CHEMISTRY LETTERS, pp. 947-950, 1982.

STUDIES ON MOLYBDO-OXIDASE MODELS: ROLE OF HEMIN OR FLAVIN FOR AIR OXIDATION OF PPh_3 BY Mo(VI) COMPLEXES OF CYSTEINE-CONTAINING PEPTIDES

Norikazu UEYAMA, Etsuo KAMADA, and Akira NAKAMURA*

Department of Macromolecular Science, Faculty of Science, Osaka University, Toyonaka, Osaka 560

Air oxidation of triphenylphosphine catalyzed by $MoO_2(cys\text{-}OMe)_2$, $MoO_2(cys\text{-}OEt)_2$, $MoO_2(cys\text{-}Met\text{-}OMe)_2$ was studied as models of molybdo-oxidase. Addition of hemin or riboflavin to the systems facilitates the catalytic activity. Redox cycle between Mo(VI) and Mo(V) proceeds smoothly with addition of the electron-transfer mediators which rapidly oxidize Mo(V) to Mo(VI).

Recently EXAFS studies of molybdo-oxidases, such as desulfo-xanthine oxidase and sulfite oxidase, have revealed probable active site structures,[1),2)] where exists a cis-dioxo-molybdenum(VI) core surrounded by three or four thiolate ligands. Analysis of EXAFS by Bordas et al. indicated that the Mo=O bond lengths and the coordination donor sets of sulfite oxidase are similar to those of $MoO_2(cys\text{-}OEt)_2$.[2)] This model complex has a weak catalytic activity for air oxidation of PPh_3.[3),4)] Further, the complex was found to require some amounts of water to realize the catalysis.[5)] Molybdo-oxidases generally contain heme or flavin depending on the identity of the substrates. By considering irreversible redox cycle associated with $MoO_2(cys\text{-}OR)_2$ in DMF, such electron-transfer mediators may assist the catalytic oxidation cycle. Actually, we have found that the addition of hemin or riboflavin facilitates the catalytic activity in air oxidation of PPh_3.

Figure 1 shows the time plots for air oxidation of PPh_3 by catalysis of $MoO_2(cys\text{-}OEt)_2$. Addition of hemin or riboflavin to the Mo(VI) complex in 1:1 molar ratio clearly enhanced the catalytic activity. No catalytic activity was observed with hemin alone for the air oxidation of PPh_3, while riboflavin possesses

activity to some extent as listed in Table I.

In the first stage, the oxidation obeyed pseudo-first order kinetics with the following equation.

$$-\frac{d[PPh_3]}{dt} = k\,[PPh_3]$$

The Table I lists rate constants (k) in the catalytic air oxidation of PPh_3 by various $Mo^{VI}O_2L_2$ (L = cys-OMe, cys-OEt, cys-Met-OMe, Ac-cys-OH) complexes. The rate enhancement with hemin amounts over ten times.

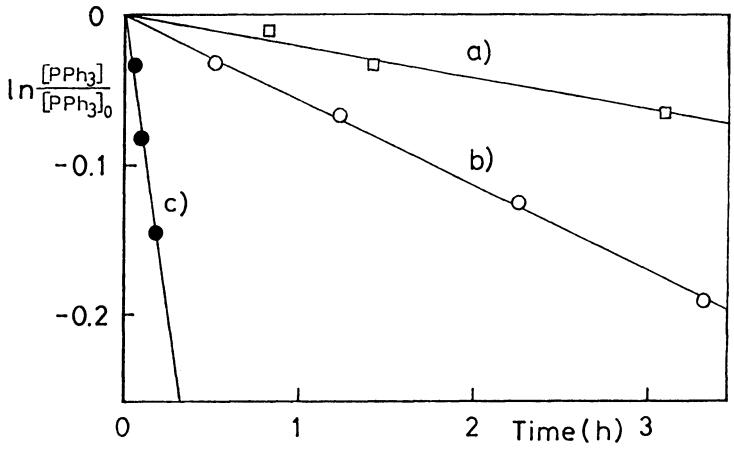

Fig. 1. Time plots for oxidation of PPh_3 by a) $MoO_2(cys\text{-}OEt)_2$, b) $MoO_2(cys\text{-}OEt)_2$/riboflavin (1:1), and c) $MoO_2(cys\text{-}OEt)_2$/hemin (1:1). Conditions: [Mo]/[PPh_3] (1:20) in DMF/H_2O (1:0.15) at 30°C.

The catalytic activity was maximally enhanced at the molar ratio of [Mo]/[hemin]= 1:1.

Table I. Rate constants of catalytic air oxidation of PPh_3

Catalysts MoO_2L_2	Solvents	Cotatalysts	k (sec^{-1})
L = cys-OEt	DMF/H_2O (1:0.15)	——	6.0×10^{-6}
cys-OEt	"	hemin	2.8×10^{-4}
cys-OMe	"	hemin	3.0×10^{-4}
ala-cys-OMe	"	hemin	1.9×10^{-4}
cys-Met-OMe	"	hemin	2.5×10^{-4}
Ac-cys-OH	"	hemin	1.7×10^{-4}
cys-OEt	"	riboflavin	1.6×10^{-5}
cys-OEt	DMF	hemin	1.2×10^{-4}
hemin	DMF/H_2O (1:0.15)	——	0
riboflavin	"	——	3.2×10^{-6}

Conditions: [Mo]/[hemin or riboflavin]/[PPh_3] = 1:1:20 at 30°C.

The electrochemical redox cycles of Mo(VI) cysteinate complexes are irreversible for the Mo(VI)/Mo(V) or Mo(VI)/Mo(IV) couple.[6] Actually the cyclic voltammogram of $MoO_2(cys\text{-}OEt)_2$ shows only a cathodic peak ($E_{p,c}$ = − 1.42 V vs SCE) in DMF. On the other hand, hemin has two reversible couples ($E_{1/2}$ = − 0.27 V and − 1.19 V vs SCE) in DMF. The cyclic voltammograms of $MoO_2(cys\text{-}OEt)_2$/hemin in both DMF and DMF/H_2O (1:0.15) exhibited the identical curves on repeated scans. Electron transfer probably occurs from the Mo(V) species to hemin owing to such close redox potentials between the Mo complex and hemin (− 1.42 V / − 1.19 V) and results in forming Fe^{II} species.

Native xanthine oxidase or sulfite oxidase is well known to have a stable, mononuclear Mo(V) species when reduced with appropriate substrates.[7] The Mo(IV) species prepared from $Mo^{VI}O_2(S_2CNR_2)_2$ immediately forms binuclear Mo(V) complexes, $Mo_2^{V}O_3(S_2CNR_2)_4$, through reaction between $Mo^{VI}O_2(S_2CNR_2)_2$ and $Mo^{IV}O(S_2CNR_2)_2$.[8] Our previous EPR study indicated that the mild reduction of $MoO_2(cys\text{-}OR)_2$ (R = Me, Et, Bzl) in DMF/H_2O results in formation of mononuclear Mo(V) species.[9] Here, the presence of water in the oxidation system prevents formation of inert binuclear Mo(V) complexes $Mo_2O_2(\mu\text{-}O)_2(cys\text{-}OEt)_2$.[5] The oxidation of the mononuclear Mo(V) species by hemin or riboflavin seems to proceed faster than that of the di-μ-oxo binuclear Mo(V) complexes.[10] However, increase of the mononuclear Mo(V) species in the above catalytic system results in dimerization of these species. Hemin plays a role in decreasing the Mo(V) concentration by the rapid oxidation.

Native xanthine oxidase has flavin and Fe_2S_2 per one Mo atom while sulfite oxidase has one heme b_5 per one Mo atom.[7] Tryptic cleavage of rat liver sulfite

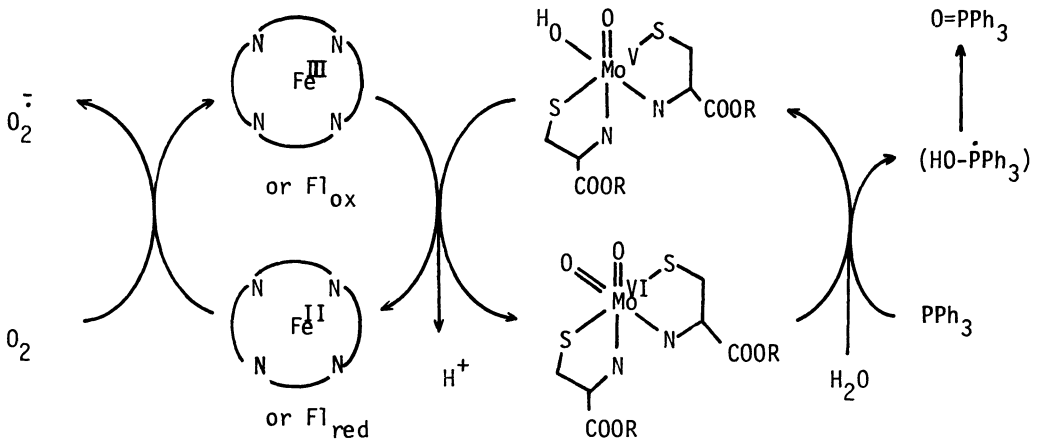

Fig. 2. Proposed scheme of air oxidation of PPh_3 by an $MoO_2(cys\text{-}OR)_2$/hemin catalyst system. Fl = riboflavin.

oxidase was reported to release heme b_5 from the Mo site and to lose the enzymatic activity.[7b] Our results also suggest that flavin or heme b_5 in native molybdo-oxidase plays a significant role in an effective electron transfer between Mo(V) and Mo(VI) species. The catalytic cycle shown in Fig. 2 postulates the redox cycle of hemin or flavin.

References

1) T. D. Tullius, D. M. Kurtz, S. D. Conradson, and K.O. Hodgson, J. Am. Chem. Soc., 101, 2776 (1979).

2) J. Bordas, R. C. Bray, C. D. Garner, S. Gutteridge, and S. S. Hasnain, Biochem. J., 191, 499 (1980).

3) G. Speier, Inorg. Chim. Acta., 32, 139 (1979).

4) A. Nakamura and N. Ueyama, "Molybdenum Chemistry of Biological Significance", ed. W. E. Newton, and S. Otsuka, (1980) Plenum Press. p. 369.

5) N. Ueyama, M. Yano, H. Miyashita and A. Nakamura, in preparation.

6) R. D. Taylor, J. P. Street, M. Minelli, and J. T. Spence, Inorg. Chem. 17, 3207 (1978).

7) a) S. Gutteridge and R. C. Bray, "Molybdenum and Molybdenum-Containing Enzymes" ed. M. Coughlan (1980), Pergamon Press, p. 223.
b) K. V. Rajagopalan, ibid, p. 243.

8) R. Barral, C. Bocard, I. Seree de Roch, and L. Sajus, Tetrahedron Lett., 1972, 1693.

9) A. Nakamura, N. Ueyama, and M. Kamachi, Chem. Lett., 1981, 19.

10) $Mo_2O_3L_4$(L=cys-OMe, cys-OEt, S_2CNEt_2) complexes are readily oxidized by air in the presence of small amounts of hemin or riboflavin (unpublished). Therefore, a mononuclear Mo(V) complex in equilibrium with $Mo_2O_3L_4$ can be regarded as species returning to the Mo(VI) complex.

(Received March 20, 1982)