as was explained in the section on the computation of ϕ_b, ϕ_b for the mixture M was obtained as the average of the values for P and Q. Strictly, it should be given by the average of the values for P′ and Q′. Numerically, however, the difference between these average values is negligible.

About the numerical value of ΔG_a^∞ little can be said at present since the chemical groupings on the surface of contact between subunits are not known. If such information becomes available, through crystal structure determinations, the value of ΔG_a^∞ will serve as a test of our ability to calculate intermolecular forces. In that connection, of course, it would be advantageous to determine ΔH_a^∞ and ΔS_a^∞ from, say, the temperature dependence of ΔG_a^∞. To that end, however, extensive further experimentation is required.

Characterization and Triplet-State Electron Paramagnetic Resonance Spectra of the Binuclear [Molybdenum(V)]$_2$–Glutathione Complex. An Example of Molybdenum–Polypeptide Complexes

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Abstract: A diamagnetic binuclear [Mo(V)]$_2$–glutathione complex, Na[Mo_2O_4(glutat)(H_2O)]·$3H_2O$, has been isolated and characterized. Infrared analysis indicates that glutathione serves as a pentadentate ligand. Epr studies in 0.2 *M* phosphate buffer solution over a pH range from 8 to 10 show that Mo(V) with glutathione forms a diamagnetic binuclear species in which the Mo_2O_4 group dissociates to form a paramagnetic binuclear species. An equilibrium between these two species exists in solution. The 11-line hyperfine splitting observed using isotopically enriched ^{95}Mo(V) has given unambiguous indication of electron-exchange coupling in the paramagnetic binuclear [Mo(V)]$_2$–glutathione complex. Under the imperfect assumption of axial symmetry, the relevant epr parameters of this paramagnetic complex are $g = 1.962$, $g_\parallel = 1.966$, $g_\perp = 1.960$, $a = 0.0029\ \text{cm}^{-1}$, $A = 0.0047\ \text{cm}^{-1}$, $B = 0.0020\ \text{cm}^{-1}$, and $D = 0.0083\ \text{cm}^{-1}$. From the zero-field splitting constant, *D*, and molecular models, the Mo–Mo distance is estimated as ~6.0 Å. A possible structure based on the intermetallic distance is proposed for the paramagnetic [Mo(V)]$_2$–glutathione complex. The biological implications for molybdenum-containing enzymes is also discussed.

Molybdenum–sulfur bonding[1–6] has been of great interest to scientists because of its biological implications. As part of our study on the molybdenum–amino acid complexes[5,6] as possible models for molybdenum–enzyme interaction, we have used glutathione, a cysteine-containing short polypeptide (γ-L-glutamyl-L-cysteinylglycine), as a ligand for our present investigation. Our previous study[5,6] on the Mo(V)–cysteine complex, $Na_2Mo_2O_4(cyst)_2\cdot 5H_2O$, indicated that the dioxo bridge can be broken by the attack of OH^- in solution, or by heat in the solid state, to form a monomeric species which gives rise to an epr signal. The lability of the dioxo bridge is created by the sulfhydryl group of the polydentate ligands and enhanced by increasing the chain length of the ligand. According to these results, glutathione appears to be a better simple ligand for this type of study.

A recent study of molybdenum(V)–thiol complexes[2] reported that no epr signal could be observed from an aqueous solution containing Mo(V) and glutathione. On the contrary, we have obtained well-defined signals from this system. Moreover, although considerable attention has been devoted to the epr study of paramagnetic binuclear complexes, such as vanadyl–tartrate[7–11] and copper–tartrate complexes,[9] no report has been made on paramagnetic molybdenum binuclear species. Here, we report the existence of a paramagnetic binuclear [Mo(V)]$_2$–glutathione complex for the first time.

In this study, a diamagnetic binuclear [Mo(V)]$_2$–glutathione complex has been isolated and characterized. The ir analysis indicates that glutathione serves as a pentadentate ligand. Epr studies show that glutathione with Mo(V) forms a diamagnetic dimer in which the Mo_2O_4 group dissociates to form a paramagnetic dimer. Its electron paramagnetic resonance spectra show that electron-exchange coupling occurs in this complex. Hyperfine splitting observed using isotopically enriched ^{95}Mo gives unambiguous indication of such coupling. The triplet state of this complex is shown to exist both in the frozen-solution epr spectrum and in the liquid-phase epr spectrum.

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Table I. Some Characteristic Ir Bands of Glutathione, $[Mo(V)]_2$–Glutathione Complexes, and Some Related Compounds

Frequency, cm^{-1}				
Glutathione	Mo(V)–glutathione	Mo(V)–cysteine[b]	Mo(V)–histidine[c]	Assignment
	3350	3430	3430	OH str (lattice water)
3130	3220	3110	3260	NH_2 str
3030		(3210)[a]	3210	
	2950	2990		OH str (coord water)
2530				SH str
1620	1635	1590	1660	COO^- str
1530			1590	
	965	955	941	Mo═O
		(970, 945, 925)[a]		
	730	735	750	Mo—O—Mo antisym str
		(730)[a]		
	470	(425)[a]	475	Mo—O—Mo sym str

[a] Reference 4. [b] Reference 3. [c] Reference 13.

Experimental Section

Materials. Stock solutions of $Mo(V)_2$ were prepared by the reduction of Na_2MoO_4 by shaking over Hg in 3 *N* HCl.[12] Glutathione and hexachloropropene were obtained from Matheson Coleman and Bell. Isotopically enriched $^{95}MoCl_5$ was synthesized by the method previously described.[6]

Preparative Procedure. The diamagnetic $[Mo(V)]_2$–glutathione complex was prepared by the following method. Glutathione (0.23 g) was dissolved in 50 ml of deaerated water at pH 7 (the pH of the water solution was adjusted with 3 *N* NaOH before addition of glutathione). Upon adding 2.5 ml of 0.5 *M* Mo(V) in a 3 *N* HCl solution to the above solution, a reddish brown solid formed; however, it redissolved when 2.5 ml of 3 *N* NaOH was added to bring the solution back to pH 7. Several minutes later, precipitation occurred again. The reaction took place in a specially designed flask under a stream of argon. On standing at room temperature for 2 hr, the dark brown solid was separated from the solution and discarded. The supernatant was treated with an equal volume of ethanol and the resulting mixture was cooled down to ice temperature under a stream of argon. Finally, the solution deposited a light brown solid which was filtered, washed with 1:1 water–ethanol solution, and then dried in air. The compound is susceptible to air oxidation in solution; however, it is extremely stable in its solid powdered form in air. For this synthesis, a slight excess of glutathione with the Mo:glutathione ratio of about 2:1 was employed to keep Mo(V) in the complex form as much as possible. Satisfactory analyses for the diamagnetic light brown complex were obtained. *Anal.* Calcd for $NaMo_2C_{10}H_{22}O_{14}N_3S$ (formula wt = 655): Mo, 29.30; C, 18.30; H, 3.35; N, 6.41. Found: Mo, 30.00; C, 18.40; H, 3.37; N, 6.22.

Attempts to grow single crystals from the isolated powdered sample for X-ray analysis were unsuccessful. Since epr studies show that an equilibrium between the diamagnetic and paramagnetic binuclear complexes exists in solution at pH 8–10, efforts to isolate the pure paramagnetic species were made; however, they failed. All we were able to isolate at that pH range was a mixture of both species.

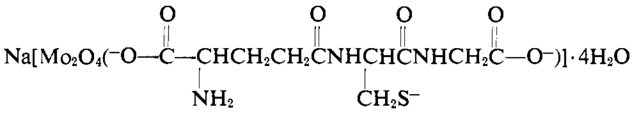
Electronic and Infrared Spectra. Since Mo(V) can be readily oxidized by air, a special apparatus[6] was used to obtain an oxygen-free sample solution for measurements of electronic spectra. Infrared and electronic spectra were determined with Perkin-Elmer Model 521 and Cary Model 14 spectrophotometers, respectively.

Epr Measurements. The samples for epr measurements were prepared in the following manner. The 0.2 *M* phosphate buffer at desired pH was deaerated with argon (99.996% purity) and a specific amount of ligand was dissolved in the buffer. A deaerated solution of Mo(V) in 3 *N* HCl (with an equal volume of deaerated 3 *N* NaOH for neutralization) was then added to start the reaction. Samples for epr measurements were removed periodically with a gas-tight syringe through a rubber diaphragm and frozen in liquid nitrogen in 3-mm i.d. bore quartz tubes under argon. The concentrations of Mo(V) and glutathione were of the order of 10^{-3} and 10^{-2} *M*, respectively. The solubility problem of Mo(V) in slightly basic solutions were overcome by employing excess ligand. A low concentration of Mo(V) was used in order to slow down the rate of any possible reduction of Mo(V) by the ligand within the time of the study.

All epr spectra were recorded with a Varian V-4502 X-band spectrometer, equipped with an automatic temperature controller, using 100-kc modulation. The *g* values were determined by comparison with the signal of powdered DPPH radical ($g = 2.0036$). For the study of reversible temperature dependence of the epr signal of the glutathione complex in solution at temperatures above 25°, a glass capillary was used as a sample cell. For the quantitative computation, the first-derivative signal was doubly integrated and compared with that of the standard compound, $K_3Mo(CN)_8$. All epr measurements on frozen samples were made at 77°K.

Results

Microanalysis. The results of microanalyses suggest the following molecular formula for the diamagnetic binuclear complex.



$$Na[Mo_2O_4(^-O-\overset{\overset{O}{\|}}{C}-\underset{\underset{NH_2}{|}}{C}HCH_2CH_2\overset{\overset{O}{\|}}{C}NH\underset{\underset{CH_2S^-}{|}}{C}H\overset{\overset{O}{\|}}{C}NHCH_2\overset{\overset{O}{\|}}{C}-O^-)]\cdot 4H_2O$$

Infrared Spectra. The characteristic ir bands of the isolated diamagnetic $[Mo(V)]_2$–glutathione complex in a KBr pellet, with their assignments, are shown in Table I. The interesting features are those bands at 965, 730, and 470 cm^{-1}, which are assigned to Mo═O, Mo—O—Mo antisymmetrical, and Mo—O—Mo symmetrical stretching modes, respectively. Complexes of Mo(V) with amino acids[3,4,13] and other ligands[14,15] are reported to show the Mo═O stretching mode in the 900–1000-cm^{-1} region, the Mo—O—Mo antisymmetrical stretching mode in the 720–730-cm^{-1} region, and the Mo—O—Mo symmetrical stretching mode in the 400–500-cm^{-1} region. The ligand bands of glutathione are all sharp and well resolved, whereas, in the complex, the ligand bands are considerably broadened. The same phenomenon was also observed in zinc–[16] and Mo(V)–cysteine[4] complexes. As compared to those of glutathione, the shifts or the absence of stretching frequencies of $-NH_2$, -SH, and $-COO^-$ groups reflect that these groups are coordinated to Mo(V). The presence of both coordinated and lattice water is suggested by O–H stretching vibrations at 2950 and 3350 cm^{-1}. The infrared spectrum does not tell whether the -NH- groups are coordinated to Mo(V). However, with the aid of the molecular model, we have assumed that the -NH- group in

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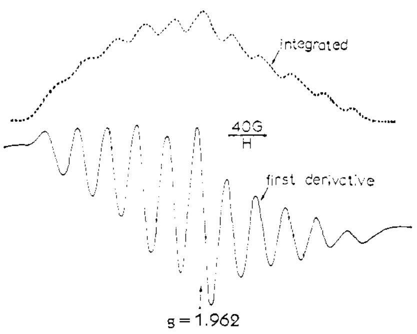

Figure 1. First-derivative epr spectrum of isotopically enriched $[^{95}Mo(V)]_2$–glutathione complexes at room temperature. $[Mo(V)]_t \approx 10^{-3}$ *M* and [glutathione] $\approx 10^{-2}$ *M*.

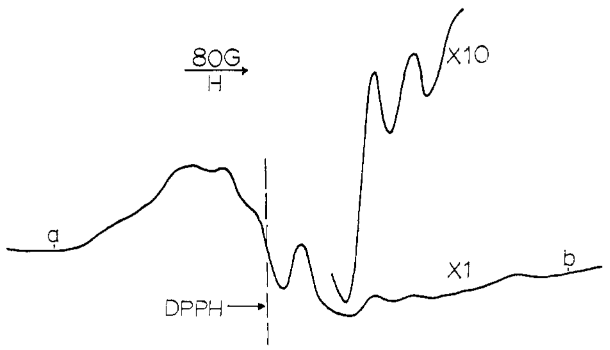

Figure 2. First-derivative epr spectrum of isotopically enriched $[^{95}Mo(V)]_2$–glutathione complexes at 77°K. $[Mo(V)]_t \approx 10^{-3}$ *M* and [glutathione] $\approx 10^{-2}$ *M*.

the glycine unit is coordinated to Mo(V), in order to complete a coordination number of six for both Mo(V) nuclei.

Electron Paramagnetic Resonance. The formation of paramagnetic species was studied in 0.2 *M* phosphate buffer over a pH range from 6 to 12. Samples were prepared directly from Mo(V) and glutathione in solution. Notice that free Mo(V) gives no epr signal in solution at this pH range because of rapid dimerization.

Mo(V) with glutathione, in solution, forms a light orange-brown complex at the concentrations employed. The epr signal was too weak to be observed in the first 15 min; however, the intensity was strengthened with increasing time and, finally, a well-defined epr signal could be obtained at 1–6 hr. This indicates possibly that a diamagnetic species is formed first, which then dissociates into a paramagnetic species. Well-defined epr signals were observed only at $8 \leq pH \leq 10$. Within this pH range, strengths of signals increase with increasing pH, reach a maximum at pH 9, and then fall off as pH is further increased. Since the solution spectra are better resolved at pH 8, most of the epr experiments were carried out at pH 8. No study was made after 20 hr because of the possible reduction of Mo(V) by the thiol-containing ligands.

Quantitative epr analyses revealed that the content of paramagnetic species never exceeds 4% of the total molybdenum. Integrated epr signals were proportional to the $[Mo(V)]_2$ concentration and independent of excess ligand. Signal intensities increase reversibly with increasing temperature, as shown in Table II.

Table II

Temp, °C	Signal height[a]	Temp, °C	Signal height[a]
25	10	75	36
45	18	65	28
65	29	45	20
75	34	25	10
93	43		

[a] Arbitrary units.

The solution epr spectrum of isotopically enriched $[^{95}Mo(V)]_2$–glutathione complexes is shown in Figure 1. The spectrum with 11 hyperfine lines is the spectrum expected from an electron interacting with two magnetically equivalent molybdenum nuclei of spin $^5/_2$. For such a system, the areas under the peaks should be in the order 1:2:3:4:5:6:5:4:3:2:1, and the peak heights obtained upon integrating the epr first derivative curves do, indeed, qualitatively follow this theoretically expected order. Unfortunately, contamination in the epr cavity itself gave a broad hump on the lower field part of the first-derivative spectrum. The interference from this hump on the lower field part of the integrated curves is clearly demonstrated. However, the intensities of those curves on the higher field part of the integrated spectrum follow the expected values reasonably well. The actual relative magnitudes cannot be evaluated because of the indeterminate areas as a result of overlap.

The isotropic *g* value and hyperfine splitting constant, *a*, are determined from the solution spectrum in Figure 1. No second-order correction was made to obtain *g* and *a*, since the spacings between each two neighboring peaks are approximately equal.

When the complex is prepared from naturally occurring Mo(V), the 11 satellites due to $^{95,97}Mo$ (nuclear spin $(I) = {}^5/_2$; natural abundance, 25%) are too weak to be observed experimentally. Presumably, they are covered by the broad intense line due to $^{94,96,98}Mo$ ($I = 0$; natural abundance, 75%), since the intensity of each of the 11 satellites is only 3% (theoretical value) of that of the line arising from $^{94,96,98}Mo$. Introduction of isotopically enriched $^{95}Mo(V)$ is necessary in this case in order to obtain the hyperfine information.

The frozen-solution epr spectrum of the isotopically enriched $[^{95}Mo(V)]_2$-glutathione complex at 77°K is shown in Figure 2. Unfortunately, no resolved spectrum could be obtained. Owing to the line broadening, a complete analysis of this spectrum is impossible to make. A similar, but worse, line broadening has been observed in the triplet-state binuclear vanadyl–tartrate complex.[10] Since frozen-solution spectra may give a measure of the magnitude of the zero-field splitting, an effort was made to obtain a well-resolved frozen-solution spectrum by using naturally occurring Mo(V) from which only peaks due to $^{94,96,98}Mo$ ($I = 0$) show up. Apparently, in the absence of nuclear spin, the spectrum is better resolved (Figure 3). This complicated spectrum suggests that there are two transitions which are expected for a triplet-state binuclear complex.[8,17] In order to obtain the structural information (see the Discussion section) regarding the paramagnetic species from the zero-field splitting constant (*D*), which can be determined from the frozen-solution spectrum, an im-

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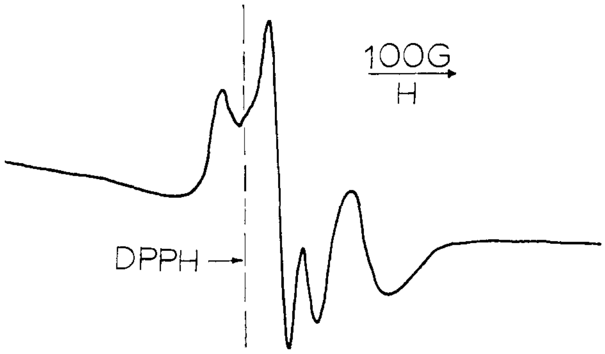


Figure 3. First-derivative epr spectrum of $[Mo(V)]_2$–glutathione complexes at 77°K. Naturally occurring Mo(V) was used. $([Mo(V)]_t \approx 10^{-3}\,M$ and [glutathione] $\approx 10^{-2}\,M$.

perfect assumption of axial symmetry ($g_{xx} \approx g_{yy}$) is made to simplify the analysis of the spectrum.

If we assume an axially symmetric system, the appropriate $S = 1$ spin Hamiltonian can be written as follows

$$\mathcal{H} = \beta[g_{\parallel}H_zS_z + g_{\perp}(H_xS_x + H_yS_y)] + AS_zI_z + B(S_xI_x + S_yI_y) + D[S_z^2 - S(S+1)/3] \quad (1)$$

where all symbols have their usual meaning.

The spectrum at $H \sim 3400$ G consists of the overlapping low- and high-field parallel and perpendicular components, with the high- and low-field parallel lines separated by approximately $2D$.

Although the position of the high-field parallel component is uncertain, we assume the two outermost peaks as the low- and high-field parallel components and then determine the $g_{\parallel}$ and D values from parallel lines through Bleaney's equation[18] for the field direction along the symmetry axis

$$\omega_0 = g_{\parallel}\beta H + 2D(M_s - {}^1/_2) + AM_I + (B^2/\omega_0)[I(I+1) - M_I{}^2 + (2M_s - 1)M_I] \quad (2)$$

which becomes the following simple form for the case (Figure 4) where nuclei have no spin.

$$\omega_0 = g_{\parallel}\beta H + 2D(M_s - {}^1/_2) \quad (3)$$

where $I = 5$; $M_s = 1, 0$; $M_I = +5, +4, \ldots, 0$; and ω_0 is the radiation frequency.

If the splitting on the upper curve of Figure 3 is considered as A, the B value, calculated on the basis of $a = (A + 2B)/3$, is less than A, which is in agreement with the fact that $A > B$ for most paramagnetic Mo(V) complexes. Finally, $g_{\perp}$ is determined from the relation $g = (g_{\parallel} + 2g_{\perp})/3$. The resultant epr parameters listed in Table III are obtained on the basis of the above uncertainty. The isotropic a value listed in Table III corresponds to the hyperfine splitting in the following spin Hamiltonian for the complex in solution, where dipolar interactions are averaged to be zero because of rapid molecular tumbling.

$$\mathcal{H} = g\beta\vec{H}\cdot\vec{S} + a\vec{I}\cdot\vec{S} \quad (4)$$

Table III. Epr Parameters of the $[Mo(V)]_2$–Glutathione Complex

g	a, cm^{-1}	$g_{\parallel}$	$g_{\perp}$	A, cm^{-1}	B, cm^{-1}	D, cm^{-1}
1.962	29×10^{-4} (32 G)[a]	1.966	1.960	47×10^{-4} (51 G)[a]	20×10^{-4}	83×10^{-4}

[a] Experimentally observed separation of hyperfine components.

According to the fact that the separation between the two perpendicular components is approximately equal to half that between the two parallel components, the positions of these two perpendicular components calculated on the basis of those parameters listed in Table III are in good agreement with the experimental observation in Figure 3. Applying the same computation to the case in which an isotope with nuclear spin was used (Figure 2), all hyperfine lines would fall in the magnetic field ranging from point a to point b, shown in Figure 2. Again, it is in good agreement with the experimental spectrum, although a detailed comparison cannot be made because of the line broadening in Figure 2.

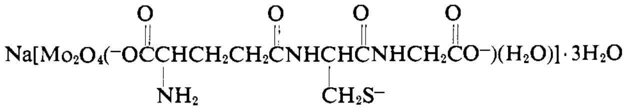
Attempts were made to use other assumptions to assign the high-field parallel component in Figure 3 for determining epr parameters; however, no better results could be obtained. Therefore, the set of epr parameters listed in Table III is considered to be as good as we can obtain within the assumption of symmetry.

Electronic Spectra. The buffer solution containing Mo(V) and no glutathione at pH 8 gives an absorption maximum at 301 nm. The $[Mo(V)]_2$–glutathione complex shows an absorption maximum at 303 nm. (ϵ ~7000) with a weak shoulder at about 580 nm. (ϵ ~75 on the basis of the dimer concentration) in phosphate buffer at pH 8–10. No such shoulder was observed in solution at pH $\leqslant 8$ where no epr signal was found. The weak transition at 580 nm probably arises from the chemical bonding between molybdenum(V) and cysteine residue in the paramagnetic species, since this weak transition was also observed in the Mo(V)–cysteine complex[6] at pH >8 and has been tentatively assigned to a transition in the paramagnetic species which is in equilibrium with the diamagnetic dimer. Notice that the same transition at 580 nm was observed and tentatively assigned to the Mo(V) chromophore in the xanthine oxidase[19] which contains Mo(V) and a cysteine residue.

When the solution containing $[Mo(V)]_2$–glutathione was exposed to air, the light orange-brown color of the solution faded gradually and eventually turned colorless, reflecting the oxidation of Mo(V) to Mo(VI) by air.

Discussion

Structure of the Isolated Diamagnetic Binuclear Complex. The results of the infrared analysis, with aid of the molecular model, indicate that glutathione may serve as a pentadentate ligand. On this basis, the structure shown in Figure 4 is proposed for the diamagnetic binuclear $[Mo(V)]_2$–glutathione complex. Therefore, the molecular formula should be properly written as follows



$$\mathrm{Na[Mo_2O_4(^{-}O\overset{O}{\overset{\|}{C}}\underset{NH_2}{\underset{|}{C}}HCH_2CH_2\overset{O}{\overset{\|}{C}}NH\underset{CH_2S^-}{\underset{|}{C}}H\overset{O}{\overset{\|}{C}}NHCH_2\overset{O}{\overset{\|}{C}}O^{-})(H_2O)]\cdot 3H_2O}$$

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(18) B, Bleaney, *Phil. Mag.*, **42**, 441 (1951).

(19) K. Garbett, R. D. Gillard, P. F. Knowles, and J. E. Stangroom, *Nature (London)*, **215**, 824 (1967).

Table IV. Intermetallic Distances from Zero-Field Splitting Parameters[a]

Binuclear complex	10^4D_{exptl}, cm^{-1}	10^4D_{pseudo}, cm^{-1}	R_{calcd}, Å[b]	R_{calcd}, Å[c]	R_{exptl}, Å[d]
$(VO)_2(d\text{-tart})_2{}^{4-}$	−335	<10	>4.15	4.19	4.35
$(VO)_2(d\text{-tart})(l\text{-tart})^{4-}$	∼−336	<10	>4.15	∼4.19	4.08
$Cu_2(C_5H_4N_5)_4 \cdot 4H_2O$	−1210 ± 50	130	2.84	2.92	2.95
$Cu_2(d\text{-tart})(l\text{-tart})^{4-}$	−572			3.85 (3.3)	3.4[f])
$[Mo(V)]_2$–glutathione[e]	83			6.0 (θ = 0°) 4.8 (θ = 90°)	(6.1[f]) (θ = 0°) (5.5–9.0[f]) (θ = 90°)[g]

[a] For the data listed in this table, see ref 9 and references cited therein. [b] Calculated from eq 6 and $D_{dd} = D_{\text{exptl}} - D_{\text{pseudo}}$. [c] Calculated from eq 6 and $D_{dd} = D_{\text{exptl}}$. [d] Metal–metal distances from X-ray data. [e] This work. [f] Estimated from molecular models. [g] Since a rotation along the C_2–C_3 axis (see Figure 5a) does not change θ too much, R_{exptl} can vary from 5.5 to 9.0 Å.

Equilibrium between Diamagnetic and Paramagnetic Binuclear Complexes. Our previous study[6] showed that the lability of the dioxo bridge in the physiological pH region is created by the sulfhydryl group of the polydentate ligands and enhanced by increasing the chain length of the ligand. Glutathione does, indeed, create stronger epr signals than cysteine does.

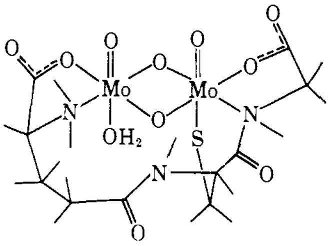

Figure 4. Proposed structure for the isolated diamagnetic binuclear $[Mo(V)]_2$–glutathione complex.

The reversible temperature dependence of the epr signals suggests two possibilities. First, an equilibrium exists between the diamagnetic and paramagnetic binuclear species, or second, thermal excitation of electrons occurred in the diamagnetic binuclear complex. In view of the slow growth of epr signals at a certain temperature in solution and the absence of epr signals from the diamagnetic species at the temperature range studied in solid state, the second possibility seems unlikely. Thermal excitation of electrons does not occur in the diamagnetic binuclear Mo(V)–cysteine complex either.[6] The first possibility is further supported by the epr results, including the 11 hyperfine lines and the low content of paramagnetic binuclear species.

The pH dependence of epr signals and the fact that intensities of epr signals are proportional to the $[Mo(V)]_2$ concentration and independent of excess glutathione are consistent with the following equilibrium

$$\underset{\text{(see Figure 4)}}{M_2L\ \text{(diamagnetic)}} \overset{OH^-}{\rightleftharpoons} \underset{\text{(see Figure 5b)}}{M_2L\ \text{(paramagnetic)}}$$

where M is Mo(V) and L is glutathione. It must be noted, however, that strong base destroys the paramagnetic species.

For a triplet-state binuclear complex, a very weak (magnetically allowed) half-field "forbidden" transition (at $H \sim 1600$ G) is expected.[8,9] No such forbidden transition ($\Delta M_s = 2$) was observed in the paramagnetic $[Mo(V)]_2$-glutathione complex. This could be interpreted in terms of small zero-field splitting. In general, the intensity of the forbidden transition is less than one hundredth of those of the full-field ($H \sim 3400$ G) allowed transitions. Attempts to increase $[Mo(V)]_2$ concentration to strengthen the intensity of the forbidden transition failed because of poor solubility of Mo(V) in basic solution.

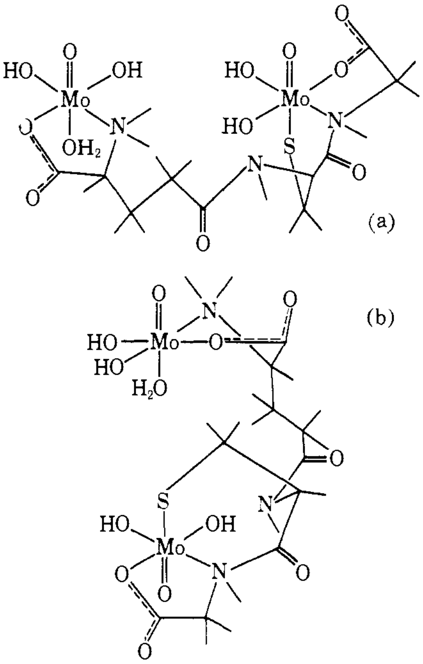

Figure 5. Proposed structures for the paramagnetic binuclear $[Mo(V)]_2$–glutathione complex.

The separation between the two full-field allowed transitions is governed by the magnitude of the zero-field splitting.[17,18] With the larger D values in copper and vanadyl complexes, these two transitions are reasonably well resolved.[8,9] With the small D value in $[Mo(V)]_2$-glutathione complexes (see Table IV), these two transitions overlap to give a complicated spectrum, as shown in Figure 3, which makes the analysis difficult.

Structural Information from Zero-Field Splitting for the Paramagnetic Species. Since the D value provides a reasonable measurement of intermetallic distances which may give structural information for the paramagnetic species, an admittedly imperfect assumption of axial symmetry was made for this system so that the D value and the metal–metal distance could be obtained without exceedingly sophisticated computation. (It is clear that accurate hyperfine and zero-field splitting parameters cannot safely be extracted from these spectra without an analysis of considerable sophistication, including a theoretical simulation of the frozen-solution spectra with general noncoincident axes for all of the magnetic interaction parameters. Nevertheless, the results of the epr on the copper[9] and vanadyl[8] tartrates, as shown in Table IV, suggest that the error in the derived epr parameters under the axial symmetry approximation is not very large.)

The magnetic dipolar interaction between two electron spins is expressed by the equation[20]

$$D_{\mathrm{dd}} = (^3/_4)g^2\beta^2\left\langle\frac{1 - 3\cos^2\theta}{r_{12}^3}\right\rangle_{\mathrm{max}} \qquad (5)$$

where r_{12} is the interelectronic distance and θ is the angle between the r_{12} vector and the magnetic field direction. Assuming that θ equals the angle between the Mo–Mo direction and the magnetic field and $1/\langle r_{12}^3\rangle = 1/R^3$, R being the Mo–Mo distance, we obtain

$$R_{\mathrm{calcd}} = (0.325g^2|1 - 3\cos^2\theta|/D_{\mathrm{dd}})^{1/3} \qquad (6)$$

According to the two most stable expected configurations of glutathione from the molecular models, if we take H to be along the Mo=O direction, θ may be ~0 or ~90°. For $\theta = 0°$ (see Figure 5b), R_{calcd} is found to be 6.0 Å, using eq 6. Similarly, R_{calcd} is found to be 4.8 Å for $\theta = 90°$ (see Figure 5a). As a comparison of R_{calcd} with R_{exptl} which is estimated from the molecular model (see Table 4), a better agreement is obtained when $\theta = 0°$. Therefore, the structure shown in Figure 5b is proposed for the paramagnetic binuclear $[\mathrm{Mo(V)}]_2$–glutathione complex.

As a matter of fact, both magnetic dipole–dipole interactions and pseudodipolar terms can contribute to the zero-field splitting constant, $D_{\mathrm{exptl}} = D_{\mathrm{dd}} + D_{\mathrm{pseudo}}$. But often (see Table IV) the pseudodipolar contributions to the zero-field splitting are negligible, although in some cases they are large. For our case, since there is no way to estimate D_{pseudo} because of the unknown energy separation between the singlet ground state and the first triplet state, the R_{calcd} value is calculated from eq 6 and $D_{\mathrm{dd}} = D_{\mathrm{exptl}}$. It must be noted that, in the case where the pseudodipolar contributions are not negligible, the R_{calcd} value obtained from eq 6 and $D_{\mathrm{dd}} = D_{\mathrm{exptl}}$ is in good agreement with R_{exptl} obtained from X-ray data (see Table IV).

Biological Implication. The striking feature of this system is that the complex meets the requirements of having two molybdenums coordinated by a biologically important sulfur-donor ligand and of having a paramagnetic species, established for xanthine oxidase (a molybdenum-containing enzyme) by epr measurements.[2,21,22] Moreover, the absorption shoulder at 580 nm of this complex could well correspond to the 580-nm transition of xanthine oxidase, which was tentatively attributed to molybdenum chromophore.

No spectra with 11 hyperfine lines have been reported for the molybdenum-containing enzymes. This does not exclude enzymatic molybdenum–molybdenum interaction, however, since if the dipole–dipole interactions (or electron-exchange rate) in the enzyme are considerably smaller (or lower) than in this complex, an epr spectrum with six hyperfine lines would be observed. If tumbling of the large molecules is sufficiently slow in solution, only a single broad line will result. From the nature of the macromolecule (apoenzyme) point of view, this seems very possible, since the two molybdenum(V) nuclei in the enzyme, after the breaking of the dioxo bridge, may be oriented, owing to the long chain length of the apoenzyme (ligand), in such a way that no electron exchange or no dipole–dipole interaction can occur.

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