Reactions of Thiols and Selenols with the Fe–Mo–S Cluster $[Fe(MoS_4)_2]^{3-}$

JOHN W. McDONALD*, G. DELBERT FRIESEN** and WILLIAM E. NEWTON†

Battelle-Kettering Laboratory, Yellow Springs, Ohio 45387, U.S.A.

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Abstract

The known complex $[Et_4N]_3[Fe(MoS_4)_2]$ has been shown by EPR and visible spectral studies to react with both thiophenol and selenophenol. The reaction results in a change in the characteristic $S = 3/2$ EPR spectrum of this species from a complex rhombic pattern to one of a very simple axial appearance. Although this effect is similar to that observed for reaction of these species with the iron–molybdenum cofactor of nitrogenase, a moiety known to consist of a Fe–Mo–S cluster species, the large excesses of reagents and the long reaction times required for complete formation of product indicate that these reactions are of questionable direct relevance to the biological system. The reaction corresponding to the EPR spectral change from rhombic to axial in the $[Fe(MoS_4)_2]^{3-}$/PhSeH system has also been partially characterized by product isolation which indicates that attack by selenol of the two terminal MoS_2 moieties in the starting material has occurred.

Introduction

The iron–molybdenum cofactor (FeMoco) is the extruded, putative N_2-reducing site of nitrogenase [1], an Fe–Mo–S cluster obtained by acid treatment of the molybdenum–iron protein of that enzyme, followed by neutralization and extraction with the organic solvent *N*-methylformamide (NMF) [2–4]. The dithionite-reduced form of both the molybdenum–iron protein and FeMoco exhibits an unusual EPR signal at low temperature which is characteristic of $S = 3/2$ magnetic ground state behavior with g values at about 4.5, 3.5 and 2.0 [5]. The similarity of the signal of the native protein to that of the extruded cluster is strong evidence that FeMoco shares close structural correlation with the site as it exists in the enzyme. The thermal stability of FeMoco in NMF indicates that synthesis of an analog for the molybdenum site of nitrogenase is feasible, and numerous attempts have been made to simulate the structural, reactivity, and/or spectroscopic properties of FeMoco via synthetic studies of Fe–Mo–S cluster complexes [6]. One such species is $[Fe(MoS_4)_2]^{3-}$, a linear trinuclear complex, which, although in no way a structural model for FeMoco, does exhibit an EPR signal characteristic of an $S = 3/2$ ground state [7–9]. The EPR signal of FeMoco is known to sharpen markedly on the addition of one equivalent of thiol [10–11] or selenol [12], becoming more similar in general appearance to that of the molybdenum–iron protein. As part of our overall efforts to study various aspects of Fe–Mo–S clusters for prototypic behavior for the molybdenum site in nitrogenase, we have studied the effect of thiophenol and selenophenol on the EPR spectrum of $[Fe(MoS_4)_2]^{3-}$ and herein report our results.

Experimental

Materials and Methods

All reactions were carried out in degassed solvents under an atmosphere of pure argon using standard Schlenk tube techniques. $[Et_4N]_3[Fe(MoS_4)_2]$ was synthesized by the literature method [9] and thiophenol (Aldrich) and selenophenol (Alfa) were used as received. Acetonitrile (MeCN) was distilled from CaH_2. Infrared spectra were obtained on a Beckman IR-20A instrument and visible spectra on a Cary 118C spectrophotometer. EPR spectra were recorded using a Varian 4502 spectrometer equipped with a Model V4560 100 kHz modulation control unit and an X-band microwave bridge. Samples were cooled with liquid helium boil-off using either an Air Products or an Oxford Instruments transfer line and dewar.

Cyclic voltammetry experiments were carried out with a three-electrode cell using MeCN as solvent and 0.1 M $[Bu_4N][BF_4]$ (Aldrich) as supporting electro-

*Author to whom correspondence should be addressed.

**Present address: Corning Glass Works, Corning, N.Y. 14831, U.S.A.

†Present address: Western Regional Research Center, USDA-ARS, Berkeley, Calif. 94710, U.S.A.

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lyte. The working electrode was a glassy carbon disk from Bioanalytical Systems (BAS), the reference electrode was an aqueous SCE separated from the sample solution by a salt bridge, and the potentiostat was a CV-1A unit from BAS. For low temperature work, the cell was immersed in a dewar cooled to –40 °C with an acetone-dry ice bath.

Visible Spectral Studies

10^{-4} to 10^{-3} M solutions of $[Et_4N]_3[Fe(MoS_4)_2]$ were placed in either a 4 ml-capacity of a 0.3 ml-capacity cell, which had pathlengths of 1.0 cm and 0.1 cm, respectively, and were equipped with septa to exclude air. Aliquots of thiophenol or selenophenol solutions in MeCN were injected into the reaction mixtures and the resulting spectra monitored at ambient temperature until invariant and then recorded. In addition, the time course of selected reactions was monitored by recording spectra at various intervals after addition of the reagent.

EPR Spectral Studies

In some cases, aliquots of the above visible spectral reaction mixtures were removed, placed in 3 mm ID quartz tubes and frozen in liquid nitrogen for later spectral analysis. For these samples, the time course of the reaction was monitored and correlated to the visible spectral change. In other cases, the semiquantitative rate of the reaction, determined from separate visible spectral studies, was used to assure that reactions of $[Fe(MoS_4)_2]^{3-}$ with thiol or selenol were complete before the samples were frozen and stored in liquid nitrogen.

Preparative Scale Reaction of $[Et_4N]_3[Fe(MoS_4)_2]$ with PhSeH

The complex (0.20 g; 0.22 mmol) was dissolved in MeCN (25 ml) and PhSeH (0.40 ml; 3.96 mmol) was added by syringe. After 2 h, the reaction mixture was filtered and the filtrate evaporated under vacuum to *ca.* 5 ml. Addition of Et_2O (45 ml) produced a tacky brown solid which solidified with time. This product (0.28 g) was isolated by filtration, washed with Et_2O, and dried *in vacuo*. *Anal.* Found: C, 38.83; H, 4.89; N, 2.24%. Attempted recrystallization of this product from MeCN/Et_2O gave only powdery samples which had almost identical IR and electrochemical data and identical EPR spectra to those of the initially isolated solids. However, typical elemental analytical data for these 'recrystallized' samples were significantly different (Found: C, 34.68; H, 4.32; N, 2.25%) from that of the crude product and difficulty was encountered in obtaining consistent C, H, and N analyses in general. In addition, neither the overall sulfur and selenium analyses nor the S/Se ratio were consistent from sample to sample (Product A: S, 10.75; Se, 20.78. Product B: S, 7.75; Se, 23.39).

Results and Discussion

Since its synthesis several years ago [7–8], the properties of the $[Fe(MoS_4)_2]^{3-}$ ion (**1**) have been investigated extensively, largely because a Fe–Mo–S moiety which exhibits an $S = 3/2$ EPR signal, similiar to that found in **1**, is known to be present in nitrogenase [5], and, as noted above, is thought to comprise the substrate-reducing site of this important enzyme [1]. Although X-ray absorption spectroscopic (XAS) experiments have conclusively shown [6] that **1** is in no way a realistic structural model for FeMoco (the synthetic cubane-type Fe_3MoS_4 clusters [13–14] mimic the XAS properties of the biological unit much more closely), nevertheless the magnetic properties of this complex in particular have been studied as they relate to those of the biological system. EPR [9] and Mössbauer [15] spectroscopy studies of **1** have suggested that the net three unpaired electrons associated with **1** arise from antiferromagnetic coupling between a single Fe(III) and two Mo(V) atoms. Recent magnetic susceptibility and magnetization data [16], however, have been interpreted in terms of the presence of Fe(I) in **1**. Thus, this complex has served as a useful prototype for characterization of spin coupling phenomena in heterometallic sulfur-containing clusters. Because of our general interest in the spectroscopy to **1** and because changes in the EPR spectrum of FeMoco were used to demonstrate [10, 12] and quantitate [11] the reaction of thiols and selenols with this $S = 3/2$ Fe–Mo–S cluster, we have studied the reactivity of these reagents with **1**, using EPR spectroscopy as the primary monitor, but also characterizing the system with other spectral and electrochemical measurements and with limited preparative studies, in order to attempt to define the nature of the products.

Figure 1 shows the EPR spectral changes elicited on addition of large excesses of thiophenol (PhSH) and selenophenol (PhSeH) to a solution of $[Et_4N]_3$-

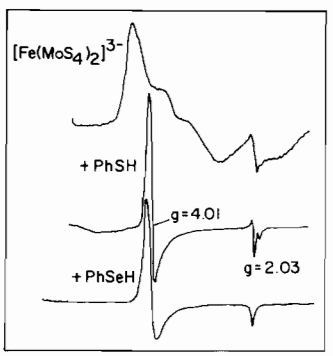

Fig. 1. EPR spectra of a *ca.* 10^{-3} M solutions of **1** in MeCN which contain no reagent (top), 1.5 M PhSH (middle), and 1.5 M PhSeH (bottom). $T = 10$ K; microwave frequency = 9.15 GHz.

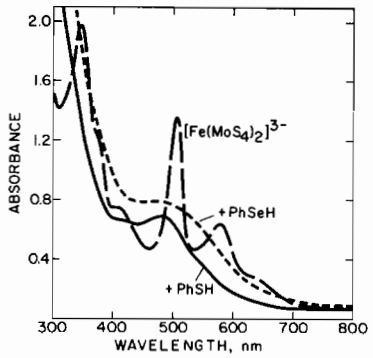

Fig. 2. Visible spectra of a *ca.* 10^{-4} M solution of **1** in MeCN containing no reagent (– – –), 1.5 M PhSH (——), and 1.5 M PhSeH (------).

$[Fe(MoS_4)_2]$ (**1**) in MeCN. It is evident that the $S = 3/2$-type spectral pattern characteristic of **1** is greatly simplified in the presence of these reagents, changing from a complex, rhombic-like appearance [9] to one which is almost perfectly axial with g values at 4.01 and 2.03. The concomitant visible spectral changes associated with this reactivity are shown in Fig. 2, with the characteristic peak pattern of **1** replaced by single broad absorption bands in the 450–500 nm region for both PhSH and PhSeH. The similarity of the final EPR and visible spectra for these two reactants suggests that the overall stoichiometry of the products for these two systems is identical except for the S/Se dichotomy.

In order to obtain the complete conversion of **1** to products, an excess of thiol or selenol was required. If less than a *ca.* 750 fold excess of PhSH or a 20 fold excess of PhSeH was added at the low concentrations of **1** used for spectral studies, both EPR and visible spectra of reaction mixtures at infinite time showed the presence of both reactant and product. As shown in Figs. 3 and 4, the reaction of **1** with PhSH was relatively slow as monitored by both EPR and visible spectroscopy. The visible spectral data was obtained in the presence of a 600-fold

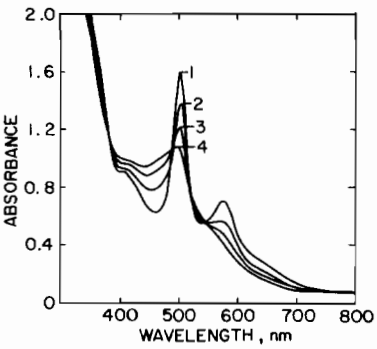

Fig. 3. Time course of the visible spectral change for reaction of a *ca.* 10^{-3} M solution of **1** in MeCN with 0.61 M PhSH: (1) 0 min; (2) 60 min; (3) 300 min; (4) 1000 min.

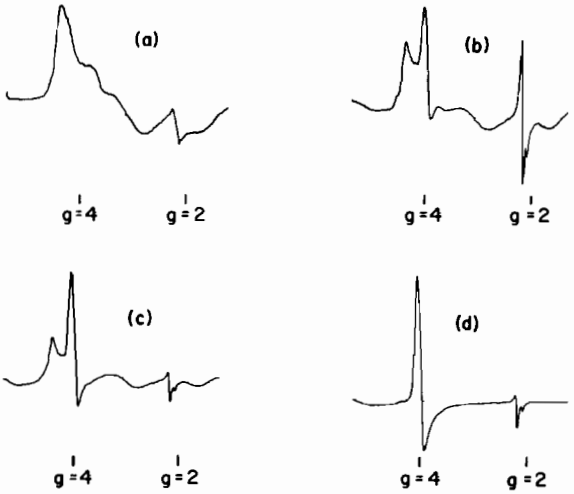

Fig. 4. Time course of the EPR spectral change for reaction of a *ca.* 10^{-3} M solution of **1** in MeCN with 1.5 M PhSH: (a) 0 min; (b) 3 min; (c) 15 min; (d) 300 min. $T = 10$ K; microwave frequency = 9.15 GHz.

excess of reagent and required *ca.* 1000 min before absorbance changes ceased. Even then, as noted above, the reaction had not gone to completion. The rate of the analogous reaction of **1** with PhSeH was much faster than for PhSH, being effectively instantaneous at a 600-fold excess of reagent. From visible spectral studies using lower concentrations of PhSeH, we estimate that the rate for the selenol is *ca.* 10 to 20 times faster than that for the thiol. It is also evident from Fig. 3 that the conversion of **1** to product is chemically 'clean', based on the presence of several isosbestic points in the visible spectral change as a function of time. In keeping with this observation, it should be noted that the overall integrity of the $S = 3/2$ spin system in **1** is clearly preserved in the products, even under the rather harsh conditions involving very high concentrations of PhSH and PhSeH and long reaction times. Thus, reaction other than simple redox behavior or decomposition of **1** is indicated in these systems.

Attempts to isolate the product from the **1**/PhSH system met with no success, apparently due to the large excess of thiol required for complete reaction. Evaporation of reaction mixtures yielded only oily residues which resisted solidification on treatment with a variety of solvents. Better results were obtained on reaction of **1** with PhSeH where, as noted above, less reagent is required for complete formation of product. Thus, after solvent removal and trituration of the residue with Et_2O, a dark brown solid (**2**) was isolated from a MeCN reaction mixture containing an 18 fold excess of PhSeH. Attempts to purify this product by recrystallization were not particularly successful, yielding only powders whose carbon, hydrogen, nitrogen, sulfur and selenium

analytical data were not consistent from sample to sample and were different from the data for the initially-isolated solid. Thus, although the data positively confirmed the presence of selenium in PhSeH-treated **1**, only limited information about the stoichiometry of **2** could be gleaned from elemental analysis. The presence of varying amounts of extraneous PhSeH in **2** could produce these inconsistent analytical data, but we have no evidence to confirm this possibility. Fortunately, EPR and visible spectra of solutions of this product in MeCN were virtually identical to those described above. So, even though the solid product is somewhat impure, information obtained from characterization of **2** thus is likely to be relevant to the product generated *in situ* from **1** plus excess PhSeH and, because of the spectral similarities, almost certainly to the product from reaction of **1** with PhSH.

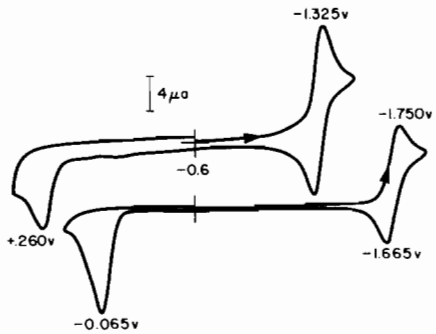

Fig. 5. Cyclic voltammograms of *ca.* 10^{-3} M solutions of **1** (bottom) and **2** (top) in MeCN at −40 °C. Scan rate = 50 mV/s.

Figure 5 shows reductive-scan cyclic voltammograms of **2** and its precursor **1** as obtained in MeCN at −40 °C. Conversely, electrochemical measurements on **2** at ambient temperatures showed an additional broad wave at *ca.* −1.1 V which increased in intensity with time, as well as decreased reversibility of the redox event at *ca.* −1.30 V, thus indicating decomposition of **2** at the higher temperature under electrochemical conditions. Two features from the voltammograms are worthy of note. First, the trace for **2** is fairly clean, containing only weak oxidation waves between −0.3 and 0.0 V in addition to the two major waves. This indicates either that the complex is relatively pure or that any major impurities, in **2** are not redox active in this voltage range. Second, the basic redox pattern of **1** (a reversible one-electron reduction at −1.70 V and an irreversible one-electron oxidation at −0.07 V) is conserved in **2**, but is shifted by *ca.* 350 mV to more positive potentials. The redox events in **1** have previously been assigned to $[Fe(MoS_4)_2]^{2-/3-}$ and $[Fe(MoS_4)_2]^{3-/4-}$ couples. The similarity of the voltammograms would seem to be consistent with the conservation of the basic Mo–S_2–Fe–S_2–Mo framework in **2**, although the evidence is obviously equivocal. In addition, it may be noted that the shift of the redox events of this postulated framework to more positive potentials is in keeping with the addition of 'softer' ligands (perhaps PhSe– or PhSeH) to the electrochemically active unit. For example, a similiar positive shift was noted for the 2−/3− couple in the heterotrinuclear complexes $[Co(WO_xS_y)_z]^{2-/3-}$ as oxygen was replaced by sulfur [17].

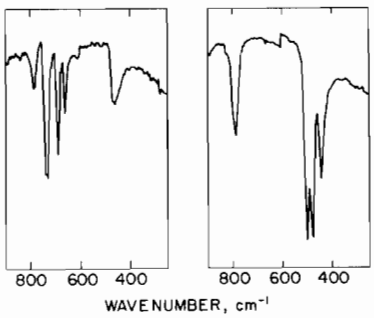

Fig. 6. Infrared spectra of **1** (right) and **2** (left) as KBr pellets.

Figure 6 shows the low-energy portion of the infrared spectra of the isolated solids **1** and **2**. On reaction with PhSeH, the characteristic strong pattern of terminal and bridging Mo–S and Mo–S–Fe bands in the 400–500 cm^{-1} region is replaced by a single broader band at 475 cm^{-1}. A number of bands due to the phenyl vibrations of PhSeH are also evident in the 650–750 cm^{-1} region. The spectrum in the M–S region is reminiscent of that displayed by the $[Cl_2FeS_2MoS_2FeCl_2]^{2-}$ ion, a linear trinuclear species [18] which contains $[MoS_4]^{2-}$ chelating two $FeCl_2$ molecules and thus has no terminal Mo–S linkages. Certainly the spectral change in Fig. 6 is strong evidence that reaction of **1** with selenophenol (and by inference thiophenol also) in some way involves attack on the two terminal MoS_2 moieties in the starting material. The presence of the 475 cm^{-1} band, which is reasonably assigned to a Fe–S_2–Mo bridge vibration, is also a good indication (and one that complements the above observations from EPR and electrochemical studies) that some sort of heteronuclear Fe–Mo–S framework remains in **2**.

Conclusions

Thiophenol and selenophenol react with $[Fe(MoS_4)_2]^{3-}$ to yield new Fe–Mo–S clusters which are structurally similar based on their EPR and visible spectral properties. The reactions are relatively slow and require an excess of reagent for complete conversion to product. Characterization of the product from the $[Fe(MoS_4)_2]^{3-}$/PhSeH system by elemental

analysis, infrared spectroscopy, and cyclic voltammetry are consistent with attack by the reagent on the terminal MoS_2 moieties of the starting material while retaining the integrity of the basic $S = 3/2$ magnetic unit. Unfortunately, efforts to characterize completely the stoichiometry of this product were unsuccessful. While the overall sharpening of the EPR spectrum of $[Fe(MoS_4)_2]^{3-}$ on addition of thiol or selenol is similar in nature to that produced by treatment of the iron–molybdenum cofactor of nitrogenase with these reagents [10–11], the large excesses of reactants and the long reaction times required for formation of products would seem to indicate that these reactions are not directly relevant to those exhibited by the biological system. However, this synthetic system has provided a starting point for studying the reactivity of these reagents with heterometallic clusters which contain both terminal and bridging sulfide atoms, species in which there is a good deal of current interest [19].

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