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The Remarkable Nucleophilic Reactivity of Molybdate[†]

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Abstract: Dianionic molybdate (pH 7.5, 27 °C, $\mu = 1.6$ M) is a good nucleophilic catalyst for the hydrolysis of *p*-nitrophenyl acetate ($k_2 = 0.26$ M^{-1} min^{-1}) and *p*-nitrophenyl thioacetate ($k_2 = 0.34$ M^{-1} min^{-1}). The solvent kinetic isotope effect for the molybdate-catalyzed hydrolysis of each ester is 1.0 (±0.1). In the presence of 0.1 M molybdate, the hydrolysis rate at pH 7.5 is increased by a factor of 50 for *p*–nitrophenyl acetate and by a factor of 80 for the thioester. The second-order rate constant for the reaction of the molybdate dianion with *p*-nitrophenyl acetate is 35-times larger than that for the reaction of the phosphate dianion in spite of the 1000-fold greater basicity of the latter species. This may reflect a greater degree of solvation of the nucleophilic site on phosphate than on the larger molybdate ion.

Acyl transfer reactions are widespread in nature, and the mechanism has been extensively studied with a vast range of nucleophilic acceptors. In a classic paper Jencks and Carriuolo[1] examined the nucleophilic reactivity of over 45 nucleophilies with *p*-nitrophenyl acetate. They showed a reasonable correlation of nucleophilic reactivity with the pK_a of the attacking group ($\beta \sim 0.8$) with the noteworthy negative deviations (by over two orders of magnitude) for the dianions phosphate and carbonate. In order to explore some of the factors responsible for this low reactivity, the reaction of a series of phosphate analogs (i.e., phosphonates) with various *p*-nitrophenyl esters was examined.[2] This study revealed an unusually small Bronsted coefficient ($\beta = 0.3$). The low reactivity and the small sensitivity of the rate constant to basicity, as well as the unusually small magnitude of the activation entropy for the bimolecular reaction,[3] have been attributed, at least in part, to the requirement for desolvation from a nucleophilic site on the oxydianion.[2] In view of this, another interesting feature appears in the data of Jencks and Carriuolo.[1] They found that although the dianions of phosphate and arsenate have nearly identical pK_as, arsenate is 5.5-times more reactive with *p*-nitrophenyl acetate than is phosphate. In order to further investigate this phenomenon, we examined the reactivity of the less basic molybdate dianion. Since arsenate and molybdate[4] can substitute for phosphate in a variety of enzymic reactions, it was of interest to examine the intrinsic reactivity of these species. Surprisingly, we found molybdate to be *more reactive* than either phosphate or arsenate with *p*-nitrophenyl acetate and its thiol ester analog.

Experimental Section

Materials. *p*-Nitrophenyl acetate was obtained from Aldrich Chemical Co. and *p*-nitrophenyl thioacetate from U.S. Biochemical Corp. The HEPES[1] buffer was obtained from Sigma Chemical Co. as a 1:1 mixture of the free acid and the sodium salt. Sodium molybdate (dihydrate) was obtained from Matheson, Coleman and Bell and stock solutions (0.5 M) were prepared by dissolving Na_2MoO_4 in buffer solutions (0.01 M HEPES, 0.1 M NaCl) and adjusting the pH to 7.5 with HCl (or DCl in D_2O). At this pH there is negligible polymerization of molybdate.[5] D_2O (99.8%) was from Sigma Chemical Co.

Methods. pH measurements were made with a Metrohm (Brinkmann) combititrator. pD values were estimated by adding 0.41 to the pH meter reading.[6] Kinetics were followed by measuring the absorbance ($\lambda = 400$ nm for *p*-nitrophenoxide or 412 nm for *p*-nitrobenzenethiolate) on either a Hewlett-Packard Model 8452A diode array spectrophotometer or a Beckman 3600 spectrophotometer. The reactions were initiated by adding 10 μL of the ester (10 mM in CH_3CN) to 1 mL of a thermally equilibrated (27 °C) buffer solution (ionic strength maintained at 1.6 M with Na_2SO_4). At the higher molybdate concentrations (0.3–0.5 M) the release of *p*-nitrophenoxide or *p*-nitrobenzenethiolate was demonstrated to follow first-order kinetics by monitoring the reaction for 9 half-lives. In general the increase in the absorbance was typically followed for at least 4 half-lives and the data analyzed by nonlinear regression to a first-order curve.[7] The internal standard deviations of the pseudo-first-order rate constants were consistently less than 1%. Rate constants were determined at least in triplicate for each reaction.

Table I. Effectors of *p*-Nitrophenyl Acetate Hydrolysis

[Na_2SO_4]	[Na_2MoO_4]	[NaCl]	μ, M	k,[a] min^{-1}
0	0	0.1	0.1	3.8 (±0.1) × 10^{-4}
0.5	0	0.1	1.6	4.2 (±0.1) × 10^{-4}
0	0.1	0.1	0.4	2.0 (±0.1) × 10^{-2}
0	0.1	1.3	1.6	1.7 (±0.1) × 10^{-2}
0.4	0.1	0.1	1.6	2.24 (±0.04) × 10^{-2}

[a] 1% v/v CH_3CN, 0.1 mM ester, 0.01 M HEPES, pH 7.5, 27 °C.

Results

In order to evaluate the influence of the reaction medium (i.e., [Na_2SO_4] and ionic strength) on the rate of hydrolysis of pNPA,[1] the concentration of Na_2SO_4 and NaCl was varied in the presence and absence of Na_2MoO_4 (0.1 M). The results (Table I) indicate negligible influence of sodium sulfate on the hydrolysis rate. The pseudo-first-order rate constants for release of *p*-nitrophenoxide or *p*-nitrobenzenethiolate from the corresponding acetate esters shows a linear dependence on the molybdate concentration (up to at least 0.5 M) at pH 7.5, 27 °C. The second-order rate constant for the molybdate-catalyzed hydrolysis is 0.26 (±0.03) M^{-1} min^{-1} with *p*-nitrophenyl acetate (pNPA) and 0.337 (±0.009) M^{-1} min^{-1} with *p*-nitrophenyl thioacetate (pNPTA). Thus, there is a significant enhanced hydrolysis rate in the presence of

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[†] Abbreviations: GPD, glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12); HEPES, *N*-2-hydroxyethylpiperizine-*N'*-2-ethanesulfonic acid; pNPA, *p*-nitrophenyl acetate; pNPTA, *p*-nitrophenyl thioacetate.

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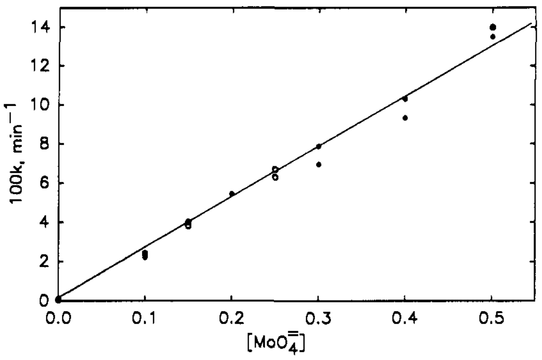


Figure 1. Molybdate-catalyzed hydrolysis of *p*-nitrophenyl acetate (27 °C, μ = 1.6 M). Reactions were carried out either in H_2O (○, pH = 7.5) or in D_2O (●, pD = 7.9). The slope of the line drawn through the points is 0.26 M^{-1} min^{-1}.

molybdate over the hydrolysis rate constants at pH 7.5 in the absence of molybdate (4×10^{-4} min^{-1} for pNPA and 5×10^{-4} min^{-1} for pNPTA). The molybdate-catalyzed hydrolysis rate constants are identical in H_2O and D_2O (Figure 1). The solvent kinetic isotope effect (SKIE = ratio of second-order rate constants in H_2O and in D_2O) is 1.0 (±0.1) for pNPA and 0.97 (±0.06) for pNPTA.

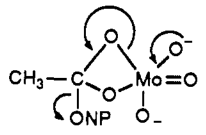
Discussion

The molybdate-catalyzed hydrolysis of esters at pH values above neutrality has not been previously reported. In this regard, it is interesting to note that Lipman and Tuttle[8] reported a 1.9-fold increase in the rate of hydrolysis of acetyl phosphate in the presence of 0.1% ammonium molybdate ($\simeq$5 mM) at pH = 4 (where the predominant species is the polymeric paramolybdate). However, at pH = 7 the same concentration of molybdate had no effect on the hydrolysis of acetyl phosphate.

A 5 mM solution of molybdate does show a detectable, though small ($\sim$3-fold), enhancement in the hydrolysis rate of pNPA at pH 7.5. Indeed, the second-order rate constant for the reaction of molybdate with pNPA (=0.26 M^{-1} min^{-1}) is 17-times larger than that for the reaction of aniline (pK_a = 4.6) with pNPA.[1] The observation of a SKIE of unity suggests that the molybdate-catalyzed hydrolysis of pNPA and pNPTA involves the nucleophilic attack of molybdate on the ester. Earlier evidence (based on enzymatic studies) has suggested that acyl molybdates, like acyl arsenates (but unlike acyl phosphates), undergo a rapid spontaneous hydrolysis (presumably involving cleavage of the oxygen–metal bond).[4] At 27 °C the second-order rate constant for the molybdate-catalyzed hydrolysis of pNPA is 35-times larger than that for the reaction of phosphate with the ester.[1] This is particularly striking in view of the low pK_{a2} (=4.1) for molybdate.[9] Indeed, based on a linear free energy relationship[2] correlating the reactivity of pNPA with the pK_{a2} (4.8–8.7) of a series of phosphonates, the calculated rate constant (at 37 °C) for a dianionic nucleophile of pK_{a2} = 4.1 ($k_2 = 1.5 \times 10^{-3}$ M^{-1} min^{-1}) is lower than the observed value for molybdate (at 27 °C) by a factor of 170. A similar calculation for the reaction with pNPTA (at 37 °C) yields a rate constant of 3.8×10^{-3} M^{-1} min^{-1} which is lower than the observed molybdolysis rate constant (at 27 °C) by a factor of 90.

There are several possible explanations for the enhanced nucleophilic reactivity of molybdate over phosphate or phosphonates. One explanation is that the larger molybdate dianion (Mo–O bond length = 1.83 Å[10]) is less extensively solvated than the phosphate dianion (P–O bond length = 1.54 Å[10]). It would thus be easier to remove a solvent molecule from a nucleophilic site on molybdate than on phosphate. This explanation would also apply to the greater reactivity of the arsenate dianion (As–O bond length = 1.75 Å[10]) than phosphate toward pNPA.[1] The observed absence of a significant SKIE on the reaction of molybdate with the esters does not necessarily rule out this explanation. Solvent isotope effects for the transfer of simple alkali salts from H_2O to D_2O are small, typically 1.1 to 1.3.[11,12] Without information about solvent isotope effects on the solvation of molybdate, and on compounds which are suitable models for the transition state, it is difficult to make predictions about the magnitude, or even the direction, of the solvation isotope effect in this reaction.

Another explanation of the greater reactivity of molybdate over that of phosphate is that the metal center could be serving as an electrophilic catalyst which stabilizes the tetrahedral intermediate in the reaction.



This is somewhat analogous to the intermediate in the aluminum alkoxide catalyzed dismutation of aldehydes into an alcohol and an ester (the Tishchenko reaction).[13]

In view of the greater reactivity of molybdate than phosphate toward both pNPA and pNPTA it is interesting to note the relative reactivities of these dianions with the thioester intermediate in the enzyme glyceraldehyde-3-phosphate dehydrogenase (GPD). The relative second-order rate constants (k_{cat}/K_m) for the reaction of the enzyme (in the presence of saturating concentrations of NAD^+ and *D*-glyceraldehyde-3-phosphate at pH = 8.5) with the dianions of phosphate, arsenate, and molybdate are 12:1.8:1.[4] This can be compared to the relative reactivity of these ions with pNPA which is 1:5.5:35. Thus, the specificity of the enzyme for phosphate over molybdate (correcting for the relative reactivity of the two nucleophiles) is 420 (=12 × 35):1. It is not unreasonable that steric factors are largely responsible for this discrimination. Nevertheless, molybdate is an excellent substrate for the enzyme. The value of k_{cat}/K_m for molybdate is 3.4×10^6 M^{-1} min^{-1}, which is larger than the second-order rate constant for the reaction of molybdate with the model thioester, pNPTA, by a factor of 10^7. GPD enhances the reactivity of phosphate by at least two orders of magnitude more than this since nucleophilic attack on the thioester intermediate may not be the rate-limiting step in the overall reaction.[14] Undoubtedly some of this enhancement comes from the fact that it is the desolvated phosphate which is bound to the enzyme. Since there are over 130 different enzymes which use inorganic phosphate as a substrate, it will be of interest to quantitate the various factors, in addition to desolvation, that these enzymes use to enhance the reactivity of phosphate.

Registry No. Na_2MoO_4, 7631-95-0; D_2, 7782-39-0; *p*-nitrophenyl acetate, 830-03-5; *p*-nitrophenyl thioacetate, 15119-62-7.

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