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Formation and Dimerization of Molybdenum(V) Monomer in Aqueous Solution

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Received February 27, 1967

Epr studies have shown molybdenum(V) monomer has a finite lifetime in aqueous solutions of acidity ranging from 2 *M* HCl to pH 6.0. The Mo(V) monomer was obtained by reduction of Mo(VI) with various reducing agents *in situ* or by addition of $(NH_4)_2MoOCl_5$. The rate of disappearance of the monomer has been studied in tartrate buffer as a function of pH and buffer concentration and a mechanism to explain the data has been proposed. The tartrate:Mo(V) ratio of the dimer complex was found to be 2:1, while the ratio for the monomer appears to be 3:1.

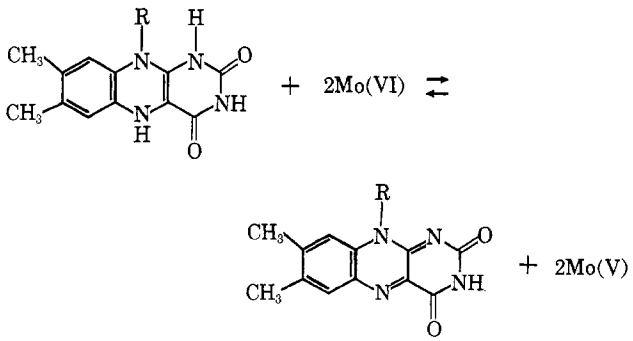
It is now well established that molybdenum is a necessary cofactor for a number of redox enzymes. Recent studies, for example, of xanthine oxidase (a molybdenum flavoenzyme) have shown that an epr signal which can be assigned to Mo(V) (a $_1$d ion) is observed in the presence of substrate.[2]

It is generally assumed that Mo(V) exists as a diamagnetic dimer in aqueous solutions in which the hydrogen ion concentration is less than 2 *M*.[3] Magnetic susceptibility and epr measurements have shown that in 4–10 *M* HCl both a monomer and a paramagnetic dimer exist. Above 10 *M* HCl only the monomer occurs, while below 2 *M* HCl all paramagnetism disappears.[3] Recent studies, however, have indicated an epr signal for Mo(V) is observed in aqueous solutions of certain thiols at pH near neutrality.[4]

During the course of an investigation of the reaction between flavins and the Mo(VI)–Mo(V) redox system at pH 6–8, an unexpected epr signal due to Mo(V) was found. The appearance of this signal has now been studied under conditions of acidity ranging from 2 *M* HCl to pH 6.0. The studies indicate the Mo(V) monomer species has a finite lifetime over a wide pH range, a result of interest for both inorganic and biochemistry.

Results

Preliminary work indicated Mo(V) monomer is formed in the reduction of Mo(VI) by reduced flavins at pH 6–7



The signal (Figure 1) was identified by its *g* value (1.92) and by comparison with a signal obtained in the buffer without flavin (see below). The Mo(V) signal was found to reach a maximum and then decay as the reaction progressed. The disappearance of the signal appears to be due to the formation of a diamagnetic dimer

$$2Mo(V) \rightleftharpoons Mo(V)_2$$

The signal at $g = 2.00$ in Figure 1 is due to flavin semiquinone, also formed as an intermediate in the reaction.

An epr signal was also observed using other reductants ($Na_2S_2O_4$, $NaBH_4$, $TiCl_3$, Hg in 2 *M* HCl) under various conditions of pH and buffer, thus eliminating the possibility that it is due to a Mo(V)–flavin semiquinone complex (such complexes are known[5] for diamagnetic metal ions such as Zn^{2+} and Cd^{2+}). Furthermore, recent work has shown that a Mo(V)–flavin semiquinone complex does indeed exist, but it is epr inactive.[6]

Conditions were sought whereby Mo(VI) could be essentially instantaneously reduced, thus producing a maximum concentration of Mo(V) monomer. This was found to occur in tartrate buffer using $TiCl_3$ as reductant. The *g* value of this signal in tartrate (1.94), its asymmetric shape (Figure 1), and the hyperfine splitting due to the nuclear abundance (25.3% Mo^{95} and Mo^{97} combined, nuclear spin $^5/_2$) are similar to those for $MoCl_5$ in concentrated HCl.[2] Signals for Mo(V) were also obtained by reduction of Mo(VI) in 1–2 *M* HCl, in 10^{-2} *M* HCl, in phosphate buffer at pH 2.0 and 6.0, in gluconate buffer at pH 2.90, and in citrate buffer at pH 6.10, indicating that reduction generally proceeds *via* the monomer. The same signals were obtained by addition of a solution of $(NH_4)_2MoOCl_5$ in 10 *M* HCl to the appropriate buffer. The Mo(V) monomer was also generated from Mo(VI) electrolytically in the epr cavity using a silver anode and a mercury cathode (Figure 2).

As expected for dimerization, the disappearance of

(1) Abstracted in part from the M.S. Thesis of M. Heydanek, Utah State University, 1966. Journal Paper No. 658, Utah State Agricultural Experiment Station.

(2) H. Beinert, R. C. Bray, and G. Palmer, *J. Biol. Chem.*, **239**, 2657 1964).

(3) C. R. Hare, I. Bernal, and H. B. Gray, *Inorg. Chem.*, **1**, 831 (1962).

(4) L. S. Meriwether, W. F. Marzluff, and W. G. Hodgson, *Nature*, **212**, 465 (1966).

(5) P. Hemmerich, D. J. Der Vartanian, C. Veeger, and J. D. W. Van Voorst, *Biochim. Biophys. Acta*, **77**, 504 (1963).

(6) J. T. Spence, M. Heydanek, and P. Hemmerich, "II International Conference on Magnetic Resonance in Biological Systems, Stockholm, 1966," A. Ehrenberg, Ed., Pergamon Press, Oxford, England, in press.

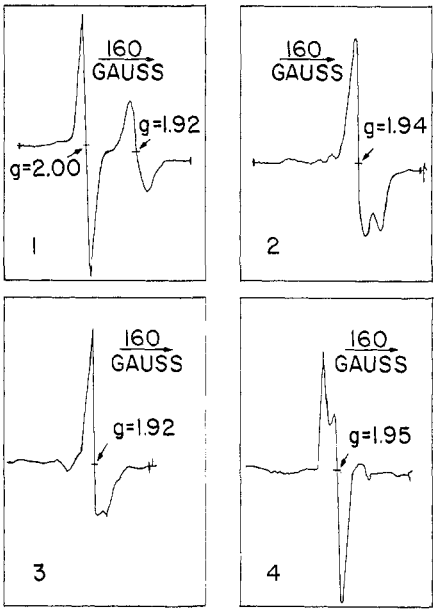


Figure 1.—Mo(V) epr signals: 1, phosphate + flavin, pH 6.40; 2, tartrate, pH 4.72; 3, phosphate, pH 2.10; 4, 2 *M* HCl. All signals measured at −196°.

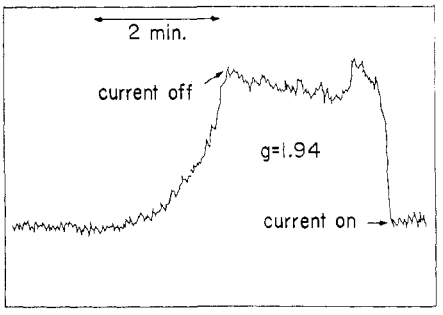


Figure 2.—Electrochemical generation of Mo(V) in epr cavity at Hg cathode. Recorder tracing of $g = 1.94$ signal peak, showing generation, steady state, and decay of signal.

the signal follows second-order kinetics for at least two half-lives. Eventually, however, an equilibrium is established, with a small monomer concentration present. The second-order rate constants were obtained by integrating the rate expression, $-d[Mo(V)]/dt = k_2[Mo(V)]^2$, and plotting $1/[Mo(V)]$ *vs.* t. As seen in Figure 3, this gives straight lines for at least two half-lives, and the rate constants for different initial concentrations of Mo(V) are in good agreement. In order to determine the effect of tartrate concentration on the rate and the equilibrium a series of experiments was made at various buffer concentrations at pH 4.72.

When the log of the second-order rate constant, k_2, was plotted against the log of the tartrate concentration, a straight line of slope −1.05 was obtained (Figure 4), thus indicating the rate expression may be written $-d[Mo(V)]/dt = k_2[Mo(V)]^2/[L]$, where [L] = tartrate buffer concentration.

Again, it was found that the higher the buffer concentration, the greater is the monomer concentration at equilibrium, and consistent values of the equilibrium constant for dimer formation were obtained by using the equation $K_D = [Mo(V)_2]/[Mo(V)]^2[L] = (3.1 \pm 0.8) \times 10^6$ at pH 4.72, $\mu = 2.84$, and 25°. This equilibrium is also pH dependent, as seen in Figure 5, with the maximum concentration of monomer occurring near pH 3.7.

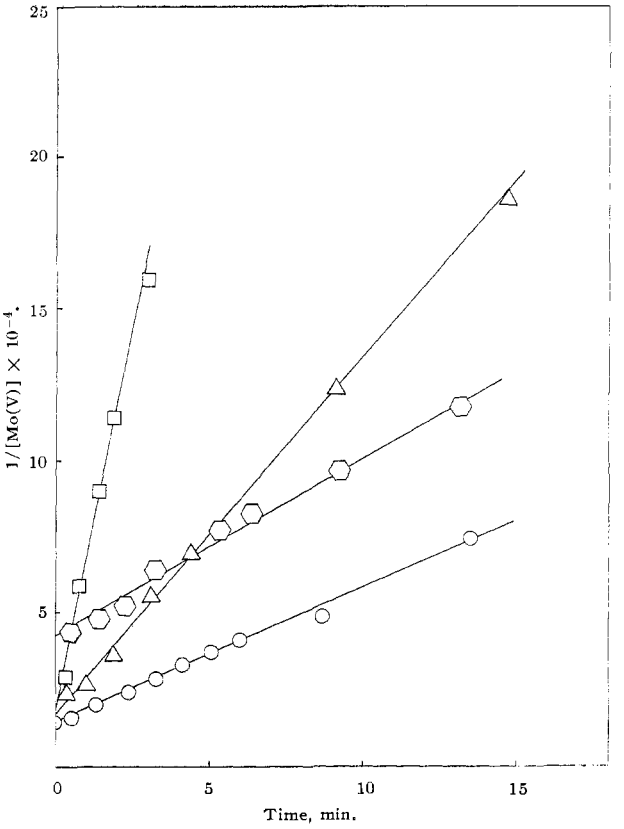


Figure 3.—Second-order plots of dimerizations of Mo(V): ○, pH 4.72, $[Mo(V)]_0 = 7.14 \times 10^{-5}$ *M*; ○, pH 4.72, $[Mo(V)]_0 = 2.38 \times 10^{-5}$ *M*; △, pH 3.15, $[Mo(V)]_0 = 5.88 \times 10^{-5}$ *M*; □, pH 2.15, $[Mo(V)]_0 = 5.00 \times 10^{-5}$ *M*. 0.25 *M* tartrate buffer, 25.0°. [Mo(V)] determined by epr.

At a constant buffer concentration, the rate was found to increase with decreasing pH. When log k_2 was plotted against pH, however, a line was obtained with a slope of 0.31, indicating a complex relationship between $[H^+]$ and rate.

An attempt was made to measure the dimerization rates in phosphate buffer, pH 6.0, and in 2 *M* HCl, but the reaction under these conditions was too rapid to obtain reliable data with conventional techniques.

The rate constants are summarized in Table I. The values of E_a and $\Delta S^{\pm}$ were obtained from an Arrhenius plot of data at four temperatures and $\Delta F^{\pm}$ was calculated from these values.

In order to obtain information concerning structures for the monomer and dimer tartrate complexes it is necessary to know the ratio of ligand to metal ion in each. Because of its low concentration and transient nature such information is difficult to obtain for the monomer species, but the dimer can be more easily studied.

The ultraviolet absorption spectrum of $Mo(V)_2$ is not altered in appearance upon addition of excess tartrate, but ϵ at 298 mμ is increased by about 30%. This

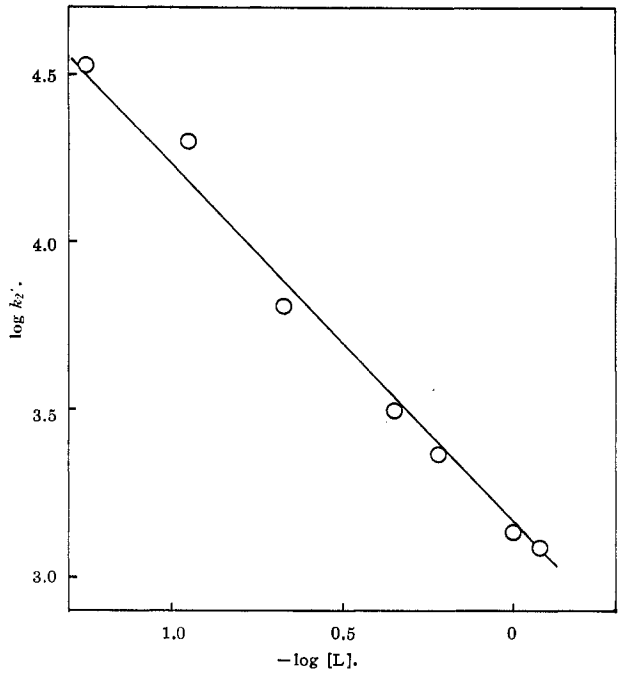


Figure 4.—Variation of rate constant, k_2', with tartrate concentration. Log k_2' is plotted *vs.* log [L]. [L] = total tartrate concentration. μ = 2.84, pH 4.72, 25°.

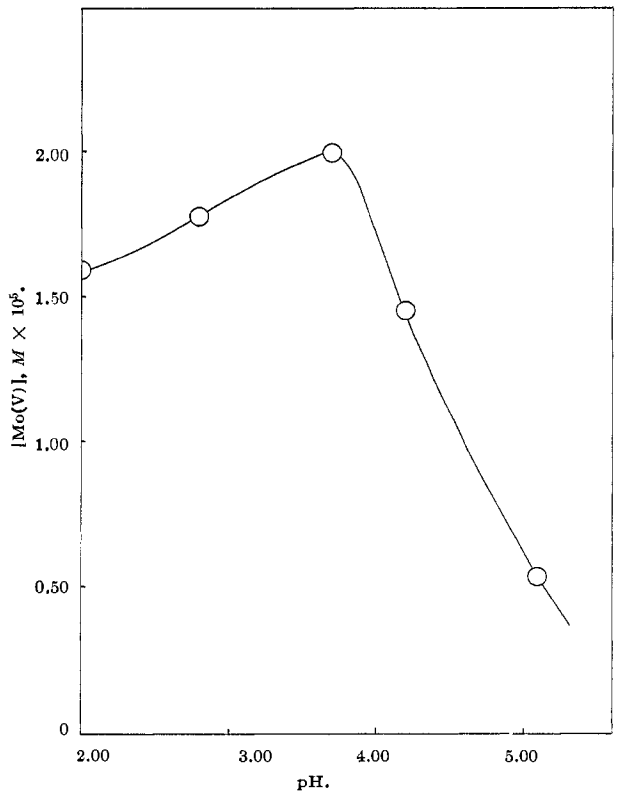


Figure 5.—Equilibrium concentration of Mo(V) as a function of pH; 0.25 *M* tartrate buffer, 25.0°. [Mo(V)] determined by epr. $2[Mo(V)_2] + [Mo(V)] = 9.20 \times 10^{-3}$ *M*.

increase was made use of in the molar ratio plot of absorbance *vs.* mole ratio ligand:metal ion of Figure 6. This method indicates a 2:1 (ligand:metal ion) complex is formed at pH 4.72.

TABLE I

RATE CONSTANTS[a]

pH	[L], *M*	μ, *M*	Temp, °C	k_2', M^{-1} min $\times 10^{-3}$
2.15	0.25	0.60	25.0	23
3.15	0.25	0.60	25.0	6.1
4.72	0.25	0.60	25.0	2.9[b]
4.72	0.25	0.60	0.0	0.98
4.72	0.25	0.60	14.6	1.6
4.72	0.25	0.60	40.0	5.0
4.72	0.056	2.84	25.0	33
4.72	0.112	2.84	25.0	20
4.72	0.225	2.84	25.0	6.6
4.72	0.450	2.84	25.0	3.2
4.72	0.60	2.84	25.0	2.4
4.72	0.80	2.84	25.0	1.7
4.72	1.00	2.84	25.0	1.4
4.72	1.20	2.84	25.0	1.2

[a] E_a = 7.1 kcal, $\Delta S^{\ddagger}$ = −0.9 eu, $\Delta F^{\ddagger}$ = 7.4 kcal, 25°, 0.25 *M* tartrate, μ = 0.60. [b] $k_2 = (2.9 \pm 0.2) \times 10^3$ M^{-1} min; standard deviation determined from eight runs at four different initial Mo(V) concentrations. All other values of k_2 are averages of duplicates.

Discussion

The results indicate both the rate of dimerization and the equilibrium concentration of monomeric Mo(V) are strongly dependent on the buffer concentration, no doubt due to the formation of a monomer complex. Since the rate of dimerization is inversely proportional to buffer concentration (Figure 4), some evidence for the nature of this complex may be obtained by a consideration of possible mechanisms for the reaction.

The simplest scheme consistent with the data involves a very fast formation of a 3:1 tartrate:Mo(V) complex, followed by a slow reaction of this complex with the 2:1 complex in equilibrium with it to form an epr inactive dimer species as shown in Scheme I.

SCHEME I

$$Mo^{V}L_3 \underset{k'_1}{\overset{k_1}{\rightleftharpoons}} Mo^{V}L_2 + L \quad \text{fast}$$

$$Mo^{V}L_3 + Mo^{V}L_2 \xrightarrow{k_2} Mo^{V}_2L_4 + L \quad \text{slow}$$

$$\frac{d[Mo^{V}_2L_4]}{dt} = k_2[Mo^{V}L_3][Mo^{V}L_2] = \frac{-d[Mo^{V}L_3]}{2dt}$$

$$K_f = \frac{[Mo^{V}L_3]}{[Mo^{V}L_2][L]} \qquad [Mo^{V}L_2] = \frac{[Mo^{V}L_3]}{K_f[L]}$$

$$\frac{-d[Mo^{V}L_3]}{dt} = \frac{2k_2[Mo^{V}L_3]^2}{K_f[L]} = \frac{k'_2[Mo^{V}L_3]^2}{[L]}$$

If K_f is large, then essentially all the Mo(V) monomer is in the form of $Mo(V)\cdot L_3$ and the signal is due almost entirely to this species. Thus, this mechanism gives the experimental rate law and explains the inverse dependence on buffer concentration.

The effect of pH on both the rate and equilibrium cannot be rationalized in any simple manner. The equilibrium concentration of Mo(V) monomer is maximum near pH 3.7, which is also the pH where the concentration of acid tartrate anion (HL^-) is maximum (pK_1 = 3.04, pK_2 = 4.36 for tartaric acid), suggesting this is the species complexing the Mo(V) monomer. When an equilibrium constant for dimer formation at

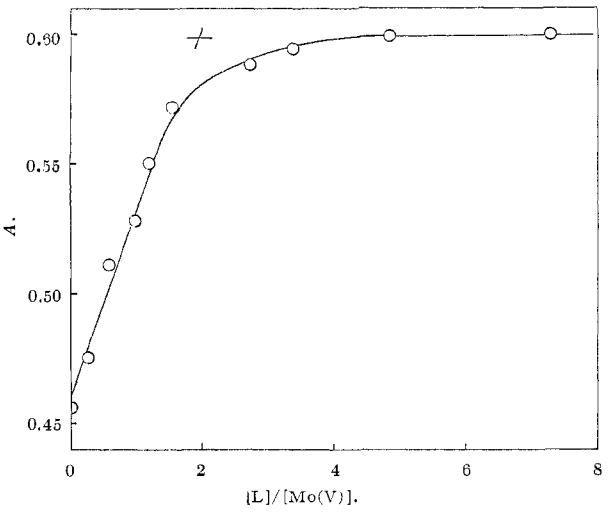


Figure 6.—Molar ratio plot for $Mo(V)_2$–tartrate complex. Mole ratio [L]/[Mo(V)] is plotted *vs.* absorbance at 298 mμ. $[Mo(V)]_T = 1.73 \times 10^{-3}$ *M*, $\mu = 2.84$, pH 4.72, 25°.

different pH is calculated on this basis ($K_D = [Mo(V)_2][HL^-]/[Mo(V)]^2$) no consistent values can be obtained, however, indicating H^+ is probably also involved in some way in the expression. Since there is no minimum in the plot of pH *vs.* log k_2 for the rate, H^+ is probably also involved in the rate-controlling step. This is not surprising, since, in addition to the pH dependence of the concentration of ligand species, the Mo(V) monomer species is most likely also pH dependent and H^+ is probably involved in both complex formation and dimerization.

It should be noted that increasing the ionic strength increases the rate of the reaction (Table I) and also affects K_D (3.1×10^6, $\mu = 2.84$; 1.4×10^7, $\mu = 0.60$).

It is not possible to deduce the detailed structures of the complexes from the data. The dimer structure, however, must allow the spins of the two Mo(V) ions to be paired, probably by interaction through an oxygen bridge, as was found to be the case with the $Mo(V)_2$–xanthate complex.[7]

The results suggest the possibility of obtaining high concentrations of Mo(V) monomer, even at biological pH, if the proper ligand can be found, a finding of considerable interest with regard to molybdenum enzymes, which contain Mo(V) in the monomeric state.[2]

(7) A. B. Blake, F. A. Cotton, and J. S. Wood, *J. Am. Chem. Soc.*, **86**, 3024 (1964).

Experimental Section

Molybdenum(VI) solutions were prepared and standardized as previously described.[8] Titanium(III) was obtained from City Chemical Co. in a 20% solution in H_2SO_4 and standardized by titration with ferric ammonium sulfate as described by Kolthoff and Elving.[9] A solution of $K_3Mo(CN)_8$ in 1 *M* H_2SO_4 was used as a standard for determining the Mo(V) monomer concentration from epr data. $K_3Mo(CN)_8$ and $(NH_4)_2MoOCl_5$ were prepared by standard methods.[10,11]

For standardization, the derivative signal obtained from the $K_3Mo(CN)_8$ was doubly integrated and compared with a double integrated signal for Mo(V) at each set of conditions. For a given buffer and pH, the height of the derivative signal, as measured from the base line, was found to be directly proportional to concentration and was used to calculate the Mo(V) concentration from the relationship between peak height and the area under the double integrated curve.

Solutions of Mo(VI) and buffer at the desired pH were deaerated with He (99.99% pure, Matheson Co.). A solution of Ti(III) that had been deaerated was then added with a hypodermic syringe through a rubber diaphragm into the reaction vessel. Samples were removed with a gas-tight syringe and frozen in liquid N_2 in quartz tubes under He. Due to the rapidity of the reaction and the difficulty of ensuring adequate stirring during the first few seconds, the time of the first sample was taken as zero time for the kinetic calculations. All epr measurements were made at −196°. For the equilibrium measurements the reaction was allowed to proceed until no further change in signal height time was observed. For studies of the effect of buffer concentration on rates and equilibria, the ionic strength was maintained constant by addition of the proper amount of acetate buffer at pH 4.72. In all other cases, NaCl was used for this purpose.

Epr measurements were made with a Varian V-4500-10A, X-band spectrometer equipped with a low-temperature accessory, using 100-kc modulation. The *g* values were determined by comparison with the signal of quinhydrone in alkaline ethanol. All measurements were made in precision bore quartz tubes.

Acknowledgment.—Thanks are expressed to Climax Molybdenum Co. of Michigan and to the Public Health Service (Grant GM 0834-04, Division of General Medical Sciences) for financial support.

(8) J. T. Spence and G. Kallos, *Inorg. Chem.*, **2**, 710 (1963).
(9) I. M. Kolthoff and E. Elving, "Treatise on Analytical Chemistry," Part II, Vol. 5, Interscience Publishers, New York, N. Y., 1961.
(10) N. H. Furman and C. O. Miller, *Inorg. Syn.*, **3**, 160 (1950).
(11) W. G. Palmer, "Experimental Inorganic Chemistry," Cambridge University Press, Cambridge, England, 1954, p 408.