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Synthesis and Reactions of Polymer-Anchored Molybdenum(V and VI) Tripeptide Complexes

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The coordination chemistry of molybdenum has been under intensive study in recent years because of its occurrence in several important redox enzymes, most notable of which is nitrogenase. The complex nature of the molybdenum enzymes and the lack of biological molybdenum chromophores with large molar absorptivities has hindered the characterization and understanding of the nature of the active sites in these enzymes. Until very recently electron paramagnetic resonance (EPR) spectroscopy [1] was the only spectroscopic technique that could be used to examine molybdenum containing enzymes. Although a wealth of information has been obtained about the molybdoenzymes from EPR spectroscopy, this technique suffers from its specificity for the molybdenum(V) oxidation state [2], an oxidation state which may only be transient while the enzyme is returning to an active oxidation state. EPR data has been obtained for a number of molybdenum enzymes with the exception of nitrogenase. Early model studies [3] using molybdenum(V) thiol complexes produced EPR spectra which were similar to those obtained for xanthine, aldehyde and sulfite oxidase and nitrate reductase [4]. These results indicated that the molybdenum found in the enzymes is likely coordinated by cysteinyl sulfur atoms. Recent EXAFS studies [5–8] confirm the presence of sulfur in the vicinity of the molybdenum and also indicate the presence of terminal molybdenum-oxo groups in the oxidized forms of xanthine oxidase [7] and sulfite oxidase [8].

In spite of numerous studies involving the $Mo(V)_2O_4(cysteine)_2^{2-}$ dimer [9], there is very little information about the binding of molybdenum to cysteine-containing peptides. Garner *et al.* [10] have reported on the synthesis, EPR characterization and reactions of monomeric molybdenum(V) complexes using cysteine dipeptides. The actual ligand is comprised of two of the dipeptides coupled by a cystine linkage. The terminal carboxyl and amino groups were chemically blocked to prevent them from coordinating to the molybdenum. This complex exhibited EPR parameters similar to those of nitrate reductase. In the absence of $NaBH_4$ it did not reduce nitrate. Non-aqueous solvents were employed to avoid molybdenum(V) dimer formation.

We have been examining the trends in reduction potentials of *cis*-dioxomolybdenum(VI) complexes coordinated by tridentate Schiff base ligands [11]. The ligands are obtained by the condensation of *o*-aminophenol and 5-X-salicylaldehyde (X = H, Br, NO_2, OCH_3). As the X-group becomes more electron withdrawing the *cis*-dioxomolybdenum(VI) complexes are more easily reduced. There is a linear correlation between the cathodic reduction potential and the Hammett σ_p parameter for the X-substituent. This control over reduction potentials is important, but we also found by cyclic voltammetry that the reductions were not reversible. The lack of reversibility is due to a rapid post-reduction chemical reaction. This reaction may involve the formation of either Mo_2O_4 (tridentate chelate)$_2$ or Mo_2O_3-(tridentate chelate)$_2$ which could then disproportionate. In either case the formation of the molybdenum(V) dimer would account for the irreversible behavior. If a catalytic redox system is to be developed, it is necessary to slow down or eliminate molybdenum(V) dimer formation. We report here on the synthesis, EPR characterization and reactions of molybdenum(V and VI) complexes prepared with ligands that are covalently attached to an insoluble polystyrene support.

Results and Discussion

Polymer-anchored transition metal complexes have been studied for about ten years especially in the area of noble metal catalysts [12]. There has also been work in this area with regard to bioinorganic model systems. Garner and coworkers [13] have reported on the reduction of MoO_2(ethyl-L-cysteinate)$_2$ mixed in a polystyrene suspension. In other work Mitchell and Taylor [14] describe molybdenum(V) complexes with a poly(iminoethylene) dithiocarbamate copolymer. Suzuki *et al.* [15] have prepared a dextran-bound binuclear molybdenum(V) cysteine complex which reduces acetylene in the presence of $NaBH_4$.

Because of our interest in potential models for molybdoenzymes, we chose to use simple peptides as the coordinating ligand. The peptides were attached to the insoluble polystyrene matrix by the method of Merrifield [16]. Chloromethylated polystyrene (1% divinylbenzene) with a chlorine content of 0.001 mol/g was used as the insoluble support. N-carbobenzoxy-protected amino acids were used to construct the tripeptide. Dicyclohexylcarbodiimide was used as the peptide coupling reagent. The peptide

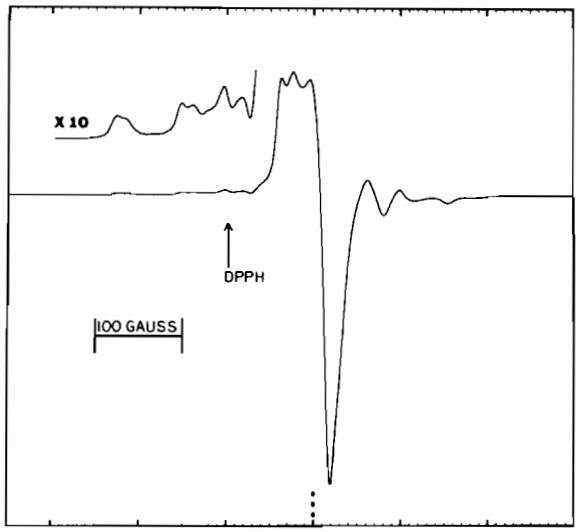
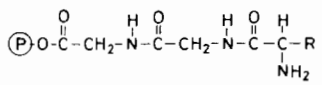
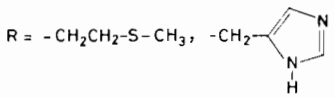


Fig. 1. Room temperature EPR spectrum of Ⓟ-GLY–GLY–MET–Mo(V). (The spectrum is essentially unchanged at 120 °C).

is attached to the polystyrene as an ester through the terminal glycine.

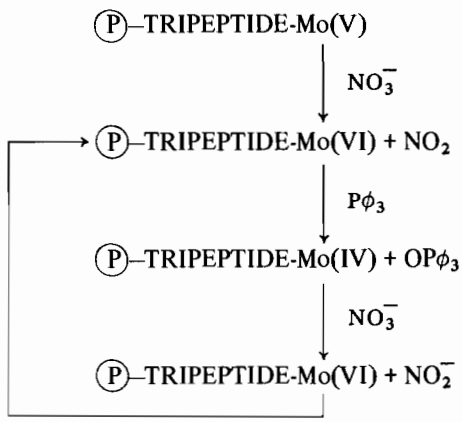




With these polymer-anchored ligands we have used MoO_2Cl_2 and $(NH_4)_2MoOCl_5$ to synthesize the peptide complexes of Mo(VI) and Mo(V) respectively. The procedure involves suspending the polystyrene-anchored peptide in dry DMF. The solvent causes considerable swelling of the polymer beads. The appropriate molybdenum salt was dissolved in dry ethanol, the solution filtered, if necessary, and then added to the polymer-anchored peptide suspension. Solvents for molybdenum(V) complex preparation were deaerated prior to use. A rapid color change was observed for the polystyrene beads as the metal salt was added. The molybdenum(VI)–tripeptide complex is lemon-yellow and the molybdenum(V) complex is tan. The molybdenum concentration is determined by atomic absorption spectroscopy. The ligand (tripeptide) to metal ratio is approximately 1:1 as determined by Volhard titration of the hydrobromide salt of the polymer-anchored tripeptide.

Examination of the tripeptide structure shows that amine and amide nitrogens as well as either the methionine sulfur or imidazole nitrogen are available to coordinate to the molybdenum. Further work is in progress to clarify the actual coordination geometry about the molybdenum. Molecular models show that these two tripeptides can function as tridentate ligands with possibly solvent, chloride, and one or two oxygens occupying the remaining coordination sites.

The molybdenum(V) tripeptide complexes are EPR active. Figure 1 shows a typical spectrum obtained at room temperature. The EPR spectrum is anisotropic at all temperatures since the molybdenum(V) complex is attached to a rigid matrix. The EPR parameters for both of the complexes are very similar. g_1 is around 1.969. g_2 and g_3 cannot be resolved and their value is around 1.942. (*cf.* for nitrate reductase [4] at low pH: g_1 = 1.999, g_2 = 1.986 and g_3 = 1.963). The g-values for these complexes are much lower than those found for nitrate reductase. This is most probably due to the fact that the molybdenum is coordinated by only one sulfur in the case of the methionine derivative and none for the hystidine complex, while in the enzyme there is evidence for three or more ligating sulfurs. The polymer-anchored molybdenum(V) complexes are stable in air toward oxidation for several days.



Scheme. Reactions of polymer-anchored tripeptide molybdenum complexes with triphenylphosphine and nitrate.

The reaction chemistry of these polymer-anchored molybdenum complexes is interesting. Both complexes react in a similar manner and these reactions are summarized in the Scheme. The molybdenum(VI) tripeptide complexes react with triphenylphosphine to produce triphenylphosphine oxide in an oxygen atom transfer reaction. This type of reaction is well documented in the literature especially for the molybdenum(VI) dithiocarbamate complexes [17–19]. This oxygen atom transfer reaction is a possible mechanism for the observed reactions of xanthine, sulfite and aldehyde oxidase and nitrate reductase. The oxygen atom transfer involves a two electron redox process. The polymer-anchored molybdenum(VI) complexes and triphenylphosphine are reacted

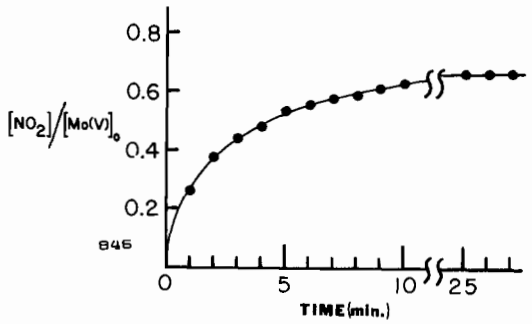


Fig. 2. Relative rate for the production of NO_2 for the reaction Ⓟ–GLY–GLY–MET–Mo(V) + NO_3^- → Products ($[Mo(V)]_o$ is the initial concentration for the polymer-anchored Mo(V) complex).

in deaerated CH_2Cl_2. The suspension is refluxed under N_2 and during the reaction the original lemon-yellow polymer beads (Mo(VI) complex) turn green. At this point triphenylphosphine oxide (identified by IR) can be isolated from the CH_2Cl_2 suspension. The green polymer beads most likely contain an oxo-molybdenum(IV) complex in analogy with those produced in the triphenylphosphine reductions of molybdenum(VI) dithiocarbamate complexes. The lemon-yellow molybdenum(VI) complex can be regenerated by adding a DMF solution of $NaNO_3$ to the suspension. NO_2^- was detected as a product of this reaction. The regenerated molybdenum(VI) complex reacts with additional triphenylphosphine to produce triphenylphosphine oxide. These reactions can be repeated through several cycles. In these reactions the molybdenum (VI) complex is acting as an oxidase model while the green molybdenum(IV) complex is acting as a reductase.

The polymer-anchored molybdenum(V) complexes react with NO_3^- in a manner similar to that described by Spence [20] for some simply molybdenum(V) complexes. During the reaction with $NaNO_3$ under N_2 the tan polymer beads containing the molybdenum(V) complex change color to lemon-yellow indicative of the molybdenum(VI) complex. A one electron oxidation of the molybdenum(V) was confirmed by detecting NO_2^- by the method of Shinn [21]. NO_2^-, however, is not the initial product of the oxidation. EPR examination of the polymer beads after reaction show complete loss of the molybdenum(V) signal and the appearance of a signal due to NO_2 trapped in the polystyrene matrix. The NO_2 then disproportionates into NO_2^- and NO_3^- under the conditions for the colorimetric determination of NO_2^-. Figure 2 shows the rate at which NO_2 is produced for the Ⓟ–GLY–GLY–MET–Mo(V) complex. It should be noted that after the initial 20 minutes almost all the available molybdenum(V) has been oxidized by the NO_3^-. Not all of the NO_2 produced is detected colorimetrically since the EPR data shows that some NO_2 is trapped in the polystyrene matrix.

This work demonstrates the utility of attaching molybdenum complexes to insoluble polystyrene supports. The synthesis allows for the isolation of monomeric molybdenum(V) complexes even in the presence of aqueous DMF. The polymer-anchored molybdenum(V) complex is stable for several days in the presence of O_2 and H_2O as shown by only a small decrease in the intensity of its EPR signal. The hydrophobic nature of the polystyrene accounts for this unusual stability in H_2O. The reactions described show that the polymer-anchored molybdenum complexes can be oxidized and reduced through several reaction cycles. This result is significant if a catalytic oxidase or reductase model is to be synthesized. We are currently extending our study of polymer-anchored molybdenum complexes.

Acknowledgement

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