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Kinetic Study of the Reaction of Di-μ-oxo-bis[oxo(L-cysteinato)molybdate(V)] with Cysteine[1]

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Reaction of the binuclear molybdenum(V)-cysteine complex, $Mo_2O_4(Cys)_2^{2-}$, with cysteine has been investigated at pH 8.0-10.0. In the absence of free cysteine, reaction of $Mo_2O_4(Cys)_2^{2-}$ is slow, but addition of cysteine promotes a reaction which is first-order in each reactant and a reaction in which cysteine is catalytically regenerated. The second-order rate constant increases with increasing pH between pH 8 and 10, and in pH 9.2 borate buffer at $\mu = 2.0$ and at 30°, $k_2 = (3.75 \pm 0.16) \times 10^{-2}\ M^{-1}\ \text{sec}^{-1}$. The products of the reaction are characterized by a weak epr signal and a visible absorption band at 580-640 nm. The products responsible for the visible absorption and epr signal are different species which are in equilibrium with one another as they display a reversible, inverse temperature dependence. The rate-determining step in the reaction is believed to be cleavage of one oxygen bridge of the $Mo_2O_4^{2+}$ structure, giving a mixture of monooxo-bridged molybdenum(V)-cysteine dimers. The visible absorption at 580-640 nm is assigned to these products which are in equilibrium with a small fraction of molybdenum(V)-cysteine monomer responsible for the epr signal.

Introduction

Molybdenum-sulfhydryl complexes have received attention in recent years as inorganic models for the active site of electron transfer in molybdenum-containing enzymes.[2-11] The ability of molybdenum-sulfhydryl complexes to catalyze the reduction of substrates (acetylene, 1-alkynes, alkyl isocyanides, azide ion[9-11]) of certain of these enzymes and the similarity of epr signals observed in the enzymes and in Mo(V) complexes[2-4] attest to the validity of these models. The most widely investigated compound has been the binuclear molybdenum(V)-cysteine complex di-μ-oxo-bis[oxo(L-cysteinato)molybdate(V)],[12-14] denoted here as $Mo_2O_4(Cys)_2^{2-}$. Although this compound is diamagnetic and has an electronic absorption only at 306 nm, aqueous solutions of molybdenum(V) and cysteine at pH 7-10 exhibit a weak epr signal and a visible absorption near 580 nm, both of which develop over a period of several hours. Huang and Haight investigated this behavior by epr and visible spectrometry in Mo(V) solutions containing a large excess of cysteine[3,4] and concluded that these characteristic features are the result of a slowly established equilibrium between the diamagnetic binuclear complex and a small fraction (~2%) of paramagnetic molybdenum(V)-cysteine monomer.

During an investigation of the electrochemistry of molybdenum-cysteine complexes[15] we observed that the epr spectrum and visible absorption described above appear at the indicated rate under the stated conditions only when free cysteine is added purposefully to a solution of $Mo_2O_4(Cys)_2^{2-}$. We found further that in the presence of cysteine the reaction proceeds with the complete consumption of $Mo_2O_4(Cys)_2^{2-}$. This behavior is substantially different than the simple dimer-monomer equilibrium indicated in the epr studies and prompted us to undertake a detailed study of the reaction of $Mo_2O_4(Cys)_2^{2-}$ with cysteine. The results of this investigation are presented in this report.

Experimental Section

Materials. Reagent grade chemicals were used without further purification. The source of cysteine was Matheson Coleman and Bell L-cysteine hydrochloride monohydrate. $Na_2Mo_2O_4(Cys)_2 \cdot 5H_2O$ was prepared following the dithionite reduction procedure of Kay and Mitchell.[14] The compound was recrystallized three times from 1:1 ethanol-water and stored *in vacuo* over P_2O_5. Satisfactory elemental analyses and visible spectra were obtained for the compound. *Anal.* Calcd for $Na_2Mo_2C_6H_{20}N_2O_{13}S_2$: C, 11.4; H, 3.2; N, 4.4; Mo, 30.5; Na, 7.3. Found: C, 11.4; H, 3.0; N, 4.4; Mo, 30.5; Na, 7.0 (ϵ $1.24 \times 10^4\ M^{-1}\ \text{cm}^{-1}$ at 306 nm). Fresh quantities of the material were prepared periodically.

Solutions were prepared from twice distilled water. Deaeration was accomplished with prepurified nitrogen, although in several instances argon was employed. No difference in behavior was observed. Triply distilled mercury (Bethlehem Apparatus Co.) was used in the hanging mercury drop electrode assembly.

Apparatus for Voltammetric Measurements. Linear-sweep voltammetric measurements were carried out with a solid-state operational amplifier potentiostat similar to that described by Goolsby and Sawyer.[16] A three-electrode circuit was employed. The working electrode was a Metrohm E 410 hanging mercury drop electrode assembly. An electrode area of 0.022 cm^2 was used for most experiments, and the electrode was renewed prior to each voltammetric trial. A coiled platinum wire isolated from the main solution by a glass frit was the auxiliary electrode. A Leeds and Northrup 117208 saturated calomel electrode (sce) was the reference electrode, and all potentials are reported relative to this half-cell. Current-potential curves were recorded on a Hewlett-Packard Model 7035B X-Y recorder. Voltammetric experiments were run in a Metrohm EA 615 water-jacketed cell, and temperature control was maintained with a circulating water bath.

Procedures for Kinetic Measurements. Solutions were prepared by adding weighed quantities of $Na_2Mo_2O_4(Cys)_2 \cdot 5H_2O$ and Cys·HCl·H_2O to deaerated borate or phosphate buffer in a volumetric flask. Ionic strength was adjusted with sodium sulfate. Solutions were diluted to volume with distilled water. The pH of each solution was measured and, as necessary, adjusted with concentrated NaOH or HCl. Solutions were deaerated for an additional 15 min before measurement, and a flow of nitrogen was maintained over the solutions at all times. Some measurements were made at ambient temperature (22 ± 2°), but in most experiments the temperature was thermostated to within ±0.2°.

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(1) Taken in part from the M.S. thesis of R. F. S., Florida Atlantic University, 1971.

(2) J. T. Spence, *Coord. Chem. Rev.*, 4, 475 (1969).

(3) T. J. Huang and G. P. Haight, Jr., *Chem. Commun.*, 985 (1969).

(4) T. J. Huang and G. P. Haight, Jr., *J. Amer. Chem. Soc.*, 92 2336 (1970).

(5) J. F. Martin and J. T. Spence, *J. Phys. Chem.*, 74, 2836 (1970).

(6) J. F. Martin and J. T. Spence, *J. Phys. Chem.*, 74, 3589 (1970).

(7) G. N. Schrauzer and G. Schlesinger, *J. Amer. Chem. Soc.*, 92, 1808 (1970).

(8) T. J. Huang and G. P. Haight, Jr., *J. Amer. Chem. Soc.*, 93, 611 (1971).

(9) G. N. Schrauzer and P. A. Doemeny, *J. Amer. Chem. Soc.*, 93, 1608 (1971).

(10) G. N. Schrauzer, G. Schlesinger, and P. A. Doemeny, *J. Amer. Chem. Soc.*, 93, 1803 (1971).

(11) G. N. Schrauzer, *Advan. Chem. Ser.*, No. 100, 1 (1971).

(12) A. Kay and P. C. H. Mitchell, *Nature (London)*, 219, 267 (1968).

(13) L. R. Melby, *Inorg. Chem.*, 8, 349 (1969).

(14) A. Kay and P. C. H. Mitchell, *J. Chem. Soc. A*, 2421 (1970).

(15) R. F. Stephenson and F. A. Schultz, unpublished work.

(16) A. D. Goolsby and D. T. Sawyer, *Anal. Chem.*, 39, 411 (1967).

It was most convenient to measure the rate of reaction from the decrease in voltammetric peak current for the reduction of $Mo_2O_4(Cys)_2{}^{2-}$. Between pH 8 and 10 $Mo_2O_4(Cys)_2{}^{2-}$ exhibited a well-defined reduction peak at −1.29 V (peak A, Figure 1). At sweep rates (v) less than 0.1 V sec^{-1} the quantity $i_p/Cv^{1/2}$ was a constant equal to 50 ± 2 A cm^3 sec$^{1/2}$ mol^{-1} V$^{-1/2}$ at a 0.022-cm^2 electrode. The peak current for $Mo_2O_4(Cys)_2{}^{2-}$ reduction was measured by scanning in the cathodic direction from −0.6 V and was corrected for a prior reduction wave (peak B, Figure 1) which developed during the reaction by visually estimating and subtracting the magnitude of this current at the potential of measurement (−1.29 V). First-order rate constants (k_1) were evaluated from a least-squares analysis of plots of log i_p for $Mo_2O_4(Cys)_2{}^{2-}$ reduction *vs.* time. A product of the chemical reaction also was reducible at the mercury electrode (peak B, Figure 1) and exhibited a voltammetric peak potential −1.03 V at pH 9.20, $\mu = 0.5$, 22°, and $v = 0.033$ V sec^{-1}. The rate constant k_1 also was determined from a plot of log $(i_{p,\infty} - i_{p,t})$ *vs.* t for the reduction of this product.

Free cysteine concentration was measured from the diffusion-controlled oxidation of the mercury electrode in the presence of cysteine. By scanning in the anodic direction from −0.6 V peaks C and D in Figure 1 are observed for the reversible couple[17]

$$Hg + 2Cys \rightleftarrows Hg(Cys)_2 + 2e^- + 2H^+$$

when cysteine is present in the solution. The peak current for the anodic wave C was a linear function of cysteine concentration.

Spectrophotometric measurements of the reaction rate also were made and employed a Cary 14 spectrophotometer equipped with a 0–0.1 absorbance slide-wire. These measurements were made in stoppered cells at ambient temperature with the sample compartment under continuous nitrogen purge. The absorbance of the reaction product was measured at 580 and 620 nm.

Visible Spectra. Visible spectra of the reaction at various stages of completion under various conditions were obtained with a Perkin-Elmer Model 202 spectrophotometer. Solutions were prepared and thermostated as in kinetic measurements and were maintained under a continuous flow of nitrogen. Samples of the reacting solution were transferred to stoppered cells by syringe, and spectra were recorded immediately. There was no indication of atmospheric oxidation during these measurements.

Epr Measurements. Qualitative epr spectra were recorded with a Varian E-3 spectrometer. Solutions were prepared by adding weighed quantities of $Na_2Mo_2O_4(Cys)_2 \cdot 5H_2O$ and $Cys \cdot HCl \cdot H_2O$ to deaerated buffer. Solutions of $\sim 10^{-3}$ M $Mo_2O_4(Cys)_2{}^{2-}$ containing no cysteine and $\sim 10^{-2}$ M cysteine were allowed to stand under nitrogen for periods up to 24 hr. Samples were withdrawn by syringe and placed in 3-mm quartz epr tubes. Spectra were recorded immediately in liquid nitrogen.

Identification of Reaction Products. The oxidation state of the product was determined by adding aliquots from completed reaction mixtures at pH 9–10 to 12 F HCl and to deaerated solutions of 8-hydroxyquinoline in ethanol. The solutions were allowed to stand under nitrogen until reaction was complete, and uv-visible spectra were recorded to determine the quantities of known molybdenum(V) complexes $MoOCl_5{}^{2-}$ [18,19] and $Mo_2O_3(C_9H_6NO)_4$[20] formed in the reactions. Analyzed $Na_2Mo_2O_4(Cys)_2 \cdot 5H_2O$ was used as a standard to construct a calibration curve for the 8-hydroxyquinoline reaction.

Numerous attempts were made to isolate and identify products from completed reaction mixtures by chromatographing solutions containing initially 0.05–0.10 M $Mo_2O_4(Cys)_2{}^{2-}$ and cysteine at pH 9–10 on a cellulose column (Bio-Rad Cellex MX) under nitrogen. A dark blue solid could be obtained following precipitation with dearated acetone, but this material did not give a satisfactory analysis.

Results

Visible Spectra and Epr Spectra. Deaerated solutions containing only $Mo_2O_4(Cys)_2{}^{2-}$ in pH 9.2 borate buffer at 22° and $\mu = 0.2$ exhibit no change in electronic spectra and no epr signal for at least 24 hr. When 10^{-2} M cysteine is added to a solution of $Mo_2O_4(Cys)_2{}^{2-}$ under these conditions, an epr signal and a visible absorption band develop simultaneously over a period of several hours. At longer times (24–48 hr) under the same conditions some reaction of $Mo_2O_4(Cys)_2{}^{2-}$ occurs in the absence of free cysteine (4–10% as estimated from the decrease in voltammetric peak current), and an increase in temperature, pH, or ionic strength increases the extent and rate of this reaction. However, the rate of reaction of $Mo_2O_4(Cys)_2{}^{2-}$ alone is slower than in the presence of cysteine, and it is clear that excess cysteine promotes formation of the epr signal and the visible absorption band. The epr signal has an average g value of 1.976 and appears to be identical with the signal observed by Huang and Haight.[3,4] No quantitative estimate of spins is made in this work, but the very weak signal indicates that only a small fraction of paramagnetic species is present.

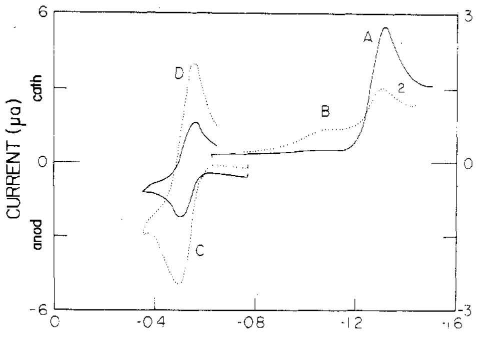

Figure 1. Linear-sweep voltammetric traces observed during reaction of $Mo_2O_4(Cys)_2{}^{2-}$ with cysteine: 1 (——), left-hand ordinate, elapsed time 5 hr; 2 (· · ·), right-hand ordinate, elapsed time 48 hr. Conditions: $[Mo_2O_4(Cys)_2{}^{2-}] = 1.06 \times 10^{-3}$ M; [Cys] = 0.96×10^{-3} M; pH 9.20 borate buffer; $\mu = 0.2$; 22°; $v = 0.050$ V sec^{-1} · electrode area 0.018 cm^2.

The color change observed during the reaction is from yellow-orange to green to blue and is the result of a visible absorption band appearing at 580 to 640 nm. Between pH 9 and 10 the absorbance in this region increases to a constant value within 10–20 hr at 30°. During the reaction the absorbance maximum shifts in the direction of shorter wavelengths, and the final position of the band occurs at shorter wavelengths with decreasing pH. Values of λ_{max} and ϵ measured at 30° after complete reaction are presented in Table IA. Between pH 9 and 10 the values of ϵ are in agreement with the value of 160 M^{-1} cm^{-1} calculated by Huang and Haight on the basis of molybdenum dimer concentration.[4] At pH <9 the final absorbance does not appear to reach a constant value, and an increase in ϵ with time and with decreasing pH is noted.

During the reaction no isosbestic point is observed between the emerging band at 580–640 nm and the diminishing shoulder of the 306-nm band of $Mo_2O_4(Cys)_2{}^{2-}$. Adjustment of pH following completion of the reaction does not lead to a reversible change of spectral properties. However, the spectral properties of the final product are temperature reversible at pH 9–10. If the temperature of the solution is increased after a constant absorbance reading at 30° is reached, a new constant absorbance reading is attained within 1–2 hr which reflects a slight shift in λ_{max} to longer wavelengths and a significant decrease in ϵ. Cooling the solution to 30° restores the earlier absorbance reading. Representative data for pH 9.2–10.0 are shown in Table IB. An increase in temperature causes a small reversible *decrease* of 10–20% in the intensity of the 580–640-nm band. Huang and Haight found that an increase in temperature caused a substantial reversible *increase* (100–200%) in the intensity of the epr signal.[4] This result leads us to the conclusion that different species, possibly in equilibrium, are responsible

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[17] I. M. Kolthoff, W. Stricks, and N. Tanaka, *J. Amer. Chem. Soc.*, 77, 4739 (1955).

[18] C. K. Jorgensen, *Acta Chem. Scand.*, 11, 73 (1957).

[19] H. B. Gray and C. R. Hare, *Inorg. Chem.* 1, 363 (1962).

[20] P. C. H. Mitchell, *J. Chem. Soc. A*, 146 (1969).

Table I

A. Visible Spectra Observed Following Reaction of $Mo_2O_4(Cys)_2^{2-}$ with Cysteine at 30°[a]

pH	Elapsed time, hr	λ_{max}, nm	ϵ,[b] M^{-1} cm^{-1}
8.4[c]	15.6	580	338[d]
8.8	22.5	595	301[d]
9.2	17.7	605	185
9.6	18.9	617	118
10.0	18.3	619	139

B. Temperature Dependence of Visible Spectra Following Reaction of $Mo_2O_4(Cys)_2^{2-}$ with Cysteine[a,e]

pH	Temp, °C	λ_{max}, nm	ϵ,[b] M^{-1} cm^{-1}
9.2	30	605	185
	50	611	150
	30	605	190
9.6	30	617	118
	45	620	111
	30	Not measd	
10.0	30	619	139
	50	628	118
	30	620	140

[a] Initial conditions: $[Mo_2O_4(Cys)_2^{2-}] = 2.0 \times 10^{-3}$ *M*, [Cys] = 1.0×10^{-2} *M*, $\mu = 2.0$ (Na_2SO_4), borate buffer. [b] Calculated on the basis of initial $Mo_2O_4(Cys)_2^{2-}$ concentration. [c] Phosphate buffer. [d] Final absorbance does not appear to reach a constant value. [e] Measurements made following attainment of constant absorbance reading at 30° at each pH; approximately 3 hr allowed between measurements at each temperature.

Table II. Kinetic Data for the Reaction of $Mo_2O_4(Cys)_2^{2-}$ with Cysteine[a]

pH	Temp, °C	$10^3 \times [Mo_2O_4\text{-}(Cys)_2^{2-}]$, *M*	$10^3 \times$ [Cys], *M*	$10^4 k_1$, sec^{-1}	$10^2 k_2$, M^{-1} sec^{-1}
9.20	30	0.35	10.0	3.84	3.84
9.20	30	0.62	10.0	3.95	3.95
9.20	30	1.00	10.0	3.69	3.69
9.20	30	1.53	10.0	3.91	3.91
9.20	30	1.00	15.0	5.41	3.61
9.20	30	1.00	4.99	1.74	3.49
9.20	30	1.00	2.51	0.96	3.82
10.02	30	1.00	10.0	6.14	6.14
9.60	30	1.00	10.0	5.21	5.21
8.90[b]	30	1.00	10.0	2.86[c]	2.86[c]
8.80	30	1.00	10.0	2.75[c]	2.75[c]
8.40[b]	30	1.00	10.0	1.20[c]	1.20[c]
9.20	40	1.00	5.00	5.58	11.2
9.20	48.5	1.00	5.00	13.0	26.0
9.20	57	1.00	2.51	15.7	62.7

[a] In borate buffer with $\mu = 2.0$ (Na_2SO_4). At pH 9.20, $E_a = 20.5 \pm 0.3$ kcal, $\Delta G^{\ddagger} = 19.8 \pm 0.4$ kcal, and $\Delta S^{\ddagger} = 0.4 \pm 0.8$ eu. [b] Phosphate buffer. [c] Evaluated from linear portion of log i_p *vs.* *t* plot ($t < 120$ min).

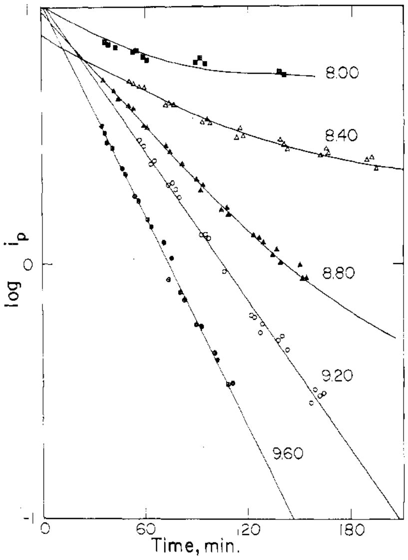


Figure 2. First-order plots of log i_p *vs.* *t* for reaction of $Mo_2O_4(Cys)_2^{2-}$ with cysteine. Conditions: $[Mo_2O_4(Cys)_2^{2-}] = 1.00 \times 10^{-3}$ *M*; [Cys] = 10.0×10^{-3} *M*; $\mu = 2.0$; 30°; $v = 0.033$ V sec^{-1}; electrode area 0.022 cm^2; pH 9.60, 9.20, 8.80 borate buffer; pH 8.40, 8.00 phosphate buffer. pH value is indicated on each curve.

for the paramagnetism and visible absorption observed during the reaction.

Kinetics of the Reaction of $Mo_2O_4(Cys)_2^{2-}$ with Cysteine. When cysteine is added to a solution of $Mo_2O_4(Cys)_2^{2-}$ above pH 8, the voltammetric peak current of the complex (peak A, Figure 1) diminishes and approaches zero as the reaction proceeds. A new voltammetric peak (B, Figure 1) appears, and its current during the reaction increases to a limiting value. Representative traces recorded during a reaction are presented in Figure 1.

Kinetic plots of log i_p for $Mo_2O_4(Cys)_2^{2-}$ reduction *vs.* *t* over a range of pH values are shown in Figure 2. In the presence of excess cysteine between pH 9.0 and 10.0 these plots are linear to ≥90% reaction and indicate first-order dependence on $[Mo_2O_4(Cys)_2^{2-}]$. Below pH 9.0 the first-order plots are initially linear but become nonlinear at later stages of the reaction. The nonlinearity occurs at successively earlier points in the reaction as pH is decreased.

Kinetic data for the reaction of $Mo_2O_4(Cys)_2^{2-}$ with cysteine are presented in Table II. The rate of reaction increases with increasing pH, cysteine concentration, and ionic strength. Comparable results are obtained in borate and phosphate buffers. The pH dependence of the reaction is much less than that expected for a reaction first-order in hydroxide ion. At pH 9.20 the rate constant k_1 exhibits first-order dependence on cysteine concentration. A plot of log k_1 *vs.* log [Cys] is linear with a slope of 1.01. The rate expression for the reaction is

$$\frac{-d[Mo_2O_4(Cys)_2^{2-}]}{dt} = k_2[Mo_2O_4(Cys)_2^{2-}][Cys]$$

The average value of k_2 (with standard deviation) at pH 9.20, $\mu = 2.0$, and 30° is $(3.75 \pm 0.16) \times 10^{-2}$ M^{-1} sec^{-1}.

A particularly interesting observation is that the reaction between $Mo_2O_4(Cys)_2^{2-}$ and cysteine maintains pseudo-first-order behavior even at low concentrations of cysteine. For example, in solutions containing ~1 m*M* $Mo_2O_4(Cys)_2^{2-}$ and ~1 m*M* cysteine second-order behavior is expected, but first-order plots are strictly linear up to at least 80% reaction at pH 9.20. The rate constant measured at low cysteine concentrations is in agreement with k_2 determined in the presence of a large excess of cysteine (runs 1-3, Table III). This result suggests that cysteine is not consumed during the reaction. This fact is established by voltammetrically monitoring free cysteine during reactions with low [Cys] : $[Mo_2O_4(Cys)_2^{2-}]$ ratios. Figure 3 shows the results of an experiment in which the concentrations of $Mo_2O_4(Cys)_2^{2-}$, cysteine, and the product reduced in peak B of Figure 1 are monitored throughout a single reaction of 1.02 m*M* $Mo_2O_4(Cys)_2^{2-}$ with 1.02 m*M* cysteine. A linear plot is obtained for log i_p *vs.* *t*, and the concentration of cysteine does not decrease during the reaction.[21]

The reaction also is characterized by the rates of appearance of the electrochemically reducible and visibly absorbing products. In Figure 3 the slope of the log $(i_{p,\infty} - i_{p,t})$ *vs.* *t* plot for product reduction is the same as the slope of the log

Table III. Kinetic Data for the Reaction of $Mo_2O_4(Cys)_2^{2-}$ with Cysteine Obtained at Low Concentrations of Cysteine and from the Rate of Appearance of Reaction Products[a]

Run[b]	$10^3[Mo_2O_4(Cys)_2^{2-}]$, M	10^3[Cys], M	$10^4 k_1$, sec^{-1}	$10^2 k_2$, $M^{-1}\ sec^{-1}$
1	0.52	16.4	1.83	1.12
2	0.89	0.96	0.115	1.20
3	1.02	1.02	0.124	1.22
4	1.02	1.02	0.125	1.23
5	0.66	29.1	3.94	1.35
6	1.02	6.3	0.56	0.90
7	0.49	14.8	1.30	0.88

[a] In pH 9.20 borate buffer at $\mu = 0.20$ and 22°. [b] Runs 1-3, from log i_p *vs.* t for $Mo_2O_4(Cys)_2^{2-}$ reduction; run 4, from log $(i_{p,\infty} - i_{p,t})$ *vs.* t for reduction of product in peak B, Figure 1; run 5, from log $(A_\infty - A_t)$ *vs.* t for visible product at 580 nm; runs 6 and 7, from log $(A_\infty - A_t)$ *vs.* t for visible product at 620 nm.

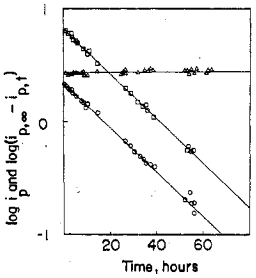


Figure 3. Plots of log i_p and log $(i_{p,\infty} - i_{p,t})$ *vs.* time for reactants and products in the reaction of $Mo_2O_4(Cys)_2^{2-}$ with cysteine: (□) log i_p *vs.* t for $Mo_2O_4(Cys)_2^{2-}$ reduction, $E_p = -1.29$ V; (△) log i_p *vs.* t for mercury electrode oxidation, $Hg + 2Cys \rightleftarrows Hg(Cys)_2 + 2e^- + 2H^+$, $E_p = -0.48$ V; (○) log $(i_{p,\infty} - i_{p,t})$ *vs.* t for product reduction, $E_p = -1.03$ V. Conditions: $[Mo_2O_4(Cys)_2^{2-}] = 1.02 \times 10^{-3}$ M; [Cys] $= 1.02 \times 10^{-3}$ M; pH 9.20 borate buffer; $\mu = 0.2$; 22°; $v = 0.050$ V sec^{-1}; electrode area 0.022 cm^2.

i_p *vs.* t plot for $Mo_2O_4(Cys)_2^{2-}$ reduction. Plots of log $(i_{p,\infty} - i_{p,t})$ and log $(A_\infty - A_t)$ *vs.* time for the electrochemically reducible and visible products, respectively, are linear and yield rate constants consistent with those obtained by following the disappearance of $Mo_2O_4(Cys)_2^{2-}$ (runs 4-7, Table III). Although the measurements regarding these products are subject to some uncertainty due to the inherently weak signals and the difficulty in establishing infinite-time values, it is apparent that the rate constants for appearance of these products are approximately equal to the rate constant for disappearance of $Mo_2O_4(Cys)_2^{2-}$.

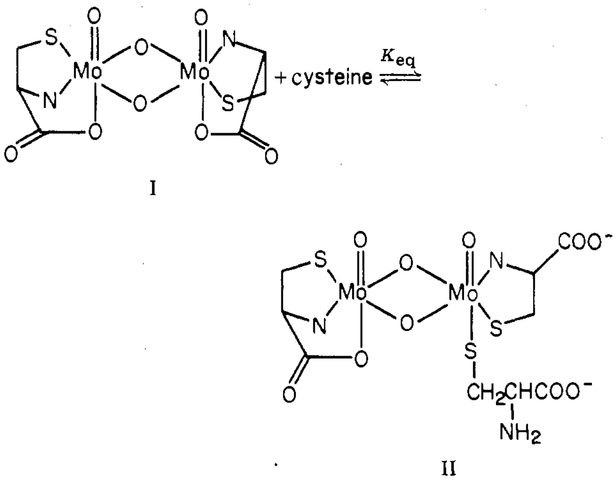
Reaction Products. The oxidation state of the reaction products is determined to be Mo(V) on the basis of chemical reaction of samples from completed reaction mixtures with 12 F HCl and 8-hydroxyquinoline. The electronic spectrum of the products in 12 F HCl is identical with that of the $MoOCl_5^{2-}$ ion,[18,19] providing quantitative evidence that the oxidation state is Mo(V). Addition of reaction products to 8-hydroxyquinoline in ethanol produces a wine red color and a strong visible absorption at 536-540 nm characteristic of the complex $Mo_2O_3(C_9H_6NO)_4$.[20] Only 75 ± 15% of the molybdenum can be accounted for as Mo(V) in these experiments; therefore, these results provide only semiquantitative confirmation of the oxidation state.

The amount of H^+ consumed or released in the reaction is determined by initiating reactions at pH 10.0-10.5 in solutions containing ~1:1 cysteine and $Mo_2O_4(Cys)_2^{2-}$ with no buffer present and measuring pH as the reaction proceeds. Under these conditions pH decreases as the reaction proceeds, and it is calculated that between 0.7 and 1.0 mol of hydrogen ion is produced for each mole of $Mo_2O_4(Cys)_2^{2-}$ consumed in the reaction at low ratios of cysteine to $Mo_2O_4(Cys)_2^{2-}$.

Discussion

A number of new facts regarding the behavior of $Mo_2O_4(Cys)_2^{2-}$ in the pH range 8-10 are apparent from this work. (1) $Mo_2O_4(Cys)_2^{2-}$ does not exist in simple equilibrium with a molybdenum(V) monomer; rather, a gross reaction between $Mo_2O_4(Cys)_2^{2-}$ and free cysteine occurs which produces an epr signal and visible color change observed in previous experiments.[3,4] (2) The chemical reaction is first order in each reactant, but cysteine is catalytically regenerated. (3) The substances responsible for the paramagnetism and the visible color change are different species, which may be in equilibrium with one another, and the small amount of paramagnetic species produced indicates that it is an intermediate or minor product of the reaction.

The experimental rate expression indicates that the sequence of events is initiated by reaction between $Mo_2O_4(Cys)_2^{2-}$ and cysteine. We believe that this reaction involves coordination of a third cysteine molecule to the complex which promotes an irreversible change from the dioxo-bridged $Mo_2O_4^{2+}$ structure to the monooxo-bridged $Mo_2O_3^{4+}$ structure. Similar reactions have been qualitatively identified by Mitchell[20] for $Mo_2O_4(C_2O_4)_2(H_2O)_2^{2-}$ in which substitution by a ligand molecule leads to cleavage of one of the oxygen bridges. On the basis of the known structures of $Mo_2O_4(Cys)_2^{2-}$ [22,23] and dimeric $Mo_2O_3^{4+}$ compounds[6,13,24] these reactions are written as (1) and (2). The observed second-order rate constant, k_2, is equal to $k_1{}'K_{eq}$.



I

II

(1)

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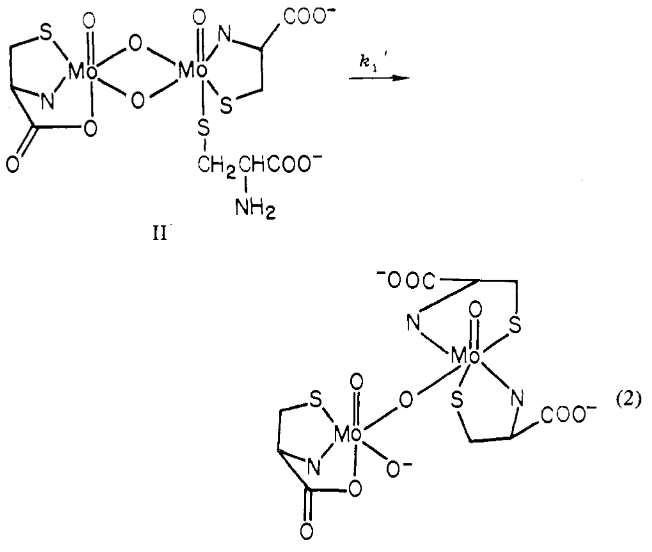
(21) The height of anodic peak C is not strictly constant during reaction at low [Cys]:$[Mo_2O_4(Cys)_2^{2-}]$ ratios. At pH 9.2-10.0 an increase of up to 30% in peak current is observed during the later stages of the reaction. This increase is attributed to oxidation of a product of the chemical reaction at the mercury electrode and not to an increase in free cysteine concentration. This can be seen from a consideration of the shift in peak potential C during the reaction. If free cysteine concentration were increasing, the potential of the electrode reaction $Hg + 2Cys \rightleftarrows Hg(Cys)_2 + 2H^+ + 2e^-$ would shift in the cathodic direction. However, during the reaction at low [Cys]:$[Mo_2O_4(Cys)_2^{2-}]$ ratios peak C shifts in the anodic direction. Furthermore, a new anodic peak approximately 100 mV anodic of the Hg-Cys wave appears after almost complete reaction. The increase in peak current C is apparently due to the formation of this material.

(22) J. R. Knox and C. K. Prout, *Chem. Commun.*, 1227 (1968).

(23) J. R. Knox and C. K. Prout, *Acta Crystallogr., Sect. B*, 25, 1857 (1969).

(24) P. C. H. Mitchell, *Quart. Rev., Chem. Soc.*, 20, 103 (1966).

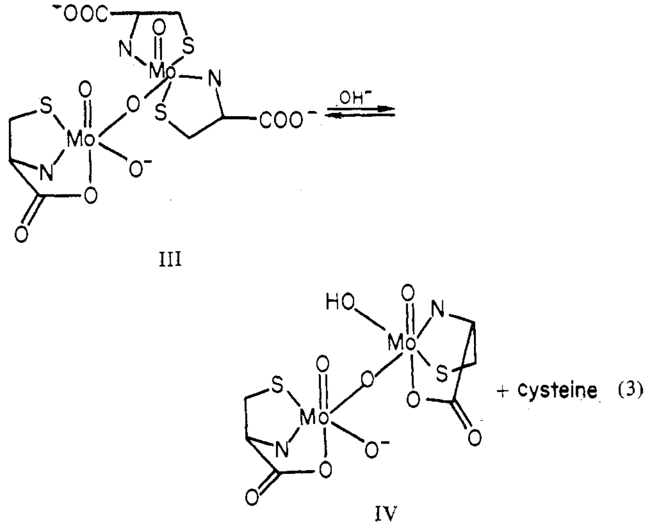


II

III

(2)

Following this structural rearrangement, replacement of the extra cysteine molecule by OH^- (eq 3) is postulated to account for the constancy of cysteine concentration and pseudo-first-order behavior observed in the experiments at low concentrations of cysteine and the decrease in pH observed in unbuffered solutions under these conditions. Further dissociation of the monooxo-bridged dimer (possibly induced also by free cysteine) can take place to produce molybdenum(V)-cysteine monomer. However, the weak epr signals observed indicate that the equilibrium in reaction 4 lies mainly to the left.



III

IV

(3)

$$\underset{\text{IV}}{Mo_2O_3(O)(OH)(Cys)_2{}^{3-}} \rightleftarrows \underset{\text{V}}{2Mo^{V}Cys} \qquad (4)$$

The products responsible for the 580-640-nm absorption band are tentatively identified as the monooxo-bridged molybdenum(V)-cysteine dimers III and IV. Presumably a mixture of products exists as the equilibrium in reaction 3 would be expected to lie to the right at low concentrations of cysteine and to the left at high concentrations of cysteine. A mixture of products also is indicated by the visible spectra (Table I) inasmuch as λ_{max} is a function of pH and extent of reaction. In addition, other electrochemically active products are observed during later stages of reaction at low cysteine concentrations. These one or more species are identified by a voltammetric reduction peak at *ca.* −1.1 V and a voltammetric oxidation peak[21] at *ca.* −0.38 V at pH 9.2. Spence and Kroneck have reported preliminary results of a study of $Mo_2O_4(Cys)_2{}^{2-}$ decomposition by epr and visible spectrometry[25] and concluded that in addition to epr-active monomers three monooxo-bridged molybdenum(V)-cysteine dimers are produced in the reaction. For lack of additional evidence on our part we conclude only that two or more monooxo-bridged dimers are produced in the reaction.

The assignment of III and IV as the visibly absorbing species at 580-640 nm is made on the basis of the inverse temperature dependence of the visible absorption and epr signals and the qualitative similarity of the visible spectra of these products with those of other monooxo-bridged Mo(V) complexes.[13,14,20] A dimeric product is indicated from analysis of absorbance-time and i_p-time curves under the same experimental conditions which shows equal rates for the appearance of visible products and disappearance of $Mo_2O_4(Cys)_2{}^{2-}$. If the visible products were monomeric species, their rate of appearance would be twice the rate of disappearance of $Mo_2O_4(Cys)_2{}^{2-}$. The overall reaction sequence described by eq 1-4 is consistent with all of the kinetic measurements at pH >9 if it is assumed that reaction 2 constitutes the rate-determining step and that only a small fraction of Mo(V) is present as monomer in reaction 4.

The conclusions reached in the kinetic study of the reaction between $Mo_2O_4(Cys)_2{}^{2-}$ and cysteine are strictly valid only between pH 9.0 and 10.0. Below pH 9.0 the reaction does not proceed to completion, and a change of mechanism may ensue. At pH 8.0 the rate of reaction is very slow. This is noted in contrast to the fact that epr signals have been observed in solutions of Mo(V) and cysteine at pH values as low as 6[3,4] and that well-defined epr signals are observed down to pH 7.[3-5] In these experiments, however, the method of preparation of the molybdenum(V)-cysteine solutions is different than in this work, and the observation of epr signals at lower pH values appears to depend on the manner of solution preparation. In general epr activity is observed at pH 6-8 when solutions are prepared by mixing Mo(V) in ~3 *F* HCl with cysteine in buffered solutions. In 3 *F* HCl Mo(V) exists primarily as a diamagnetic and presumably dioxo-bridged dimer,[26-28] but small amounts of paramagnetic dimer are present and, at slightly higher acidities, paramagnetic monomer as well.[26-28] It is possible that the low-pH epr signals peculiar to this method of solution preparation arise from the small amounts of non-dioxo-bridged species introduced with the Mo(V) which may subsequently react with cysteine. When $Mo_2O_4(Cys)_2{}^{2-}$ is added as a solid chelate to excess cysteine at pH 7.50, as done in an experiment by Martin and Spence,[5] no epr signal is observed. In the present work the solid chelate is used exclusively. We believe that the behavior of $Mo_2O_4{}^{2+}$ complexes can be validly investigated only be employing the pure isolated complexes known to contain this structural unit. This is particularly critical in view of the small fraction of epr-active material occurring in the molybdenum(V)-sulfhydryl systems.

In previously studied rearrangements of dioxo-bridged to monooxo-bridged Mo(V) complexes,[20] bridge cleavage occurs following substitution of ligand molecules foreign to the

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(25) J. T. Spence and P. Kroneck, Abstracts, 1st Rocky Mountain Regional Meeting of the American Chemical Society, Ft. Collins, Colo., June 30-July 1, 1972, No. INOR-20.

(26) L. Sacconi and R. Cini, *J. Amer. Chem. Soc.*, **76**, 4239 (1954).

(27) G. P. Haight, Jr., *J. Inorg. Nucl. Chem.*, **24**, 663 (1962).

(28) C. R. Hare, I. Bernal, and H. B. Gray, *Inorg. Chem.*, **1**, 831 (1962).

initial complex. The reaction of $Mo_2O_4(Cys)_2^{2-}$ with cysteine, however, is an interesting situation in that the structural change is catalyzed by a molecule that is also a ligand in the complex. Cleavage of the dioxo structure in molybdenum(V) complexes produces monooxo-bridged and monomeric species that appear to be more reactive than the original material. In the case of monooxo-bridged molybdenum(V)-thioglycolate complexes, reduction by sulfhydryl compounds is found to occur.[6] For Mo-EDTA complexes the following sequence of reactivity and ease of reducibility is found:[29] monomeric > monooxo-bridged > dioxo-bridged.

Registry No. $Na_2Mo_2O_4(cysteine)_2 \cdot 5H_2O$, 22723-22-4; cysteine, 52-90-4.

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[29] F. A. Schultz and D. T. Sawyer, *J. Electroanal. Chem.*, 17, 207 (1968).

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Proton Nuclear Magnetic Resonance Studies of the Electronic Structure of Outer-Sphere Reducing Agents of Chromium(II). IV. Solvation of Tris(α-diimine) Chelates

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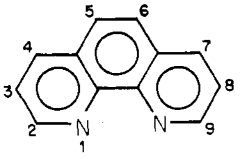
Received February 21, 1973

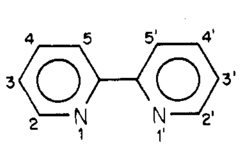
The anomalous proton nmr line broadening of the alkyl substituents in tris(*o*-phenanthrolines) of chromium(II) is interpreted in terms of a specific solvent–solute interaction which is characteristic of such complexes with reducing metal ions. The ligand line width study reveals that this interaction is highly specific to the 4,7 positions and is most important in aqueous solution. This solvent–solute interaction is postulated to involve some form of hydrogen bonding of the solvent to pockets of electron density on the ligand which result from extensive M→L π back-bonding. Analysis of the solvent line width in mixed aqueous–methanolic solutions reveals a distinct preference for water molecules penetrating within the "ionic radius" in the cationic chelates of the reducing chromium(II) ion but not for the more redox-stable nickel(II) ion.

Introduction

We have been interested in using proton nmr for investigating paramagnetic complexes of low-valent metal ions[2-4] inasmuch as this technique[5] is especially well suited for elucidating the anticipated important contributions to the electronic structure from metal-to-ligand π charge transfer.[6] Such π back-bonding has been considered to be characteristic[6] of a particularly interesting class of complexes of low-valent metal ions, the outer-sphere reducing agents.[7] The particular reducing agents of interest have been the tris chelates[2,3] of chromium(II) with substituted *o*-phenanthrolines, I (phen), and α,α'-bipyridines, II (bipy), and their mixed-ligand chelates,[8] whose redox properties,[9] magnetic susceptibilities,[10] electronic spectra,[11] and bonding[2,3,8,12-15] have been the subject of recent interest.



I



II

Analysis of the isotropic shifts for these low-spin (d^4, $S = 1$) chelates has shown[2,12] that they are primarily contact in origin, resulting from both L→M and M→L π charge transfer,[8,12,13] the latter mechanism imparting the facile outer-sphere reducing properties of these chelates. The detailed description of the spin-transfer mechanism was achieved[8,12,13] only after investigating the proton nmr spectra of mixed-ligand chelates, for which it was possible not only to characterize the bonding and assign the orbital ground state of each complex[8,12-13] but also to determine the relative π-acceptor abilities[12,13] of a variety of substituted diimines. It was demonstrated[8] that the M→L delocalized π spin density was centered predominantly at the 4,7 positions of phen (or 4,4' positions in bipy), which were postulated[12,13]

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[1] (a) University of California; Fellow of the Alfred P. Sloan Foundation, 1972–1974. (b) Harvey Mudd College.

[2] (a) G. N. La Mar and G. R. Van Hecke, *J. Amer. Chem. Soc.*, 91, 3442 (1969); (b) G. N. La Mar and G. R. Van Hecke, *Inorg. Chem.*, 9, 1546 (1970).

[3] G. N. La Mar and G. R. Van Hecke, *J. Amer. Chem. Soc.*, 92, 3021 (1970).

[4] G. N. La Mar, E. O. Sherman, and G. A. Fuchs, *J. Coord. Chem.*, 1, 289 (1971).

[5] D. R. Eaton and W. D. Phillips, *Advan. Magn. Resonance*, 1, 103 (1965).

[6] L. E. Orgel, "Introduction to Transition Metal Chemistry: Ligand Field Theory," Methuen, London, 1960, Chapter 9.

[7] H. Taube, "Electron Transfer Reactions of Complex Ions in Solution," Academic Press, New York, N. Y., 1970, pp 28–35.

[8] G. N. La Mar and G. R. Van Hecke, *J. Amer. Chem. Soc.*, 94, 9042 (1972).

[9] J. H. Sutter and J. B. Hunt, *J. Amer. Chem. Soc.*, 91, 2107 (1969).

[10] A. Earnshaw, L. F. Larkworthy, K. C. Patel, K. S. Patel, R. L. Carlin, and E. G. Terezakis, *Inorg. Chem.*, 6, 2125 (1967); P. M. Lutz, G. J. Long, and W. A. Baker, Jr., *ibid.*, 8, 2579 (1969).

[11] E. Konig and S. Herzog, *J. Inorg. Nucl. Chem.*, 32, 585 (1970).

[12] G. N. La Mar and G. R. Van Hecke, *J. Amer. Chem. Soc.*, 94, 9049 (1972).

[13] G. N. La Mar, *J. Amer. Chem.*, 94, 9055 (1972).

[14] G. N. La Mar and G. R. Van Hecke, *J. Magn. Resonance*, 4, 384 (1971).

[15] G. N. La Mar and G. R. Van Hecke, *J. Chem. Phys.*, 52, 5676 (1970).