is present. The effect of coordination and porphine structure on the reduction mechanism still needs to be pursued. In addition, the study of the mechanism for the reduction of NO^- to hydroxylamine and ammonia has just begun, and further work will be necessary to verify the important steps in the reduction mechanism. This work is in progress in our laboratory.

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Raman-Scattering Properties of $Mo^V{=}O$ and $Mo^V{=}S$ Complexes as Probes for Molybdenum-Containing Enzymes

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The two monomeric Mo(V) complexes $LMoOCl_2$ and $LMoSCl_2$ having the ligand L = hydrotris(3,5-dimethyl-1-pyrazolyl)borate have been studied by Raman spectroscopy. Characteristic stretching frequencies for the $\{Mo{=}E\}^{3+}$ unit (E = O, S) in the solid state are observed at 957 cm^{-1} for $\nu(Mo{=}O)$ and at 525 cm^{-1} for $\nu(Mo{=}S)$. Skeletal frequencies of the remaining $\{LMoCl_2\}$ moiety are not strongly affected by substitution of an oxo versus a sulfido ligand. Enhancement profiles obtained in backscattering from solid samples of the two chromophores show the $LMoSCl_2$ compound to have a distinctive profile with maximum scattering at ~570 nm. However, the solution optical spectrum exhibits no absorption at this wavelength. The profile for the oxo analogue is rather constant in the visible range but suggests increased scattering intensity for $\nu(Mo{=}O)$ when approaching the UV region.

Introduction

Terminal oxo groups are a characteristic property of high-valent molybdenum complexes and occur in such common cores as $\{Mo^{IV}O\}^{2+}$, $\{Mo^{V}O\}^{3+}$, $\{Mo^{VI}O_2\}^{2+}$, and $\{Mo^{VI}O_3\}$.[2] Compounds containing one or more terminal sulfido groups are also frequently seen. The $\{Mo{=}S\}$ moiety is well-known for molybdenum in the oxidation states IV and VI, but only multinuclear Mo(V)-sulfido complexes had, until recently,[3] been available.[2c,4] Mononuclear mixed oxo-sulfido Mo(VI) complexes have also been reported.[5] Molybdenum complexes containing either $\{Mo{=}S\}$ or $\{Mo{=}O\}$ have been the subject of intense spectroscopic studies since the discovery of a high-valent molybdenum center in a number of oxomolybdoenzymes.[4,6] All molybdoenzymes, except nitrogenase, possess a common molybdenum center, the Mo cofactor, that represents the enzymatically active site in these proteins.[7] This cofactor is proposed to contain a pterin ring that is chelated to the Mo atom via two sulfur atoms from a dithiolene group.[7]

A common feature of oxomolybdoenzymes is their ability to carry out an oxo-transfer reaction between the Mo center and the substrate, with Mo cycling through the oxidation states VI, V, and IV. The reactions catalyzed are formally hydroxylations of the substrate. Xanthine oxidase has been shown to contain one terminal oxo and one sulfido ligand in its resting, oxidized Mo(VI) state.[8] In the fully reduced state, the enzyme has a Mo(IV) center with one terminal oxo group. The transient Mo(V) state is believed to also contain one terminal oxo group.[8] Since X-ray crystallographic data are not yet available, the main source of information concerning the nature of the molybdenum site stems from only limited spectroscopic techniques, including EXAFS,[9,10] EPR,[11] and, potentially, NMR spectroscopy. All three oxidation states of sulfite oxidase have been studied by EXAFS,[12] but only the paramagnetic Mo(V) state can be detected by EPR spectroscopy.[11-14] ^{95}Mo NMR spectroscopy has proven useful in distinguishing dioxo, oxo-sulfido, and disulfido Mo(VI) complexes;[15] however, results from molybdoenzymes have not yet been realized. Thus, a considerable need for other spectroscopic probes for the molybdenum center exists.

Raman spectroscopy, especially resonance Raman spectroscopy, has proven to be a powerful technique for probing the metal sites of a variety of metalloproteins.[16] Previously, only a few Raman studies had been reported for oxomolybdoenzymes[17,18] and for reasonable models of these enzymes.[19-21] The paucity of Raman data for these molybdenum systems is due, in part, to the added

(1) (a) Oregon Graduate Institute of Science and Technology. (b) University of Arizona.
(2) (a) Mitchell, P. C. H. *Q. Rev., Chem. Soc.* **1966**, 103. (b) Mitchell, P. C. H. *Coord. Chem. Rev.* **1966**, *1*, 315. (c) Stiefel, E. I. *Prog. Inorg. Chem.* **1977**, *22*, 1.
(3) Young, C. G.; Enemark, J. H.; Collison, D.; Mabbs, F. E. *Inorg. Chem.* **1987**, *26*, 2925.
(4) Garner, C. D.; Bristow, S. In *Molybdenum Enzymes*; Spiro, T. G., Ed.; Wiley: New York, 1985; p 343.
(5) Wieghardt, K.; Hahn, M.; Weiss, J.; Swiridoff, W. *Z. Anorg. Allg. Chem.* **1982**, *492*, 164.
(6) (a) Wilson, G. L.; Kony, M.; Tiekink, E. R. T.; Pilbrow, J. R.; Spence, J. T.; Wedd, A. G. *J. Am. Chem. Soc.* **1988**, *110*, 6923. (b) Farchione, F.; Hansen, G. R.; Rodriguez, C. G.; Bailey, T. D.; Baghi, R. N.; Bond, A. M.; Pilbrow, J. R.; Wedd, A. G. *J. Am. Chem. Soc.* **1986**, *108*, 831.
(7) Johnson, J. L.; Rajagopalan, K. V. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 6856.
(8) (a) Hille, R.; Massey, V. In *Molybdenum Enzymes*; Spiro, T. G., Ed.; Wiley: New York, 1985; p 443. (b) Bray, R. C. *Q. Rev. Biophys.* **1988**, *21*, 5992.
(9) Cramer, S. P.; Wahl, R.; Rajagopalan, K. V. *J. Am. Chem. Soc.* **1981**, *103*, 7721.
(10) Bordas, J.; Bray, R. C.; Garner, C. D.; Gutteridge, S.; Hasnain, S. S. *Biochem. J.* **1980**, *191*, 499.
(11) Bray, R. C. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1980**, *51*, 107.
(12) George, G. N.; Kipke, C. A.; Prince, R. C.; Sunde, R. A.; Enemark, J. H.; Cramer, S. P. *Biochemistry* **1989**, *28*, 5075.
(13) (a) Dowerah, D.; Spence, J. T.; Singh, R.; Wedd, A. G.; Wilson, G. L.; Farchione, F.; Enemark, J. H.; Kristofzski, J.; Bruck, M. *J. Am. Chem. Soc.* **1987**, *109*, 5655. (b) Hinshaw, C. J.; Spence, J. T. *Inorg. Chim. Acta* **1986**, *125*, L17.
(14) George, G. N.; Prince, R. C.; Kipke, C. A.; Sunde, R. A.; Enemark, J. H. *Biochem. J.* **1988**, *256*, 307.
(15) Minelli, M.; Enemark, J. H.; Wieghardt, K.; Hahn, M. *Inorg. Chem.* **1983**, *22*, 3952.
(16) For example: *Biological Applications of Raman Spectroscopy*; Resonance Raman Spectra of Heme and Metalloproteins, Vol. 3; Spiro, T. G., Ed.; Wiley: New York, 1988.
(17) Willis, L. J.; Loehr, T. M. *Biochemistry* **1985**, *24*, 2768.
(18) Garner, C. D.; Porter, T. G.; Wynn, C. H.; Alexander, R. S. Unpublished results cited in ref 4.
(19) Ueyama, N.; Nakata, M.; Araki, T.; Nakamura, A.; Yamashita, S.; Yamashita, T. *Inorg. Chem.* **1981**, *20*, 1934.
(20) (a) Willis, L. J.; Loehr, T. M.; Miller, K. F.; Bruce, A. E.; Stiefel, E. I. *Inorg. Chem.* **1986**, *25*, 4289. (b) Willis, L. J.; Loehr, T. M. *Spectrochim. Acta* **1987**, *43A*, 51.
(21) Lincoln, S. E.; Loehr, T. M. *Inorg. Chem.* **1990**, *29*, 1907.

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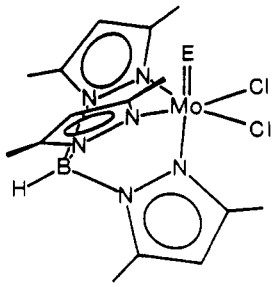

Figure 1. Perspective view of the structure in $LMoECl_2$ (E = O, S).

spectral complexity introduced by the presence of multiple metal-containing prosthetic groups in most molybdoenzymes and, in part, to the absence of suitable series of molybdenum model compounds for systematic investigation of the Raman characteristics of the proposed {Mo=O} and {Mo=S} fragments. Recently, there has been renewed interest in Raman spectral investigations of the molybdenum cofactor, and several studies have been reported since the original submission of this paper. Oertling and Hille[22] have used Raman spectroscopy to probe the complex of reduced xanthine oxidase with violapterin. Gruber et al.[23] have provided Raman spectroscopic evidence in DMSO reductase for the proposed dithiolene moiety of the Mo cofactor, and Subramanian et al.[24] reported a Raman study of dithiolene models of these molybdoenzymes.

This paper presents a Raman study of two monomeric Mo(V) model complexes, $LMoOCl_2$ and $LMoSCl_2$, that are nearly perfect models for studying solely the influence of the {Mo=O} or {Mo=S} group while keeping the remaining $\{LMoCl_2\}$ moiety constant. Both molecules adopt a nearly octahedral geometry with symmetry C_s imposed by the tridentate ligand, L = hydrotris(3,5-dimethyl-1-pyrazolyl)borate, a dichloro unit cis to $\{Mo{=}E\}^{3+}$ (E = O, S), and either a terminal oxo or sulfido substituent in the sixth position as the distinguishing feature (Figure 1).[3,25] The sterically bulky tridentate ligand has been used to stabilize a wide range of mononuclear oxo Mo(V) complexes,[25] and it also yielded the first isolable mononuclear sulfido Mo(V) complex, $LMoSCl_2$.[3]

$LMoOCl_2$ and $LMoSCl_2$ are expected to have distinctive vibrational spectroscopic frequencies arising from their {Mo=O} and {Mo=S} groups. Extensive vibrational spectroscopic assignments exist for the characteristic Mo=O vibrations in the ~850–1050-cm^{-1} region for Mo(V) and Mo(VI) complexes, and many of these have been definitively established with ^{18}O-isotope substitution.[2c,20,21] A mass effect alone would predict that ν(Mo=S) will be found in the 650–800-cm^{-1} range. From infrared (IR) spectra, the vibrational frequencies of ν(Mo=O) and ν(Mo=S) were reported as 957 and 523 cm^{-1}, respectively, for the $LMoOCl_2$ and $LMoSCl_2$ compounds.[3,25] Although the latter frequency is lower than expected on the basis of mass, ν(Mo=S) modes are typically assigned to bands above but near 500 cm^{-1}.[26] In the present work, analogous ν(Mo=E) modes have been observed by Raman spectroscopy. Raman has a distinct advantage over IR spectroscopy in that information on the electronic structure of the {Mo=O} and {Mo=S} groups may be obtained via the resonance phenomenon.[27] Vibrational Mo=O stretching modes have been shown to be subject to resonance Raman (RR) enhancement.[19,24] The knowledge of the ν(Mo=O) and ν(Mo=S) as well as the excitation profile of the molybdenum chromophore in the model compounds will be of considerable importance in predicting the optimum excitation wavelength to be used in future protein studies.

Table I. Electronic Absorption Data for $LMoOCl_2$ and $LMoSCl_2$[a]

compd	λ_{max}, nm (ϵ, M^{-1} cm^{-1})
$LMoOCl_2$	705 (50), 435 sh (~1000), 337 (5200)
$LMoSCl_2$	1010 (50), 450 (880), 310 (6980)

[a] From ref 3.

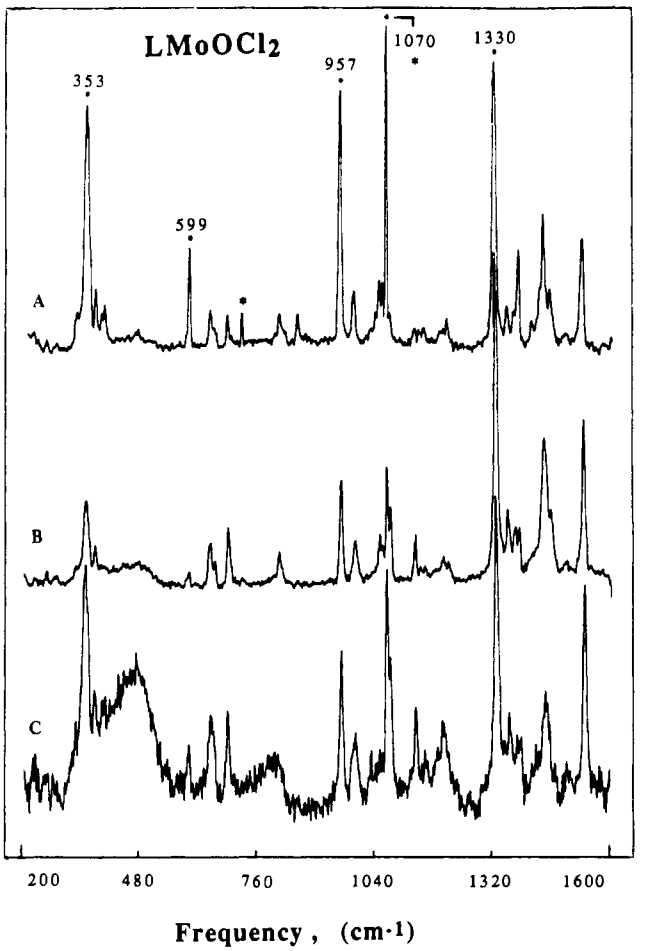

Figure 2. Raman spectra of $LMoOCl_2$ obtained with (A) 514.5-, (B) 457.9-, and (C) 413.1-nm excitation with an incident power at the sample of ~22 mW. Spectra are sums of three, three, and five scans, respectively, at an instrumental resolution of 5 cm^{-1} and a scan rate of 1 cm^{-1}/s. All spectra have been subjected to background subtraction and five-point smoothing. Asterisks mark modes of the $NaNO_3$ internal standard.

Experimental Section

Materials. The preparation and characterization of $[HB(3,5\text{-}Me_2pz)_3]MoOCl_2$ and $[HB(3,5\text{-}Me_2pz)_3]MoSCl_2$ have been reported previously.[3,25]

Spectroscopy. Raman spectra were obtained on solid samples in standard melting point capillaries maintained at 90 K by use of a copper cold finger inserted into a Dewar flask filled with liquid nitrogen (~150° backscattering). Excitation was provided by Spectra-Physics lasers (164-05 Ar and 2025-11 Kr) and a dye laser (Coherent 599-01 with rhodamine 6G pumped with the 514.5-nm line of a Coherent Innova 90-6 Ar laser). Spectra were collected on a computer-interfaced Jarrell-Ash spectrophotometer equipped with an ORTEC Model 9302 amplifier/discriminator and an RCA C31034 photomultiplier tube. Samples were prepared by mixing exact amounts of the molybdenum sample with $NaNO_3$ as an internal standard for frequency and intensity prior to loading in capillaries. Molar scattering factors were calculated from peak area (cut-and-weigh method) of the sample relative to that of $\nu_1(NO_3^-)$ at ~1070 cm^{-1}.

Results

The electronic absorption spectra of CH_2Cl_2 solutions of $LMoOCl_2$ and $LMoSCl_2$ are summarized in Table I.[3] In both

(22) Oertling, W. A.; Hille, R. *J. Biol. Chem.* **1990**, *265*, 17446.
(23) Gruber, S.; Kilpatrick, L.; Bastian, N. R.; Rajagopalan, K. V.; Spiro, T. G. *J. Am. Chem. Soc.* **1990**, *112*, 8179.
(24) Subramanian, P.; Burgmayer, S.; Richards, S.; Szalai, V.; Spiro, T. G. *Inorg. Chem.* **1990**, *29*, 3849.
(25) Cleland, W. E., Jr.; Barnhart, K. M.; Yamanouchi, K.; Collison, D.; Mabbs, F. E.; Ortega, R. B.; Enemark, J. H. *Inorg. Chem.* **1987**, *26*, 1017.
(26) (a) Hahn, M. Dr. nat. Dissertation, Ruhr-Universität Bochum, 1983. (b) Hadjikyriacou, A. I.; Coucouvanis, D. *Inorg. Chem.* **1987**, *26*, 2400. (c) Young, C. G.; Roberts, S. A.; Ortega, R. B.; Enemark, J. H. *J. Am. Chem. Soc.* **1987**, *109*, 2938.
(27) Müller, A.; Diemann, E. *J. Chem. Phys.* **1974**, *61*, 5469.

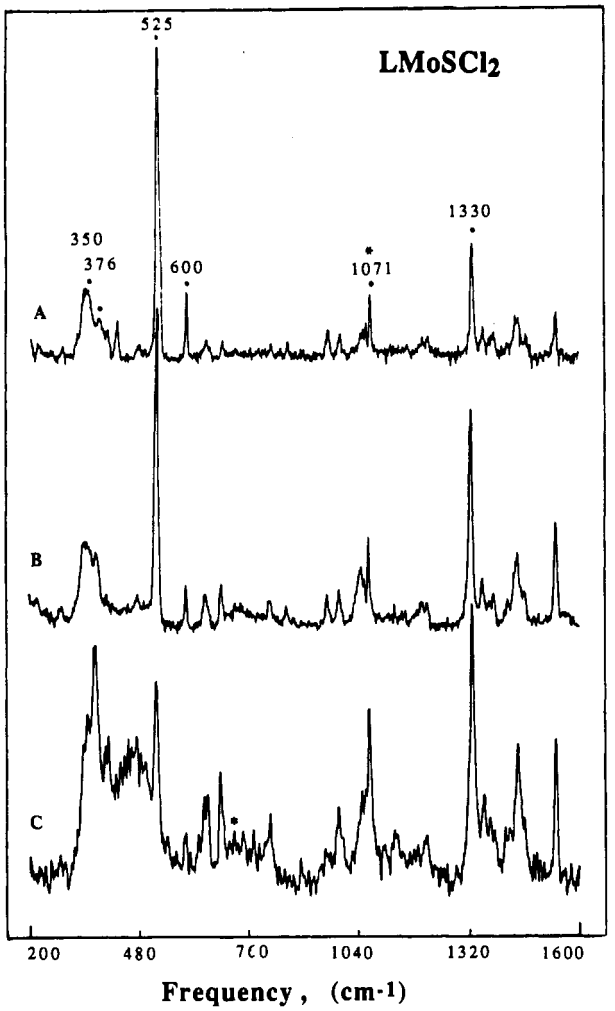

Figure 3. Raman spectra of $LMoSCl_2$ obtained with (A) 594.2-, (B) 514.5-, and (C) 457.9-nm excitation. Note the resonance enhancement of the Mo=S stretching mode with long-wavelength excitation. Spectra are sums of five scans collected at a scan rate of 1 cm^{-1}/s and a resolution of 6 cm^{-1}. Smoothing was applied only to the bottom spectrum. Asterisks mark modes of the $NaNO_3$ internal standard.

complexes, the lowest energy bands are the d → d transitions that are observed at 705 and 1010 nm, respectively. They are distinctively different as expected for a {Mo=O}- or {Mo=S}-containing complex.[3,25,28] The energy difference between the two d → d bands is consistent with a weaker π-donor ability of the sulfido compared to the oxo ligand and is supported by molecular orbital calculations.[3] The spectra become very similar in the near-UV region, where the intense absorption bands are assigned to charge-transfer transitions involving the Cl, O, and S ligands in the two Mo(V) complexes.[3]

The Raman spectra of $LMoOCl_2$ and $LMoSCl_2$ are displayed in Figures 2 and 3 and show strong resemblance with respect to the overall spectral pattern, as expected given the nearly identical structural environments. The only major difference lies in the appearance of distinct bands at 957 cm^{-1} for ν(Mo=O) and at 525 cm^{-1} for ν(Mo=S). These modes are among the most intense features in their respective Raman spectra.

The Raman spectra of $LMoOCl_2$ obtained with 514.5-, 457.9-, and 413.1-nm excitations are shown in Figure 2. All display three very intense features at 353, 957, and 1330 cm^{-1}. These are assigned predominantly to stretching modes of Mo—Cl and Mo=O and an internal ligand mode from the tridentate $[HB(3,5\text{-}Me_2pz)_3]^-$ ligand, respectively. A fourth high-intensity band at 1070 cm^{-1} is the ν_1 mode of the added nitrate ion. From the relative intensity of the Mo=O stretch with respect to that of the

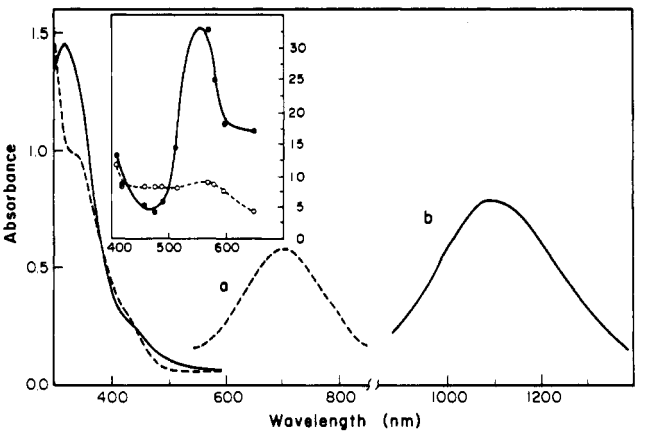

Figure 4. Resonance Raman enhancement profile for the Mo=S stretch (●) at 525 cm^{-1} in $LMoSCl_2$ and the Mo=O stretch (○) at 957 cm^{-1} in $LMoOCl_2$ (insert) and electronic absorption spectra in CH_2Cl_2 of $LMoOCl_2$ (---) and $LMoSCl_2$ (—): (a) $A \times 50$; (b) $A \times 80$. Absorption spectra were taken from ref 3, where ϵ (M^{-1} cm^{-1}) values were reported.

internal standard, molar scattering values for ν(Mo=O) were calculated. The resulting enhancement profile for Mo=O is depicted in Figure 4. As there are no distinct features in the low-energy range of the electronic absorption spectrum, the intensity of the ν(Mo=O) mode remains relatively constant and gives a nearly flat profile between 400 and 600 nm. Some enhancement of the Mo=O mode occurs when the near-UV region is approached, reaching a maximum value of ~12 with 406.7-nm excitation.[29] The intensity may increase further with UV excitation. Studies of the electronic properties of $[MoOCl_4]^-$ show that charge-transfer transitions fall at even higher energy. For example, Gray and co-workers[30] showed that transitions involving $\pi(O) \rightarrow$ Mo CT occur at $\lambda < 300$ nm. That work suggests that enhanced Mo=O intensities should be observed with higher excitation energies. Unfortunately, both of the complexes proved to be fluorescent under these conditions, and no measurable Raman spectra could be obtained for either complex with UV excitations.

The Raman spectrum of $LMoSCl_2$ (Figure 3) has two strong features at 525 and 1330 cm^{-1}. The former is attributed to the Mo=S stretching mode, and the latter is identical with the band observed in the oxo complex and assigned to a ligand mode.[31] Interestingly, the RR behavior of the ν(Mo=S) stretch is quite different from that of the ν(Mo=O) mode in $LMoOCl_2$. The increased intensity of the Mo=S stretch relative to that of the standard is clearly seen when one compares I(525 cm^{-1}) to I(1070 cm^{-1}) in Figure 3A,B, revealing the significantly greater intensity with orange-red excitation. Maximum enhancement is obtained at ~570 nm (Figure 4). Here, the relative molar scattering factor

(28) Money, J. K.; Folting, K.; Huffman, J. C.; Collison, D.; Temperly, J.; Mabbs, F. E.; Christou, G. *Inorg. Chem.* **1986**, *25*, 4583.

(29) Determination of the correct intensity for the internal standard at λ <457.9 nm was complicated owing to overlap of $\nu_1(NO_3)^-$ with resonance-enhanced bands at ~1080 and 1060 cm^{-1} from $[HB(3,5\text{-}Me_2pz)_3]^-$ that, moreover, exhibited variable intensity as $f(\lambda)$. Molar scattering factors at 413.1 and 406.7 nm are therefore less reliable.

(30) (a) Ballhausen, C. J.; Gray, H. B. *Inorg. Chem.* **1962**, *1*, 363. (b) Winkler, J. R.; Gray, H. B. *Comments Inorg. Chem.* **1981**, *20*, 257.

(31) Assignment of the ~1330-cm^{-1} feature to an overtone or combination band involving $\{Mo=E\}^{3+}$ is unlikely because it is seen in both samples. It is more likely to be a ring-stretching mode of the pyrazoles of the $[HB(3,5\text{-}Me_2pz)_3]^-$ ligand. In 3,5-dialkylpyrazole, these are observed at 1600–1450 cm^{-1} but may shift in the metal–ligand species.[32] However, no such bands are seen in the free ligand, in $K[HB(3,5\text{-}Me_2pz)_3]$, or in other oxo Mo(V) complexes, LMoO(XY) (where X and Y are thiolates or phenolates),[33] thereby strongly suggesting that the ~1330-cm^{-1} frequency is linked, in an as yet unknown manner, to the presence of the Cl^- ligands. Since the 1330-cm^{-1} band is seen in both compounds, it would appear to be independent of the presence of either the oxo or sulfido group. The intensity of the band was noted to be remarkably enhanced with 457.9-nm excitation in both $LMoOCl_2$ and $LMoSCl_2$ but decreased with near-UV excitation.

(32) Dollish, F. R.; Fateley, W. G.; Bentley, F. F. *Characteristic Raman Frequencies of Organic Compounds*; Wiley: New, York, 1974; p 229.

(33) Backes, G.; Loehr, T. M.; Enemark, J. H. Unpublished results.

for the Mo=S mode is calculated to be 33, which is almost 3 times the value of that of the Mo=O mode at its optimum excitation wavelength. As seen for ν(Mo=O), near-UV excitation also results in slight resonance enhancement. A comparison with the electronic absorption spectrum of $LMoSCl_2$ shows that the observed maximum in the excitation profile does not correspond to any major absorption bands.

Discussion

A feature common to both the Mo=O and Mo=S groups is that they are relatively weak Raman scatterers. Optimal molar scattering values for ν(Mo=O) in $LMoOCl_2$ reach ~12 (400–650-nm interval) relative to $NaNO_3$. Although the number of Mo=O complexes investigated by Raman spectroscopy is limited, low Raman scattering intensities do seem to be a typical property of oxo Mo(IV–VI) species.[19-21,24] This is in strong contrast to doubly and triply bridged dinuclear ferric complexes containing the Fe–O–Fe moiety that are ~30 times more enhanced at their optimum excitation wavelength than these Mo=O complexes.[34,35] Similarly, the intensity of ν_s(Mo–O–Mo) of the linearly bridged dinuclear Mo(V) complex $[HB(pz)_3MoOCl]_2O$ is also much greater and undergoes strong resonance enhancement whereas the terminal Mo=O modes are relatively weak and show no significant resonance effects.[21] This large difference could be due to the fact that the terminal oxo → Mo CT transitions do not have appropriate excited-state distortions for effective resonance enhancement of the Mo=O vibrational mode. Alternatively, strong enhancement of the Mo=O stretch might only be achieved with excitation much deeper into the UV region.[27]

No previous Raman data have been available for assessment of the scattering properties of the {Mo=S} moiety. The RR excitation profile maximum for $LMoSCl_2$ is observed at ~570 nm (Figure 4). Absence of a distinct absorption at this wavelength may mean that only a minor, unresolved band is responsible for resonance enhancement at this energy; such a situation has been observed in many μ-oxo-bridged dinuclear iron complexes.[34] Alternatively, an interference may be present. The latter is made plausible by the dispersive shape of the profile having a minimum at ~470 nm, which could indicate possible interference between scattering contributions from states <430 nm and unresolved components near ~470 nm. A similar explanation has recently been advanced to account for the excitation profile of an Mo–S(thiolate) complex.[24]

The low-frequency region is expected to show the stretching frequencies of the $\{MoCl_2\}$ moiety. The Raman spectrum of $LMoOCl_2$ is dominated by a strong band at ~353 cm^{-1} and is in the correct region for Mo–Cl stretching vibrations.[21,36] Curve fitting shows that this peak has two components located at 356 and 350 cm^{-1}. The RR spectrum of $LMoSCl_2$ displays a similar set of bands at 367 and 354 cm^{-1}. A recent Raman study from our laboratory on μ-oxo-bridged dinuclear Mo(V) complexes assigned a similar pair of bands in this region to the symmetric and asymmetric $\{MoCl_2\}$ stretching modes, respectively.[21] Quantitation of the Mo–Cl intensity at 353 cm^{-1} in $LMoOCl_2$, which takes into account both the symmetric and the asymmetric stretches, shows that this vibration is intensified with near-UV excitation and reaches a molar scattering value of ~20 relative to nitrate.

Replacement of a terminal oxo by a sulfido ligand does not result in profound differences in the Raman spectroscopic properties of the remaining $\{LMoCl_2\}^{2+}$ moiety. This suggests that the ligand vibrations within this group are more or less independent of the presence of a terminal oxygen or sulfur ligand and, furthermore, are largely decoupled from {Mo=E} modes. An important observation is that the two chromophores have different enhancement behaviors. Although both {Mo=E} complexes are relatively weak Raman scatterers, ν(Mo=S) shows a definitive and significant RR enhancement maximum at ~570 nm, which is not observed for ν(Mo=O). Both Mo=O and Mo=S modes show increased intensities with UV excitation.

Resonance Raman spectroscopy should thus be considered as a potentially useful technique for future protein studies, since the Mo=O and Mo=S stretching modes have characteristic frequencies that are subject to RR enhancement. An {Mo=S} functionality has been postulated to exist in certain forms of xanthine oxidase.[8,22] The ~30-fold enhancement of ν(Mo=S) relative to NO_3^- in the present study suggests that such a vibrational mode might be observed in a xanthine oxidase sample having a concentration ≥1 mM. Mo=O intensities are likely to be disappointingly weak unless UV excitation can be successfully employed or there is an influence by another chromophoric group. For example, in a recent study by Spiro and associates, the Mo=O band of an Mo=O thiolate complex was seen to be resonance enhanced by coupling to an intense CT band at 410 nm.[24] Raman experiments on xanthine oxidase[17] and preliminary work on sulfite oxidase[18] have been described, but they have not yet been successful with respect to detection of Mo=O or Mo=S stretching frequencies.

The new report on a xanthine oxidase–violapterin complex has shown several RR bands associated with the product analogue by excitation into a low-energy Mo(IV)–violapterin CT band (λ_0 = 676.4 nm).[22] Although no ^{18}O-sensitive vibrations attributable to Mo=O could be found, some low-frequency bands were ascribed to the Mo center.[22] Additionally, we note that neither of the previous xanthine oxidase RR investigations employed a λ_0 close to the 570-nm enhancement maximum that we have observed for ν(Mo=S) in $LMoSCl_2$ in the present study. Only wavelengths to the red and blue of this value have been used. The second new study on DMSO reductase also used low-energy near-infrared excitation to probe the resonance Raman properties of the proposed Mo–dithiolene complex of the cofactor.[23] These workers used ^{34}S-enriched media to assign two bands at 383 and 352 cm^{-1} (reduced enzyme) and at 370 and 350 cm^{-1} (oxidized enzyme) to the Mo–dithiolene vibrations; additional low-energy vibrational modes (<450 cm^{-1}) were also observed and associated with the Mo center.[23] It is clear that studies of model compounds coupled with a new look at chromophoric derivatives of molybdoenzymes will play an increasingly important role in future resonance Raman spectroscopic investigations of molybdoenzymes.

Acknowledgment. This work was supported by grants from the National Institutes of Health (GM 18865 to T.M.L. and GM 37773 to J.H.E.), which are gratefully acknowledged.

(34) Sanders-Loehr, J.; Wheeler, W. D.; Shiemke, A. K.; Averill, B. A.; Loehr, T. M. *J. Am. Chem. Soc.* **1989**, *111*, 8084.

(35) Scattering intensities of the Fe–O–Fe complexes were based on sulfate as an internal standard; however, the Raman scattering intensities of NO_3^- and SO_4^{2-} are comparable, and no correction for this difference has been taken into account in the present discussion.

(36) (a) Nakamoto, K. *Infrared and Raman Spectra of Inorganic and Coordination Compounds*; Wiley: New York, 1986; p 324. (b) Collin, R. J.; Griffith, W. P.; Pawson, D. *J. Mol. Struct.* **1973**, *19*, 531.