine, was also identified as an oxidized product, as described below. It was necessary to examine the reactivity of cystine toward Mo(V). Because of the limited and pH-dependent solubility of cystine in H_2O,[18] the experiment was conducted in basic solution. In the presence of 2 mM dipic, 2 mL of 2.4×10^{-4} M cystine purged with Ar was mixed with 50 μL of 1.4×10^{-4} M Mo(V) at pH 11.13. Over 3000 seconds, there was only a 21% absorbance decrease at pH 11.13, from which it can be concluded that cystine is unreactive on the time scale of the oxidation of cysteine.

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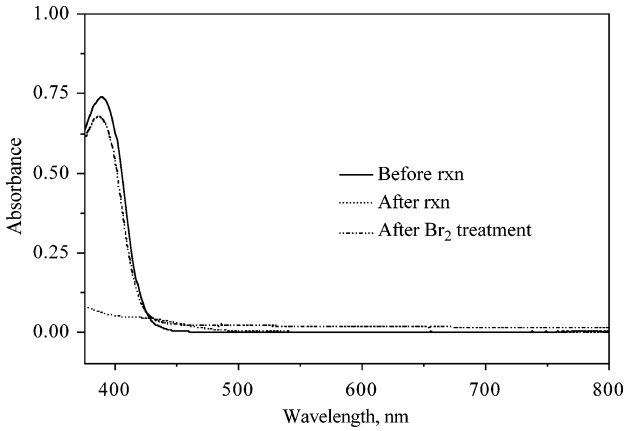

Figure 2. Overlaid UV−vis spectra of Mo-containing solutions: Mo(V) solution before reaction, product solution, and product solution after Br_2 treatment. The reaction was conducted with 4.96×10^{-4} M Mo(V) and 8.3×10^{-4} M cysteine in the presence of 1 mM dipic at pH 2.9, $\mu = 0.1$ M (NaCl), and 25 °C.

As is described below, cysteinesulfinate is another reaction product. Its reactivity toward Mo(V) was examined by mixing 2 mL of 2.0×10^{-4} M cysteinesulfinate containing 2 mM dipic with 50 μL of 5.5×10^{-3} M Mo(V) at pH ~11. The Mo(V) was consumed in about 7 min, which shows that the oxidation of cysteinesulfinate can also be neglected in the studies of cysteine oxidation.

The stability of 1.72×10^{-4} M Mo(V) was examined at pH 11.4: less than 10% of Mo(V) decomposed over 100 minutes. Therefore, the decomposition of Mo(V) at this pH[19,20] can be ignored under the conditions in this study.

Stoichiometry. The Mo-containing product was identified spectrophotometrically and electrochemically. For the spectrophotometric study, a solution of 9.92×10^{-4} M Mo(V) in 0.1 M NaCl was mixed with an equal volume of 1.66 mM cysteine solution in the presence of 1 mM dipic in 0.1 M NaCl at pH 2.9. Figure 2 shows the Mo(V) reactant spectrum (corrected for dilution) and the spectrum of the product mixture. After volume correction, the absorbance of product solution treated with ~1.7 mL of Br_2/CH_3CN was restored to 0.736 at 388 nm; the corresponding corrected initial absorbance was 0.739. These results show that the Mo(V) is completely consumed in the reaction with excess cysteine. The product spectrum is consistent with a conversion to $[Mo(CN)_8]^{4-}$. Full recovery by treatment with Br_2 is also consistent with a complete conversion to $[Mo(CN)_8]^{4-}$. OSWVs of the above Mo-containing solutions are shown in Figure S-5 (Supporting Information). The peak potentials are essentially identical for the Mo(V) reactant and the product solution, and the peak currents after correction for dilution are also essentially identical. Consistent with prior literature reports,[21] we find that cysteine is electrochemically inactive. These electrochemical results confirm that $[Mo(CN)_8]^{4-}$ is produced quantitatively in the reaction of Mo(V) with excess cysteine.

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^{1}H NMR spectroscopy was used to identify the cysteine-derived products of the reaction with Mo(V). Because the solubility of cystine is dependent on the pH,[18] identification of cystine was performed in basic solution to prevent its precipitation. A sample was prepared with 10.73 mM cysteine and 7.06 mM Mo(V) in D_2O in the presence of 1 mM dipic at pH 8.94 (adjusted by 40% $NaOD_{(D2O)}$). The ^{1}H NMR spectrum of the product solution taken right after the reaction is shown in Figure 3. Because the chemical shifts of many of the peaks are pH-dependent, products were identified by spiking the sample with known compounds. This procedure led to the identification of both cystine and cysteinesulfinate as major reaction products. Besides the assigned peaks, two groups of small unidentified multiple peaks also were observed, and their integrated intensities increased ~70% during 1 h. These unidentified peaks are attributed to species that are produced after the formation of cystine and cysteinesulfinate.

The ^{1}H NMR spectrum shown in Figure 3 is unsuitable for a quantitative determination of the product yields because of the low solubility of cystine at that pH, 9. A similar experiment at a higher pH was performed by recording two ^{1}H NMR spectra, the first (Figure S-6 in the Supporting Information) for a solution of 28.5 mM cysteine in D_2O in the presence of 2 mM dipic with 5.1 mM DSS as a reference. This solution was then mixed with an equal volume of 14.67 mM Mo(V) in D_2O, after which, 50 μL of 34 mM $NaOD_{(D2O)}$ was added to prevent the precipitation of cystine (pH 12.5). The ^{1}H NMR spectrum of this product solution is shown in Figure S-7 (Supporting Information). The difference in the integrals of the quartet at 3.5 ppm in the two spectra indicates a 5.07 mM consumption of cysteine, and thus, the consumption ratio, $n_{Mo(V)}/n_{cysteine}$, is calculated as 1.45. The consumption ratio was also determined by using Ellman's method[3,11] to measure $[cysteine]_{tot}$: A solution of 1.76×10^{-4} M cysteine was mixed with 6.68×10^{-5} M Mo(V) in a phosphate buffer at pH 10.8 in the presence of 1 mM dipic on the stopped-flow spectrophotometer. An analysis of the product mixture yielded a consumption ratio of 1.42 ± 0.08, which is in agreement with the NMR result. In the presence of 2 mM PBN, the consumption ratio was found to be 1.52, which implies that PBN has no significant effect on the stoichiometry.

In view of the quantitative conversion of $[Mo(CN)_8]^{3-}$ to $[Mo(CN)_8]^{4-}$, the qualitative identification of cystine and cysteinesulfinate as major products, and the unreactivity of these two products, it is evident that the reaction stoichiometry can be described by eqs 4 and 5 occurring in parallel. The unreactivity of cystine (described above) rules out the possibility that the cysteinesulfinate yield arises from the successive oxidations of cysteine and cystine.

$$4[Mo(CN)_8]^{3-} + HSCH_2CH(NH_3^+)CO_2^- + 2H_2O \rightarrow 4[Mo(CN)_8]^{4-} + {}^-O_2SCH_2CH(NH_3^+)CO_2^- + 5H^+ \quad (4)$$

$$2[Mo(CN)_8]^{3-} + 2HSCH_2CH(NH_3^+)CO_2^- \rightarrow 2[Mo(CN)_8]^{4-} + {}^-O_2CCH(NH_3^+)CH_2SSCH_2CH(NH_3^+)CO_2^- + 2H^+ \quad (5)$$

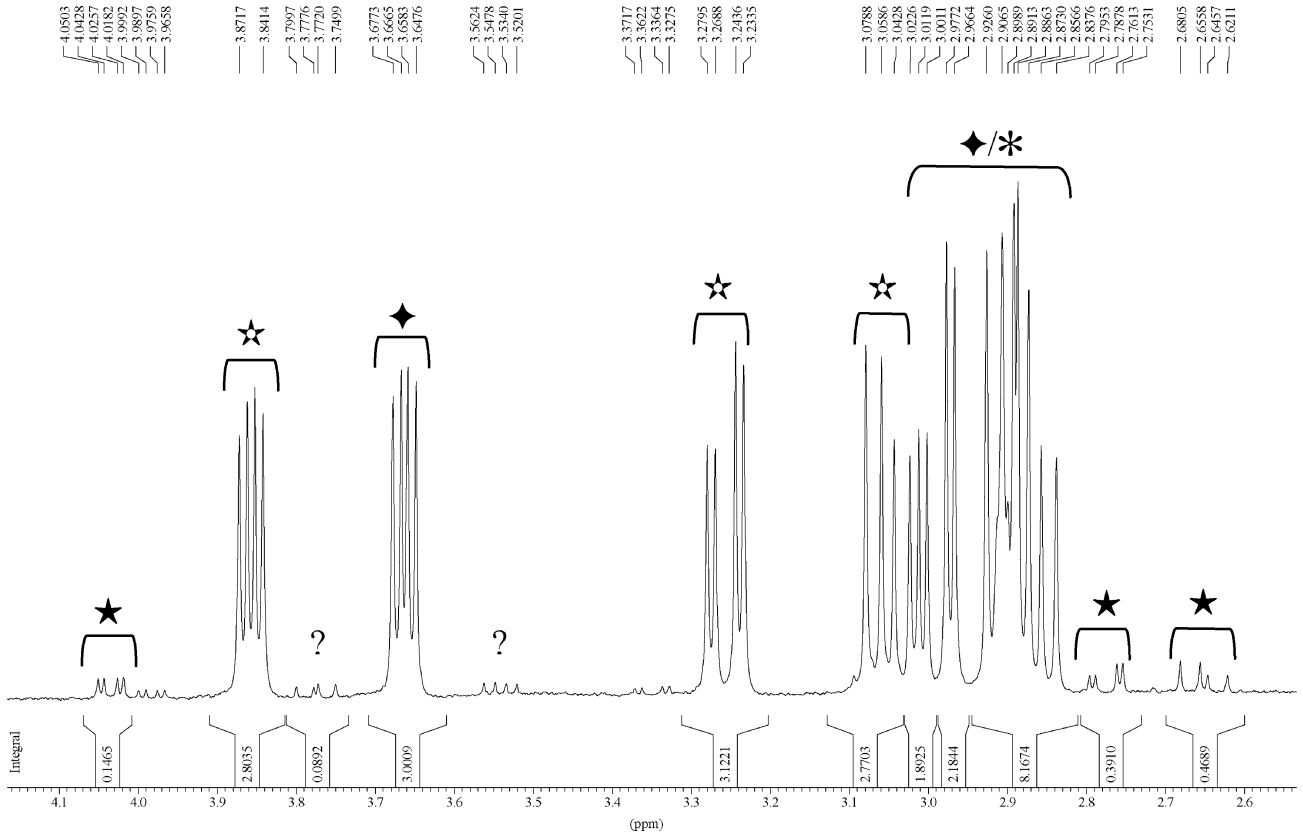

Figure 3. ^{1}H NMR spectrum of the product solution of 10.73 mM cysteine oxidized by 7.06 mM Mo(V) in D_2O in the presence of 1 mM dipic at pH 8.94 with DSS as a reference. (*) DSS, reference; (♦) cysteine; (☆) cystine; (★) cysteinesulfinate; (?) unassigned peaks that grow in after the reaction.

On the basis of the consumption ratio of ~1.5, the contributions of eqs 4 and 5 to the overall reaction are 40 and 60%, respectively.

Kinetics. Reactions with various concentrations of cysteine, 0.501−50.0 mM, oxidized by 4.7×10^{-5} M Mo(V) in 0.02 M acetate buffer with 1 mM dipic and 2 mM PBN present at 0.1 M ionic strength (sodium triflate) and pH 4.6 display excellent pseudo-first-order kinetics. Apparent rate constants, k_{obs}, with the corresponding $[cysteine]_{tot}$ at pH 4.6 are summarized in Table S-2 (Supporting Information). The plot of k_{obs} versus $[cysteine]_{tot}$ shown in Figure 4 is linear with a slope of $(5.05 \pm 0.02) \times 10^{-2}$ M^{-1} s^{-1}, which shows that the rate law is first-order with respect to $[cysteine]_{tot}$, as in eq 6.

$$-d[Mo(V)]/dt = k_{obs}[Mo(V)] = k[cysteine]_{tot}[Mo(V)] \quad (6)$$

A study of the pH dependence of the kinetics was performed with various cysteine concentrations under pseudo-first-order conditions over the pH range of 3.48−12.28 in the presence of 2 mM PBN and 1 mM dipic at 0.1 M ionic strength (CF_3SO_3Na). Selected buffers at a 2 mM concentration were employed to maintain the pH values. To prevent possible complications caused by the decomposition of Mo(V) at a high pH[22] or the protonation of the cyano ligand coordinated on the metal center,[23,24] no reaction was performed at a pH higher than 12.5 or lower than 3. All kinetic data are collected in Table S-3 (Supporting Information). The contour of the complicated plot of $\log(k_{obs}/[cysteine]_{tot})$ versus pH shown in Figure 5 contains three sections corresponding to the reactivity of cysteine at various pHs. As the pH increases, the reactivity increases until reaching pH 8 (~pK_{a2}). The reactivity remains steady up to a pH of about 10 (~pK_{a3}) then drops until it reaches another plateau. At this pH, dianion cysteine is the dominant species.

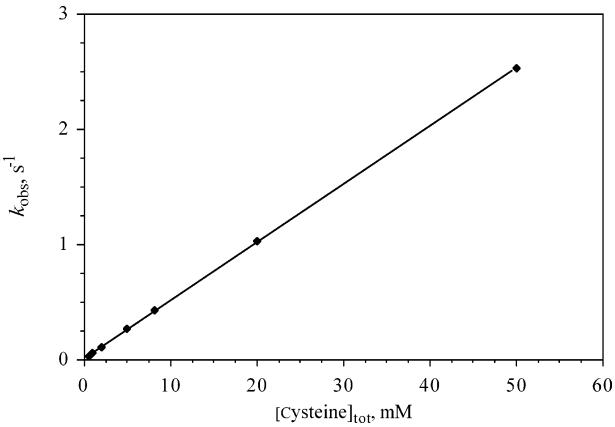

Figure 4. Plot of k_{obs} vs $[cysteine]_{tot}$. $[Cysteine]_{tot} = (0.501-50.0)$ mM, $[Mo(V)]_0 = 4.7 \times 10^{-5}$ M, [dipic] = 1 mM, [PBN] = 2 mM in 0.02 M acetate buffer with the ionic strength controlled at 0.1 M by sodium triflate at pH 4.6 and 25 °C.

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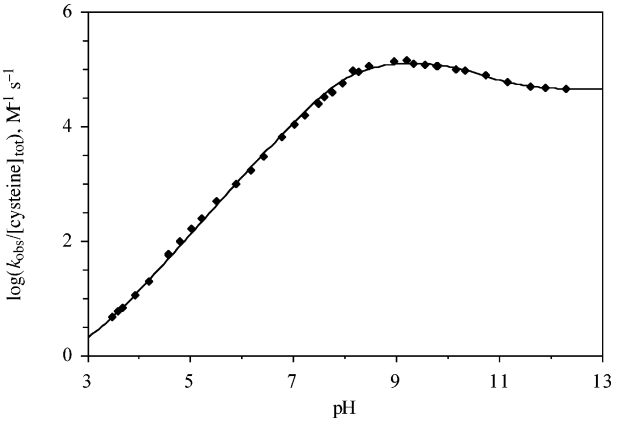

Figure 5. Plot of log(k_{obs}/[cysteine]$_{tot}$) vs pH. Data from Table S-3 (Supporting Information). Symbol ♦ represents k_{obs} at various pHs ranging from 3.48 to 12.28. The solid line is the least-squares fit of eq 8.

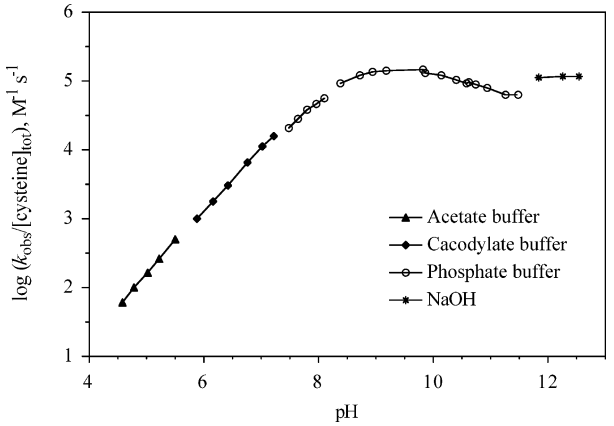

Figure 6. Kinetic salt effects illustrated by a plot of log(k_{obs}/[cysteine]$_{tot}$) vs pH at μ = 0.1 M. Four different buffers were used at 0.02 M concentration, leading to various Na^+ concentrations.

In principle, each protonation state of cysteine can react with $[Mo(CN)_8]^{3-}$ through kinetically distinguishable terms, as in eq 7:

$$\frac{k_{obs}}{[cysteine]_{tot}} = \frac{k_1[H^+]^3 + k_2K_{a1}[H^+]^2 + k_3K_{a1}K_{a2}[H^+] + k_4K_{a1}K_{a2}K_{a3}}{[H^+]^3 + K_{a1}[H^+]^2 + K_{a1}K_{a2}[H^+] + K_{a1}K_{a2}K_{a3}} \quad (7)$$

where k_1-k_4 represent the reactivity of protonated, neutral, monoanionic, and dianionic cysteine species, respectively. A nonlinear least-squares fit of the data in Table S-3 (Supporting Information) to eq 7 with pK_{a1} held at the literature value of 1.9 shows that eq 7 can represent the data very accurately. The derived parameters are $k_1 = 1.0 \times 10^{-7} \pm 72$ M^{-1} s^{-1}, $k_2 = 1.0 \pm 1.7$ M^{-1} s^{-1}, $k_3 = (1.42 \pm 0.08) \times 10^5$ M^{-1} s^{-1}, $k_4 = (4.5 \pm 0.4) \times 10^4$ M^{-1} s^{-1}, pK_{a2} = 8.03 ± 0.03, and pK_{a3} = 10.4 ± 0.2. The large uncertainty in k_1 means that this parameter is unresolved by the data, and it indicates an upper limit of 72 M^{-1} s^{-1} for k_1. The values of pK_{a2} and pK_{a3} are both in agreement with the literature values, 8.18 and 10.3, respectively.[16]

Excluding the k_1 term, as in eq 8, is equally good in representing the data, and the fit is shown in Figure 5.

$$\frac{k_{obs}}{[cysteine]_{tot}} = \frac{k_2K_{a1}[H^+]^2 + k_3K_{a1}K_{a2}[H^+] + k_4K_{a1}K_{a2}K_{a3}}{[H^+]^3 + K_{a1}[H^+]^2 + K_{a1}K_{a2}[H^+] + K_{a1}K_{a2}K_{a3}} \quad (8)$$

The derived parameters are the same as those from the fit to eq 7 except for a slight adjustment to k_2, the new value being 1.0 ± 0.5 M^{-1} s^{-1}. This result indicates that the k_2 term is marginally significant. Exclusion of both the k_1 and k_2 terms yields a fit that deviates systematically at low pHs. Accordingly, eq 8 best represents the data, and it implies that cysteine reacts primarily via its anionic forms from pH 3.5 to 12.3.

Specific Salt Effects. A significant kinetic salt effect is revealed by a series of experiments at high buffer concentrations. These experiments consist of a pH-dependent study, as described above, at 0.1 M ionic strength ($NaCF_3SO_3$), but with 0.02 M buffers. The data, illustrated in Figure 6 as a plot of log(k_{obs}/[cysteine]$_{tot}$) versus pH, show discontinuities at the points where the buffer switches from sodium cacodylate to sodium phosphate and again where sodium phosphate switches to sodium hydroxide. These discontinuities correspond to changes in the identity of the anion and also to changes in the concentration of Na^+, because phosphate is polybasic. Further evidence for this effect is given by experiments in which the ionic strength is maintained at 0.10 M while the background electrolyte is changed from $NaCF_3SO_3$ to Na_2SO_4 (Table S-4, Supporting Information). These experiments (at pH 7.3 and also at pH 11.4) show a ~30% reduction in k_{obs} in Na_2SO_4 media while the Na^+ concentration is reduced by a similar amount. The proportionality of k_{obs} to $[Na^+]$ suggests that the salt effect is due to the cation. Changing the cation from Li^+ to Na^+ and K^+ while maintaining the ionic strength at 0.1 M in $CF_3SO_3^-$ media further demonstrates a specific cation effect, as shown in Table S-5 (Supporting Information). The rate constant k_{obs} increases in the order $Na^+ < Li^+ < K^+$ at pH 7.5, whereas the order is $Li^+ < Na^+ < K^+$ at pH 11.5.

A specific cation effect is not unexpected for this reaction, given the high electrostatic repulsion between the −3 charge of the oxidant and the negative charge of the reactive forms of cysteine. Similar effects were previously reported for the reactions of $[Mo(CN)_8]^{3-}$ with OH^-, $S_2O_3^{2-}$, and SO_3^{2-}.[22,25,26] It is quite likely that the full rate law for the oxidation of cysteine by $[Mo(CN)_8]^{3-}$ is first order with respect to $[Na^+]$. However, rather than attempt to resolve this effect from the effects of varying ionic strengths, we have elected to minimize its importance by the use of low buffer concentrations and by avoidance of phosphate buffers. These constraints on the solution compositions enable the reaction to be studied at a constant ionic strength and a constant Na^+ concentration. Thus, the values reported above for k_3 and k_4 most likely include unresolved cation dependencies.

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Discussion

The oxidation of cysteine by $[Mo(CN)_8]^{3-}$ is no exception to the rule that trace copper ion catalysis dominates the reactions of aliphatic thiols with outer-sphere oxidants. However, this report is the first to show that the direct oxidation of cysteine by a typical outer-sphere reagent can be studied by the judicious use of a metal-binding ligand.

We have not attempted to determine the rate law for the copper-catalyzed reaction because the rates are too fast to measure when meaningful amounts of copper are added. It is reasonable, however, to speculate that the mechanism involves the formation of a copper−cysteine complex, as has been suggested for the oxidations by ferricytochrome c and by $[Co_2(O_2)(CN)_{10}]^{5-}$.[7,27] We find, as before in our studies of the oxidation of TGA,[2,3] that dipic is a convenient and effective inhibitor of the copper catalysis. Presumably, dipic acts as an inhibitor by binding copper ions and, thus, rendering them inactive. EDTA, which is often used as an inhibitor of trace-metal ion catalysis, is unsuitable in the present study because of its susceptibility to oxidation and because of the prior demonstration that complexes of EDTA can themselves catalyze redox reactions of this type.[28]

The role of PBN in the direct oxidation of cysteine apparently is rather insignificant. The addition of PBN has no significant effect on the consumption ratio, and it slows the reaction by only ~20%. Its principal effect is to yield improved first-order kinetics. We infer that PBN scavenges some minor reactive radicals that are short-chain carriers. Similar results and inferences were obtained in a prior study of the reaction of TGA with $[Mo(CN)_8]^{3-}$.[2] DMPO, another nitrone spin trap, reacts with OH radicals 10-fold faster than with cysteinyl radicals.[29,30] PBN, in general, is less reactive than DMPO, so it is reasonable to propose that PBN is not an effective scavenger of cysteinyl radicals under the conditions in the present study. Alternatively, it is possible that PBN reacts reversibly with cysteinyl radicals, as is the case for other nitrones.[31] In either case, the kinetics and stoichiometry of the oxidation of cysteine by $[Mo(CN)_8]^{3-}$ would not be perturbed by the presence of PBN. A further concern is that cysteine might react directly with PBN, as it has been documented that cysteine can react with other nitrones.[31] The equilibrium constant for the addition of cysteine to DEPMPO is only ~0.03 M^{-1};[31] if the equilibrium constant for addition to PBN is similar, then the concentration of free cysteine will not be significantly reduced by this reaction. We conclude that the reactions of PBN need not be included in the mechanism of the reaction of cysteine with $[Mo(CN)_8]^{3-}$.

The following mechanism is proposed:

$$HSCH_2CH(NH_3^+)CO_2^- \rightleftharpoons {}^-SCH_2CH(NH_3^+)CO_2H \qquad K_i \tag{9}$$

$${}^-SCH_2CH(NH_3^+)CO_2H + Mo(V) \rightarrow Mo(IV) + {}^{\bullet}SCH_2CH(NH_3^+)CO_2H \qquad k_a \tag{10}$$

$${}^-SCH_2CH(NH_3^+)CO_2^- + Mo(V) \rightarrow Mo(IV) + {}^{\bullet}SCH_2CH(NH_3^+)CO_2^- \qquad k_b \tag{11}$$

$${}^-SCH_2CH(NH_2)CO_2^- + Mo(V) \rightarrow Mo(IV) + {}^{\bullet}SCH_2CH(NH_2)CO_2^- \qquad k_c \tag{12}$$

$$RS^{\bullet} + RS^- \rightleftharpoons RSSR^{\bullet -} \qquad K_{rad}, k_{o2}, k_{-o2} \tag{13}$$

$$RSSR^{\bullet -} + Mo(V) \rightarrow RSSR + Mo(IV) \qquad k_{o3} \tag{14}$$

$$RS^{\bullet} + Mo(V) + H_2O \rightarrow RSOH + Mo(IV) + H^+ \qquad k_{o4} \tag{15}$$

$$2RSOH \rightarrow RS(O)SR + H_2O \tag{16}$$

$$RS(O)SR + OH^- \rightarrow RSO_2^- + RSH \tag{17}$$

If the steady-state approximation is applied to the concentrations of $RS^{\bullet}$ and $RSSR^{\bullet -}$, the following rate law can be derived.

$$-\frac{d[Mo(V)]}{dt} = \frac{2(k_a K_{a1} K_i [H^+]^2 + k_b K_{a1} K_{a2} [H^+] + k_c K_{a1} K_{a2} K_{a3})}{[H^+]^3 + [H^+]^2 K_{a1} + K_{a1} K_{a2} [H^+] + K_{a1} K_{a2} K_{a3}} [cysteine]_{tot} [(Mo(V)] \tag{18}$$

This rate law has the same form as the empirical rate law (eq 8), and thus, the following results are obtained from the empirical parameters reported below eq 7: $2k_a K_i = 1.0 \pm 0.5\ M^{-1}\ s^{-1}$, $2k_b = (1.42 \pm 0.08) \times 10^5\ M^{-1}\ s^{-1}$, and $2k_c = (4.5 \pm 0.4) \times 10^4\ M^{-1}\ s^{-1}$.

In the above mechanism, the three rate-limiting steps correspond to electron transfer from the thiolate forms of cysteine. The thiyl radical so generated can react with cysteine rapidly to form the corresponding well-known disulfide radical anion, as in eq 13.[32] Further oxidation of the disulfide radical anion yields cystine, as in eq 14, whereas oxidation of the thiyl radical yields cysteinesulfenate, which disproportionates[33,34] to cysteinesulfinate (eqs 15−17).

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In its neutral and monoanionic forms, cysteine can exist as various tautomers.[35−37] The predominant form of neutral cysteine is the zwitterion, $HSCH_2CH(NH_3^+)CO_2^-$, and the minor tautomers are $HSCH_2CH(NH_2)CO_2H$ and $^-SCH_2CH(NH_3^+)CO_2H$. From data reported by Kallen and as summarized by Reuben and Bruice,[36,37] tautomerization of the major zwitterion to the thiolate form occurs with $pK_i = 5.5$, whereas tautomerization to the amine form is somewhat less unfavorable. The monoanion is predominantly $^-SCH_2CH(NH_3^+)CO_2^-$, and its minor tautomers are $HSCH_2CH(NH_2)CO_2^-$ and $^-SCH_2CH(NH_2)CO_2H$. Tautomerization of the monoanion to the thiol form is only weakly disfavored ($K = 0.4$),[35] but the carboxylic acid form must be quite inaccessible. Significantly different microscopic equilibrium constants were reported by Patel and Williams,[38] but they must be in error because they disagree with the highly reliable macroscopic pK_a value reported for the cysteine monoanion.

The value for pK_i estimated above combined with the (marginally significant) measured value for $2k_aK_i$ leads to a value of 1.6×10^5 M^{-1} s^{-1} for k_a. This gives a trend of $k_a > k_b > k_c$, which is as expected if all three rate constants refer to electron transfer to $[Mo(CN)_8]^{3-}$ from thiolate forms of cysteine having overall charges of 0, −1, and −2, respectively. Consistent with this picture, the cationic form of cysteine is completely unreactive because it has no thiolate tautomer. The neutral form of cysteine is only marginally reactive because the reactive state is the minor tautomer. The two anionic forms are highly reactive because they are both thiolates, and the dianionic form is somewhat less reactive {with $[Mo(CN)_8]^{3-}$} than the monoanion because of the large electrostatic repulsion. In contrast, $[Mo(CN)_8]^{3-}$ oxidizes thioglycolic acid increasingly rapidly with successive deprotonations because the major tautomer of the monoanion is the thiol.

Further insight into the mechanism can be gained by a consideration of driving forces as revealed by one-electron redox potentials. The oxidant is moderately strong, with $E^\circ = 0.77$ V for the $[Mo(CN)_8]^{3-/4-}$ redox couple at $\mu = 0.1$ M. The nature of the cysteine potentials is less definitive. A prominent review indicates that $E^{\circ\prime} = 0.920$ V at pH 7.[39] The actual source of the data, however, reports results only for thiols other than cysteine. For cysteine, per se, the only reported potential is 0.73 ± 0.05 V at pH 13.[40] This result was obtained by measurement of the electron-transfer equilibrium constant between the cystine radical anion (actually the Cys−Gly peptide) and tyrosine at pH 9.15 ($K_{tyr} = 3.3 \times 10^{-3}$ M).

$$\text{Tyr} + \text{RSSR}^{\bullet-} \rightleftharpoons \text{Tyr/O}^\bullet + 2\text{cysteine} \qquad K_{tyr} \quad (19)$$

The cysteine radical potential was derived from this equilibrium constant by the use of a literature value for K_{rad} ($= 6 \times 10^3$ M^{-1} at this pH) and a literature value of E° ($= 0.60 \pm 0.05$ V) for the tyrosine system at pH 13. However, it is now considered that K_{rad} differs by about a factor of 6 from the value used[41] and that E° for the tyrosine system at pH 13 is considerably higher than 0.60 V (0.73 ± 0.02 V).[42−44] Moreover, it is unclear how these results were extrapolated to pH 13.

If the measured value for K_{tyr} is accepted, then for the reaction

$$\text{Tyr} + \text{cys}^\bullet \rightleftharpoons \text{Tyr/O}^\bullet + \text{cysteine} \qquad K_{et} \quad (20)$$

one obtains the relationship $K_{tyr} = K_{et}/K_{rad}$. The use of an interpolated value[41] of 0.9×10^3 M^{-1} for K_{rad} leads to $K_{et} = 3.0$. The cysteinyl radical is fully deprotonated at pH 9.15 ($pK_a = 8.26$);[32] because cysteine and tyrosine have very similar pK_a values (∼10.3), the value of K_{et} will remain as 3.0 at pH 13. The use of the revised tyrosine potential then leads to 0.76 ± 0.02 V for the $cysS^\bullet/cysS^-$ reduction potential at pH 13. This result is in good agreement with potentials reported for other aliphatic thiyl radicals.[45]

An estimate of the free energy change for electron transfer from the cysteine dianion to $[Mo(CN)_8]^{3-}$ (eq 12) can be made by the use of pertinent reduction potentials. For $cysS^\bullet/cysS^-$, we use $E_f = 0.76$ V, as derived above, and for $[Mo(CN)_8]^{3-/4-}$, we use our measured value of 0.77 V. The derived value for ΔG° is −1 kJ mol^{-1}, corresponding to an equilibrium constant of 1.5 for reaction 12. This calculation shows that electron transfer from the cysteine dianion to $[Mo(CN)_8]^{3-}$ is virtually thermoneutral and that the forward and reverse rate constants are both about 2×10^4 M^{-1} s^{-1}. This low rate constant for the back reaction, the reduction of the thiyl radical, is consistent with a prior flash photolysis study in which $C_2H_3S^\bullet$ was shown to oxidize $[Fe(CN)_6]^{4-}$ with $k = 2 \times 10^7$ M^{-1} s^{-1}.[46] Qualitatively, this result implies that the intrinsic barrier to electron transfer from $cysS^-$ to form $cysS^\bullet$ should be rather small. It also provides a justification for treating the electron-transfer steps as irreversible processes in deriving rate law 18, because the subsequent radical reactions (eqs 13−15) can be much faster than the slow reverse electron-transfer process.

Application of the Marcus cross correlation, eqs 21−24,[47,48] with respective rate constant k_c, leads to an estimate

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of the self-exchange rate constant of the $^{\bullet}SCH_2CH(NH_2)CO_2^-/^-SCH_2CH(NH_2)CO_2^-$ couple

$$k_{12} = (k_{11}k_{22}K_{12}f_{12})^{1/2}W_{12} \quad (21)$$

$$\ln f_{12} = \frac{[\ln K_{12} + (w_{12} - w_{21})/RT]^2}{4[\ln(k_{11}k_{22}/Z^2) + (w_{11} + w_{22})/RT]} \quad (22)$$

$$W_{12} = \exp(-w_{12} - w_{21} + w_{11} + w_{22})/2RT \quad (23)$$

$$w_{ij} = 4.23Z_iZ_j/[r(1 + 0.328r\sqrt{\mu})] \quad (24)$$

where k_{12} is the observed cross electron-transfer rate constant, $k_c = (2.3 \pm 0.2) \times 10^4$ M^{-1} s^{-1}, and k_{11} and k_{22} are the self-exchange rate constants of the $^{\bullet}SCH_2CH(NH_2)CO_2^-/^-SCH_2CH(NH_2)CO_2^-$ and $[Mo(CN)_8]^{3-/4-}$ redox couples. According to Table S-7 in the Supporting Information of Metelski and Swaddle's report, k_{22} is independent of ionic strength when the ionic strength is lower than 0.3 M in the presence of only counterion K^+ without additional electrolyte present.[49] Na^+ was observed with the similar effect, as was K^+, on the self-exchange rate of $[Mo(CN)_8]^{3-/4-}$.[49] It is reasonable to use 2.7×10^4 M^{-1} s^{-1} as k_{22} in the calculation.[23] A value of 1×10^{11} M^{-1} s^{-1} is used for the collision frequency Z. Z_i and Z_j are ionic charges on the reactants, R is the ideal gas constant (1.987×10^{-3} kcal mol^{-1}), and r is the center-to-center distance between reactants while in contact. The radii of $[Mo(CN)_8]^{3-}$ and $^-SCH_2CH(NH_2)CO_2^-$ are 4.7 Å[2] and 3.0 Å, respectively, estimated from Corey−Pauling−Koltun models. With the above parameters, the experimental value of k_{12} (2.3×10^4 M^{-1} s^{-1}) and the estimated value for K_{12} (1.5), the self-exchange rate constant k_{11} for the $^{\bullet}SCH_2CH(NH_2)CO_2^-/^-SCH_2CH(NH_2)CO_2^-$ couple is calculated as 5.4×10^3 M^{-1} s^{-1}. This rate constant is similar to the corresponding value derived previously for the $^-SCH_2CO_2^-/^{\bullet}SCH_2CO_2^-$ system (1.5×10^5 and 2.8×10^3 M^{-1} s^{-1}).[1,2] It is also consistent with the small internal reorganizational energy that one would predict for the formation of the cysteinyl radical, where the unpaired electron is sulfur-centered.[50]

The above discussion, in terms of Marcus theory, is dependent on the reaction having an outer-sphere electron-transfer mechanism. An inner-sphere mechanism is also conceivable, in which cyanide could bridge between molybdenum and the cysteine sulfur, although we have no evidence for such an intermediate. Further studies with other typical outer-sphere oxidants might distinguish between these two possibilities.

As is noted above, the value of k_c likely includes an unresolved sodium cation dependence, which arises because Na^+ acts as an electrostatic buffer to reduce the repulsion between dianionic cysteine and $[Mo(CN)_8]^{3-}$. Strictly speaking, such a Na^+ effect is inconsistent with the model used in deriving the Marcus cross relationship for electron-transfer rate constants. However, the second-order self-exchange rate constant for the $[Mo(CN)_8]^{3-/4-}$ self-exchange reaction likewise contains an unresolved cation dependence.[49] As a reasonable approximation, these effects should substantially cancel when the Marcus cross relationship is used with such unresolved rate constants for both the cross reaction and one of the self-exchange reactions.

Very recently, a paper by Nekrassova et al. has appeared describing electrochemical studies on $[Mo(CN)_8]^{4-}$ and the oxidation of cysteine by electrogenerated $[Mo(CN)_8]^{3-}$.[51] One of the reported findings is that $[Mo(CN)_8]^{3-}$ undergoes aquation with a rate constant of 0.03 s^{-1}. If correct, this result would imply that virtually all of our Mo(V) solutions would have become fully aquated prior to our stopped-flow measurements. We dispute this result of Nekrassova et al. on various grounds. For one thing, the evidence is based on a cyclic voltammetry (CV) scan-rate-dependent reduction of the peak current for the reduction of electrogenerated $[Mo(CN)_8]^{3-}$; if aquation were the explanation, one would expect to see the development of a new wave for the reduction of $[Mo(CN)_7(H_2O)]^{2-}$, but no such wave was reported. Second, it has previously been shown that $[Mo(CN)_8]^{3-}$ undergoes aquation only in acidic media,[17] whereas the results of Nekrassova et al. were obtained at pH 9. A conceivable explanation for the observations of Nekrassova et al. is that $[Mo(CN)_8]^{3-}$ undergoes base-induced reduction, as reported by Marchaj et al.[17] Third, our CV measurements on $[Mo(CN)_8]^{3-}$ and $[Mo(CN)_8]^{4-}$ are virtually identical, indicating that $[Mo(CN)_8]^{3-}$ does not undergo aquation at neutral pH.

The other problematic result from the study of Nekrassova et al. is the evaluation of a rate constant of 9×10^4 mol^{-1} cm^3 s^{-1} ($= 9 \times 10^1$ M^{-1} s^{-1}) for the oxidation of cysteine by $[Mo(CN)_8]^{3-}$ at pH 10,[51] a result which is in complete disagreement with ours. Nekrassova et al. took no precautions against copper ion catalysis of this reaction, so we are highly skeptical of the significance of their result.

Conclusions

With the use of dipic as a Cu-catalysis inhibitor, the direct oxidation of cysteine by $[Mo(CN)_8]^{3-}$ can be studied. Oxidation yields cystine and cysteinesulfinate through parallel paths that diverge after the rate-limiting step. The rate-limiting step is electron transfer to generate the cysteinyl radical and $[Mo(CN)_8]^{4-}$. Only the thiolate forms of cysteine are reactive. A complex pattern of reactivity as a function of pH arises from the concurrent influences of the pK_a's of cysteine, the tautomerizations of the various forms, and the electrostatic repulsion between the anionic oxidant and the anionic states of cysteine. This electrostatic repulsion leads to a significant specific "inert" salt effect. The electron-transfer process itself is virtually thermoneutral and rather rapid; its rate constant is consistent with a low predicted internal reorganizational energy for the conversion of a thiolate to a thiyl radical.

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Acknowledgment. We thank professors Dave Armstrong (University of Calgary) and Holly Ellis (Auburn University) for helpful discussions. The contributions from some unusually diligent anonymous referees are also appreciated. This material is based upon work supported by the National Science Foundation under Grant 0138142. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

Supporting Information Available: Tables of various effects on the oxidation of cysteine by Mo(V), tables of kinetic data for the oxidation of cysteine, UV−vis spectra and CVs of $K_4[Mo(CN)_8]$ and $Cs_3[Mo(CN)_8]$, a reaction trace of the oxidation of cysteine by Mo(V), OSWVs of Mo-containing solutions, and ^1H NMR spectra of cysteine and the product solution of the reaction of cysteine with Mo(V) in the presence of dipic. This material is available free of charge via the Internet at http://pubs.acs.org.

IC050427C