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SPECTROSCOPIC STUDIES OF MOLYBDENUM COMPLEXES
AS MODELS FOR NITROGENASE

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MASTER

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Spectroscopic Studies of Molybdenum Complexes
as Models for Nitrogenase

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ABSTRACT

Because biological nitrogen fixation requires Mo, there is an interest in inorganic Mo complexes which mimic the reactions of nitrogen-fixing enzymes. Two such complexes are the dimer $\text{Mo}_2\text{O}_4(\text{cysteine})_2^{2-}$ and trans- $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$ (dppe = 1,2-bis(diphenylphosphino)ethane).

The H^1 and C^{13} NMR of solutions of $\text{Mo}_2\text{O}_4(\text{cys})_2^{2-}$ are described. It is shown that in aqueous solution the cysteine ligands assume at least three distinct configurations. A step-wise dissociation of the cysteine ligand is proposed to explain the data.

The Extended X-ray Absorption Fine Structure (EXAFS) of trans- $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$ is described and compared to the EXAFS of $\text{MoH}_4(\text{dppe})_2$. The spectra are fitted to amplitude and phase parameters developed at Bell Laboratories. On the basis of this analysis, one can determine 1) that the dinitrogen complex contains nitrogen and the hydride complex does not and 2) the correct Mo-N distance. This is significant because the Mo in both complexes is coordinated by four P atoms which dominate the EXAFS. A similar sort of "interference" is present in nitrogenase due to S coordination of the Mo in the enzyme. This model experiment indicates that, given adequate signal to noise ratios, the presence or absence of dinitrogen coordination to Mo in the enzyme may be determined by EXAFS using existing data analysis techniques.

A new reaction between $\text{Mo}_2\text{O}_4(\text{cys})_2^{2-}$ and acetylene is described to the extent it is presently understood. A strong EPR signal is observed,

suggesting the production of stable Mo(V) monomers. EXAFS studies support this suggestion. The Mo K-edge is described. The edge data suggests Mo(VI) is also produced in the reaction. UV spectra suggest that cysteine is released in the course of the reaction.

Chapter 1

INTRODUCTION

This thesis describes three projects: 1) an NMR study of $\text{Mo}_2\text{O}_4(\text{cysteine})_2^{2-}$ in solution; 2) an EXAFS study of trans- $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$ and $\text{MoH}_4(\text{dppe})_2$; 3) a broad study of a reaction between $\text{Mo}_2\text{O}_4(\text{cys})_2^{2-}$ and acetylene, featuring UV-VIS, EPR, and EXAFS data.

While these projects appear disparate, they are linked by an interest in biological nitrogen fixation, and, more specifically, the role of Mo in nitrogenase. Both $\text{Mo}_2\text{O}_4(\text{cys})_2^{2-}$ and trans- $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$ have been used as models for the Mo site in nitrogenase. ^{6,7}

This introduction will first review what is known about the Mo site in nitrogenase. Next, the model studies of Schrauzer and Chatt will be discussed. Finally, Extended X-ray Absorption Fine Structure (EXAFS) will be described, and its value in studying Mo in nitrogenase discussed.

Nitrogenase

A number of bacterial organisms have the capacity to reduce N_2 to NH_3 . This reaction is mediated by the enzyme nitrogenase. In addition to fixing nitrogen, nitrogenase catalyzes the multi-electron reduction of many other classes of compounds containing triple bonds. The most important of these is the reduction of acetylene to ethylene, the

reaction by which nitrogenase activity is assayed.

Nitrogenase is composed of two proteins. The larger component, known as Component I or MoFe component, has a molecular weight of between 220,000 and 270,000 and contains about 2 Mo, 20-32 Fe, and 20-30 acid labile S. The smaller component, Component II or Fe component, weighs between 55,000 and 75,000 daltons and contains 4 Fe and 4 acid labile S.

Recently, Burris¹ has proposed that the small Fe component is a specific reducing agent for the large MoFe component. The MoFe component, then, is responsible for the reduction of substrate.

Nitrogenase has an absolute requirement for Mo. A small Mo-Fe cofactor has been isolated from Component I². This cofactor contains 8 Fe, and 6 acid labile S per Mo. The cofactor has restored activity to the inactive nitrogenase produced by certain mutants of Azotobacter vinelandii.

In addition, inorganic nitrogen-fixing systems have been devised (see below). For a number of these systems, Mo is the only essential metal. No system employing only Fe has succeeded in fixing nitrogen or mimicking the reactions of nitrogenase.

All of this suggests that Mo plays a central role in the reduction of substrate, and is likely to be involved in the binding of substrate.

Cramer³ concluded from EXAFS studies that the Mo in nitrogenase is bound by 4-5 S atoms. This is consistent with what is known of the Mo-Fe cofactor. Little else is known about the Mo because other spectroscopic techniques (e.g., UV-VIS and EPR) have failed to see signals from the enzyme that may be assigned to Mo.⁴

Inorganic Model Systems

Chatt has recently reviewed advances in Mo chemistry which relate nitrogen fixation.⁵ This review covers the model systems covered here plus all other important nitrogen fixing systems.

Schrauzer⁶ has designed systems around $\text{Mo}_2\text{O}_4(\text{cysteine})_2^{2-}$ which mimic many of the reactions of nitrogenase, although at much lower rates. The essential components of his systems are the Mo dimer, a reducing agent, and basic pH. For example, at pH 9 in the presence of BH_4^- the dimer catalyzes the reduction of acetylene to ethylene, the reaction by which nitrogenase activity is assayed. Schrauzer has elaborated on this basic system, adding Fe-S clusters, ATP, and other cofactors or reducing agents. He reports some reduction of N_2 to NH_3 (through hydrazine) for these more refined systems.

Chatt⁷ has done extensive work with a variety of Mo- N_2 complexes. From trans- $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$ (dppe = 1,2-bis(diphenylphosphino)ethane) he has produced--through ligand reduction-- $\text{MoBr}_2(\text{N}_2\text{H}_2)(\text{dppe})_2$, $\text{MoBR}(\text{N-NH}_2)(\text{dppe})_2^+$ and $\text{MoBr}(\text{N}_2\text{H})(\text{dppe})_2$. Using related Mo- N_2 complexes with different phosphine ligands he has also produced stable complexes involving every intermediate protonation between N_2 and NH_3 . Finally, treatment of trans- $\text{Mo}(\text{N}_2)_2(\text{PPH}_2\text{CH}_3)_4$ with H_2SO_4 in methanol, followed by base distillation, has produced NH_3 in good yield. The extent of reduction of the N_2 ligand in these systems is a function of the phosphine ligands. Thus, trans- $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$ (the compound examined in this thesis) does not produce NH_3 when treated with H_2SO_4 , but stops at $\text{Mo}(\text{NNH}_2)\text{SO}_3\text{H}(\text{dppe})_2^+$. In addition, the reduction to NH_3 appears to follow a different route in W analogues.

It should be emphasized that Schrauzer's system is catalytic, while Chatt's is not. Thus, while Chatt's work demonstrates the ability of Mo to bind and activate N_2 , it is probably not very biological. Schrauzer's system may more closely resemble the chemistry of the enzyme, although Cramer's finding³ that the Mo in nitrogenase is bound almost entirely by S casts doubt on this, also. In fairness to Schrauzer's system, however, it should be noted that the analogue $Mo_2O_2S_2(cys)_2^{2-}$ exists¹⁵ though it has not been shown to be active in a Schrauzer-type system.

In spite of the fact that these compounds' chemistry may differ from that of the enzyme Mo, they remain interesting. Indeed, the differences between these two systems and the mechanism of the enzyme must give us new insight into the chemistry of Mo and dinitrogen in a very general way. Also, as will be detailed later, the EXAFS of trans-Mo(N₂)₂(dppe)₂ can be expected to resemble the EXAFS of the enzyme in an important way, even though the chemistry must be very different.

Extended X-ray Absorption Fine Structure (EXAFS)

An EXAFS experiment is simply the taking of an X-ray absorption spectrum. One measures absorption as a function of photon energy.

Because conventional X-ray tubes emit high intensity beams only at their characteristic frequencies, it is difficult to obtain spectra over wide energy ranges⁸. For this reason the X-ray source for the experiments described in this thesis was the synchrotron radiation generated by the Stanford Synchrotron Radiation Laboratory. The synchrotron beam has high intensity over a broad band of energies, making it an excellent source for our purposes.

The experimental set-up is illustrated schematically in Figure 1. The monochromator is a rapidly tunable two-crystal (Si 400) apparatus

under computer control. The monochromatic X-ray beam passes from the monochromator first into an ion chamber where the initial intensity, I_0 , is measured. It then goes through the sample and into another ion chamber to measure the final intensity, I .

The photon energies scanned are in the region of excitation and ionization of a core electron of the atom of interest. In this work we are interested in the excitation and ionization of the 1-s electron of Mo--the "Mo K-edge". This involves photon energies of 20-21KeV.

As the photon energy approaches the excitation threshold, or edge, a sharp rise in absorption is observed (Figure 2). Initially this absorption is due to bound-state transitions such as 1-s to 4-d, 5-s, and 5-p. Immediately following this region the absorption exhibits complicated structure not fully understood but perhaps related to band structure in crystals. Beginning about 30eV above the edge, however, the absorption exhibits an oscillatory behavior as one scans out in energy. These oscillations⁹ are the EXAFS.

The theory of EXAFS has made great gains over the past five years.^{9,10} At high enough photon energies, the 1-s electron is excited into the continuum (Figure 2). The outgoing photoelectron may be thought of as a spherical wave propagating away from the central atom. This wave will be partially backscattered by neighboring atoms, and the outgoing and scattered parts of the wave then interfere with each other. This interference modulates the absorption through the transition matrix element $|\langle \Psi_1 | H' | \Psi_f \rangle|^2$, where Ψ_1 is the 1-s core electron initial state, H' is the dipole operator, and Ψ_f ($= \Psi(\text{out}) + \Psi(\text{scattered})$) is the photoelectron wavefunction. Because Ψ_1 is localized at the atomic core, the integral is large only at the core and, therefore, only the value of

Ψ_F at the core contributes to the absorption probability.

As the photon energy increases, the energy of the photoelectron increases, and the wavelength of the photoelectron decreases. The change in photoelectron wavelength, of course, changes the self-interference pattern, so that at some energies there is constructive interference at the core and the absorption goes through a maximum, while at other energies there is destructive interference and the absorption goes through a minimum.

A simple model, in which the absorbing atom is surrounded by a single shell of identical scattering atoms, gives for the EXAFS scattering cross section, $\chi(k)$,

$$\chi(k) = (N/kR^2)e^{-r^2k^2} |f(\pi,k)|^2 \sin(2kR + \alpha(k))$$

Here k is the photoelectron's wave vector, $k = \sqrt{\frac{2m(E_{\text{photon}} - E_{\text{binding}})}{\hbar^2}}$.

N is the number of scattering atoms, R is their distance from the absorbing atom, $f(\pi,k)$ is the backscattering amplitude, $e^{-r^2k^2}$ is a Debye-Waller type factor, and $\alpha(k)$ is the phase difference due to the electron's interaction with the atomic potentials.

A Fourier transform of $\chi(k)$ yields a radial distribution of atoms around the absorbing atom. The peaks of the radial distribution function will be shifted by α_{FT} due to $\alpha(k)$. It appears that $\alpha(k)$ is characteristic of the particular absorber-scatterer pair, independent of the rest of the molecular environment.¹¹ It is possible to look at known structures, determine $\alpha(k)$ between various pairs of atoms, and use this $\alpha(k)$ to determine unknown structures. Cramer¹² had a great deal of success predicting the structures of Mo complexes in this fashion.

Lately, Lee and coworkers^{10d,e,f} have calculated and parameterized phase shifts and amplitudes from first principles. This thesis makes use of these calculations, and their usefulness and generality will be commented upon. Cramer has also developed parameterized phase shifts and amplitude parameters,^{3,12} although these are empirical, not theoretical.

Generally one hopes to extract two types of information from EXAFS: 1) the radial distribution of atoms around the absorbing atom; 2) the identity and number of neighboring atoms. The radial distribution is contained in the frequency of the EXAFS. The identity and number of neighboring atoms is contained in the amplitude function: lower Z scatterers' oscillations are larger at low k and damp out quickly; higher Z scatterers' oscillations are smaller at low k but persist to higher k; and, of course, the more scatterers, the larger the oscillations. It is generally easy to distinguish scatterers of very different Z (Mo and N, say), but not really possible at this time to distinguish with certainty between very-close-Z scatterers (S and P).

The data are analyzed by two basic techniques: Fourier transform and curve fitting. Fourier transform is useful for qualitative results. Major changes in the coordination sphere are immediately apparent in the Fourier transform radial distribution function. However, for high precision distances and estimates of coordination numbers (in addition to identifying neighboring atoms), the data must be fitted to theoretical models.

EXAFS has unique potential in the study of metal centers in metalloproteins such as nitrogenase.¹³ It is element specific, allowing examination of only the metal of interest without interference from

other metals present or from the C, N, H backbone of the protein. An EXAFS spectrum may be taken on a sample in any phase. Thus the protein may be studied under biologically relevant conditions: in aqueous solution and even in the presence of important cofactors or substrate. For the problem at hand, the Mo in nitrogenase, EXAFS is a direct probe of the environment of the Mo in active, undenatured nitrogenase.

EXAFS can also probe inorganic chemical reactions.¹⁴ Again, there is the advantage of obtaining structural information from solutions, allowing study of species which cannot be isolated as solids or single crystals. The last section of this thesis will illustrate this approach.

EXAFS Data Analysis

EXAFS data are analyzed by two techniques: 1) Fourier transform and 2) fitting the data with a theoretical or empirical model function. The Fourier transform yields a radial distribution function describing the distance between the absorbing atom and neighboring atoms.¹⁶ This is most useful for obtaining qualitative results and also provides a starting point for curve-fitting analysis. Detailed information such as accurate distances, coordination numbers, and scatterer identity can generally only be extracted by fitting the data to a model.

A problem in EXAFS data analysis is that the data collected are not just EXAFS. It is EXAFS superimposed on a "baseline" absorption cross-section, μ_0 . The EXAFS cross section, $\chi(k)$, is defined as¹⁷

$$\chi(k) = \frac{\mu - \mu_0}{\mu_0}$$

where μ is the measured absorption cross-section from

$$\frac{I}{I_0} = e^{-\mu x}$$

Further, the data are collected as a function of photon energy, while $\chi(k)$ depends on the photoelectron wave vector, $k = \left(\frac{2m}{\hbar^2} (E_{\text{photon}} - E_0) \right)^{1/2}$, where m is the electron mass and $\hbar$ is Planck's constant.

Before analysis may begin, then, one must first remove the background, μ_0 , and select an appropriate E_0 to define k .

Background removal is the most difficult and poorly defined part of the analysis. There are actually two sources of background. The first is the intrinsic μ_0 , the monotonic decrease in the absorption cross-section referred to as the "free atom" cross-section. This is the absorption we would observe if the absorbing atom were isolated in space. There is no way to separate this from the EXAFS introduced by neighboring atoms; both must be measured simultaneously. There is, however, a model for this background¹⁸ so that, in principle, it may be calculated and removed. Unfortunately, the second source of background is the instrument, and this background can not be calculated. The experiment is not done with a split beam, and the I_0 and I ion chambers are not perfectly matched, so that as the photon flux decays a time-dependent background is introduced.

The basic assumption made in background removal is that the background is of low frequency. This is reasonable because, 1) there is no reason to expect high-frequency oscillations in these sources and 2) very low frequencies correspond to distances which are unreasonably short for bond distances. It must be borne in mind, however, that there may be pathological cases in which some special effect introduces a background of higher frequency.

In this work, the background function is generated by a smoothing routine first developed by Ferrel Lytle. The EXAFS oscillations are

smoothed out of the data by replacing each data point with the average of all the points within a small region of the data point of interest. In this work, the average was taken over $1.2\text{\AA}^{-1}$ on either side of the point of interest. This smoothing algorithm was applied once to the data (in k -space), then applied to the output function, and finally applied to this twice-smoothed output, generating what will be referred to as an S^3 background function. This was subtracted from the data, the data were multiplied by k^3 , and another S^3 background function was generated and removed. The data were then multiplied by k^{-3} to generate the final EXAFS function to be analyzed.

The choice of E_0 for the conversion to k -space depended on whether the file was being prepared for Fourier transform or curve-fitting analysis (although FT's are generated in the course of curve-fitting). Theoretically, E_0 is the photon energy required to create a "free" photoelectron with zero kinetic energy. There is no consensus as to how this may be determined. Fortunately, for the purposes of Fourier analysis, the choice of E_0 does not appear to be critical. Cramer,¹⁹ in fact, found it adequate to choose the same E_0 for all the Mo complexes he studied. There is, however, every reason to expect E_0 to vary from compound to compound. Assigning the highest edge peak as a transition to the 5p Mo orbital, Moore's atomic spectral tables suggest complete ionization occurs about 6eV above this peak.²⁰ Therefore, unless otherwise noted, all Fourier transforms presented here were generated from data with E_0 selected 6eV above the highest edge peak. E_0 in the curve-fitting routines developed by Bell Labs²¹ is a free parameter. Its starting value is determined by requiring the real part of the Fourier transform (as generated by a Fast Fourier Transform algorithm) to peak

at the same frequency as the power spectrum.

Once the EXAFS has been isolated, the data processing diverges, depending on whether the data are to be Fourier transformed or curve-fitted. Fourier transformation will be discussed first.

Before the data are Fourier transformed, it is truncated at the low k end. At low k the scattering of the photoelectron is complicated by band effects and multiple scattering. The Fourier transforms presented here, unless otherwise noted, were truncated at $3\text{\AA}^{-1}$. The data were also truncated at the high k end due to a large "glitch" in the spectra near $16\text{\AA}^{-1}$. The truncation at both ends was smoothed by the application of a Kaiser window function of 3.²² The window function reduces sidelobing in the Fourier transform caused by the truncation.

After truncation, the data are Fourier transformed. It is sometimes of interest to weight the data before FT Fourier transforming. Generally the data are multiplied by k^n , $n=0,1,2,3$. The Fourier transform is denoted $\phi_n(R)$. Because heavy scatterers' oscillations tend to extend to higher k , weighting the data by powers of k increases the contribution of heavy scatterers to $\phi_n(R)$. By observing the relative changes of peak amplitudes as a function of the k weight, one may sometimes distinguish those peaks due to light scatterers from those due to heavy scatterers. In addition, because the unweighted EXAFS decays to zero at higher k , k -weighting, which increases the high- k oscillations, tends to increase the resolution of the Fourier transform.

Curve-fitting starts with the Fourier transform. One first determines which peaks in $\phi_3(R)$ one wishes to model. Then the data are Fourier filtered. Again, a window function is used--this time in R -space--to reduce truncation artifacts. This window, the Hamming window,

is the same shape as a cosine from peak to trough.

The data are fitted to the model developed by Teo and Lee.²¹ The theoretical formula for the EXAFS cross section is:

$$\chi(k) = \frac{1}{k} \sum_i (N_i/R_i^2) |f_i(\pi, k)|^2 e^{-\sigma_i^2 k^2} \sin(2kR_i + \alpha_i(k))$$

where the summation is over shells of identical atoms at a distance R_i . Teo and Lee have ab initio calculations of $f_i(\pi, k)$ and $\alpha_i(k)$. The fit parameters are σ_i , R_i , and E_0 (which determines k) as nonlinear parameters and the quantity (N_i/R_i^2) as a linear parameter. Teo and Lee's calculated amplitudes and phases are published as lists of the value of each of these functions at various values of k . There are three types of functions calculated: 1) contribution of the absorbing atom to α_i ; 2) contribution of the backscattering atom to α_i ; and 3) the backscattering amplitude $f_i(\pi, k)$ of the backscattering atom. There are, in turn, two sets of these functions calculated: one using Hermann-Skillman wavefunctions as the basis set, the second using Clementi-Rossi wavefunctions. These calculations were not done on all elements, and in those cases where an element of interest was not tabulated, its parameters were linearly interpolated. The fitting program generated continuous amplitude and phase functions by fitting a continuous curve, piecewise, through the tabulated values.

The Fourier transform and background removal programs employed in this work were developed by Alan S. Robertson. The curve-fitting programs were developed by Jon A. Kirby.

The inflection points of edges were determined by linearizing the data to 3 points/eV (the raw data were approximately 1 point/eV), determining the first derivative at each point, and taking the peak of

the first derivative as the inflection point. The point-by-point derivative was determined as follows: a cubic was fitted through 11 points (3.7eV) on either side of the point of interest, and the analytic derivative of this cubic was evaluated at the point.

Figure Captions

Figure 1: Schematic diagram of experimental arrangement for the collection of EXAFS absorption spectra.

Figure 2: Mo K-edge absorption spectrum of Na_2MoO_4 with schematic of possible corresponding K-electron transitions, illustrating the bound-state transition "edge" region and the continuum EXAFS region.

Figure 1

XBL 764-5807

Figure 2

XBL 764-5809

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Chapter 2

AN NMR STUDY OF THE STATE OF $\text{Mo}_2\text{O}_4(\text{Cys})_2^{2-}$ IN AQUEOUS SOLUTION¹Abstract

The state of the di- μ -oxo-bis(oxo(L-(+)-cysteinato)molybdate(V)) dianion ($\text{Mo}_2\text{O}_4(\text{cys})_2^{2-}$) in aqueous solution has been studied with ^{13}C and ^1H NMR spectroscopy. It was found that three species of the cysteine ligand exist simultaneously in a ratio 10:3:1. These species are in slow equilibrium with each other thereby giving rise to separate NMR signals, but making chromatographic separation impossible. Our data are consistent with a model that invokes stepwise dissociation of the carboxyl and amino groups from the molybdenum. In view of the complexity of the aqueous solution of this molecule, studies of its reactions must be performed with caution.

Introduction

The di- μ -oxo-bis(oxo(L-(+)-cysteinato)molybdate(V)) dianion ($\text{Mo}_2\text{O}_4(\text{cys})_2^{2-}$) (Fig. 1) has been the subject of considerable study.² Much of this interest stems from its use by Schrauzer as the central component of an inorganic model system which mimics the reactions of nitrogenase.³ Schrauzer's studies primarily involved the kinetics of the consumption of substrates and the production of products. From these

studies he has inferred detailed reaction mechanisms revolving around the formation of Mo(IV) monomers. A limitation of this work is that the $\text{Mo}_2\text{O}_4(\text{cys})_2^{2-}$ complex was not studied directly.

A complete understanding of the chemistry of $\text{Mo}_2\text{O}_4(\text{cys})_2^{2-}$ must be based on knowledge of its structure in solution. A starting point is provided by the crystal structure determined by Knox and Prout.⁴ Among the highlights of this structure are the tridentate configuration of the cysteine ligands (Fig. 1) and a relatively long Mo-O (carboxyl) bond of 2.3Å. This weak bond is attributed to a combination of the "trans effect" of the molybdenyl oxygen and the steric restrictions imposed by cysteine's limited flexibility. This contortion of the cysteine also lengthens the C(α)-C(carboxyl) bond by .035 Å over that observed in the free ligand.

The weak chelation by the carboxyl in the crystal suggests that in solution the carboxyl may dissociate and be replaced by water in the coordination sphere. Kay and Mitchell⁵ proposed a dynamic exchange between carboxyl and water based on acid titration of the complex and UV, IR, and ^1H -NMR spectroscopy.

We have examined the ^{13}C -NMR and reexamined the ^1H -NMR of the complex in solution using a higher field spectrometer than that employed by Kay and Mitchell. Our results indicate the existence of at least three different types of cysteine ligands. Although the data to date are not conclusive as to the structures of the three species, they are consistent with a stepwise dissociation of the cysteine from tridentate to bidentate(carboxyl dissociated) to an amino-dissociated form.

Experimental

$\text{Na}_2\text{Mo}_2\text{O}_4(\text{cys})_2^{2-} \cdot 5\text{H}_2\text{O}$ was prepared by the method of Kay and Mitchell^{2c} and recrystallized three times from ethanol/water. Sodium molybdate

(Baker), sodium dithionite (Mallinkrodt), and L-cysteine hydrochloride (Sigma) were used without further purification. Analysis:

($C_6H_{20}Mo_2N_2Na_2O_{13}S_2$) C,H,N,O,S; Mo: calculated 30.4; found 29.9.

NMR Samples were approximately 0.1M dimer in unbuffered D_2O . Solutions of the dimer were kept under nitrogen or argon atmospheres using standard techniques. The pH was adjusted with HCl, DCl, or NaOH and measured with a Corning pH electrode. The readings were not corrected for isotope effects.

NMR spectra were obtained on a custom-built spectrometer⁶ employing a Bruker 6.3 T magnet and a Nicolet 1180 computer. All data were obtained using pulsed Fourier transform methods. Decoupling and saturation transfer experiments were performed using a gated homonuclear decoupler. T_1 values were calculated from the spectra following the standard 180° - τ - 90° sequence⁷ and the J resolved 2-D spectra⁸ from a series of 90° - τ - 180° sequences. The software used for all data reduction was the standard NTCFT program supplied by Nicolet, Inc.

Results

¹³C-NMR: The uncoupled, natural abundance ¹³C-NMR spectrum of the dimer in aqueous solution is presented in Figure 2. Three sets of peaks are present: a triplet ($J = 148$ Hz), three doublets ($J = 148$ Hz), and a singlet (not shown) about 70 ppm downfield of the doublets. The triplet is assigned as the β -carbon⁹, split by coupling to the two β -protons. The doublets are assigned as α -carbons, each split by a single β -proton. The singlet is assigned as the carboxyl carbon.

A ¹H-NMR spectrum of the dimer at 270 MHz in D_2O is illustrated in Figure 3. The assignments are also shown in the figure and listed in

Table I. These assignments will be discussed later in this paper and are introduced now to facilitate the description of the results.

Table I lists the chemical shifts and intensities of the four peaks in the proton spectrum as well as those of free cysteine. Homonuclear decoupling experiments were undertaken to ascertain the connections between the various lines of the ^1H spectrum. During the course of these decoupling experiments it was noted that saturation transfer among some resonances occurred. We took advantage of this to study the interconversion rates among these resonances. Table II lists the results of the relaxation time and saturation transfer measurements. The experiments were performed at 22°C and only the steady state saturation levels were measured. Furthermore, due to the overlap of some lines, we could perform only a few of the measurements that are necessary in order to calculate the rate constants and true relaxation values in what we subsequently found to be a three-site equilibrium.¹⁰

Decoupling in the $\alpha(\text{I,II})$ region has two effects on the spectrum. First, the small splitting of $\beta_2(\text{I})$ collapses. Second, the $\alpha(\text{III})$ loses much of its intensity. Decoupling $\alpha(\text{III})$ greatly reduces the intensity of $\alpha(\text{I,II})$, but does not affect $\beta_2(\text{I})$. Decoupling $\beta_2(\text{I})$ sharpens $\alpha(\text{I,II})$ and changes the complex multiplet at 2.97 ppm from 5 to 4 apparent peaks. Decoupling the complex multiplet collapses the large $\beta_2(\text{I})$ splitting, reduces the intensity of $\beta_2(\text{I})$, and decreases the resolution of $\beta_2(\text{I})$ enough to smear out the small splitting.

In order to determine whether the splittings of the features at 4.46 and 3.59 ppm arose from chemical shift differences or J-couplings, we resorted to J-resolved two-dimensional spectroscopy.⁸ In the 2-D spectrum

the $\alpha(\text{I})$ and $\alpha(\text{II})$ resonances were centered on the $J = 0$ line, indicating that they were, to our resolution, singlets, and thus assignable to chemically distinct species. The $\beta_2(\text{I})$ quartet (Fig. 4) was arranged diagonally across the $J = 0$ line, diagnostic of a single proton split into a quartet.⁸ The multiplet was too complex to be interpreted further.

Figure 5 displays the ^1H -NMR spectra obtained at higher temperatures. Unfortunately, the peaks become obscured by the water peak as the temperature is increased. However, the β peaks are seen to first broaden, followed by the appearance of a doublet ($J = 12$ Hz) in the complex β peak. At 70° this doublet is collapsed by decoupling at the broad $\beta_2(\text{I})$ peak at 3.4 ppm.

In addition to spectra at ambient temperature of the dimer dissolved in D_2O at $\text{pH}^*6.7$ ($\text{pH}^* = \text{pH}$ reading uncorrected for isotope effects), spectra were also taken after titration with HCl to $\text{pH}^*4.5$ and 3.0. The resulting spectra are shown in Figure 6. There is a broadening of all the peaks except $\alpha(\text{III})$, which instead undergoes a shift to lower field.

Discussion

The ^{13}C -NMR proves the existence of at least three states of the cysteine ligand. These three states are seen in the three NMR lines of the α carbon, but not in the β or carboxyl signals.

The interpretation of the proton spectrum starts with the assumption of three cysteine species. Table I compares the chemical shifts of the dimer ligands with that of free cysteine. The dimer peaks at 4.46 and 3.90 ppm are near the normal α proton region. The observed saturation transfer between these peaks confirms that they are all α protons. The skewed nature of the peak at 4.46 ppm suggests two overlapping signals. The two-dimensional NMR suggests that this skewing does not

arise from spin coupling, but rather arises from overlapping singlets. Labeling the three cysteine species as I, II, and III, we arbitrarily assign the labels such that $\alpha(I)$ and $\alpha(II)$ are the overlapping signals at 4.46 ppm with species I representing the more abundant species. $\alpha(III)$, then, is the signal at 3.90 ppm.

Also by analogy with free cysteine (Table I), the multiplet at 2.97 ppm is assigned to β -type protons. Decoupling and 2-D experiments establish that the quartet at 3.59 ppm is coupled to $\alpha(I \text{ or } II)$ ($J = 3.3$ Hz) and to the β multiplet ($J = 12.6$ Hz) and that it is a single species. This indicates that the quartet is one of two inequivalent β protons, and we will label it β_2 . The quartet, arising from only one β proton, ought to have the same intensity as the corresponding α proton in the same species. Hence, the quartet must correspond to only one of the two species under the 4.46 ppm peak, and the intensity indicates it must be the dominant species I. Thus the quartet is fully assigned as $\beta_2(I)$. By elimination, the multiplet at 2.97 ppm is assigned as the overlapping signals of all the remaining protons: $\beta_1(I, II, III)$ and $\beta_2(II, III)$.

These assignments are consistent with the intensities observed. There is an approximate 2:1 ratio between all β 's and all α 's. These assignments are also consistent with the temperature dependence of the NMR spectrum (Fig. 5). Because all β_1 's lie within the multiplet, their maximum spread in frequency is approximately 20 Hz. $\beta_2(I)$, however, is about 170 Hz from the center of the multiplet where the other β_2 's reside. Hence, as we heat the sample and increase the exchange rates among species I, II, and III, the β_1 's will reach the fast exchange limit at a lower temperature than will the β_2 's. At 70°C the β_1

multiplet collapses into a doublet with 12 Hz splitting which further collapses into a singlet upon decoupling at $\beta_2(I)$. The quartet and multiplet move toward each other as the temperature is raised owing to the averaging among the β_2 's. At higher temperatures we expect the β_2 's to reach the fast exchange limit downfield of the β_1 doublet.

The saturation transfer experiment corroborates our interpretation that in solution there exists at least three different species. There is slow exchange among the α protons and among the β protons. From the saturation transfer to $\beta_2(I)$ (when all other β 's are saturated) we calculate $k_{I,II} + k_{I,III} = 7 \text{ s}^{-1}$ at 22°C. (In arriving at this result we have taken the measured relaxation rate as the true one, an approximation that is valid when all apparent β relaxation rates are equal.¹⁰ The other rate constants are difficult to extract from our experiments, but a simple line-width measurement shows that the sum of the off-constants ($k_{II,I} + k_{II,III}$ and $k_{III,I} + k_{III,II}$) are less than 20 s^{-1} .

The assignment of physical structures to species I, II, and III is ambiguous based on the present evidence. However, a model with which the data are consistent is illustrated in Figure 7. The cysteine is postulated to undergo a stepwise dissociation, acting as a tridentate ligand (species I), a bidentate ligand with the carboxyl dissociated (species II), and as either a bi- or monodentate ligand with the amino group dissociated (species III).

The tridentate form is that found in the crystal and must be considered the leading candidate for the dominant solution species, I. Furthermore, we may rationalize the inequivalence of the β -protons of species I as resulting from their proximity to the fixed carboxyl group. Species II with its dissociated carboxyl group provides nearly equal

environments to the β protons, which therefore have similar chemical shift values. As a tridentate ligand, the $C(\alpha)-COO^-$ bond is stretched,⁴ which provides a rationale for the slight α -proton shift between species I and II.

We believe species III involves dissociated amino group. This conclusion is based on the large shift of α (III) away from α (I, II). Because the amino nitrogen is bound directly to the α carbon, it can be expected to influence the α proton more strongly than the carboxyl oxygens, thus producing a larger shift when the amino dissociates.

We do not believe species III is free cysteine. The ^{13}C -NMR shows only one β carbon, making dissociation of sulfur difficult to rationalize. In addition, we have taken the spectrum of the complex in the presence of excess cysteine. The resulting spectrum, particularly in the α (III) region, appears as a superposition of the free cysteine spectrum (a clean α triplet) on the dimer spectrum.

The pH dependence of the spectrum supports our model. Increasing (H^+) should, through protonation of the carboxyl, increase the rate of interconversion between species I and II, and shift the equilibrium toward species II (carboxyl dissociated). All peaks arising from species I and II are seen to broaden as the pH is decreased to 3.0. We attribute this broadening to the decreasing lifetimes of species I and II as the interconversion rate increases. There is also a small change in the intensity ratio between $\beta_2(I)$ and the β multiplet. The multiplet containing $\beta_2(II)$ grows, shifting the $\beta_2(I) : \beta$ ratio from 1:1.9 at $pH^* 6.7$ to 1:2.2 at $pH^* 3.0$. This change must be viewed cautiously since integration of broad peaks is imprecise.

The α (III) peak shifts as the pH is lowered, moving from 3.90 ppm at pH* 6.7 to 3.99 ppm at pH* 3.0. We have observed that the α proton of free cysteine has a similar pH dependence, moving from 3.96 ppm near neutral pH to 4.31 ppm at pH below 2. The pH behavior of the free ligand is attributed to the fractional protonation of the carboxyl: the proton comes and goes fast enough to give sharp lines in the NMR spectrum, and the observed chemical shift of the α proton is the weighted average of the protonated and unprotonated carboxyl species. As previously suggested, it may be that in the dimer, species III has both the amino and carboxyl dissociated, so that acid titrates the carboxyl and shifts the α (III) proton in the same way. Again, we caution that though suggestive, this does not eliminate the possibility of bound carboxyl in species III.

In summary we have definite evidence for the presence of three distinct, interconverting states of cysteine ligation to Mo. This leads to the possibility of six dimer states. In general, then, we may expect that any model system containing this complex may exhibit complicated behavior. For example, Corbin, Pariyadath and Steifel¹² observed that minor changes in the ligands of the complex or minor changes in the buffer have profound effects on the product distribution and/or catalytic activity of the dimer. Ott and Schultz¹³ likewise found that the electrochemical behavior of solutions of the dimer varied with buffer type, buffer composition, and pH in such a way as to make it difficult to substantiate any single reaction mechanism. Tam and Swinehart^{2d} recently observed that the reaction of the dimer with O_2 depends strongly on the supporting electrolyte and solvent. All of these factors -- ligand variations, buffer type, buffer composition, pH, supporting electrolyte, and solvent -- are likely to alter the relative concentrations

of the various dimer species. If, as seems probable, the various species have different catalytic properties or electrochemical properties, then the observed complicated behavior is to be expected. Such studies may warrant reexamination to determine exactly which dimer species is involved in the processes studied.

Acknowledgement

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TABLE I

Chemical shift (ppm from Dss)		Relative intensities of dimer signals		Assignments
<u>Dimer</u>	<u>Free Cys</u> ¹¹			
4.46	3.56-4.31	1.1		$\alpha(I)$, $\alpha(II)$
3.90		.3		$\alpha(III)$
3.59	2.98-3.16	1.0		$\beta_2(I)$
2.97	2.80-3.11			$\beta_1(I, II, III)$; $\beta_2(II, III)$

TABLE II

Relaxation rates and Overhauser Enhancements of Mo-cys Dimer at 22°C

$f_{\alpha(I,II)}(\alpha_{III})$	= -0.9	$R_{\alpha(I,II)}$	= 0.74 s ⁻¹
$f_{\alpha(III)}(\alpha_I, \alpha_{II})$	< -0.9	$R_{\alpha(III)}$	= 1.0 s ⁻¹
$f_{\beta_2(I)}(\text{all } \beta)$	= -0.7	$R_{\beta(\text{all})}$	= 2.9 s ⁻¹

The notation follows Perrin and Johnston J. Mag. Res. 33, 619 (1979)

$$f_i(j) = (I_i(j) - I_i^0) / I_i^0$$

$I_i(j)$ = intensity of i when j is saturated;

I_i^0 = equilibrium intensity

$$R_i = 1/T_{1i}$$

Figure Captions

- Figure 1: The structure of $\text{Mo}_2\text{O}_4(\text{cys})_2^{2-}$.
- Figure 2: A portion of the room temperature proton-coupled C-13 NMR spectrum of $\text{Mo}_2\text{O}_4(\text{cys})_2^{2-}$ approximately .1M in D_2O , pH*6.7. The three doublets are assigned as α -carbons in three different species.
- Figure 3: Room temperature proton NMR spectrum of $\text{Mo}_2\text{O}_4(\text{cys})_2^{2-}$ approximately .1M in D_2O pH*6.7. Peaks are labeled with their assignments.
- Figure 4: 2-D J-resolved NMR of $\beta_2(\text{I})$. The sample was approximately .1M $\text{Mo}_2\text{O}_4(\text{cys})_2^{2-}$ in D_2O , pH*6.7.
- Figure 5: Proton NMR spectrum of $\text{Mo}_2\text{O}_4(\text{cys})_2^{2-}$ at 40° , 60° , and 80°C . The sample was approximately .1M dimer in D_2O , pH*6.7.
- Figure 6: pH titration of $\text{Mo}_2\text{O}_4(\text{cys})_2^{2-}$. The sample was approximately .1M dimer in D_2O , room temperature.
- Figure 7: Proposed structure of the interconverting cysteine states present in aqueous solutions of $\text{Mo}_2\text{O}_4(\text{cys})_2^{2-}$.

XBL 80I-4022

Figure 1

XBL 799-5024

Figure 2

Figure 3

XBL 798-4989A

Figure 4

XBL 798-4992

Figure 5

XBL 798-4990

Figure 6

XBL 80I-402I

Figure 7

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Chapter 3

Trans-Mo(N₂)₂(dppe)₂ EXAFS

Introduction

The role of Mo in the enzyme nitrogenase has been a mystery largely due to lack of spectroscopic probes. Nitrogenase contains a great deal of Fe, and these contribute to UV-VIS and EPR spectra of the enzyme, obscuring the contribution--if any-- of Mo.¹

The recent availability of synchrotron radiation has permitted the application of Extended X-ray Absorption Fine Structure (EXAFS) to the study of Mo in nitrogenase. EXAFS spectra are element specific, and reflect only local structure around the element under study. This gives EXAFS unique potential in the study of metal centers in metalloenzymes. Cramer and Hodgson² have been particularly active in this area, recently publishing a report suggesting the Mo in nitrogenase is bound by S atoms.

Joe Smith examined the EXAFS of nitrogenase in the presence and absence of substrate.³ He hoped to observe binding of substrate to Mo. The data, however, were inconclusive. Part of the problem was that the heavy S atoms dominate the EXAFS, making it difficult to sort out a possible contribution from the light (and poorly scattering) N.

The question arises: can one hope to observe such a light atom entering a coordination sphere dominated by heavy atoms? In other words, could Smith's experiment--given significantly better signal to noise--determine conclusively whether or not nitrogen binds to the Mo μ -nitrogenase, or is the EXAFS technique and data analysis not μ ed and sensitive enough to detect such a change?

To test this, the EXAFS spectra of trans-bis-dinitrogen-bis(1,2-bis(diphenylphosphino)ethane)Mo(0), abbreviated trans-Mo(N₂)₂(dppe)₂, and tetrahydrido-bis(1,2-bis(diphenylphosphino)ethane)Mo(IV), abbreviated MoH₄(dppe)₂, were taken. The crystal structure of the dinitrogen compound is known.⁴ The crystal structure of a similar hydride--MoH₄(PPH₂CH₃)₄-- is also known.⁵ Figure 1 illustrates the configuration of trans-Mo(N₂)₂(dppe)₂, and Table I lists relevant bond distances of both compounds. It should be noted that the phosphines of the hydride are arranged tetrahedrally, rather than in a plane as in the dinitrogen complex. EXAFS, however, is sensitive only to the distance between the absorbing and scattering atoms, not their stereochemistry (except in the special cases of "hidden" scatterers⁶ and oriented samples). The similarity of Mo-P bond distances leads us to assume the contribution of the phosphorus to the EXAFS will be nearly identical in both compounds. The difference between the two spectra should be attributable to the dinitrogen ligands (the hydride atoms are such poor scatterers that their contribution to the EXAFS should be negligible).

Phosphorus, of course, is next to sulfur in the Periodic Table. Its electron scattering power should be comparable to that of sulfur. The problem of identifying the nitrogen in the midst of the phosphorus is similar to the problem Smith faced in trying to identify substrate binding

to Mo in nitrogenase. Thus, these two compounds simulate Smith's substrate/no substrate experiment. Our ability to distinguish between the EXAFS of these compounds based on the presence/absence of dinitrogen ligands will indicate the feasibility not only of Smith's experiment, but also of any EXAFS experiment attempting to identify a light atom in the presence of heavy atoms.

EXAFS Spectra

Figure 2 illustrates the Mo K-edge X-ray absorption spectra of $\text{MoH}_4(\text{dppe})_2$ and $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$. Figure 3 illustrates the EXAFS, $\chi(k)$, after the background has been removed. In both spectra the EXAFS oscillations persist to $16\text{\AA}^{-1}$ and beyond. This is due to the heavy phosphorus scatterers. Comparing the two spectra closely, it is seen that they are almost identical except for the presence of a "beat" near $7\text{\AA}^{-1}$ in the trans- $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$ spectrum. This beat is due to the N_2 ligands. The EXAFS frequency of the nitrogen scatterers should be somewhat different from the frequency of the dominating phosphorus scatterers due to the different bond lengths and phase shifts. The addition of the two frequencies produces the observed beat. There is no beat in the $\text{MoH}_4(\text{dppe})_2$ because the H atoms scatter electrons so poorly that the EXAFS amplitude of the H atoms is near zero.

Fourier Transforms

The power spectra of the Fourier transforms of the EXAFS are shown in Figure 4. Both transforms are dominated by a main peak at an apparent distance $R_{\text{FT}} = 1.93\text{\AA}$. In addition there are small peaks near $1\text{\AA}$. Finally, there are other small features beyond $2.5\text{\AA}$.

The longest bond distance is the first coordination sphere of either compound is near $2.5\text{\AA}$. Typically, the Fourier transform phase shift, α_{FT} , shifts peaks to an apparent R_{FT} which is shorter than the true R .⁷ Therefore, the peaks beyond $2.5\text{\AA}$, if real, represent atoms not bound directly to Mo. These features will not be analyzed here.

It is difficult to completely subtract the background from an absorption spectrum. Often residual background appears as a peak in the Fourier transform at or below $1\text{\AA}$. Attempts to fit the $1\text{\AA}$ peaks to N parameters failed to yield reasonable results. The $1\text{\AA}$ peaks were therefore assigned as background artifacts.

The main peak in each transform certainly represents the four P atoms. By a process of elimination, it appears that the nearest N atoms ($2.01\text{\AA}$ from Mo) are buried in the main peak of the trans- $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$ transform.

Comparing the two transforms, it is seen that there are only minor differences between the two. It is tempting to speculate about the significance of these differences -- for example, the peak around $2.7\text{\AA}$ in the trans- $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$ transform is near where one would expect the far N atom (about $3\text{\AA}$ from Mo) to appear, assuming a phase shift of a few tenths of an Angstrom. However, the potential for artifacts from either the background or the truncation of the EXAFS before Fourier transforming is great enough to cast considerable doubt on any conclusions based on such minor features. Using only the transforms, then, it would be difficult if not impossible to distinguish between the two samples with any confidence.

Curve-fitting

Both the trans-Mo(N₂)₂(dppe)₂ and MoH₄(dppe)₂ EXAFS were fitted to the model functions calculated by Lee and coworkers at Bell Laboratories.¹⁰ The analysis of the main peaks in the two spectra began by isolating the main peak through Fourier filtering. Figures 5 and 6 illustrate the window applied to the power spectrum of each. The peak is brought smoothly to zero by applying a Hamming window⁸ on either side (Figures 7 and 8). The peak is then back-transformed to k-space to yield the filtered EXAFS (Figures 9 and 10). The filtered EXAFS of both samples were then weighted by k⁰ or k³ and fitted to a) a single phosphorus shell and b) a phosphorus shell and nitrogen shell. The results are tabulated in Tables IA-IC and plotted in Figures 11-17.

Looking first at the single shell phosphorus fits of the MoH₄(dppe)₂ data, it is seen that R_{Mo-P} is within .03Å of the crystallographic distance for both the k⁰ and k³ weightings. The positive σ obtained in the k⁰ weighted fit is the result of the interdependence of σ and the other elements of the amplitude envelope.

On the other hand, the 2-shell fit of the MoH₄(dppe)₂ data yields a negative amplitude for the nitrogen shell when weighted by k⁰, and an R_{Mo-N} nearly equal to R_{Mo-P} when weighted by k³. The program seems to be having difficulty incorporating a (nonexistent) nitrogen shell into the fit, responding with unphysical amplitude parameters or forcing the Mo-N distance to be equal to the one frequency actually present: the Mo-P distance.

The single shell phosphorus fits of the trans-Mo(N₂)₂(dppe)₂ also resulted in reasonable values for R_{Mo-P}. This is less surprising when one remembers that the heavy phosphorus atoms dominate the EXAFS, so

that leaving out the nitrogen shell introduces only a small error (in the case of the $\text{MoH}_4(\text{dppe})_2$ the error in leaving out the H atoms is even smaller because the H atoms are such poor electron scatterers). The amazing accuracy of the k^3 fit ($\pm .01\text{\AA}$) is due to the fact that the k^3 weighting emphasizes the persistent oscillations of the P shell and de-emphasizes the rapidly damped N oscillations, thus further reducing the error.

In contrast to the $\text{MoH}_4(\text{dppe})_2$, the trans- $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$ 2-shell fits look quite good. $R_{\text{Mo-P}}$ is within .01Å of the crystallographic distance in both the k^0 and k^3 weighted fits. $R_{\text{Mo-N}}$ is within .02Å of the crystallographic distance when the data are weighted by k^0 (essentially no weighting at all, the nitrogen and phosphorus contributions in their "natural" ratio). $R_{\text{Mo-N}}$ is accurate only to .05Å when the data are multiplied by k^3 (emphasizing phosphorous over nitrogen). These results are quite respectable considering the dominance of the phosphorus oscillations.

In summary then, single phosphorus shells can be fitted satisfactorily to either sample due to the dominance of the phosphorus scattering. However, the 2-shell phosphorus-nitrogen fit only succeeded with the trans- $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$. Thus the 2-shell fit succeeded in distinguishing between the presence and absence of nitrogen despite the interference of the phosphorus. This suggests that Joe Smith's experiment, examining nitrogenase EXAFS with and without substrate, should be able to determine whether or not nitrogen enters the first coordination sphere of Mo.

Edges

The Mo K-edges of $\text{MoH}_4(\text{dppe})_2$ and trans- $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$ are detailed in Figure 18. The inflection points are listed in Table II and the

first derivatives of the edges are plotted in Figure 19.

The first inflection point of trans-Mo(N₂)₂(dppe)₂ is about 2eV higher in energy than that of MoH₄(dppe)₂. The beam-line resolution is about 1eV and the data points were spaced about 0.7eV apart across the edge, so that this difference is just larger than the experimental error. The closeness of the edge energies despite the disparity of formal oxidation states (IV for the hydride, 0 for the dinitrogen) is probably due to the phosphine ligands. Cramer² found that when Mo is ligated to many sulfurs, the edge inflection energy is relatively insensitive to changes in the formal oxidation state of the Mo. Such Mo-S complexes were found to have inflection point energies of about 20010-11eV, essentially equal to (within experimental error) the energy observed for the dinitrogen complex (20010.3eV).

Because the inflection point of trans-Mo(N₂)₂(dppe)₂ is somewhat higher than that of MoH₄(dppe)₂, it appears that the dinitrogens are more oxidizing than the hydrides. This oxidizing effect is due to the "push-pull" bonding mechanism⁹ between the Mo and N₂: the dinitrogens donate electrons to Mo through a sigma bond and draw electrons from the Mo d-orbitals into the N₂ π* molecular orbital.

There is also an interesting but difficult to interpret difference between the shapes of the MoH₄(dppe)₂ edge and the trans-Mo(N₂)₂(dppe)₂ edge. The dinitrogen complex edge exhibits a clear shoulder, while the hydride complex edge has a less well resolved shoulder -- if indeed it has a shoulder at all.

These early shoulders are generally assigned as 1s → π* (bound state) transitions. They arise in Mo = 0 systems due to the mixing of

Mo 4d orbitals with O p orbitals. Cramer² observed that the presence of a molybdenyl oxygen (Mo=O) generally results in a shoulder which he assigned as $1s \rightarrow \pi^*$. Such a transition into the π system of the Mo-N=N system is quite conceivable. An analogous transition in the hydride is more difficult to see. Thus the availability of a π bonding system in the dinitrogen complex may be responsible for the difference in the edge shapes. However, the present data are really inadequate to understand fully this edge effect. The phosphines, for example, may also participate in π bonding to Mo, and the different geometries of the phosphine ligands -- tetrahedral in the hydride, planar in the dinitrogen complex -- may also be the source of the different edge shapes. A study of the edges of a series of analogous compounds would be necessary to say for certain what the edge shapes are attributable to.

Experimental

General: Many of the compounds prepared and some of the starting materials are air or moisture sensitive, particularly in solution. Unless otherwise noted, all reactions were carried out anaerobically using a standard bench-top technique. Solutions were manipulated in Schlenk glassware, and a gas-vacuum line employing BASF Catalyst R3-11 (oxygen removal) and molecular sieve (water removal) was used to degas glassware and solutions. Usually, N_2 gas was employed as the inert atmosphere, although argon was used whenever there was some possibility of reaction with N_2 .

Organic solvents were prepared as follows: THF distilled from Na/benzophenone; benzene distilled from CaH_2 ; methanol and ethanol distilled from Mg turnings; propionitrile distilled from P_2O_5 ; dichloromethane distilled from P_2O_5 . THF and benzene were degassed by freeze-

vacuum-flush-thaw cycles (at least three times). Other solvents were degassed by simple vacuum-flush cycles (at least three times). Hexane (Mallinckrodt Analytical Reagent) was used without further purification.

Among the starting materials, it should be noted that MoCl_5 and MoCl_4 are extremely air and moisture sensitive, and must be transferred under an inert atmosphere.

Infrared spectra were obtained on a Perkin-Elmer 283. Samples were run as KBr pellets. Analyses were performed by the analytic laboratory of the Chemistry Department, University of California, Berkeley.

$\text{MoCl}_4(\text{dppe})$: This compound was most conveniently prepared as described in the Ph.D. thesis of J. Gladysz.¹² 9.9g MoCl_4 (Atomergic Chemetals) was suspended in 700ml THF. 16.6g dppe (1,2-bis (diphenylphosphino)ethane, Alfa-Ventron) was added to the suspension, and $\text{MoCl}_4(\text{dppe})$ precipitated as a brick red solid (44% yield). It is important to suspend the MoCl_4 first, before adding dppe. Occasionally, the MoCl_4 reacted with the THF before the addition of dppe, forming a green solution. This did not affect the subsequent reaction with dppe. The rate of reaction was variable, and it was best to allow several hours for maximum yield. The $\text{MoCl}_4(\text{dppe})$ solid is air stable and may be filtered in the open and washed with reagent grade hexane.

An alternate preparation¹³ starts with MoCl_5 . This, added to propionitrile, yields $\text{MoCl}_4(\text{EtCN})_2$ as a brown precipitate.¹⁴ Stirring is recommended during this reaction because the product otherwise forms large, very hard blocks. The $\text{MoCl}_4(\text{EtCN})_2$ is then refluxed in benzene with dppe to form $\text{MoCl}_4(\text{dppe})$.¹⁵

Mo(N₂)₂(dppe)₂: The EXAFS sample was prepared by the method of George and Siebold.¹³ The general scheme was to mix 1.25g dppe and 2.5g MoCl₄(dppe) in 30ml THF, add 20g 2% Na-Hg¹⁶ and stir under dinitrogen for six hours.

The reaction flask is illustrated in Figure 20. It was found that rubber tubing is permeable enough to oxygen over the course of six hours to reduce the yields. For this reason lengths of rubber tubing leading to the flask were kept to a minimum, and the input gas was introduced through copper tubing. The exhaust gas was run through an oil bubbler to protect the system from back diffusion of air.

At the end of the reaction 50ml of THF was added to the flask to replace solvent evaporated by the gas flow and to dissolve additional product. The product, dissolved in the THF, was drawn off through a thin stainless-steel tubing. On one end of this tubing a short glass tube was epoxied. A piece of filter paper was then tied over the glass end with copper wire. The reaction flask and the receiving flask were both stoppered with rubber septa through which the tubing ran. The filtered end was submerged in the THF suspension, and vacuum applied to the receiving flask, pulling over the THF.

Filtering was always slow due to the very fine but heavy silt produced in the reaction flask. This silt quickly clogged the filter, reducing the flow to drop by drop. Use of more conventional sintered glass filtering tubes--even with filter aid--did not help, and proved considerably more awkward and difficult to clean up. Likewise, changing filters was of no help, because the new filter would clog almost immediately. Additionally, changing filters exposed the solution to air.

It was found best to simply wait, though it took seven hours or more to finish.

Once filtered, the THF was removed with a rotary evaporator. The residue was then dissolved in benzene, the benzene filtered to remove insoluble residues, and the product precipitated with chilled methanol (ten times the volume of benzene). George and Siebold report using only 5ml benzene to dissolve gram quantities of the complex. This author found it necessary to use about 50ml benzene. Final yield was 0.89g, crude.

Just prior to the experimental run, the orange crystals were recrystallized from benzene/methanol. $\nu(\text{N}\equiv\text{N})$ was observed at 1980cm^{-1} (KBr). Calculated for $\text{C}_{52}\text{H}_{48}\text{MoN}_4\text{P}_4$: C, 65.83%; H, 5.10%; Mo, 10.11%; N, 5.90%; P, 13.06%. Found: C, 65.56%; H, 5.38%; Mo, 10.7%; N, 5.68%; P, 12.94%.

Chatt¹⁷ has developed an alternate synthesis using $\text{MoCl}_3(\text{THF})_3$ as starting material and Mg turnings as reductant. Much higher yields are claimed, and the Mg reduction is much less messy than the Na-Hg. This author attempted this route, but without success, perhaps due to insufficient activation of the Mg with I_2 vapor.¹⁸ It should be noted that Chatt, in Reference 18, recommends a change in solvent (to benzene) in working up the reaction (THF/ether is used in Reference 17).

$\text{MoH}_4(\text{dppe})_2$: There is some confusion in the literature over the formulation of this compound. Hidai and coworkers¹⁹ bubbled H_2 through a benzene solution of $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$, obtaining yellow crystals they formulated as $\text{MoH}_2(\text{dppe})_2$. Archer and George²⁰ studied an analogous compound with more soluble phosphine ligands and, on the basis of ^1H and ^{31}P NMR, concluded the correct formula was $\text{MoH}_4(\text{dppe})_2$. Meakin and

coworkers²¹ reduced $\text{MoCl}_4(\text{dppe})$ with BH_4^- in the presence of additional dppe, formulating their yellow crystals as $\text{MoH}_4(\text{dppe})_2$. Again, this formulation was based on NMR studies.

This author carried out the H_2 gas reaction of Hidai, and Ninon Kafka repeated Meakin's synthesis. The IR of both products was identical in every feature ($\nu(\text{Mo-H})=1748\text{cm}^{-1}$ and 1816cm^{-1} , KBr). This author feels the NMR work is strong evidence, and favors the $\text{MoH}_4(\text{dppe})_2$ formulation.

The EXAFS sample was the material produced by N. Kafka following Meakin. It was recrystallized as pale yellow crystals from benzene/ethanol. Calculated for $\text{C}_{52}\text{H}_{52}\text{MoP}_4$: C, 69.64%; H, 5.80%; Mo, 10.71%; P, 13.84%. Found: C, 71.80%; H, 6.21%; Mo, 10.20%; P, 12.98%. The combination of high C,H and low Mo,P is attributed to incomplete solvent removal. Unfortunately, the time pressure of scheduled beam time made it impossible to dry the sample further. The only impurities that might affect the EXAFS would be appreciable amounts of Mo species other than the desired product. The agreement between the IR spectra for both syntheses and the clean appearance of the crystals after recrystallization argues against such impurities.

Collection of EXAFS data: trans- $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$ and $\text{MoH}_4(\text{dppe})_2$ were run as solids. 460mg of trans- $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$ and 490 mg of $\text{MoH}_4(\text{dppe})_2$ were mixed with cellulose and pressed into $1\frac{1}{2}$ " diameter pellets. The pellets were mounted on plastic slide holders.

The data were collected by placing the pellet holders in a styro-foam box cooled by liquid nitrogen boil-off. The samples were mounted on a lucite carrousel which rotated the samples in and out of the beam. This arrangement allowed the changing of samples without disturbing the flow of cold nitrogen or opening the box, enabling us to collect data

at virtually the same temperature for both samples. Cooling was desirable to reduce thermal disorder in the samples, yielding higher resolution EXAFS. The air temperature inside the box was monitored with a thermocouple and varied slowly from -85 to -90°C.

Each individual scan collected data for one second per point. Scans were repeated at least ten times for each sample. Across the edge region, points were spaced approximately every 0.7eV. Data were collected to $k > 17\text{\AA}^{-1}$, but were analyzed to only $k=15.85\text{\AA}^{-1}$ due to a large "glitch" at that point, extending over many points. This glitch was present in every spectrum of every sample, and probably represented a flaw in the crystal monochromator.

SSRL: EXAFS data were obtained at the Stanford Synchrotron Radiation Laboratory (SSRL). SSRL has distributed the following specifications for Station IV-2 (Wiggler Magnet), the station at which the data was taken.

Number of Mrad: ~2 (unfocused)

Monochromator: (400)Si, 2 crystal, rapidly tunable, computer controlled.

Spectral Range: 2.8-40.0KeV (with a variety of crystals).

Resolution: $\lambda 1\text{eV}$

Approximate Spot Size: ~1-2mm high by 35mm wide.

The wiggler magnet should increase the critical energy to about 1GeV higher than that in SPEAR (electron storage ring). Data were collected with SPEAR running at just over 2GeV.

TABLE IA
Crystal Distances, Å

trans-Mo(N₂)₂(dppe)₂

Mo-P₁ 2.445

Mo-P₂ 2.462

Mo-N₁ 2.014

Reference: T. Uchida, Y. Uchida, M. Hidai, T. Kodama
Acta Crystallogr., Sect. B, 31, 1197 (1975).

MoH₄(Pφ₂CH₃)

Mo-P₁ 2.433

Mo-P₂ 2.503

Mo-H 1.70

Reference: P. Meakin, L. J. Guggenberger, W. G. Peet,
E. L. Muetterties, J. P. Jesson, J. Amer. Chem.
Soc., 95, 1467 (1973).

TABLE IB
Curve-Fitting Results
Single Shell Mo-P Fits

<u>Weight=k⁰</u>	<u>trans-Mo(N₂)₂(dppe)₂</u>	<u>MoH₄(dppe)₂</u>
σ	-0.001321	0.001908
$R_{\text{Mo-P}}, \text{\AA}$	2.492	2.444
E_0, eV	20011.2	20010.1
Amplitude	0.2349	0.2310
<u>Weight=k³</u>		
σ	-0.003468	-0.002796
$R_{\text{Mo-P}}, \text{\AA}$	2.449	2.420
E_0, eV	20003.3	20005.6
Amplitude	0.3111	0.3290

TABLE IC
Curve-Fitting Results
Two Shell Mo-P-N Fits

<u>Weight=k⁰</u>	<u>trans-Mo(N₂)₂(dppe)₂</u>	<u>MoH₄(dppe)₂</u>
σ_P	-0.002268	-0.004076
σ_N	-0.003588	-0.01191
$R_{Mo-P, \text{\AA}}$	2.457	2.429
$R_{Mo-N, \text{\AA}}$	2.030	2.124
E_{OP}, eV	20004.9	20008.7
E_{ON}, eV	20021.0	20009.9
Amplitude P	0.2981	0.365
Amplitude N	0.1285	-0.2036

<u>Weight=k³</u>		
σ_P	-0.004220	-0.000110
σ_N	0.001186	.002976
$R_{Mo-P, \text{\AA}}$	2.453	2.418
$R_{Mo-N, \text{\AA}}$	2.063	2.414
E_{OP}, eV	20004.4	20003.8
E_{ON}, eV	20009.1	19998.7
Amplitude P	0.3525	0.5114
Amplitude N	0.1336	0.4799

TABLE II

Edge Results, Inflection Points, eV

$\text{Mo}(\text{N}_2)_2(\text{dppe})_2$	20010.3
	20031.3
$\text{MoH}_4(\text{dppe})_2$	20007.9
	20019.6
Na_2MoO_4	20005.6
	20021.0
	20035.7

Figure Captions

- Figure 1: Structure of trans-bis(dinitrogen)-bis[1,2-bis(diphenylphosphino)ethane]Mo(0), abbreviated trans-Mo(N₂)₂(dppe)₂.
- Figure 2: Mo K-edge absorption spectra of MoH₄(dppe)₂ (upper trace) and trans-Mo(N₂)₂(dppe)₂ (lower trace) plotted in energy space from 19.7 to 21.2 keV.
- Figure 3: Isolated EXAFS, $\chi(k)$, of MoH₄(dppe)₂ (upper trace) and trans-Mo(N₂)₂(dppe)₂ (lower trace) plotted in photoelectron k-space from 3.0 to 16.0 Å⁻¹.
- Figure 4: k³-weighted Fourier transforms, $\phi_3(R)$, of the EXAFS of MoH₄(dppe)₂ (upper trace) and trans-Mo(N₂)₂(dppe)₂ (lower trace) plotted in R space from 0.0-5.0 Å.
- Figure 5: $\phi_3(R)$ of trans-Mo(N₂)₂(dppe)₂ with superimposed square window illustrating region to be isolated for curve-fitting.
- Figure 6: $\phi_3(R)$ of MoH₄(dppe)₂ with superimposed square window illustrating region to be isolated for curve-fitting.
- Figure 7: $\phi_3(R)$ of trans-Mo(N₂)₂(dppe)₂ with superimposed dots (...) illustrating final isolated peak for curve-fitting obtained after application of Hamming window.
- Figure 8: $\phi_3(R)$ of MoH₄(dppe)₂ with superimposed dots (...) illustrating final isolated peak for curve-fitting obtained after application of Hamming window.
- Figure 9: EXAFS, $\chi(k)$, of trans-Mo(N₂)₂(dppe)₂ before Fourier filtering (...) and after Fourier filtering (—). Filtered $\chi(k)$ is the back transform of the isolated peak in Figure 7.

- Figure 10: EXAFS, $\chi(k)$, of $\text{MoH}_4(\text{dppe})_2$ before Fourier filtering (···) and after Fourier filtering (—). Filtered $\chi(k)$ is the back-transform of the isolated peak in Figure 8.
- Figure 11: Fourier filtered EXAFS of trans- $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$ weighted by k^0 (···) and corresponding single shell P fit (—).
- Figure 12: Fourier filtered EXAFS of $\text{MoH}_4(\text{dppe})_2$ weighted by k^0 (···) and corresponding single shell P fit (—).
- Figure 13: Fourier filtered EXAFS of trans- $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$ weighted by k^3 (···) and corresponding single shell P fit (—).
- Figure 14: Fourier filtered EXAFS of $\text{MoH}_4(\text{dppe})_2$ weighted by k^3 (···) and corresponding single shell P fit.
- Figure 15: Fourier filtered EXAFS of trans- $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$ weighted by k^0 (···) and corresponding two-shell P-N fit (—).
- Figure 16: Fourier filtered EXAFS of trans- $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$ weighted by k^3 (···) and corresponding two-shell P-N fit (—).
- Figure 17: Fourier filtered EXAFS of $\text{MoH}_4(\text{dppe})_2$ weighted by k^3 (···) and corresponding two-shell P-N fit.
- Figure 18: Linearized energy-space K-edges of $\text{MoH}_4(\text{dppe})_2$ (upper trace) and trans- $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$ (lower trace) plotted from 19.95 to 20.05 keV.
- Figure 19: First derivatives of the K-edges of $\text{MoH}_4(\text{dppe})_2$ (upper trace) and trans- $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$ (lower trace) plotted from 19.97 to 20.07 keV.
- Figure 20: Reaction vessel used in synthesis of trans- $\text{Mo}(\text{N}_2)_2(\text{dppe})_2$. N_2 gas is introduced through the tapered bubbler as shown.

Figure 20: (Continued)

The exhaust bubbler is connected by a short piece of rubber tubing to an oil bubbler to protect the reaction mixture from oxygen if the N_2 flow is interrupted. The valved side arm is connected to the Schlenck vacuum/gas line. The mixture is stirred with a magnetic stir bar.

Figure 1

trans-Bis (dinitrogen)-bis[1,2-bis (diphenylphosphino) ethane] Mo(O)

XBL 764-5805

XBL 815-4589

Figure 2

XBL 85-4590

Figure 3

XBL 815-4598

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

Figure 10

Figure 11

Figure 12

Figure 13

Figure 14

Figure 15

Figure 16

Figure 17

XBL 815-4591

Figure 18

XBL 815-4592

Figure 19

Figure 20

_XBL 7910-5033

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Chapter 4

REACTION OF $\text{Mo}_2\text{O}_4(\text{cysteine})_2^{2-}$ WITH ACETYLENEIntroduction

The $\text{Mo}_2\text{O}_4(\text{cysteine})_2^{2-}$ anion (Figure 1) is a much studied complex. This interest arises from its use as a model system for nitrogenase.¹ At basic pH in the presence of strong reducing agents, $\text{Mo}_2\text{O}_4(\text{cysteine})_2^{2-}$ catalyzes the reduction of a number of nitrogenase substrates, notably acetylene to ethylene (see Figure 1). Schrauzer¹ studied this reaction through the kinetics of ethylene production. On the basis of these studies, he concluded that the active catalyst was a monomer, and he suggested the reduced catalyst was Mo(IV).

Spence and coworkers² studied another reaction of the dimer: high pH solutions of the dimer turn from orange to blue over periods ranging from hours to days depending on the dimer concentration. Spence looked at the kinetics of the color change, polarographs of solutions of the dimer, and the reaction of the blue species with flavin. He proposed the mechanism shown in Figure 2 to explain his results: one of the dimer's oxygen bridges is hydrolyzed by OH^- , producing one blue species (labeled II in Figure 2); II goes on to exchange cysteine ligands for buffer to produce III. The initial hydrolysis is catalyzed by cysteine

(thus the rate dependence on dimer concentration: the more dimer initially, the more III produced, releasing more catalyzing cysteine). Excess cysteine may be added to shift the equilibrium away from III toward II.

Lately, LaMache³ studied this color change electrochemically, concluding the change to blue actually represents a disproportionation of the dimer to MoO_4^{2-} and an undefined Mo(III) product.

It should also be noted that Haight⁴ observed an EPR signal in the blue solutions which correspond to 2% of the total Mo in solution. This he attributed to an equilibrium amount of Mo(V) (the dimer couples the odd electrons).¹⁵

This paper reports the observation of a direct reaction between $\text{Mo}_2\text{O}_4(\text{cysteine})_2^{2-}$ and acetylene. This reaction occurs at basic pH. No reductant is used. The reaction is manifested by an inhibition of the color change from orange to blue and the concurrent appearance of a strong EPR signal, stronger than the signal observed by Haight in the blue solutions referred to above. Extended X-ray Absorption Fine Structure (EXAFS) studies show that the final product(s) is a Mo monomer. Mo K-edge studies suggest that the EPR-active product is Mo(V), not Mo(III). As yet, however, the data are insufficient to describe fully the final product.

Experimental

$\text{Mo}_2\text{O}_4(\text{cysteine})_2^{2-}$ was prepared as previously described in Part I. EPR and VIS-UV studies were performed on solutions approximately $5 \times 10^{-3}\text{M}$ in dimer, 0.1M in borate buffer, pH 9.2. Acetylene was bubbled through concentrated H_2SO_4 to remove acetone. All solutions were handled under Ar or acetylene using standard techniques.

EPR measurements were made on a Varian E-9 EPR spectrometer. VIS-UV measurements were made on a Cary 118 spectrometer.

Collection of EXAFS data: $\text{Na}_2\text{Mo}_2\text{O}_4(\text{cys})_2 \cdot 5\text{H}_2\text{O}$ was run as a cellulose peller, 510mg in a $1\frac{1}{4}$ " diameter disk. Solutions were run in lucite cells of 2cm path length with kapton windows. The cells were flushed with argon earlier in the day, then sealed. The samples were kept in the tubes in which they had been mixed--sealed under the appropriate atmosphere with rubber septa--until it was time to take the spectrum. An aliquot of the sample was then withdrawn from the tube and injected into the cell with a syringe.

The pH7.1 solution was .04M in dimer and had been prepared one week before the experimental run. Experience with such solutions, stored under argon, indicated good stability over considerably longer periods.

The pH9.2 (.1M borate buffer) samples were prepared as a single stock solution .04M in dimer and .01M in excess cysteine. This stock solution was split in half, one portion stored under argon, the other stored under acetylene. The acetylene gas was bubbled through H_2SO_4 to remove acetone. The samples were approximately one day old when run.

Each individual scan collected data for one second per point. Scans were repeated at least four times for each sample. Across the edge region, points were spaced approximately every 0.7eV. Data were collected to $k > 17\text{\AA}^{-1}$, but were analyzed to only $k = 15.85\text{\AA}^{-1}$ due to a large "glitch" at that point, extending over many points. This glitch was present in every spectrum of every sample, and probably represented a flaw in the crystal monochromator.

All samples were run at room temperature.

Observations

Solutions $5 \times 10^{-3} \text{M}$ in dimer at pH 9.2 under an argon atmosphere turn from orange ($\lambda_{\text{max}} = 306 \text{nm}$) to blue in about four days. The final blue spectrum is shown in Figure 3, spectrum a. It is characterized by the broad absorption from 550-600nm. A similar solution kept under acetylene rather than argon turns pale yellow with a greenish tint after four days (Figure 3, spectrum b). Notice that spectra a and b in Figure 3 are plotted on different absorbance scales, so that the dimer/Ar(spectrum a) absorbance is 5 times stronger than that of the dimer/acetylene (spectrum b). The UV spectrum of the final dimer/acetylene solution is shown in Figure 4. It exhibits features around 285 and 230nm.

If a blue dimer/argon solution (pH 9.2, $5 \times 10^{-3} \text{M}$ in dimer) is flushed with acetylene, the blue color fades in the course of a week to pale yellow, and the UV spectrum becomes identical to that of an original dimer/acetylene spectrum. Reflushing the now yellow solution with argon produces no further reaction.

The dimer/acetylene solution develops an EPR signal as it changes from orange to yellow (Figure 5). No quantitative EPR studies were performed. The final signal intensity of the dimer/acetylene solution is an order of magnitude stronger than that present in blue dimer/argon solutions of identical concentrations. Hyperfine structure due to coupling with the 25% $\text{Mo}^{95,97}$ is observed ($a=37.5 \text{G}$). A g-value of 1.989 was measured using DPPH as a standard.

Gas chromatography of the atmosphere over a yellow dimer/acetylene solution showed no evidence of ethylene.

All of the above changes in color and the development of the EPR signal are accelerated by the addition of excess cysteine.

Mo K-edge EXAFS spectra were taken of $\text{Na}_2\text{Mo}_2\text{O}_4(\text{cysteine})_2 \cdot 5\text{H}_2\text{O}$, $\text{Mo}_2\text{O}_4(\text{cysteine})_2^{2-}$ (pH 7.1, under argon), dimer/argon (pH 9.2, after the color change), and dimer/acetylene (pH 9.2, after the color change). Figures 6 and 7 illustrate the data. Figure 8 is an expansion of the edge regions. Table I lists the edge inflection points. Figure 9 is a plot of the isolated EXAFS. Figure 10 plots the Fourier transform power spectra. The details of the analysis process are discussed in Part II of this thesis.

Discussion

The above observations are not adequate to characterize fully this reaction between $\text{Mo}_2\text{O}_4(\text{cysteine})_2^{2-}$ and acetylene. However, the EPR and EXAFS data indicate the final product is monomeric. The dimer is EPR silent because the single d-electrons of the two Mo(V)'s are coupled through a direct Mo-Mo bond. Breaking the dimer into monomers, of course, breaks the coupling, and if the Mo remains Mo(V) an EPR signal would appear. While no quantitative EPR has been done yet, the intensity of the signal (ten times that of the blue solutions) suggests that a significant portion of the Mo present (perhaps 20-30%, extrapolating from Haight's measurements⁴) is EPR active after the reaction with acetylene.

The EXAFS data support the conclusion that the acetylene breaks up the dimer. Looking first at the isolated EXAFS in Figure 9, one sees that the oscillations of the dimer/acetylene sample damp out much more quickly than that of the other samples. This rapid damping is typical

of light-atom scatterers,⁵ while the persistent oscillations of the other three samples are typical of heavy scatterers such as another Mo.

The Fourier transform radial distribution functions (Figure 10) are difficult to interpret exactly because the Mo is coordinated by so many different atoms at various distances. Table II lists the ligating atoms and their distances from Mo in the $\text{Na}_2\text{Mo}_2\text{O}_4(\text{cysteine})_2 \cdot 5\text{H}_2\text{O}$ crystal.⁶ The coordination in solution is even more complicated due to changes in the conformation of the cysteine ligand (see Part I of this thesis). Nevertheless, the Mo-Mo distance of $2.57\text{\AA}$ ⁹ suggests that the Mo peak should appear in the Fourier transform at an R_{FT} a little greater than $2\text{\AA}$. This, combined with a detailed study of the amplitude envelope of the feature at $2.2\text{\AA}$ ⁹ led Cramer to assign that peak to Mo in the spectrum of the crystalline dimer.⁷ Whether this peak is a "pure" Mo peak is not obvious, since the Mo-S distance of $2.49\text{\AA}$ ⁹ is only $0.08\text{\AA}$ ⁹ less than the Mo-Mo distance.¹³ Nevertheless, it does appear reasonable that the Mo is a significant part of the $2.2\text{\AA}$ ⁹ feature. In the dimer/acetylene spectrum, this feature is almost completely attenuated, consistent with the monomerization of much of the dimer and perhaps loss of the S coordination as well (discussed below).

The observed EPR active species is probably Mo(V). Mo(III), a d^3 , $S = \frac{3}{2}$ system, generally produces no observable EPR signal at room temperature due to relaxation processes.¹⁴ Since no low temperature EPR studies were attempted, the presence of Mo(III) cannot be definitively ruled out, however.

Examination of the Mo K-edge inflection points (Table I) reveals that, within experimental error, the first inflection point does not

vary among the four samples and is the same as the first inflection point in Na_2MoO_4 . Cramer⁸ assigns this pre-edge shoulder (and first inflection point) as the "1s-4d" bound-state transition as enhanced by mixing the 4d Mo level with p character from the molybdenyl oxygen ($\text{Mo}=\text{O}$) π -system. Cramer believes this shoulder is diagnostic of the presence of molybdenyl oxygen. If this assignment is correct, one would expect the energy of this bound-state transition to remain relatively constant, as observed. Accepting this assignment implies that a significant portion of the dimer/acetylene products retain the molybdenyl oxygen.

The second inflection point of the dimer/acetylene sample is at significantly higher energy than the dimer/pH7.1 sample. Superficially, this edge-shift suggests oxidation. Indeed, the second inflection point of the dimer/acetylene sample is, within experimental error, at the same energy as the second inflection point of Na_2MoO_4 . (Table I).

There is a problem in interpreting this edge position, however. The dimer/acetylene sample is likely to contain several Mo species. This is so because, first of all, it is unlikely that the dimer reaction with acetylene goes cleanly to completion. Secondly, the EPR signal is most likely Mo(V), while the edge suggests Mo(VI).

The difficulty is that the inflection point of a mixed sample does not have the same physical significance as the inflection point of a pure sample. The observed edge of a mixed sample is the sum of the edges of the single components. It is shown in the appendix to this chapter that the inflection point -- or points -- of the sum may be different from the inflection points of the components. If the inflection points of the components are close enough to "interfere"

with each other, the inflection points of the mixture will appear at an energy between the energies of the highest and lowest inflection points of the components. If one species dominates the mixture, the inflection point of the mixture will tend toward the inflection point of the dominant species.

Because the inflection point of the dimer/acetylene sample has moved significantly toward Mo(VI), it follows that a considerable portion of the Mo in the sample has been oxidized. This does not necessarily mean there has been a formal change in oxidation state. Drastic changes in coordination may have drastic effects on the electron density of the Mo, even though the formal oxidation state remains unchanged. Such a change in electron density would result in an edge shift.

Nevertheless, the possibility that some fraction of the Mo has been oxidized to Mo(VI) cannot be ignored. If one postulates this as an explanation of the edge shift, one must go further and identify the oxidant. Since no ethylene was detected, the only oxidant remaining is other Mo atoms. It appears from the data available, then, that there is a disproportionation involved here. This means either Mo(III) or Mo(IV) should be present in the sample. Low temperature EPR would determine whether Mo(III) is present. However, on the present data it is not possible to say which, if either, reduced Mo is present.

There is clearly a relationship between the reaction converting the dimer to the blue species and the present reaction with acetylene, but again nothing definitive can be said. The dimer/argon sample exhibits the same -- within experimental error -- edge shift as the dimer/acetylene sample (Table I). The EXAFS of the blue solution reveals that the "Mo peak" is attenuated relative to the lower R peaks, but is not

as weak as in the dimer/acetylene sample (Figure 10).

Again, the edge shift observed in the dimer/argon sample suggests the presence of Mo(VI), which in turn suggests a disproportionation. But again, the data are insufficient to say whether Mo(III) or Mo(IV) is produced. Thus, the present data are consistent with LaMache's mechanism of disproportionation to Mo(III), but are insufficient to prove it.

The continued presence -- albeit attenuated -- of the "Mo peak" likewise does not rule out a disproportionation. The products may include Mo(III) dimers. See, for example, Ott and Schultz¹¹, where they claim to produce Mo(III) dimers electrochemically. However, the attenuated "Mo peak" also fits well with Spence's view of the "blue" products: a weakening of the link between the Mo's -- by Spence's partial hydrolysis -- would lead to greater movement between the Mo's; this would increase the effective Debye-Waller factor and reduce the Mo amplitude considerably.⁷ Going simply from the solid, crystalline state into solution causes such an effect as shown by a comparison of the Fourier transforms of the solid pellet dimer and the pH7.1 dimer solution (Figure 10).

Understanding this "blue reaction" may be the key to understanding the acetylene reaction. Since the blue products, whatever they are, appear to react with acetylene in the same way as the original dimer (as evidenced by the identical UV spectra of the final products), the blue products may be intermediates to the reaction with acetylene. This is further suggested by other parallels: both reactions require basic pH, and both reactions are catalyzed by cysteine.

A final point is that considerable cysteine may be freed in the course of the reaction with acetylene. The UV spectrum exhibits a 230nm feature (Figure 4). This is the region in which cysteine absorbs at pH 9.¹² The fact that it is a shoulder on a larger peak prevents a precise statement of the intensity of this peak, but it is high enough to account for almost all the cysteine present in the original dimer.

Spence² attributed the appearance of free cysteine to the exchange of cysteine ligands for buffer. A similar process could be involved in the acetylene reaction. However, a disproportionation to MoO_4^{2-} would also release a great deal of cysteine ligand. Whatever the mechanism, the release of cysteine is also supported by the EXAFS envelope of the dimer/acetylene sample (Figure 9) which damps out quickly enough to suggest that even the sulfur is gone.

It is interesting here to mention some recent results of Schrauzer's¹⁰ in which he finds the dimer reduces acetylene in strongly alkaline solutions (4M NaOH in 20% ethylene glycol/water). Schrauzer proposes a disproportionation of the dimer to Mo(IV) and Mo(VI) to explain his results. He assumes the solutions are EPR silent, relying on Haight's report⁴ that the EPR signal of the blue dimer/argon solutions disappears at pH 12.

In the present study, of course, much milder reaction conditions are employed, so that there may be little connection between the present observations and Schrauzer's studies. Nevertheless, it would be interesting actually to check for an EPR signal in Schrauzer's solutions. It may be that in the more mildly alkaline solutions, a stable Mo-acetylene complex forms, while in the more extreme conditions of Schrauzer's

experiments the acetylene is actually reduced to ethylene. This points up the possibility that understanding the presently observed Mo-acetylene may lead to an understanding of the dimer's catalytic role in reducing acetylene to ethylene.

In summary, then, it is definitely concluded that at basic pH, $\text{Mo}_2\text{O}_4(\text{cysteine})_2^{2-}$ reacts with acetylene, producing monomeric Mo products. A significant portion of these monomers are EPR active, most likely Mo(V). Shifts in the edge inflection points suggest Mo(VI) may be produced. There is also evidence that the cysteine dissociates from the Mo. The mechanism may parallel that of the "blue reaction" studied by Spence and LaMache, with the acetylene finally stabilizing Mo monomers. The mechanism of this reaction and the structure of any Mo-acetylene adducts may shed light on the mechanism of the catalytic reduction of acetylene by Mo.

TABLE I

Edge Results, Inflection Points, eV

$\text{Na}_2\text{Mo}_2\text{O}_4(\text{cysteine})_2 \cdot 5\text{H}_2\text{O}/\text{Pellet}$	20006.3
	20016.3
$\text{Mo}_2\text{O}_4(\text{cysteine})_2^{2-}/\text{pH}7.1$	20005.6
	20016.6
Dimer/ C_2H_2	20006.9
	20020.6
Dimer/Ar	20006.9
	20019.6
Na_2MoO_4	20005.6
	20021.0
	20035.7

TABLE II
 Crystal Distances, Å
 $\text{Na}_2\text{Mo}_2\text{O}_4(\text{cysteine})_2 \cdot 5\text{H}_2\text{O}$

Mo-Mo	2.569
Mo-S	2.49
Mo-N	2.23
Mo-O _a	2.30
Mo-O _t	1.71
Mo-O _b	1.95, 1.91

Reference: J. R. Knox, C. K. Prout, Acta Crystallogr.,
Sect. B, 25, 1857 (1969).

Figure Captions

- Figure 1: $\text{Mo}_2\text{O}_4(\text{cysteine})_2^{2-}$ structure and the simplest Schrauzer model system for the reduction of C_2H_2 to C_2H_4 .
- Figure 2: Spence's mechanism for the conversion of the orange dimer into blue species in basic solution.
- Figure 3: Visible spectra of $\text{Mo}_2\text{O}_4(\text{cysteine})_2^{2-}$ dimer solutions. Spectrum "a" (—) is a four-day-old $5 \times 10^{-3}\text{M}$ dimer solution in pH 9.2 buffer under an argon atmosphere (dimer/Ar sample). Spectrum "b" (---) is a solution identical to "a" but maintained under an acetylene atmosphere (dimer/ C_2H_2). Note that the spectra are not plotted on the same absorbance scale, so that the dimer/Ar solution has an absorbance roughly five times higher than that of the dimer/ C_2H_2 sample. Thus the dimer/Ar solution appears blue, while the dimer/ C_2H_2 sample is greenish yellow.
- Figure 4: UV spectrum of four day-old $5 \times 10^{-3}\text{M}$ dimer solution in pH 9.2 borate buffer under an acetylene atmosphere (dimer/ C_2H_2).
- Figure 5: EPR signal of a dimer/ C_2H_2 solution. The g value is 1.989 (DPPH standard). Hyperfine coupling constant $a = 37.5\text{G}$.
- Figure 6: Mo k-edge absorption spectra of $0.4\text{M Mo}_2\text{O}_4(\text{cysteine})_2^{2-}$ at pH 7.1 (upper trace) and $\text{Na}_2\text{Mo}_2\text{O}_4(\text{cysteine})_2 \cdot 5\text{H}_2\text{O}$ in a cellulose pellet (lower trace).
- Figure 7: Mo K-edge absorption spectra of solutions of $.04\text{M Mo}_2\text{O}_4(\text{cysteine})_2^{2-}$ at pH 9.2 (borate buffer) and about $.01\text{M}$ in excess cysteine. The solutions are about one day old. The upper trace sample was kept under argon. The lower trace sample was kept under acetylene.

Figure 8: Mo k-edges of the four samples of Figures 6 and 7. From top to bottom: dimer pellet; dimer/pH 7.1; dimer/Ar; dimer/C₂H₂.

Figure 9: EXAFS, $\chi(k)$, of the four samples of Figures 6 and 7. From top to bottom: dimer/C₂H₂; dimer/Ar; dimer/pH 7.1; dimer pellet.

Figure 10: k^3 Fourier transform power spectra, $\phi_3(r)$, of the four samples of Figures 6 and 7. From top to bottom, dimer/C₂H₂; dimer/Ar; dimer/pH 7.1; dimer pellet.

SCHRAUZER MODEL SYSTEM

XBL 764-5806

Figure 1

Figure 2

XBL 799-5022

Figure 3

XBL 799-5021

Figure 4

XBL 799-5023

Figure 5

XBL 815-4593

Figure 6

XBL 815-4594

Figure 7

XBL 815-4595

Figure 8

XBL 815-4596

Figure 9

XBL 815-4597

Figure 10

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Appendix

The Relationship between the Inflection Point Observed in the K-Edge of a Mixed Sample and the Inflection Points of the Pure Components.

The following discussion is a rough, intuitive discussion of where the inflection point in the "K-edge" of a sample composed of several absorbing species may be expected to appear relative to the inflection points of the components. The basic conclusion reached will be that the position of the inflection point or points of the mixture is a sort of weighted average of the positions of the inflection points of the pure components. The important "weighting" parameters are the separation of the inflection points of the components and the relative concentrations of the components.

The shape of the continuous absorption curve at an x-ray "edge" is that of an arctangent function.¹ The first derivative of an arctangent is a Lorentzian. The inflection point of the edge corresponds to the peak of the Lorentzian.

If one has a two component mixture $s(E) = f(E) + g(E)$, the absorption curve of the mixture will be the sum of the individual absorption curves. (Figure A-1). The first derivative of the sum will be the sum of the component first derivatives, $s'(E) = f'(E) + g'(E)$. (Figure A-2). Thus, $s'(E)$ is simply the sum of two Lorentzians.

The behavior of the sum of two Lorentzians as the peaks of the Lorentzians move closer together is well known from other forms of spectroscopy including NMR.

Suppose $f(E)$ and $g(E)$ are defined as follows:

$$f(E) = A(1 + \frac{2}{\pi} \arctangent(\frac{E - E_{01}}{w_1}))$$

$$g(E) = B(1 + \frac{2}{\pi} \arctangent(\frac{E - E_{02}}{w_2}))$$

Then, $f'(E)$ and $g'(E)$ will be:

$$f'(E) = A(\frac{2}{\pi}) \frac{1/w_1}{1 + (E - E_{01})^2/(w_1)^2}$$

$$g'(E) = B(\frac{2}{\pi}) \frac{1/w_2}{1 + (E - E_{02})^2/(w_2)^2}$$

Now w_1 and w_2 are the half-width at half maximum of $f'(E)$ and $g'(E)$, respectively. The quantities $2w_1$ and $2w_2$ will be taken to be the "edge width" of $f(E)$ and $g(E)$, respectively. The degree of "overlap" of the edges will be taken to be the ratio $2(w_1 + w_2)/(E_{02} - E_{01})$.

If this ratio is much less than 1, the edges may be considered "separate", and $s(E)$ will have inflection points at E_{01} and E_{02} , the inflection points of the components, to a very good approximation. As this ratio approaches 1, the inflection points of $s(E)$ (maxima of $s'(E)$) will shift away from E_{01} and E_{02} and toward the center of gravity of the two Lorentzian peaks. The minimum of $s'(E)$ will also be an inflection and will be located at the center of gravity. As the ratio exceeds 1, the center of gravity becomes a maximum and the "maxima" of the two components are washed out, so that $s(E)$ has only a single inflection point at the center of gravity. This sequence is illustrated in Figures A-3 to A-5 for the case where $A = B$ and $w_1 = w_2$, so that the center of gravity is at $(E_{01} + E_{02})/2$.

Where f and g are different absorbing elements, the edges will generally be far enough apart to assume there is no distortion of the component inflection points. This is important since many interesting samples contain more than one element. If all of these edges interacted appreciably, the edge inflection points would lose all meaning.

Likewise, where f and g are close enough to "interfere", they will generally be different species of a single element. The magnitudes A and B will depend on the total absorption cross-sections of f and g . Since f and g are the same element, the atomic cross-sections will be nearly identical. Thus, A and B will depend upon the relative concentrations of f and g . If the concentration of f is much higher than that of g , A will be much greater than B , and the $g'(E)$ maximum will be washed out by the tail of $f'(E)$ without shifting the maximum of $f'(E)$ appreciably. (Figure A-6). Thus $s(E)$ will have an inflection point near E_{01} , the inflection point of the dominant species. This is also an important result since no sample is 100% pure. If small amounts of impurities appreciably shifted the inflection points, again the points would have little meaning.

Finally, of course, where f and g are different species of a single element present in comparable concentrations, the inflection points of the mixture will be significantly different from the inflection points of the components. If the component edges are separated by more than the combined widths of the edges, the components' inflection points will survive but appear shifted toward a new inflection point at the center of gravity. If the component edges are within the combined widths of the two edges, the mixture will show only a single inflection point at

the center of gravity. Thus, the position of the inflection points of the mixture will be a sort of weighted average of the positions of the inflection points of the components.

Appendix Figure Captions

- Figure A-1. $s(E)$ (—), $f(E)$ (···), and $g(E)$ (— · —), where $E_{01} = 20000\text{eV}$, $E_{02} = 20100\text{eV}$, $w_1 = w_2 = 25\text{eV}$, $A = B = 0.5$.
- Figure A-2. $s'(E)$ (—), $f'(E)$ (···), and $g'(E)$ (— · —), where parameters are as in Figure A-1.
- Figure A-3. $s'(E)$ (—), $f'(E)$ (···), and $g'(E)$ (— · —), where $E_{01} = 20000\text{eV}$, $E_{02} = 19000\text{eV}$, $w_1 = w_2 = 25\text{eV}$, $A = B = 0.5$.
- Figure A-4. $s'(E)$ (—), $f'(E)$ (···), and $g'(E)$ (— · —), where $E_{01} = 20000\text{eV}$, $E_{02} = 20050\text{eV}$, $w_1 = w_2 = 25\text{eV}$, $A = B = 0.5$.
- Figure A-5. $s'(E)$ (—), $f'(E)$ (···), and $g'(E)$ (— · —), where $E_{01} = 20000\text{eV}$, $E_{02} = 20005\text{eV}$, $w_1 = w_2 = 25\text{eV}$, $A = B = 0.5$.
- Figure A-6. $s'(E)$ (—), $f'(E)$ (···), and $g'(E)$ (— · —), where $E_{01} = 20000\text{eV}$, $E_{02} = 20050\text{eV}$, $w_1 = w_2 = 25\text{eV}$, $A = 10B = 0.5$.

XBL 814 - 4543

Figure A-1

XBL 814-4548

Figure A-2

XBL 814-4547

Figure A-3

XBL 814-4546

Figure A-4

XBL 814-4545

Figure A-5

XBL 814-4550

Figure A-6

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