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Heteropoly Molybdate Complexes of Flavin Mononucleotide and Some Other Phosphate Esters

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Heteropoly molybdates and tungstates have long been used as electron-dense stains for electron microscopy [1], and in recent years have attracted much attention as electron-acceptors in studies of photosynthesis [2–10] and as antiviral agents [11–15]. The modes of interaction of polyoxometalates with organic molecules and biopolymers is therefore of considerable interest, and we are currently investigating the chemistry of novel organo-polyoxometalate complexes and of derivatized polyanions [16, 17]. We report here the formation of stable heteropoly molybdate complexes with some phosphate esters and nucleotides. These complexes are of interest in connection with the well-known catalytic effect of molybdate(VI) upon phosphate ester hydrolyses [18], and they suggest new processes for the incorporation of polymetalate clusters into biological systems. Furthermore, to our knowledge, there has been no previous report of a complex between flavin mononucleotide and molybdenum(VI) although much attention has been devoted to flavin–molybdenum interactions [19].

The new complexes $[(ROPO_3)_2Mo_5O_{15}]^{4-}$, are formed rapidly at room temperature in aqueous solution at pH 2.5–4.0 and appear to be structural analogues of the pentamolybdobisphosphonate complexes that we have described elsewhere [20]. Salts of complexes of β-glycerophosphate, riboflavin-5′-phosphate (flavin mononucleotide, FMN), adenosine-5′-monophosphate (AMP), and uridine-5′-monophosphate (UMP) have been isolated and characterized by chemical analysis, infrared and ^1H and ^{31}P NMR spectroscopy. Spectroscopic evidence for analogous complexes of DL-α-glycerophosphate, glucose-6-phosphate, pyrophosphate, and adenosine-5′-diphosphate (ADP) has been obtained.

The solid complexes are precipitated from stoichiometric [21] mixtures of phosphate ester and sodium molybdate ([P] ~ 0.02 *M*) acidified to pH 3–4, by addition of cesium or guanidinium chloride. The products are recrystallized from aqueous acetate buffers, pH 3.5–4.5. In the case of the β-glycerophosphate complex the more soluble guanidinium and potassium salts crystallize after partial evaporation of the solvent [22]. Typical analyses: Found (calcd.): *β-glycerophosphate complex, guanidinium salt,* $C_{10}H_{38}N_{12}P_2Mo_5O_{27}\cdot 3H_2O$: C, 8.72 (8.87); H, 3.07 (2.83); N, 12.28 (12.41); Mo, 35.38 (35.43); H_2O, 4.00 (3.99)%. *Potassium salt,* $K_4C_6H_{14}P_2Mo_5O_{27}\cdot 4H_2O$: C, 6.23 (5.59); H, 1.65 (1.72); P, 4.76 (4.80); Mo, 37.65 (37.23); H_2O, 5.02 (5.58)%. *FMN complex, guanidinium salt,* $C_{38}H_{62}N_{12}P_2Mo_5O_{33}\cdot 8H_2O$: C, 21.7 (22.66); H, 3.64 (3.89); N, 13.89 (13.75); Mo, 24.75 (23.83); H_2O, 6.86 (7.15)%. *Cesium salt,* $Cs_4C_{34}H_{38}N_8P_2Mo_5O_{33}\cdot 5H_2O$: C, 18.64 (18.15); H, 2.06 (2.15); N, 4.95 (4.98); P, 2.95 (2.75); Mo, 22.48 (21.32); H_2O, 3.82 (4.00)%. *AMP complex, guanidinium sodium salt,* $NaC_{23}H_{42}N_{19}P_2Mo_5O_{29}$: C, 16.11 (17.11); H, 2.94 (2.93); N, 15.16 (16.49); P, 3.87 (3.84); Mo, 30.10 (29.76); H_2O, 0.05 (0.0)%. *UMP complex, cesium salt:* $Cs_4C_{18}H_{22}N_4P_2Mo_5O_{27}\cdot 4H_2O$: C, 10.56 (10.99); H, 1.77 (1.54); N, 2.71 (2.85); P, 3.11 (3.15); H_2O, 3.99 (3.66)%.

The infrared spectra of all the complexes in the P–O and Mo–O stretching region (1200–600 cm^{-1}) are similar both in the solid state (KBr disc) and in solution (D_2O) (Fig. 1). The pattern of band positions and relative intensities closely parallels those of the molybdophosphonates, $(RP)_2Mo_5O_{21}^{4-}$ and molybdophosphates, $P_2Mo_5O_{23}^{6-}$ [20], from which we conclude that the oxometalate structure is the same in all these complexes. The dissymmetric (C_2) structure of the $P_2Mo_5O_{21}$-cluster [23, 24], a ring of edge- and corner-shared MoO_6 octahedra capped on each side by tripod phosphate groups, is shown in Fig. 2.

The complexes are, remarkably, non-labile on the NMR time-scale, and are of moderate stability. For example, 90-MHz ^1H-NMR spectra of β-glycerophosphate-molybdate solutions (P:MO > 2:5) at pH 3.2 show separate signals for the free ester (αCH_2OH doublet at 3.74 ppm) and complex (multiplet [25] at 3.89–3.94 ppm) at temperatures up to 80 °C. NMR spectra of stoichiometric solutions (P:Mo = 2:5) at pH 3.4, μ = 1.0 ($NaClO_4$), were measured at different phosphate concentrations. At [P] = 10^{-3} *M*, about 50% of the phosphate was complexed, a result confirmed by UV spectral measurements [26]. At [P] = 0.01 *M* about 90% complexation had occurred and at [P] = 0.02 *M* no free ester was detected. For the case of FMN, complex formation in solution at pH 3 was demonstrated by NMR shifts and splittings of the isoalloxazine ring protons and methyl groups at 7.5–7.8 and 2.3–2.5 ppm and by a downfield shift (0.3 ppm) in the ^{31}P NMR spectrum. Attempts to prepare an analogous complex with the reduced nucleotide, $FMNH_2$, either directly, or by electrolytic reduction of solutions of the oxidized heteropoly complex, were unsuccessful owing to the simulta-

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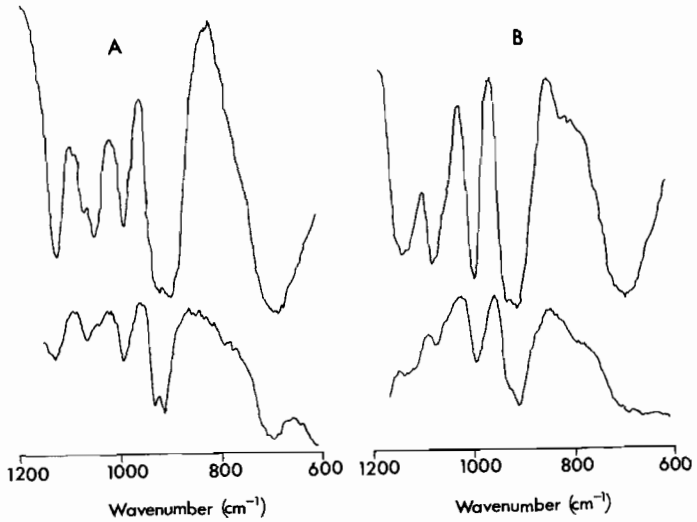

Fig. 1. Infrared spectra of β-glycerophosphate (A) and AMP (B) complexes. Upper spectra: $K_4[(C_3H_7O_3PO_3)_2Mo_5O_{15}]\cdot 4H_2O$ and $Cs_4[(C_{10}H_{12}N_5O_4PO_3)_2Mo_5O_{15}]\cdot 4H_2O$ in KBr pellets. Lower spectra: Solutions of phosphate ester (0.25 *M*) and sodium molybdate (0.625 *M*), in D_2O, acidified to pD 3.5 (A) and 3.7 (B) with DCl. Path length, 0.05 cm.

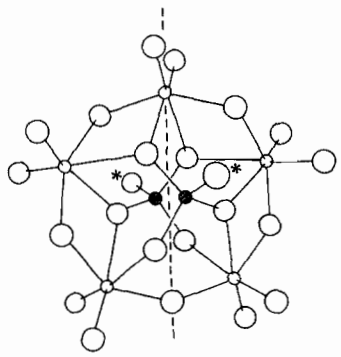

Fig. 2. Proposed structure of the heteropoly oxometalate cluster in $(ROPO_3)_2Mo_5O_{15}^{4-}$ complexes, showing pseudo-C_2 axis (broken line). Small circles, Mo and P atoms. Large open circles, O atoms. Asterisks indicate oxygen atoms to which R-groups are attached.

neous reduction of molybdenum [27] and destruction of the P_2Mo_5 cluster.

Although it was possible to isolate pure 2:5 complexes of the nucleotide monophosphates AMP and UMP, analogous precipitation experiments with ADP and ATP lead to apparent mixtures with lower phosphate contents than anticipated. Preliminary ^{31}P NMR spectra of ADP + Mo and ATP + Mo solutions at pH 3.5–4.5 show that partial hydrolysis of terminal phosphate groups has occurred (diminution of resonances at 22 and 10.5 ppm [28] and appearance of new monophosphate resonances at *ca* 0 ppm). These results are consistent with the known sensitivity of di- and triphosphates towards molybdate [18]. That a heteropoly complex is probably implicated in the molybdate-catalyzed hydrolysis of pyrophosphate is suggested by the pH profile of the hydrolysis which shows a pronounced maximum at pH 2–3 in the presence of molybdate [18]. The ^{31}P NMR spectrum of a 2:5 solution of pyrophosphate and molybdate at pH 3.5 shows equal resonances at 7.5 and 10.3 ppm attributed to the $(O_3POPO_3)_2Mo_5O_{15}$-complex [29] and resonances at −1.3 and −0.1 ppm from protonated $(OPO_3)_2$-$Mo_5O_{15}^{6-}$ and phosphate ions respectively.

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