

## INCORPORATION OF SULFIDE AND SELENIDE IONS INTO DITHIOL–MOLYBDENUM COMPLEXES

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(First received 4 November 1974; in revised form 10 February 1975)

**Abstract**—The molybdenum complexes of several dithiols, such as dithiothreitol (DTT) and 1,4-butanedithiol, showed two visible peaks by the incorporation of inorganic sulfur. In difference from other thiol-molybdenum complexes, DTT-molybdenum complex(I) incorporates inorganic selenium without release of elemental selenium and the absorption maxima were remarkably shifted toward longer wavelength than in the case of sulfur complex. The molar ratio of sulfur and selenium to molybdenum was found to be 1:1, and the maximal incorporation of sulfur and selenium occurred in pH range from 7.8 to 9.3, and from 7.3 to 8.2, respectively. The complex(I) in the solid state was inactive for EPR measurements. In an aqueous solution, the complex(I) showed an EPR absorption at  $g = 1.979$ , which had about 0.5% of electron spin per total molybdenum. Upon the addition of sulfur or selenium to the complex(I), however, the EPR signal disappeared. On the basis of these results, dithiol-molybdenum(V) dimer complex with sulfide and selenide bridges was considered to be less readily converted to paramagnetic monomer than dimer with oxide bridge.

### INTRODUCTION

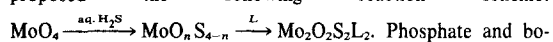
THE chromophore of 2Fe–2S\* ferredoxins which consists of two iron atoms, two labile sulfur atoms and four cysteine residues, is believed to be a binuclear iron–sulfur cluster,  $[\text{Fe}_2\text{S}_2(\text{SR})_4]$ . We have previously observed that several thiol-iron complexes incorporate sulfide and selenide, and exhibit similar optical absorption and EPR characteristics of ferredoxins[1, 2]. The thiol-molybdenum complexes have been investigated as an interesting model for molybdenum-containing enzymes, such as xanthin oxidase and nitrogenase[3–6]. Therefore, the incorporation of inorganic sulfur and selenium into dithiol-iron and -molybdenum complexes is of particular interest in the context of iron-sulfur proteins and molybdenum-containing enzymes. This paper deals with the incorporation of sulfide and selenide ions into various dithiol-molybdenum complexes. Our hopes were (1) to know the difference of reactivity between iron and molybdenum; and (2) to gain some insight into the specificity of the metal complexes toward incorporation of inorganic sulfur and selenium.

### EXPERIMENTAL

**Materials** Dithiothreitol (DTT) was obtained from Sigma. 1,2-Ethanedithiol, 1,4-butanedithiol, 1,6-hexanedithiol, 1-thioglycerol, 2-mercaptoethanol and dimercaptosuccinic acid were purchased from Tokyo Kasei, and 1,3-propanedithiol, 1,5-pentanedithiol and 1-mercaptopyrrolidylglycine (1-MPG) were obtained from K & K, as reagent grade materials. Generally, the reduction of sodium molybdate by excess thiol ligands or sodium dithionite was utilized on the reaction. In some cases, the standard molybdenum(V) solution prepared by the reduction of sodium molybdate by shaking over Hg in 3N HCl[7], was used. The DTT-molybdenum complex was isolated according to the following procedure by reference to the method of Mitchell[8]. Sodium molybdate (484 mg) in water was added to DTT (312 mg) in sodium hydroxide solution. The reaction solution was filtered

after 0.5 hr and ethanol was added to the filtrate to give an yellow-orange precipitate. After a few minutes the solid was filtered off, washed with ethanol and dried *in vacuo* over  $\text{P}_2\text{O}_5$  (Anal. Calcd. for  $\text{Na}_2[\text{Mo}_2\text{O}_4(\text{C}_4\text{H}_8\text{O}_2\text{S}_2)_2]$ : C, 15.85; H, 2.66; S, 21.15; Mo, 31.65; Na, 7.58. Found: C, 15.32; H, 2.74; S, 20.59; Mo, 31.88, Na, 7.20%). The complex gave green color with conc. HCl, indicating the presence of molybdenum(V)[9]. Certainly, this complex was also obtained by reacting DTT with molybdenum pentachloride and sodium hydroxide. Generally, in order to obtain complexes of molybdenum(VI) with thiol ligands, it is necessary to minimize reduction of molybdenum(VI) by the ligand. This is achieved by rapid isolation of the complexes and by keeping the acidity of the solution as low as possible. With excess DTT as well as 2-mercaptoethanol[8], however, reduction was too rapid to permit isolation of a molybdenum(VI) complex. Further, hydrogen sulfide was passed through an aqueous solution of the complex  $\text{Na}_2[\text{Mo}_2\text{O}_4(\text{C}_4\text{H}_8\text{O}_2\text{S}_2)_2]$  (300 mg) for 3 hr. The solution was evaporated to dryness and gave a dark brown solid. The complex was filtered off and washed with cold ethanol (Anal. Calcd. for  $\text{Na}_2[\text{Mo}_2\text{O}_2\text{S}_2(\text{C}_4\text{H}_8\text{O}_2\text{S}_2)_2]$ : C, 15.05; H, 2.53; S, 30.13; Mo, 30.06. Found: C, 14.71, H, 2.77; S, 30.86; Mo, 30.79%).  $\text{K}_3\text{Mo}(\text{CN})_6$  was prepared by the method of Furman and Miller[10].

**Methods.** The incorporation of sulfide and selenide into dithiol-molybdenum complexes was determined by the optical spectral change induced by the addition of sodium sulfide (or selenious acid) to the mixture of excess dithiol and sodium molybdate in an aqueous solution. In the case of DTT, the absorption spectrum obtained in this manner was almost agreement with that of isolated complex  $\text{Na}_2[\text{Mo}_2\text{O}_2\text{S}_2(\text{C}_4\text{H}_8\text{O}_2\text{S}_2)_2]$ . Therefore,  $\text{Mo}_2\text{O}_2\text{S}_2$  appears to be predominant species, although species  $\text{MoO}_n\text{S}_{4-n}$  and  $\text{Mo}_2\text{O}_n\text{S}_{4-n}$  may be also present in an aqueous solution. Mitchell *et al.*[8] have also proposed the following reaction scheme:



Phosphate and borate buffers were used for the adjustment of pH. Optical absorption spectra were recorded by the use of a Shimadzu spectrophotometer, model Double-40R. The EPR sample was prepared by mixing of thiol ligand (5.0 M) and sodium molybdate (0.5 M), or thiol ligand (5.0 M), sodium molybdate (0.5 M) and sodium sulfide (or selenious acid, 0.5 M) at pH 8.5. EPR

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spectroscopy was performed by the use of a Japan Electron spectrometer (model ME-3X) operating with 100 KHz magnetic field modulation. The  $g$ -value was standardized using 2,2'-diphenylpicrylhydrazyl (DPPH) and the number of spins was estimated by comparison with standard  $K_3Mo(CN)_6$ . All EPR observations were carried out at room temperature.

## RESULTS AND DISCUSSION

**Optical characteristics of dithiol-molybdenum complexes and their sulfur and selenium complexes.** Table 1 summarizes the optical absorption characteristics of the dithiol-molybdenum complexes. Among these ligands, 1,2-ethanedithiol, dimercaptosuccinic acid and 1,3-propanedithiol formed very stable five- and six-membered chelates with molybdenum, and did not afford any spectral change upon the addition of sulfide. While, the molybdenum complexes of 1,4-butanedithiol and DTT, showed two visible peaks by the incorporation of sulfide. In these dithiol-molybdenum complexes, a blue shift of the absorption maxima at about 350 and 450 nm was linearly observed in accord with the increase of methylene numbers in the dithiol ligands. This spectral property is consistent with thiol ligand  $\rightarrow$  molybdenum charge-transfer assignment for these absorption bands.

Table 1. Spectral data of various dithiol-Mo complexes

	$\lambda_{max.}, nm (\epsilon, \times 10^3)^{c)}$	
a) 1,2-Ethanedithiol-Mo	397(2.0)	495(0.4)
1,3-Propanedithiol-Mo	375(2.2)	473(0.5)
1,4-Butanedithiol-Mo	345(3.2)	443(0.5)
1,4-Butanedithiol-Mo-S	325(2.7)	395(1.2)
1,5-Pentanedithiol-Mo	336(3.3)	422(0.4)
1,5-Pentanedithiol-Mo-S	325(2.9)	390(1.2)
1,6-Hexanedithiol-Mo	326(3.2)	412(0.3)
1,6-Hexanedithiol-Mo-S	320(2.6)	375(1.0)
b) Dithiothreitol-Mo	338(3.1)	440(0.5)
Dithiothreitol-Mo-S	320(3.1)	390(1.4)
Dithiothreitol-Mo-Se	335(3.2)	458(1.6)
Dimercaptosuccinic acid-Mo	395(2.2)	485(0.5)
2-Mercaptoethanol-Mo	335(2.8)	430(0.4)
2-Mercaptoethanol-Mo-S	325(3.2)	388(1.5)
1-Thioglycerol-Mo-S	337(3.0)	392(1.5)
1-Mercaptopropionylglycine-Mo	345(2.9)	440(0.4)
1-Mercaptopropionylglycine-Mo-S	325(3.0)	390(1.3)

a) These data were obtained by mixing dithiol ligand(300 mM) and  $Na_2MoO_4$  (1.0 mM), or dithiol ligand(300 mM),  $Na_2S$  (1.0 mM) and  $Na_2MoO_4$  (1.0 mM) in the presence of sodium dithionite in 50%(v/v) ethanol solution containing borate buffer(pH 8.8).

b) These data were obtained in an aqueous solution at pH 8.5.

c) Extinction coefficients were expressed per gram of Mo.

The relation between the difference in wavelength of absorption maxima and methylene numbers of the dithiols in dithiol-molybdenum and dithiol-iron[11] complexes, is shown in Fig. 1. This suggest that the affinity of sulfide ion is highest when the size of the chelate ring is eight in molybdenum complexes, whereas when that is seven in iron complexes. On the basis of the results on cysteine related compounds, Key *et al.*[8] indicated that the molybdenum(V) complexes of sulfur-donor ligands have strong peak at  $26000 \sim 30000\text{ cm}^{-1}$  and sulfur-bridged molybdenum(V) complexes show additional peak or shoulder at  $20000 \sim 24000\text{ cm}^{-1}$ . In contrast with thiol-iron complexes, the addition of selenide ion to most of thiol-molybdenum complexes induces release of elemental selenium. When selenide was added to DTT-molybdenum complex, however, DTT-molybdenum complex clearly incorporates selenide and

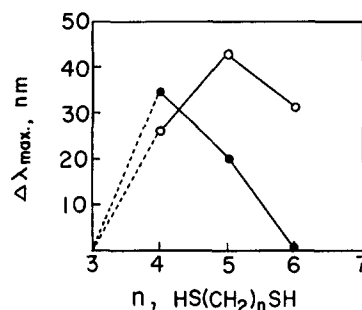


Fig. 1. Differences in wavelength of absorption maxima between dithiol-metal and dithiol-metal-sulfide complexes. ○, Mo complex; ●, Fe complex[11].

the absorption maxima were remarkably shifted toward longer wavelength compared with those of the sulfide complex. In the case of DTT-iron complexes, a red shift (25 nm) was observed upon selenide substitution of sulfide[12]. The test for exchangeability of the selenide with the sulfide in DTT-molybdenum-sulfide complex was carried out by the addition of selenide ion. In this system, the sulfide was predominantly substituted by the selenide. This strongly indicate that selenium atoms are more easily incorporated into DTT-molybdenum complex than sulfur atoms.

The solvent effect on the absorption spectra of DTT-molybdenum complexes was investigated in mixture of 80% organic solvent and 20% water (v/v). Any shift of the principal peak was not observed, regardless of presence or absence of sulfide (or selenide). On the contrary, in the case of DTT-iron complexes[12], the solvent-induced red shifts of the absorption peaks were proportionally observed to the dipole moment of organic solvents, and the environmental changes of the iron chromophore induced by organic solvents were suppressed by the coordination with sulfide or selenide. Of interest is the different behavior on the solvent effect between dithiol-iron and -molybdenum complexes, in connection with the stability of the metal chromophore.

**Stoichiometry and pH effects on the sulfide- and selenide-incorporation.** The ratio of molybdenum to sulfide (or selenide) in the dithiol complexes was determined by the molar ratio method. As shown in Fig. 2, equimolar sulfide or selenide to molybdenum was incorporated into DTT-molybdenum complex. The effects of pH on the incorporation of sulfide and selenide into DTT-molybdenum complex are shown in Fig. 3. The optimum pH range (pH 7.8 ~ 9.3) for the sulfide-

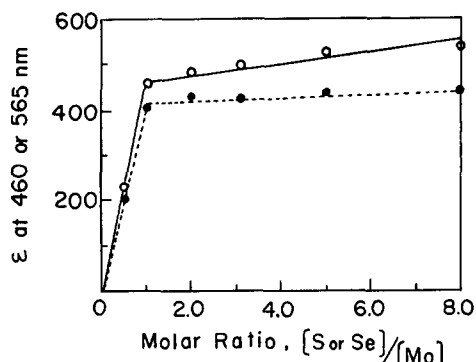


Fig. 2. Change in molar absorptivity with ratio of sulfide or selenide to molybdenum.

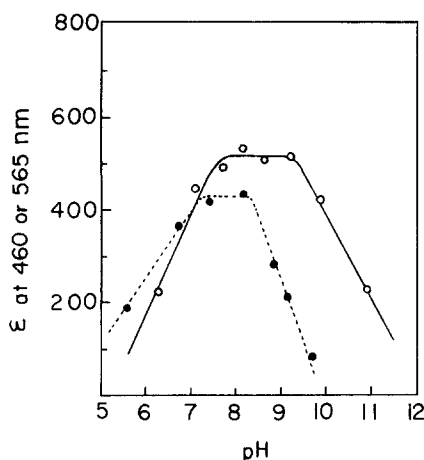


Fig. 3. Effect of pH on incorporation of sulfide (○) or selenide (●) into DTT-Mo complex.

incorporation is a little more alkaline than that (pH 7.3–8.2) for the selenide-incorporation. This may be attributed to the weaker acidity of hydrogen sulfide than hydrogen selenide.

**EPR spectra.** The solution of DTT-molybdenum complex showed a EPR absorption at  $g = 1.979$ , which would be attributed to pentavalent molybdenum ion (Fig. 4). Mitchell *et al.* have assigned signals at  $g = 1.97$ – $1.98$  to thiol-molybdenum(V) complexes and signals at  $g > 1.99$  to thiol-molybdenum(III) complexes [13].

At room temperature in liquid solution, the anisotropies are averaged to zero, and the EPR spectrum may be described by the following spin Hamiltonian.

$$\mathcal{H} = g\beta H \cdot S + aI \cdot S.$$

The average  $g$ -value and hyperfine splitting constant ( $a$ -value) are determined from the solution spectrum. Table 2 shows EPR parameters from DTT-molybdenum complexes, together with those of 1-MPG-molybdenum complexes. Intensities (i.e. electron spin per molybdenum) were calculated by the EPR signals with potassium octacyano-molybdate(V) as calibrant. EPR signal from DTT-molybdenum complex has larger  $g$ -value and smaller  $a$ -value than that from 1-MPG-molybdenum complex. This suggest that electron delocalization from molybdenum to thiol ligand is more extensive in the case of DTT in comparison with 1-MPG. Quantitative analyses revealed that the content of paramagnetic species in

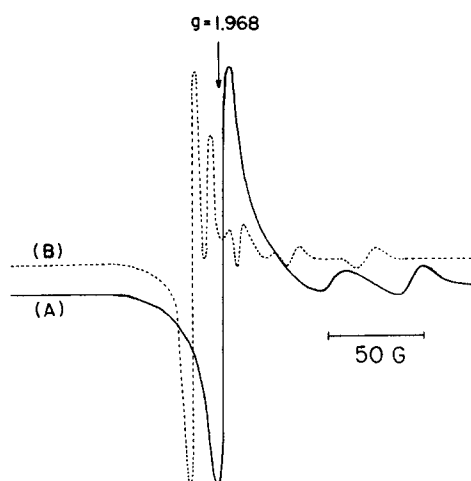


Fig. 4. EPR spectra of 1-MPG-Mo(A) and DTT-Mo(B) complexes. Conditions of EPR spectroscopy: microwave power, 6 mW; frequency, 9.280 GHz; modulation amplitude, 6.3 G; time constant, 0.3 sec; room temperature.

DTT-molybdenum(V) and 1-MPG-molybdenum(V) complexes never exceed 0.5 and 3% of the total molybdenum, respectively. Further, there were no EPR signals from the solid DTT- and 1-MPG-molybdenum complexes. The result of microanalysis suggests the following molecular formula for the isolated DTT-molybdenum complex, which is considered to be diamagnetic binuclear complex.



In an aqueous solution, hence, the binuclear DTT- and 1-MPG-molybdenum(V) complexes may dissociate in part into a paramagnetic, apparently mononuclear, molybdenum(V) species. It is well-known that an equilibrium between dimers and monomers exists in solution for cysteine-molybdenum(V) complex [14]. In addition, the  $g$ - and  $a$ -values observed for DTT- and 1-MPG-molybdenum complexes are very close to those of xanthine oxidase ( $g_{av} = 1.977$ ,  $a = 34$  G), except for the high proportion of paramagnetic molybdenum(V) species (ca. 50%) in the molybdenum-containing enzyme.

Upon the addition of sulfide (or selenide) to the solution of DTT- and 1-MPG-molybdenum(V) complexes, EPR signals disappear, based on the formation of binuclear disulfide(or diselenide)-bridged molybdenum(V) complex. The observation indicates that molybdenum(V) dimer

Table 2. EPR signals from thiol-molybdenum complexes

Complexes	EPR Signals <sup>a)</sup>			Electron Spin
	$g_{av}$	$a(G)$	$\Delta H(G)$	Molybdenum (%)
1-MPG-Mo <sup>b)</sup>	1.968	none		3
1-MPG-Mo <sup>c)</sup>		40	12	
1-MPG-Mo-S <sup>c)</sup>		none		
DTT-Mo <sup>b)</sup>	1.979	none		0.5
DTT-Mo <sup>c)</sup>		32	7	
DTT-Mo-S <sup>c)</sup>		none		
DTT-Mo-Se <sup>c)</sup>		none		

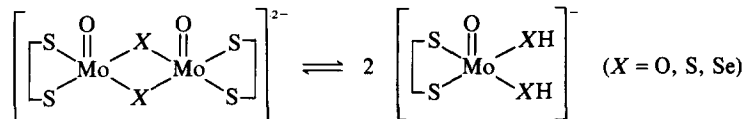
a) The  $g_{av}$ ,  $a$  and  $\Delta H$  represent average  $g$ -value,  $a$ -value ( $^{95,97}\text{Mo}$ -hyperfine splitting) and line width of major absorption, respectively.

b) solid

c) solution

d) Unfortunately, EPR spectrum of 1-MPG-Mo-Se complex was not obtained because of release of elemental selenium.

with sulfide or selenide bridges is less readily converted to paramagnetic monomer than dimer with oxide bridge. Further, the charge-transfer bands which appear to be characteristic of the  $\text{MoX}_2\text{Mo}$  group, shift to shorter wavelength in the order of  $\text{O} > \text{S} > \text{Se}$  in DTT-molybdenum complexes (see Table 1). In the following equilibrium, therefore, the dissociation of dimers presumably follows the order,  $\text{O} > \text{S} > \text{Se}$ .



In dithiol-molybdenum-sulfide(or selenide) complexes, the molybdenum-sulfur coordination structure would be similar to that of the iron-sulfur chromophore of  $2\text{Fe}-2\text{S}^*$  ferredoxins. Recently, one of us was successful in preparing the cobalt- and ruthenium-substituted proteins from adrenal iron-sulfur protein[15]. Metal substitution for iron in iron-sulfur proteins will provide useful information on protein specificity. On the basis of the present model reaction for sulfide- and selenide-incorporation into dithiol-molybdenum complexes, we are investigating the molybdenum substitution of adrenal iron-sulfur protein.

**Acknowledgement**—We are grateful to Prof. K. Ishizu for valuable discussion and helpful advice in EPR measurements.

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