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A Mini Review on Some Aspects of the Biochemistry of the Micronutrient Molybdenum (VI), (Mo^{6+})

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Mini Review

Abstract

This mini-review touches on some of the most important aspects of the biochemistry of the second row transition metal, micronutrient, molybdenum (VI) (Mo^{6+}). Molybdenum can exist in metal complexes in a variety of oxidation states ranging from the metallic oxidation state of 0 to the most oxidized form of +6. To date, it is believed that molybdenum is taken up by living cells as the molybdate anion $[MoO_4]^{2-}$. There are not many reviews in the literature that cover the current topic. There are a total of about 50 Mo-Containing Enzymes/Proteins. Alongside the detailed literature review, we are also presenting the reactions of aqueous Mo^{6+} with the organic ligand Malic Acid (MA). It appeared that, the reaction of Mo^{6+} with MA in aqueous solutions at 25°C in 0.1 M ionic strength ($NaNO_3$) formed a reaction mixture that released a large number of hydrogen ions, or protons (H^+); 17 H^+ to be exact. This observation is not surprising for such complex behavior of such complex metal ion in aqueous solutions. This mini-review is a contribution to celebrate the 85th birthday of Professor Mostafa El-Sayed; at department of chemistry of the Georgia Institute of Technology, Atlanta, Georgia, USA.

Keywords: Aqueous solutions; Molybdenum-containing-enzymes; Malic acid; Mo^{6+}; Nitrogenase; Potentiometry.

1. Introduction

Among all micronutrients, Molybdenum possesses very unique characters. It is the only second row transition metal that has a tangible biological activity, it exists in a wide variety of oxidation states (ranging from 0 to +6) and it is a required co-factor for at least four dozen enzymes [1]. There are a limited number of reviews or mini-reviews that have appeared with the biology/biochemistry of molybdenum in mind [1-3], particularly in aqueous solutions. However, the meticulous and thorough 75 page review by Hille et al. [2] is a great reference for the biochemistry of molybdenum of which they cited 536 other bio-molybdenum-related research articles.

Herein, we have conducted detailed literature research to prepare for this min-review and found the following three facts: (1) Not many research articles dealt with the reaction of Mo^{6+} and aqueous solutions; (2) The chemistry of molybdenum is extremely complex; and (3) Billions of years ago, nature understood the uniqueness of molybdenum biochemistry that scientists only recently have recognized [1-20]. Figure 1 of the supplementary materials shows the details of this library search of the molybdenum research articles and review articles that were found within all American Chemical Society (ACS) Journals. It is noteworthy that there are a total of 44 journals within all ACS publication domains, which publishes thousands of research papers and reviews monthly.

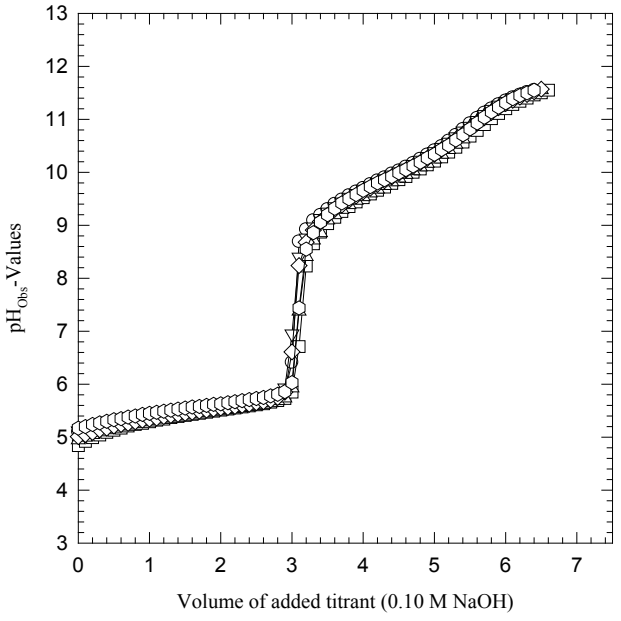

Figure 1. Potentiometric titration graph of free Mo^{6+} in aqueous solutions, 25°C, I=0.10 M ($NaNO_3$). Six replicas show data presission.

There are at least four dozens known molybdenum-containing-enzymes and or proteins (molybdo-enzymes); Nitrogenase being the most well-known among all of them within the biology and chemistry audience. Herein, we are going to mention a dozen as example of these known molybdo-enzymes: (1) Nitrogenase, (2) Nitrate Reductase, (3) Xanthine Oxidase or Xanthine Dehydrogenase, (4) Pyrimidine Oxidase/Aldehyde Oxidase, (5) Trimethylamine Oxide Reductase, (6) Formate Dehydrogenase (7) Carbon Monoxide Oxoreductase/Carbon Monoxide Dehydrogenase, (8) Pyridoxal Oxidase, (9) Sulfite Oxidase, (10) Biotin Sulfoxide Reductase, (11) Dimethyl Sulfoxide Redutase, and (12) Tetrathionite Reductase. Table 1 catalogues all of these enzymes. Some of these Molybdenum-containing-enzymes were isolated from bacteria (particularly cyanobacteria), fungi, yeast, plants, or mammals. For more details refer to references 1-3 and all 694 references mentioned therein. This current mini-review will focus on the discussion of the Molybdenum-containing-enzymes "Nitrogenase".

Nitrogenase, isolated from N_2-fixing bacteria, is the enzyme catalyzing the reduction of molecular N_2 to ammonia (NH_3). In addition to the reduction of molecular nitrogen N_2, Nitrogenase can also reduce a variety of small, unsaturated substrates including the reduction of C_2H_2 to C_2H_4, N_2O to N_2 and H_2O, N_3^- to NH_4^+ and N_2, and HCN to CH_3NH_2 [17,18]. Nitrogenase is composed of two proteins, dinitrogenase (FeMo protein) and dinitrogenase reductase (Fe protein). Dinitrogenase reductase is a dimer of two identical α_2 subunits that bridge one ferredoxin-like $(Fe_4S_4)^{2+/1+}$ cluster. Dinitrogenase is a $\alpha_2\beta_2$ tetramer, and it carries a unique iron-molybdenum cluster that contains Fe, Mo and S with one cage-like $MoFe_7S_9$ homocitrate cluster per α, β-pair. It is believed that the substrate molecules (such as molecular nitrogen, N_2) bind inside this FeMo cluster [4-8]. The Mo center is octahedrally coordinated to three sulfides (μ_3-S ligands), a histidine imidazole side chain, and two oxygen atoms from homocitrate. Supplementary Figure 2 shows the structure formula of the active site of the FeMo cofactor in the N_2 fixing enzyme; Nitrogenase.

Burris and coworkers demonstrated that citrate substitutes for homocitrate in nitrogenase of a nifV

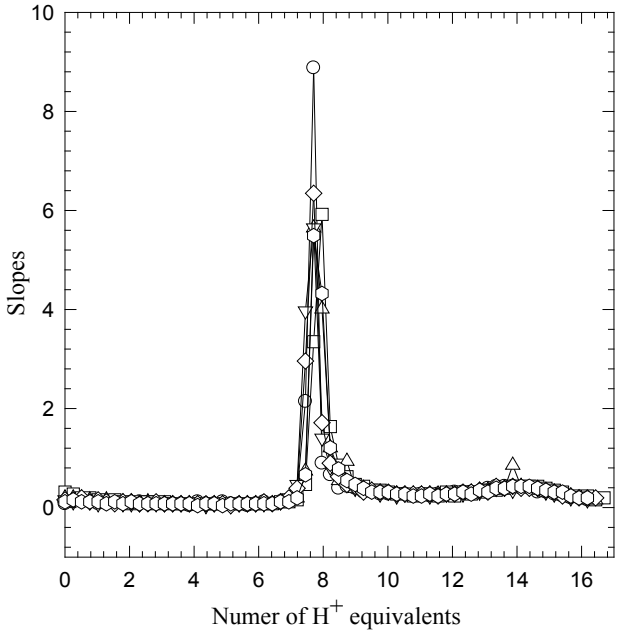

Figure 2. Number of proton equivalent versus slopes for the six free Mo^{6+} potentiometric titration.

Table 1. Enzyme commission numbers and functions of all molybdenum containing enzymes mentioned in this mini-review.

Serial No.	Enzyme/protein	Enzyme Commission # (EC)	Action/Function
1	Nitrogenase	1.18.6.1	Converts nitrogen to ammonia (N_2 to NH_3)
2	Nitrate Reductase	1.7.99.4	Converts nitrate to nitrite (NO_3^- to NO_2^-)
3	Xanthine Oxidase	1.17.3.2	Converts Xanthine to Uric acid
4	Pyrimidine Oxidase/Aldehyde Oxidase	1.2.3.1	Converts Aldehydes to Carboxylic acids
5	Trimethylamine Oxide Reductase	1.6.6.9	Converts Trimethylamine-N-Oxide to trimethylamine
6	Formate Dehydrogenase	1.2.1.2	Converts Formate to CO_2
7	Carbon Monoxide Oxoreductase/Carbon Monoxide Dehydrogenase	1.2.99.2	Converts CO to CO_2
8	Pyridoxal Oxidase	1.2.3.8	Converts Pyridoxal to Pyridoxoic acid
9	Sulfite Oxidase	1.8.3.1	Converts Sulfite to Sulfate (SO_3^{2-} to SO_4^{2-})
10	Biotin Sulfoxide Reductase	-	Converts Biotin Sulfoxide to Biotin
11	Dimethyl Sulfoxide Redutase	1.8.5.3	Converts Dimethyl Sulfoxide to Dimethyl sulfide [$(CH_3)S{=}O$ to $(CH_3)_2S$]
12	Tetrathionite Reductase	-	Converts Tetrathionite to Thiosulfite ($S_4O_6^{2-}$ to $S_2O_3^{2-}$)

mutant of *Klebsiella pneumonia* [9]. In dinitrogenase, the homocitrate ligand is coordinated to the Mo center via the central carboxyl and the central hydroxyl group. We have visited this subject a decade ago with our detailed speciation article [19]. Molybdenum is believed to be taken up by organisms as the molybdate anion MoO_4^{2-}. This would be essential for the biosynthesis of the final FeMo cofactor from an oxomolybdenum citrate precursor [13-16]. Tricarboxylic acids, citric and homocitric acids may play an essential role in the mobilization of molybdenum during cofactor biosynthesis and the mobilized oxomolybdenum tricarboxylate fragment must undergo reduction, exchange the oxo ligands for the sulfide ligands, and merge with the rest of the nifB. While the precise role of tricarboxylic acids in the biosynthesis of the FeMo cofactor and the mechanism of dinitrogenase reduction is still regarded as poorly understood [15], the elucidation of the role played by the tricarboxylic acids has been pursued with great interest [9-16,19].

Many researchers, including ourselves, have attempted to mimic the reaction of Mo^{6+} with that of mono-hydroxyl poly-carboxylates (citrate, homocitrate and malate) [9-16,19-23]. Herein, we show the outcome of the reactions of the simple mono-hydroxyl di-carboxylate (Malate) in the form of the protonated Malic acid (H_2MA) with Mo^{6+} in aqueous solutions under ambient conditions in 0.1 M ionic strength ($NaNO_3$).

2. Experimental Section

2.1 Materials and method

Aqueous solutions of D,L-Malic acid (H_2MA) were prepared using 99% purity (Sigma reagent grade), formula weight 134.09 $g.mol^{-1}$. Aqueous molybdenum (Mo^{6+}) solutions were prepared from the crystalline ammonium salt of molybdic acid, $(NH_4)_6Mo_7O_{24}.4H_2O$, 83% MoO_3, formula weight 1235.9 $g.mol^{-1}$, also Sigma reagent grade. The primary (1°) standard potassium hydrogen phthalate (KHP, 99.99%) and the solid sodium hydroxide pellets (NaOH, 98%) were purchased from Fisher Chemical Company. We have standardized the sodium hydroxide (NaOH) solution to the fourth digits to the right of the decimal point. All pH values were measured using Thermo-Orion Membrane Advanced ISE/pH/mV/ORP meter (model 720A+) connected to the accurate-combination Orion-glass electrode in 0.1 $mol.L^{-1}$ ionic strength. The 0.10 M ionic strengths of all solutions were adjusted by the addition of 10% v/v of 1.0 M $NaNO_3$ solution.

2.2 Potentiometry

The detailed methods used to carry potentiometric titrations are well established in the literature [19,21-24]. For each individual experiment, H_2MA solutions were first added to the titration vessel, followed by the addition of the specified amount of Mo^{6+} solution. The final total volume of the reacting species was 100 mL. NaOH solution was added in 100 µL increments by means of the calibrated and accurate, 100 µL total capacities, Eppendorf micro-pipette. Each individual potentiometric titration took about 3.5 h to complete.

3. Results and Discussion

Figure 1 shows the potentiometric titration plots of free Mo^{6+}. The free H_2MA titration curve has been published in previous studies [24,25-27]. It is established that H_2MA releases two protons out of the two carboxylic acid groups. Additionally, the hydroxyl group can participate in metal ion chelation to form two fused six-membered and five-membered very robust ring chelates [19,25-27]. Figure 2 shows the potentiometric titration plots of Mo^{6+} in the form of number of proton equivalents released (x-axis) versus the first derivatives or slopes (y-axis) of the experimental pH-values (the inflection points appeared at 7.78 ± 0.10, n=6). This observation parallels that of the previous study of Mo^{6+} with citric acid which is consistent with the literature values [19].

Figure 3 is the double plots of free H_2MA in which the potentiometric titrations of six replicas in the form of volume of added titrant versus experimental pH-values are overlaid with the number of proton equivalents on the x-axis versus the slopes on the y-axis. A similar titration plateau was observed in our previous studies [25,27]. This particular titration graph is the very same graph that has been collected alongside the reaction of the hexavalent molybdenum (Mo^{6+}) in its reaction with malic acid (H_2MA); see

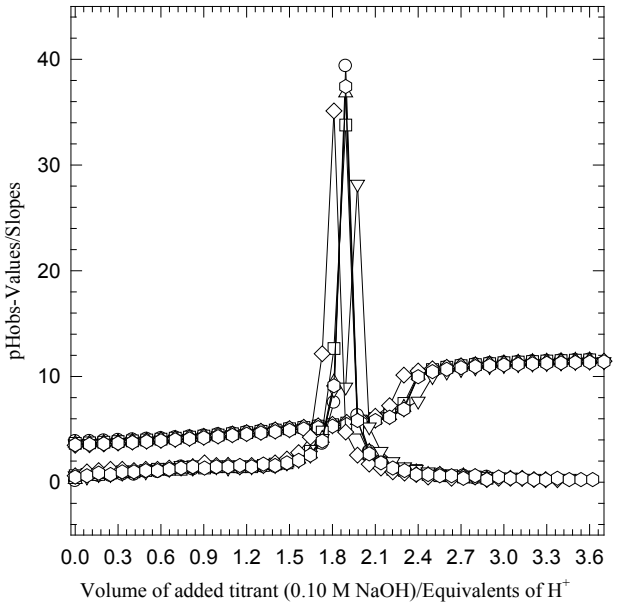

Figure 3. Potentiometric titration graph of six replicas of free Malic Acid in aqueous solutions, 25°C, I=0.1 M ($NaNO_3$)

below for details of the Mo^{6+} reaction with H_2MA.

Figure 4 shows the titration plateaus of the reaction mixture of Mo^{6+} with MA in 1:3 M ratios. Excess amount of malic acid was selected for three reasons: (1) to avoid metal ion hydrolysis; (2) to avoid the formation of any oligomeric metal-complexes that were formed when the ratio of the Mo^{6+} ion was in excess [21-23]; and (3) for ease of pipetting 2.00 mL H_2MA with 2.00 mL Mo^{6+}, which gave this 1:3 molar reaction ratios. After calculating the number of proton

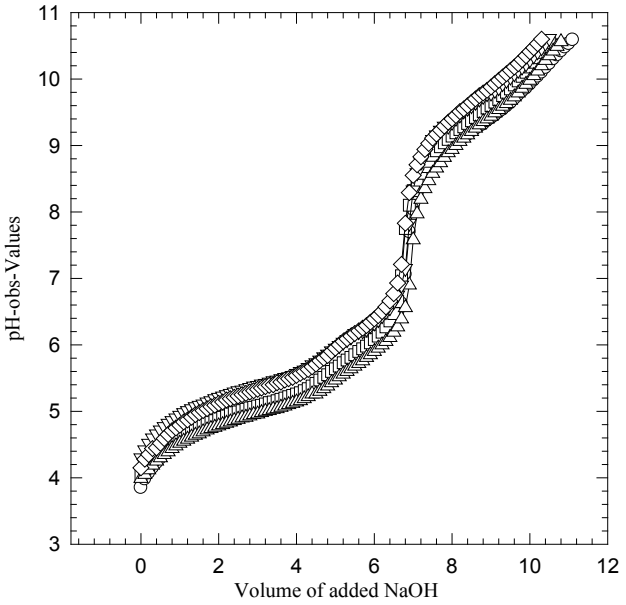

Figure 4: Potentiometric titration of Mo^{6+} : Malic acid in 1:3 ratios. 25 °C, I =0.10 M ($NaNO_3$)

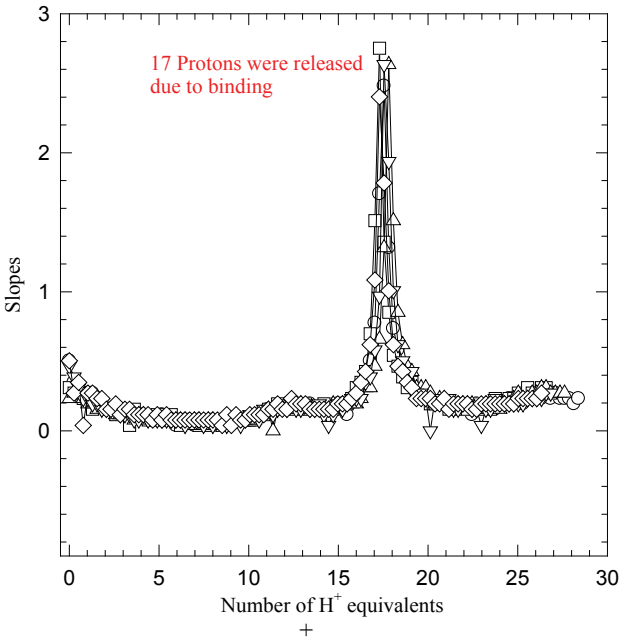

Figure 5. Number of H^+ equivalent versus slopes of the Mo^{6+}: Malic acid titrations in 1:3 ratio shown in Figure 4. More than 17H^+ were released due to the binding of Mo^{6+} to MA

equivalents of the reactions of Mo^{6+} with H_2MA in 1:3 M ratios (Figure 5), it appeared that ~ 17 proton equivalents were released into the solution (17.01 ± 1.22, n=6). This observation is in a very good agreement that one might expect for the release of a net 7.78 proton equivalents from the free mole of Mo^{6+} in addition to the net of nine protons total out of the three available protons to be released per malic acid molecule (i.e., 9H^+ from the three moles of H_2MA plus 7.78 from the free Mo^{6+} ion). In n Figure 5, it shows the values which are equal to 16.78 proton equivalents.

Figure 5 confirms that the malic acid is releasing three H^+ equivalents although it is referred to as H_2MA in the literature [19,24-27]. This is due to the central hydroxyl group upon chelation to Mo^{6+} (or any metal ion for that matter) releases its proton. It is concluded from this data that the main complexes that have been formed in solution are those according to equation 1.

$$3\ H_2MA + Mo^{6+} \leftrightarrow [MoO_2(H_{-1}MA)(OH)_2]^{3-} + [MoO_2(H_{-1}MA)_2]^{4-} + 17\ H^+ \quad (1)$$

4. Conclusion

The current experimental data presented in this mini-review is novel. When the detailed literature reviews were searched for the reactions of H_2MA with the hexavalent molybdenum ion Mo^{6+}, only few papers were found [21-23]. In these studies, they all agreed that the aqueous solution chemistry of Mo^{6+} is not that simple. They also agreed on the formation of the simple one to one complexes $[MoO_2(MA)(OH)_2]^{3-}$, $[MoO_2(MA)_2]^{4-}$, $[Mo_2O_5(MA)_2(OH)_2]^{4-}$ with stability constant values of Logβ of 8.17, 13.89 and 22.31, respectively [22]. When the crystal structure was published two years later after these initial aqueous attempts in 1983 [21], it turned out that what crystallized was the bis-mono-protonated complex which has the following molybdenum malate complex formula: $[MoO_2(Hmal)_2]^{2-}$ [21]. We do not believe that we are observing this bis-mono-protonated complex. What concerns us here is not whether we are observing the formation of the di- anion, the tri-anion, or the tetra-anion molybdenum-malate complex. We are also not concerned with whether it is the mono-protonated or the de-protonated or the (hydroxo) molybdenum-malate complex. However, in this study the significant revelation is that we have identified the total number of proton equivalents released into the solution which gives the same binding mode as seen in the crystal structure and the previous equilibrium studies [21-24].

The detailed and the most trusted reviews of the molybdoenzymes by researchers [1-3] stressed that the bi-dentate binding mode of the mono-

hydroxyl poly-carboxylates, whether it is in the form of citrate or homocitrate, is the dominant mode of binding. Here in, we are stressing that the formed complex of Mo^{6+} with malic acid released a net of 17 proton equivalents which can only be accounted for by the binding of Malate in a bi-dentate or a tri-dentate fashion (the potentiometric titration is lacking supplying this information). The stoichiometry given in the equilibrium shown in equation (1) is only accounted for by the formation of a mixture of the two proposed molybdenum-malate complexes depicted, i.e., $[MoO_2(H_{-1}MA)(OH)_2]^{3-}$ + $[MoO_2(H_{-1}MA)_2]^{4-}$. This mixture of the two complexes released a net of 17 H^+. It is noteworthy that these two complexes are consistent with the ones identified by others [21-24].

5. Acknowledgement

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