J. Am. Chem. Soc. 1989, 111, 2111-2115

Reduction of Nitrate to Nitrite by Molybdenum-Mediated Atom Transfer: A Nitrate Reductase Analogue Reaction System

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Abstract: The kinetics of the oxygen atom transfer reaction $Mo^{IV}O(L\text{-}NS_2)(DMF) + NO_3^- = Mo^{VI}O_2(L\text{-}NS_2) + NO_2^- +$ DMF was investigated in DMF solution ($L\text{-}NS_2$ = 2,6-bis(2,2-diphenyl-2-mercaptoethyl)pyridine(2−)). The reaction is quantitative and well-behaved when conducted in the presence of an excess of nitrate and ca. 1.5 equiv of sulfamic acid, which rapidly scavenges nitrite that otherwise bleaches the Mo chromophores. It is characterized by saturation kinetics in which nitrate reversibly forms a substrate–Mo(IV) complex that generates products in a first-order pathway with $k_1 = (1.49 \pm 0.05) \times 10^{-3}\ s^{-1}$ at 295.5 K, $\Delta H^{\ddagger} = 23.7 \pm 0.6$ kcal/mol, and $\Delta S^{\ddagger} = 8.0 \pm 2.0$ eu. The moderate activation entropy suggests that the ground and transition states are structurally similar. The activation enthalpy is indistinguishable from previously reported values for the reductions of *S*-oxide and *N*-oxide substrates, which also follow pseudo-first-order kinetics. Inasmuch as the difference in S–O (Me_2SO) and N–O (pyridine *N*-oxide) bond energies is about 14 kcal/mol, the essentially constant activation enthalpies indicate that the transition state is reached without significant substrate bond weakening. The recently introduced thermodynamic reactivity scale for oxo transfer as applied to substrates with N–O bonds is discussed. This work contributes the only well-documented reduction of nitrate to nitrite mediated at a Mo(IV) atom and demonstrates that reduction of nitrate by atom transfer is a plausible (but unproven) pathway in the mechanism of action of nitrate reductases.

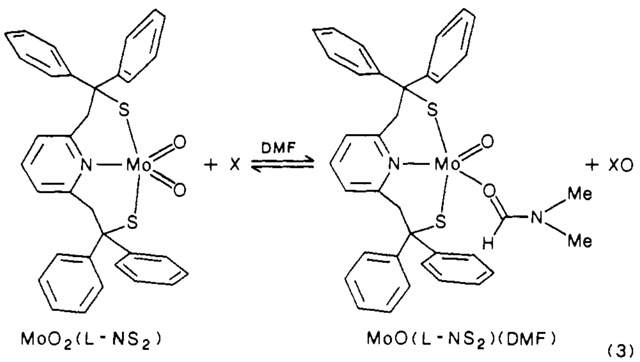
Among the more familiar molybdoenzymes, often referred to as the molybdenum hydroxylases,[2-5] are the nitrate reductases.[6,7] These enzymes catalyze reaction 1, with NAD(P)H or ferredoxins as common electron donors. The obligatory role of molybdenum

$$NO_3^- + 2H^+ + 2e^- \rightleftharpoons NO_2^- + H_2O\ (E_0' = +420\ \text{mV}) \qquad (1)$$

in enzymic catalysis has been definitively demonstrated by the reconstitution to full activity of the inactive nitrate reductase from the *Neurospora crassa* mutant *nit-1* with molybdenum cofactor released from other molybdoenzymes.[8-10] The cofactor is a complex of molybdenum and molybdopterin.[11] A recent EXAFS study of *Chlorella vulgaris* nitrate reductase suggested the minimal coordination units $Mo^{VI}O_2(SR)_{2,3}$ and $Mo^{IV}O(SR)_{3,4}$ in the oxidized and dithionite- and NADP-reduced forms, respectively.[12] Two of the anionic sulfur ligands presumably derive from the ene–dithiolate chelate group on the side chain of the pterin.[11] By the EXAFS criterion, the molybdenum coordination units in these states of the enzyme resemble those of liver sulfite oxidase.[13] Nitrate reductase from *E. coli* does not appear to fit into this structural pattern, there being evidence for only one oxo group in the oxidized state and none in the dithionite-reduced form.[12] While the somewhat unexpected results for this enzyme may be subject to further examination, the enzymes do differ in being dissimilatory (*E. coli*) and assimilatory (*Chlorella*). Conceivably, their catalytic sites may not be conserved even though they both catalyze the same reaction.

In this laboratory, we have been engaged in the development of analogue reaction systems for molybdoenzymes.[14] Some of these enzymes we have termed oxotransferases to indicate the possibility that substrate oxidation or reduction may occur without the intervention of any exogenous reactant, as in reaction 2. In earlier experiments, we have developed the analogue reaction system 3 whose component Mo(IV,VI) complexes have been designed to prevent formation of a μ-oxo Mo(V) dimer and to approach the biological coordination units such as are found in sulfite reductase and *Chlorella* nitrate reductase. This system is quite effective in oxidizing tertiary phosphines and reducing *N*-oxides and *S*-oxides,[15-18] some of which are enzyme substrates. The purpose of these investigations is to elucidate the kinetics and mechanism of oxidation or reduction of enzyme substrates or pseudosubstrates, with the intention of providing plausible pathways for enzymic reactions.

$$Mo^{VI}O_2L_n + X \rightleftarrows Mo^{IV}OL_n + XO \qquad (2)$$



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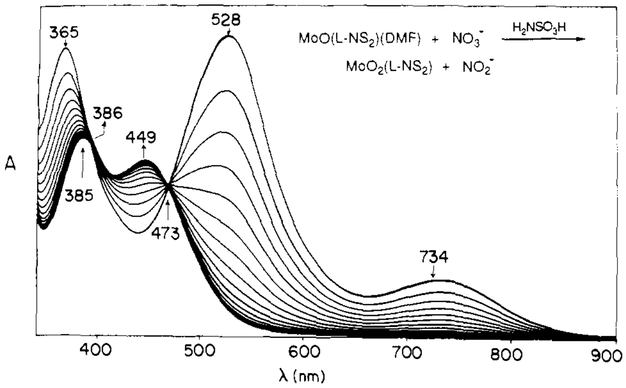

Figure 1. Spectral changes in the reaction of 0.82 mM MoO(L-NS$_2$)(DMF), 3.0 equiv of (n-Bu$_4$N)(NO$_3$), and 1.0 equiv of sulfamic acid in DMF solution at 21 °C. Spectra were recorded every 4 min at a scan rate of 600 nm/min. (These spectra were recorded on a Perkin-Elmer Lambda 4C spectrophotometer.)

Abiological reduction of nitrate by molybdenum has been accomplished with Mo(III)[19,20] and Mo(V).[21-29] In a number of these systems the initial reaction product is not nitrite but nitrogen dioxide. There is a single, brief claim of the reaction of nitrate with Mo(IV) to afford nitrite and Mo(VI);[29] supporting details were not provided. With use of reaction system 3, we have devised an analogue reaction system for nitrate reductase and demonstrated that reduction occurs by atom transfer. The details of this reaction and related observations of nitrate reduction are the subject of this report. The broader matter of oxo transfer reactions mediated at molybdenum and other metal centers is treated elsewhere.[30]

Experimental Section

Preparation of Compounds. MoO$_2$(L-NS$_2$) and MoO(L-NS$_2$)(DMF) (L-NS$_2$ = 2,6-bis(2,2-diphenyl-2-mercaptoethyl)pyridine(2-)) were synthesized as previously described,[31] with the minor modification of 10:1 chloroform/DMF (v/v) as the solvent for the reduction of MoO$_2$(L-NS$_2$) with Ph$_3$P. In this medium, MoO(L-NS$_2$)(DMF) precipitates and is obtained in a form more readily soluble in DMF.

Kinetics Measurements. All reactions were carried out under strictly anaerobic conditions in solutions of DMF (Burdick & Jackson). The solvent was stored over 3–4 Å molecular sieves for several days and degassed immediately before use with two freeze–pump–thaw cycles. (n-Bu$_4$N)(NO$_3$) (Fluka) and sulfamic acid (primary standard, G. F. Smith) were used as received. (Et$_4$N)(NO$_2$) was prepared by cation exchange of NaNO$_2$ in water on a column of Bio-Rad AG 50W-X 50–100 mesh resin that had been loaded with Et$_4$NOH in methanol and then washed to neutral pH with water. The product was isolated by removal of water from the eluate.

Nitrate reduction reactions were monitored spectrophotometrically with use of a Cary 219 or Varian 2390 spectrophotometer and a cell compartment thermostated to ±0.5 °C. Absorption spectra such as those presented in Figure 1 demonstrate the oxidation of MoO(L-NS$_2$)(DMF) to MoO$_2$(L-NS$_2$) in the presence of nitrate and sulfamic acid. The reaction systems consisted initially of 0.52–0.84 mM MoO(L-NS$_2$)(DMF), 8–115 equiv of (n-Bu$_4$N)NO$_3$, and 1.5 equiv of sulfamic acid and were examined at four temperatures in the range 281–317 K. Sulfamic acid was introduced to scavenge nitrite, which otherwise bleaches the Mo complexes. The Mo(IV) + sulfamic acid solutions were equilibrated in the cell at the temperature of measurement prior to the addition of a concentrated nitrate solution. Spectra of MoO(L-NS$_2$)(DMF) and MoO$_2$(L-NS$_2$) have been given earlier[31] and appear in Figure 1. The Mo(IV) spectrum is slightly temperature dependent in that λ_{max} for the visible band varies from 528 to 536 nm over the temperature range of measurement. The reactions were followed at these maxima. At each of the four temperatures, at least five runs under pseudo-first-order conditions were carried out. Observed rate constants were determined from plots of ln $(A_t - A_\infty)$ vs time, which were linear for at least 2.5 half-lives. Nonlinearities at longer times may be due in part to the small quantities of water generated in the scavenging reaction of nitrite with sulfamic acid. In the spectrophotometric monitoring of reactions, perfect isosbestic points could not be obtained in the absence of sulfamic acid. In control experiments at the temperatures and concentrations of the kinetics runs, sulfamic acid altered the apparent extinction coefficients of the Mo(IV) and Mo(VI) complexes by <2% over a minimum of 3 half-lives. In an additional control at room temperature, the spectrum of MoO$_2$(L-NS$_2$) was unchanged in the presence of 4.5 equiv of sulfamic acid and 100 equiv of (n-Bu$_4$N)NO$_3$ over the course of 10 h.

The analysis of data and the estimation of errors in rate constants and activation parameters follow our previous procedures.[15,18] In determining the rate constants k_1, the function $\chi^2 = \sum[V_i^{obsd} - V_i^{calcd}]^2/\sigma(V_i^{obsd})^2$, involving observed and calculated reaction velocities, was minimized as described earlier.

Dinitrogen, resulting from the reaction of sulfamic acid and nitrite, was identified and analyzed on a Varian 3700 gas chromatograph equipped with a column containing 5 Å molecular sieves. Analyses were performed at 27 °C with He carrier gas. Peak heights of gas samples from reaction system 3 were compared to those from reactions of standard solutions of (Et$_4$N)(NO$_2$) and sulfamic acid in DMF. All solutions were maintained under an argon atmosphere.

Results and Discussion

The first indication that Mo(IV), in at least one coordination environment, might be capable of reducing nitrate to nitrite by atom transfer came from the pioneering thermochemical work of Watt et al.[32] in 1977. Their evaluation of the enthalpy of reaction 4 in 1,2-dichloroethane predicted a modest driving force or, at the least, approximate thermoneutrality. The report several years earlier of the occurrence of this reaction in chloroform solution at ambient temperature[33] was not confirmed by the results of Durant et al.[26] in 1977, who observed more complicated products. Recently, we have demonstrated intermetal oxo transfer reaction 5.[16] This shows that MoO(L-NS$_2$)(DMF) is a stronger

$$\mathrm{MoO(S_2CNEt_2)_2 + NO_3^- \rightarrow MoO_2(S_2CNEt_2)_2 + NO_2^-} \quad \Delta H = -4.4 \pm 4.2 \text{ kcal/mol} \tag{4}$$

$$\mathrm{MoO(L\text{-}NS_2)(DMF) + MoO_2(S_2CNEt_2)_2 \rightarrow MoO_2(L\text{-}NS_2) + MoO(S_2CNEt_2)_2} \tag{5}$$

oxo acceptor than MoO(S$_2$CNEt$_2$)$_2$, a desirable feature in an attempt to reduce, by atom transfer, the relatively stable nitrate ion. Further, in the course of the development of reaction system 3 in this laboratory, it was observed that MoO(L-NS$_2$)(DMF) was oxidized to MoO$_2$(L-NS$_2$) by excess nitrate in DMF.[15] The system was complicated by a rapid bleaching reaction between nitrite and the Mo complexes. However, a plot of ln $(A_t - A_\infty)$ vs t was approximately linear over several half-lives and further analysis suggested a first-order rate constant $k_1 \approx 1 \times 10^{-3}$ s^{-1} at 296 K.

Kinetics of Nitrate Reduction. In this investigation, we have devised an effective system for the reduction of nitrate by Mo(IV) in the form of reaction 3 in the presence of a small excess (1.5 equiv/Mo) of sulfamic acid, which acts as a scavenger for nitrite. The rapid, quantitative reaction 6 proceeds in aqueous acid solution[34] and has been used to discharge nitrite in several aqueous

$$\mathrm{HNO_2 + H_2NSO_3H \rightarrow N_2 + HSO_4^- + H_3O^+} \tag{6}$$

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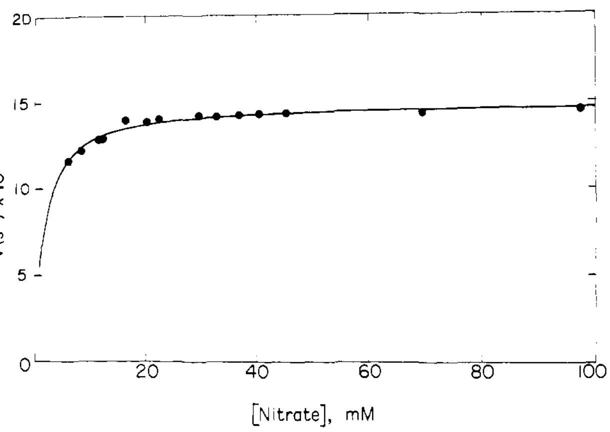


Figure 2. Dependence of the rate of the reaction of 0.84 mM MoO(L-NS_2)(DMF) and 7.4–116 equiv of (n-Bu_4N)(NO_3) in DMF solution at 22.5 °C on the nitrate concentration. Each run contained 1.5 equiv of sulfamic acid.

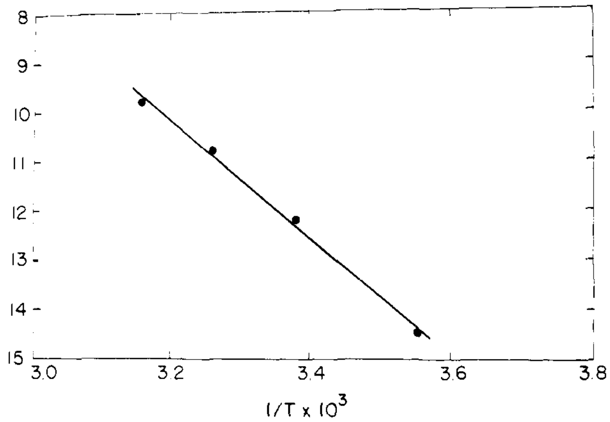
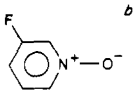


Figure 3. Eyring plot of the rate constants for reaction 3 with X = NO_3^-.

nitrate reduction systems based on Mo(III)[20] and Ru(II).[35] The solubility and stability of sulfamic acid in DMF[36] suggested a similar use in reaction system 3, and this approach has eliminated any secondary reactions between Mo complexes and nitrite.

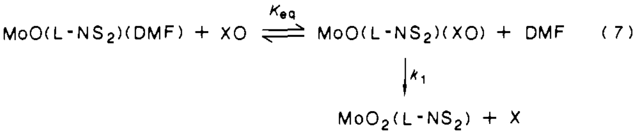
The spectrophotometric course of reaction 3 with XO = NO_3^- and the ratio $[NO_3^-]_0$:$[Mo(IV)]_0$ = 3.6:1 in the presence of 1.0 equiv of sulfamic acid/Mo is presented in Figure 1. As the spectrum of the Mo(IV) complex, with maxima at 365, 528, and 734 nm, diminishes with time, tight isosbestic points at 386 and 473 nm are observed, and the final spectrum, with λ_{max} = 385 and 449 nm, is identical with that, and demonstrates quantitative formation, of $MoO_2(L\text{-}NS_2)$. As shown in Figure 2, the system exhibits saturation kinetics at sufficiently high substrate concentration. Under these conditions, the reaction is first order in the Mo(IV) complex as shown by linear plots of ln $(A_t - A_\infty)$ vs time, from which the observed rate constants were obtained. N-Oxide and S-oxide substrates also display saturation kinetics.[15,18]

Table I. Kinetics Data for Substrate Reduction by Oxo Transfer in Reaction System 3 (DMF Solution)

substrate	T, K	10^3k_1, s^{-1}	$\Delta H^‡$, kcal/mol	$\Delta S^‡$, cal/(deg·mol)
NO_3^- [a]	281.5	0.146 ± 0.008		
	295.5	1.49 ± 0.05		
			23.7 ± 0.6	8.0 ± 2.0
	306.6	6.3 ± 0.3		
	316.6	17.6 ± 1.0		
 [b]	297.5	1.60 ± 0.08	23.4 ± 1.4	7.2 ± 2.0
$(p\text{-}FC_6H_4)_2SO$ [b]	297.5	1.40 ± 0.07	22.1 ± 1.3	2.6 ± 1.6
Me_2SO [c]	296	1.50 ± 0.03	d	d

[a] This work. [b] Reference 18. [c] Reference 15. [d] Not determined.

Nitrate reduction in reaction 3 can be interpreted in terms of scheme 7 (XO = NO_3^-), which is analogous to that for the reduction of XO = N-oxides and S-oxides.[15,18] Reaction is initiated by reversible substrate binding followed by irreversible intramolecular oxo transfer with rate constant k_1; product X = NO_2^- is rapidly scavenged. The scheme is described by rate law 8 (Y = DMF + XO), from which $[MoO(L\text{-}NS_2)Y]_t = [Mo]_0 \exp(-Vt)$.[15] Values of K_{eq} and k_1 were obtained by fitting the observed rate data to eq 9 for the reaction velocity. The best fit curve is plotted with the rate data in Figure 2. Over the range 282–317 K, K_{eq} varies from 4.4 × 10^3 to 21 × 10^3. Activation parameters were obtained by a least-squares fit of k_1 values to the Eyring eq 10,[37] as shown in Figure 3.



$$\begin{tikzcd} \mathrm{MoO(L\text{-}NS_2)(DMF) + XO} \arrow[r, shift left, harpoon, "K_{eq}"] & \mathrm{MoO(L\text{-}NS_2)(XO) + DMF} \arrow[l, shift left, harpoon] \arrow[d, "k_1"] \\ & \mathrm{MoO_2(L\text{-}NS_2) + X} \end{tikzcd} \qquad (7)$$

$$d[MoO(L\text{-}NS_2)Y]/dt = -k_1[MoO(L\text{-}NS_2)(XO)] = \\ -k_1\{K_{eq}[XO]/(K_{eq}[XO] + [DMF])\}[MoO(L\text{-}NS_2)Y] \quad (8)$$

$$V = k_1\{K_{eq}[XO]/(K_{eq}[XO] + [DMF])\} \quad (9)$$

$$k_1 = (k_BT/h)\exp[\Delta S^‡/R - \Delta H^‡/RT] \quad (10)$$

Rate constants at four temperatures and activation parameters for nitrate reduction, and for the reduction of N-oxide and S-oxide substrates,[15,18] are collected in Table I. The value k_1(295.5 K) = 1.49 (5) × 10^{-3} s^{-1} is essentially indistinguishable from k_1(297.5 K) = 1.60 (8) × 10^{-3} s^{-1} for the reduction of 3-fluoropyridine N-oxide in reaction 3.[18] Rate constants for reductions of S-oxides are practically the same, falling in the narrow (1.4–1.7) × 10^{-3} s^{-1} range at ambient temperature.[15,18]

Reaction Stoichiometry. Spectrophotometric experiments such as that in Figure 1 have established that reaction 3 results in quantitative oxidation of Mo(IV) to Mo(VI). Gas chromatographic analysis of the product of reaction 6 in the nitrate reduction system 3 resulted in the identification and quantitation of dinitrogen. In systems containing, initially, [MoO(L-NS_2)(DMF)] = 1.0–2.5 mM, 1.5–11 equiv of sulfamic acid, and 2–4 equiv of nitrate, 80 ± 5 mol % of dinitrogen based on complete oxidation of Mo(IV) and the stoichiometry of reaction 6 was observed.

Other Observations. Several additional observations in DMF solutions are pertinent to the course of nitrate reduction in reaction system 3. MoO(L-NS_2)(DMF) (ν_{MoO} 946 cm^{-1} [38]) did not undergo isotope exchange with excess $H_2{}^{18}O$ over a period of 2 h. In contrast, a 1 mM solution of $MoO_2(L\text{-}NS_2)$ when treated with 10 equiv of $H_2{}^{18}O$ reacted quickly and isotope exchange was complete within ca. 2.5 h. The infrared spectrum of the unlabeled complex (ν_{MoO} 945, 909 cm^{-1}) changed progressively to that of the $Mo^{16}O^{18}O$ species (ν_{MoO} 933, 878 cm^{-1}) and then to the fully labeled $Mo^{18}O_2$ complex (ν_{MoO} 898, 864 cm^{-1}).[39] Reduction of partially labeled $MoO_2(L\text{-}NS_2)$ with Ph_3P[15] gave MoO(L-NS_2)(DMF) whose spectrum revealed ν_{MoO} 900 cm^{-1} for the $Mo^{18}O$ complex.

A 2 mM solution of MoO(L-NS_2)(DMF) containing 2 equiv of (n-Bu_4N)(HSO_4) underwent a decrease in absorbance of 5% over 30 min. Addition of 2 equiv of water slightly accelerated the decrease (1–2%) over 5 h. Neither bisulfite nor water cause

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(37) Equation 10 as previously given[17] mistakenly contains a ln term. The correct equation was used in the data analysis.

(38) All spectra were recorded as KBr pellets.

(39) $MoO_2(S_2CNEt_2)_2$ has been enriched in ^{17}O by similar means: Miller, K. F.; Wentworth, R. A. D. *Inorg. Chem.* **1979**, *18*, 984.

Table II. Selected Thermodynamic Data for the Reaction X + $^1/_2O_2$ = XO

X	XO	ΔH,[a] kcal/mol
N_2(g)	N_2O(g)	+20
PhCH=NPh(g)	PhCH=N(O)Ph(g)	−4[b]
C_5H_5N(g)	C_5H_5NO(g)	−13[c]
PhN=NPh	PhN=N(O)Ph	−17[b]
NO_2^-(aq)	NO_3^-(aq)	−25
C_2H_4(g)	C_2H_4O(g)	−25
Me_2S(g)	Me_2SO(g)	−27
NO_2^-	NO_3^-	−32[d]
$MoO(S_2CNEt_2)_2$	$MoO_2(S_2CNEt_2)_2$	−35[d,e]
$MoO(L\text{-}NS_2)(DMF)$	$MoO_2(L\text{-}NS_2)$ + DMF	[f]
2PhSH	PhSSPh + H_2O	−54
SO_3^{2-}	SO_4^{2-}	−64[d,e]
H_2(g)	H_2O(l)	−68[e]

[a] Data from ref 30; other references cited contain recent thermochemical data from which ΔH was calculated with ΔH = 59.55 kcal/mol for $^1/_2O_{2(g)} = O_{(g)}$. [b] Reference 40. [c] Reference 41. [d] 1,2-Dichloroethane solution. [e] Reference 32. [f] See text.

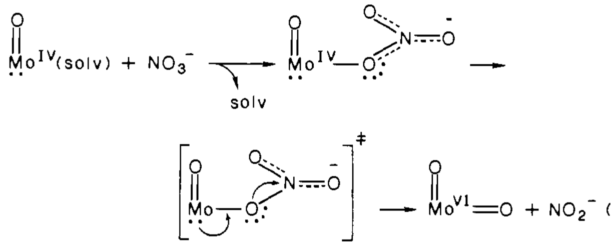
any appreciable oxidation of the Mo(IV) complex; indeed, such oxidations by atom transfer are thermodynamically unfavorable (vide infra).

These observations demonstrate the differing lability of oxygen atoms in the Mo(IV,VI) complexes to oxidic isotopic exchange. Because anaerobic DMF solutions of $MoO(L\text{-}NS_2)(DMF)$ are indefinitely stable, they also require that the source of oxygen atoms in the formation of $MoO_2(L\text{-}NS_2)$ in reaction 3 is nitrate.

Thermodynamic Considerations. Earlier we have introduced a thermodynamic reaction scale for oxo-transfer reactions.[16,30] Briefly, the scale consists of reactions (couples) of the type X + $^1/_2O_2$ = XO. When their ΔH values are tabulated in descending order, the scale may be used in the manner of a table of standard potentials. The reduced member of a given couple can reduce the oxidized member of any other couple with a larger ΔH, and conversely. A partial listing of couples is given in Table II. The enthalpy change for the $MoO(L\text{-}NS_2)(DMF)/MoO_2(L\text{-}NS_2)$ reaction is unknown, but its value can be bracketed. Because of the occurrence of reaction 5 and the reduction of $MoO_2(L\text{-}NS_2)$ to $MoO(L\text{-}NS_2)(DMF)$ by benzenethiol,[16] $-54 < \Delta H < -35$ kcal/mol. Consequently, the reduction of nitrate by $MoO(L\text{-}NS_2)(DMF)$ should be spontaneous, as observed. The heat of reaction should not exceed ca. −22 kcal/mol if the ΔH value for NO_2^-/NO_3^- in 1,2-dichloroethane is taken as the more appropriate for DMF solution.

The more extensive compilation of X/XO reaction enthalpies, available elsewhere,[30] contains many predictions as to the spontaneity of oxo-transfer reactions mediated (or not mediated) at $Mo^{IV}O$ and $Mo^{VI}O_2$ centers. Acceptance and utilization of the reactivity scale for reactions not yet attempted or otherwise in question will be enhanced by demonstration that the enthalpy data are sufficiently accurate for correct predictions. This is a matter of importance, for the data were not necessarily obtained under the conditions in which they will be applied, and estimated uncertainties in the data have not always been reported. We note that the reaction of nitrate in reaction system 3 is correctly predicted. Among other cases involving N–O bonds, recent thermochemical results lead to the predictions that PhCH=N(O)Ph[40] and pyridine *N*-oxide[41] should oxidize $MoO(L\text{-}NS_2)(DMF)$ to $MoO_2(L\text{-}NS_2)$. Both of these predictions have been confirmed.[16,18] Further, the deoxygenation of epoxides to olefins by $MoO(S_2CNEt_2)_2$, recently demonstrated by Moloy,[42] is predicted to be spontaneous based on the enthalpy datum for ethylene/ethylene oxide. Lastly, from the data in Table II, the oxidation of $MoO(L\text{-}NS_2)(DMF)$ by water or sulfate is unfavorable on an enthalpy basis by >10–14 kcal/mol. The actual values are probably larger because ΔH = +30 kcal/mol for the reaction $MoO(S_2CNEt_2)_2 + H_2O(l) = MoO_2(S_2CNEt_2)_2 + H_2(g)$.[32]

Reaction Pathway. Activation parameters for the reduction of nitrate in reaction 3 are listed in Table I, where they are compared with those for the reduction of 3-fluoropyridine *N*-oxide and bis(*p*-fluorophenyl) sulfoxide. In reaction pathway 11, reversible substrate binding is followed by intramolecular atom transfer with rate constant k_1. The moderate activation entropy of 8.0 ± 2.0 eu suggests that the structure of the transition state resembles that of the ground-state substrate complex and that, in particular, the Mo–ONO_2 bond is retained.



(11)

The data of Table II predict the enthalpy change for reaction 12, which provides the best available value for the difference in S–O and N–O bond energies in *S*-oxides and aromatic *N*-oxides.

$$C_5H_5NO(g) + Me_2S(g) = C_5H_5N(g) + Me_2SO(g) \qquad \Delta H = -14 \text{ kcal/mol} \tag{12}$$

Similarly, but apparently less accurately owing to differences in phases, it may be estimated that the N–O bond in nitrate is ca. 5 and 19 kcal/mol stronger than those in Me_2SO and pyridine *N*-oxide, respectively. The activation enthalpy of 23.7 ± 0.6 kcal/mol for the reduction of nitrate is indistinguishable from the values for the other two substrates, which may be likened to pyridine *N*-oxide and Me_2SO. The essential equality of activation enthalpies for these three substrates argues against any significant expression of S–O or N–O bond energies in reaching the transition state, and it certainly eliminates rupture of these bonds from that process. The indicated transition state is similar to those depicted for *S*-oxide and *N*-oxide reduction. These were suggested[18] prior to the availability of the dissociation energy of pyridine *N*-oxide,[41] which has permitted evaluation of the enthalpy of oxidation of pyridine (Table II). It is probable that such representations overstate the degree of substrate bond-breaking in the transition state. One possibility is that the transition state is different, involving a structural change of the adduct complex prior to the commencement of Mo–O bond strengthening and N/S–O bond weakening.[43]

Summary. This research demonstrates that nitrate can be reduced to nitrite by atom transfer with concomitant oxidation of $Mo^{IV}O$ to $Mo^{VI}O_2$. The coordination environment is consistent with the minimal coordination units in (at least) *Chlorella vulgaris* nitrate reductase.[12] The mean Mo–S distance in the enzyme

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(43) It has been recognized that nitrate should bind in a cis position to the oxo atom with an orientation that allows interaction of its π^* MO with the filled (Mo(IV)) or half-filled (Mo(V)) d-orbital, in order that electron and atom transfer occur.[6,22,44] We have not been able to obtain diffraction-quality single crystals of $MoO(L\text{-}NS_2)(DMF)$ or its adducts. However, the structure shown in reaction system 3, with the substrate binding site in the equatorial plane of a distorted trigonal bipyramid,[31] is clearly related to that proven for $MoO_2(L\text{-}NS_2)$.[31] If any significant structural change is required to reach the transition state, it may be a small rearrangement in metal coordination geometry and/or an alteration in the twist-boat conformation of the chelate rings in order to allow reorientation of bound substrate. Another possibility is that $MoO(L\text{-}NS_2)(DMF)$ or its adduct, as $[Zn(L\text{-}NS_2)]_2$,[45] is a bis(thiolate-bridged) dimer, steric crowding notwithstanding. Dissociation to a monomer, or at least the rupture of one bridge, would then be required for the reaction to proceed. The small activation entropies for reduction of 3-FC_5H_4NO and especially the sulfoxide in Table I are not notably compatible with this pathway. Moreover, at 0.5–2.0 mM in DMF, the concentration range of the kinetics measurements, the compound obeys Beer's law with the same spectrum as that of the originally isolated DMF adduct.[31]

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compares closely with the values of 2.412 (2) and 2.419 (2) Å in $MoO_2(L\text{-}NS_2)$.[31] We conclude that nitrate reductase activity by atom transfer is a feasible enzymic reaction pathway. Direct oxo transfer has now been proven to occur, by oxygen labeling studies, in the mechanism of action of xanthine oxidase.[46] Similarly, oxo transfer to and from the catalytic site of *Nitrobacter agilis* nitrite dehydrogenase is required by the results of oxygen and nitrogen isotope labeling.[47] However, it has not been established whether this is a molybdoenzyme.

Lastly, it is noted that Williams[48] in 1973 perceived the possibility of nitrate reduction by atom transfer. Wentworth[49] in 1976 explicitly recognized the minimal reaction $Mo^{IV}O + NO_3^- = Mo^{VI}O_2 + NO_2^-$ as a possible enzymic process. Subsequently, this reaction has been incorporated into mechanistic proposals for nitrate reductase.[6,44] The present work contributes the only well-documented atom transfer reaction of this type in a model system. It, together with our earlier results in analogue reaction systems[15-19] and the isotope labeling study of xanthine oxidase,[46] constitutes a growing base of support for the proposition that many (if not all) reactions of the molybdenum hydroxylases involve one step of atom transfer.[14]

Acknowledgment. This research was supported by National Science Foundation Grant CHE 85-21365. We thank E. W. Harlan for useful discussions and Professor K. V. Rajagopalan for a preprint of ref 11b.

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Gold–Rhodium and Gold–Iridium Hydride Clusters

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Received June 24, 1988

Abstract: The hydrides MH_3(tripod) (M = Rh and Ir; tripod = triphos and triars) react with $[Au(L)]^+$ cations to give heterometallic species of the types $[(tripod)MH_3Au(L)]^+$, type **A**, $[(tripod)MH_3\{Au(L)\}_2]^{2+}$, type **B**, and $[(tripod)MH_2\{Au(L)\}_3]^{2+}$, type **C**. The complexes of type **A** can be described as containing one M–H–Au unit which, however, is still dynamic at −90 °C. Similar behavior is observed for the complexes of type **B** where two M–H–Au units are present. The complexes of type **C** contain only two hydride ligands. The X-ray crystal structure of $[(triphos)RhH_2\{Au(PPh_3)\}_3](CF_3SO_3)_2$ shows the presence of a $RhAu_3$ tetrahedron with the rhodium capped by triphos, while each gold atom is coordinated to one PPh_3. The hydride ligands could not be located, but it is proposed that they may occupy two $RhAu_2$ triangular faces. Crystal data: space group $R3c$, $a = 20.343$ (3) Å, $c = 44.937$ (7) Å, $Z = 6$, $V = 16104.9$ Å^3, ρ(calcd) = 1.486 g cm^{-3}, and $R = 0.049$.

Interest in transition-metal clusters has centered around the use of these species as models for heterogeneous catalysts.[2] Recent studies have also shown how unusually large clusters begin to mimic the properties of bulk metals.[3] Obviously, this is an area in which the rational synthesis of clusters would be beneficial. The stoichiometric incorporation of certain metal fragments in clusters would not only change the size of the clusters but may influence the subsequent physical and chemical properties of the cluster.

A significant role in the synthesis of gold and gold heterometallic clusters has been played by the fragment, $[Au(L)]^+$ (L = phosphine or other two-electron donors). Gold clusters can be made by $NaBH_4$ reduction of $[Au(L)]^+$ units.[4] Similarly, treatment of transition-metal hydrides by $[Au(L)]^+$ yields gold heterometallic clusters.[5]

Our interest in the synthesis of hydride-bridged heterometallics produced from metal hydrides and unsaturated, electrophilic metal complexes[6] prompted the synthesis and structural characterization of one of the first gold hydrides, $[(PPh_3)_3H_2Ir(\mu\text{-}H)Au(PPh_3)]^+$ (I).[7] Since this initial report, several other bimetallic aggregates resulting from rhodium and iridium trihydrides and group 11 electrophiles have been reported.[8] The fact that $IrH_3(PPh_3)_3$ can be isolated as mer and fac isomers provides the unique opportunity to study cluster formation as a function of geometry. While two group 11 electrophiles are supported on a meridional hydride template,[8d] the facial hydride template can accommodate up to three, all with rather short contacts to one another.[9] Thus, the facial arrangement appears to better facilitate cluster growth.

With this in mind, we have investigated the addition of successive equivalents of gold(I) phosphine and arsine units to the facial trihydrides, MH_3(tripod) (where M = Rh or Ir and tripod = $CH_3C(CH_2EPh_2)_3$, E = P (triphos) or As (triars)). As a result of this study we now report (1) the synthesis and characterization of gold heterometallic clusters containing one, two, and three

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