Phosphoesterase activity of polyoxomolybdates: diffusion ordered NMR spectroscopy as a tool for obtaining insights into the reactivity of polyoxometalate clusters†

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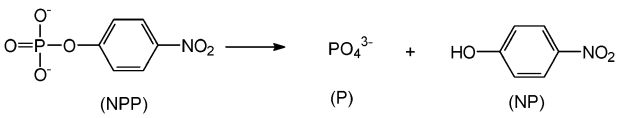
Diffusion ordered NMR spectroscopy (DOSY NMR) is shown to be an excellent tool for observing reactive transients in the hydrolysis of the phosphatase model substrate (*p*-nitrophenyl)-phosphate (NPP) promoted by polyoxomolybdate.

Polyoxometalates represent a large group of negatively charged metal-oxide clusters that contain the early transition metals in their high oxidation states. The diversity in their structures and compositions offers a broad versatility in terms of chemical and physical properties, resulting in numerous potential applications.[1] Due to their strong acidity and redox properties, several heteropolyacids have found important applications in the field of homogeneous and heterogeneous catalysis.[1b] In the last decade there has been also a growing interest in the biological activity of polyoxometalates, especially after it was shown that many of them exhibit antiviral, antibacterial, and antitumor activities.[2] Among these biologically active clusters, the polyoxomolybdate $[Mo_7O_{24}]^{6-}$, is particularly noteworthy mentioning, due to its potent *in vivo* anti-tumor activity against a range of different cancer cells, including pancreatic cancer cells, which are extremely resistant toward most of the known therapeutic agents.[3] Although the biological activity of polyoxometalates is well documented, the molecular origins of the mode of action remain largely unknown. The rich solution chemistry and tendency to form clusters of different nuclearities depending on concentration, temperature, and pH, complicates the thorough analysis and understanding of their reactivity.

In our quest for understanding the biological activity of polyoxomolybdates at molecular level, we have recently discovered its unprecedented hydrolytic activity towards phosphodiesters.[4] Although the kinetic studies strongly suggested heptamolybdate $[Mo_7O_{24}]^{6-}$ as the hydrolytically active complex, the detailed analysis of the reactive species was hindered by the low NMR sensitivity of ^{95}Mo and ^{17}O nuclei, and by the weak complexation of phosphodiesters to $[Mo_7O_{24}]^{6-}$. Considering the established biological activity of $[Mo_7O_{24}]^{6-}$, understanding its mode of interaction with biologically relevant molecules and model systems are of substantial interest. In this work we describe the unique application of diffusion ordered NMR spectroscopy (DOSY NMR) for obtaining insights into the hydrolytic activity of $[Mo_7O_{24}]^{6-}$ towards commonly used model monophosphate (*p*-nitrophenyl)phosphate (NPP).

Hydrolysis of monophosphates is one of the most important reactions in biological systems, being involved in several essential processes such as signal transduction, energy storage and replication of genetic material.[5] In recent decades, a range of simple metal complexes that exhibits remarkable activity toward phosphoester bond cleavage has been designed.[6] Although different in their design, all the hydrolytically active metal complexes have several essential features in common: they are positively charged, which is presumably essential for the favorable electrostatic interactions with the negatively charged phosphate group, they contain free coordination sites for the binding of phosphoryl oxygen, and they have coordinated water or hydroxide which may act as an effective nucleophile.[6] In strong contrast, even though the $[Mo_7O_{24}]^{6-}$ cluster misses all these features, with its high negative charge, coordination saturation, and lack of coordinated water or hydroxide, we demonstrate that it promotes likewise efficiently the hydrolysis of the phosphoester bond in NPP (Scheme 1).

The reaction between equimolar amounts of $Na_6[Mo_7O_{24}]$ and NPP (100 mM, pD 5.5, 50 °C) was conveniently monitored by ^1H NMR spectroscopy. The first-order rate constant ($k_{obs} = 7.8 \pm 0.1 \times 10^{-5}\ s^{-1}$) for the cleavage of the P–O bond was calculated from the appearance profile of the *p*-nitrophenol (NP) ^1H resonances. The ^{31}P NMR spectra recorded shortly upon mixing of NPP and $[Mo_7O_{24}]^{6-}$ indicated that NPP was fully bound into two types of complexes **A** and **B** which were present in *ca.* 9 : 1 ratio. During the hydrolysis course, the concentrations of **A** and **B** decreased, and two new species were detected in the ^{31}P NMR spectra: an intermediate with a ^{31}P resonance at −2.1 ppm, unambiguously assigned to the $H_2PO_4^-$ ion, and species **C**, which at the reaction end was the only phosphorus containing species to remain in solution (Fig. 1). The latter has the same spectroscopic features as a separately prepared pentamolybdodiphosphate ion $Mo_5P_2O_{23}^{6-}$, which typically forms in mildly acidic solutions



Scheme 1

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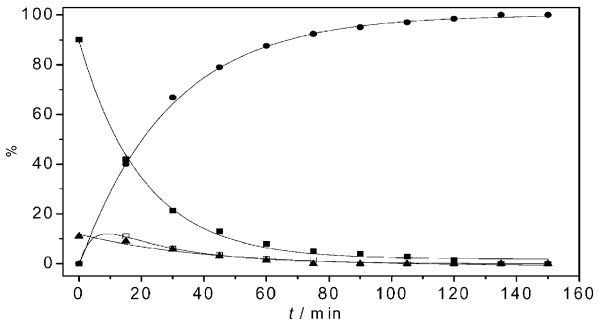

Fig. 1 Progress of the reaction of a mixture of NPP and $[Mo_7O_{24}]^{6-}$ in D_2O at 50 °C (100 mM, pD 5.5) monitored by ^{31}P NMR spectroscopy using $(CH_3)_3PO$ as a 0 ppm reference. Fractions of the complex **A** (■, $\delta = -3.82$ ppm), **B** (▲, $\delta = -6.46$ ppm), C (●, $\delta = -1.27$ ppm) and $H_2PO_4^-$ (□, $\delta = -2.10$ ppm) are shown as a function of time.

containing molybdate and phosphate ions.[7] The $Mo_5P_2O_{23}^{6-}$ induces no observable cleavage of the phosphoester bond in NPP, even after prolonged reaction times. The nature of the reactive species **A** and **B** is however difficult to assess on the sole basis of their ^1H and ^{31}P NMR shifts. Similarly, the IR, UV-Vis and Raman spectra indicated a change in $[Mo_7O_{24}]^{6-}$ structure, but were inconclusive as to the composition of the formed complexes.

The diffusion correlation peaks in 2D diffusion-ordered (DOSY) NMR spectra, which combine a chemical shift scale and a diffusion coefficient scale, can provide a straightforward assignment of the various molecular species present in the mixture on the basis of their specific diffusion coefficient.[8] An advantage of this methodology is its potential to reveal and unravel interactions between various species in solution, even in reaction transients. Consequently, once a model system is analyzed structurally, the correlation between the diffusion coefficient and the hydrodynamic radius, in combination with well-known shape factors, allows discriminating between different possible cluster structures.[9] In order to assess the feasibility of this DOSY based strategy for the identification of polyoxometalate transients in reaction courses, we have investigated first the interaction of the molybdate with phenylphosphonate (PhP), which is a hydrolytically inactive analogue of NPP. The binding of PhP to molybdate has been previously studied by combination of ^{31}P NMR, potentiometry and large-angle X-ray scattering.[10] These studies identified the major complex as the $[(PhP)_2Mo_5O_{21}]^{4-}$ polyanion, consisting of five MoO_6 octahedra coupled by one-corner- and four edge-shared units to form a ring structure, which is capped on either side by two phosphate moieties.

According to Lyxell *et al.*,[10b] mixing PhP with molybdate at pH 5.3, results in the formation of two new species, $[(PhP)_2Mo_5O_{21}]^{4-}$ (92%) and $[(PhP)Mo_7O_{25}]^{4-}$ (8%), which are in mutual fast exchange on the ^{31}P NMR time scale. Our own investigations confirm this exchange to be likewise fast on the ^1H NMR time scale. Thus, our DOSY measurements provide a single averaged diffusion coefficient, 4.2×10^{-10} $m^2\ s^{-1}$, to be compared with that of PhP, 6.4×10^{-10} $m^2\ s^{-1}$. Taking into account the prolate shape (3.30 and 8.44 Å) of $[(PhP)_2Mo_5O_{21}]^{4-}$ and the spherical shape of $[(PhP)Mo_7O_{25}]^{4-}$ (4.80 Å) species,[9a] and the estimated molar fractions of, respectively 92 and 8%,[10b] the calculated averaged diffusion coefficient of these complexes ($D_{calc} = 4.3 \times 10^{-10}$ $m^2\ s^{-1}$), appears in good agreement with the experimentally determined diffusion coefficient ($D_{exp} = 4.2 \times 10^{-10}$ $m^2\ s^{-1}$), which illustrates convincingly the accuracy of diffusion coefficient estimations for this type of clusters on the basis of their size and dimensions. Similarly, the experimentally determined diffusion coefficient of the PhP ligand agreed very well with the diffusion coefficient calculated assuming a spherical shape for PhP (Table 1).

Table 1 Comparison of experimental and calculated diffusion coefficients for the species detected by DOSY NMR. The estimated maximum error on the diffusion coefficient is 0.1×10^{-10} $m^2\ s^{-1}$

Compound	$D_{exp}/10^{-10}$ $m^2\ s^{-1}$	$D_{calc}/10^{-10}$ $m^2\ s^{-1}$
PhP	6.4	6.6
$[(PhP)_2Mo_5O_{21}]^{4-}$ (92%)	4.2	4.3
$PhPMo_7$ (8%)		4.4
NPP	5.5	5.8
NPP_2Mo_5		3.9
$NPPMo_6$		4.9
$NPPMo_7$		3.9
Dimer		3.0
Complex **A**	4.1	3.9
Complex **B**	2.8	3.0
NP	5.7	6.0

In the following step the diffusion behavior of a solution containing NPP and molybdate was investigated. For the sake of comparison, the concentrations of the NPP and molybdate were taken identical to those used in the hydrolytic reaction. During the hydrolysis of NPP, three different species were detected by DOSY NMR, each displaying its own diffusion coefficient. The major species (complex **A**) formed in solution immediately upon mixing is present in 90% and is characterized by ^1H NMR signals at 8.23 and 7.32 ppm. It has an averaged diffusion coefficient of $D_{exp} = 4.1 \times 10^{-10}$ $m^2\ s^{-1}$, which is significantly lower than the $D_{exp} = 5.7 \times 10^{-10}$ $m^2\ s^{-1}$ measured for free NPP. The second minor species (complex **B**) present in 10%, gives NMR signals at 7.82 and 8.28 ppm, the latter overlapping strongly with the corresponding resonance of complex **A**, and displays a diffusion coefficient of 2.8×10^{-10} $m^2\ s^{-1}$, indicating that NPP is associated with even a larger cluster. The third species, which is formed in the course of the hydrolytic reaction, gives ^1H NMR signals at the same frequencies as NP (8.17 and 6.98 ppm). Good agreement with the diffusion coefficient of NP unambiguously points to identification of this species as nitrophenol, the expected product of NPP hydrolysis.

Considering the structural analogy between PhP and NPP ligands, the major complex **A** is expected to have $(NPP)_2Mo_5O_{21}^{4-}$ structure, possibly in fast exchange with $(NPP)Mo_7O_{25}^{4-}$, which is further supported by the good agreement between the measured (4.1×10^{-10} $m^2\ s^{-1}$) and estimated (3.9×10^{-10} $m^2\ s^{-1}$ as the weighted average of 3.86 and 3.94×10^{-10} $m^2\ s^{-1}$) diffusion coefficients for these species. The analogous pentamolybdodiphosphate type structure also forms in mildly acidic solutions containing molybdate and nucleotides.[11]

Previous studies of molybdophenylphosphonate solutions suggested that besides the major pentamolybdodiphosphate

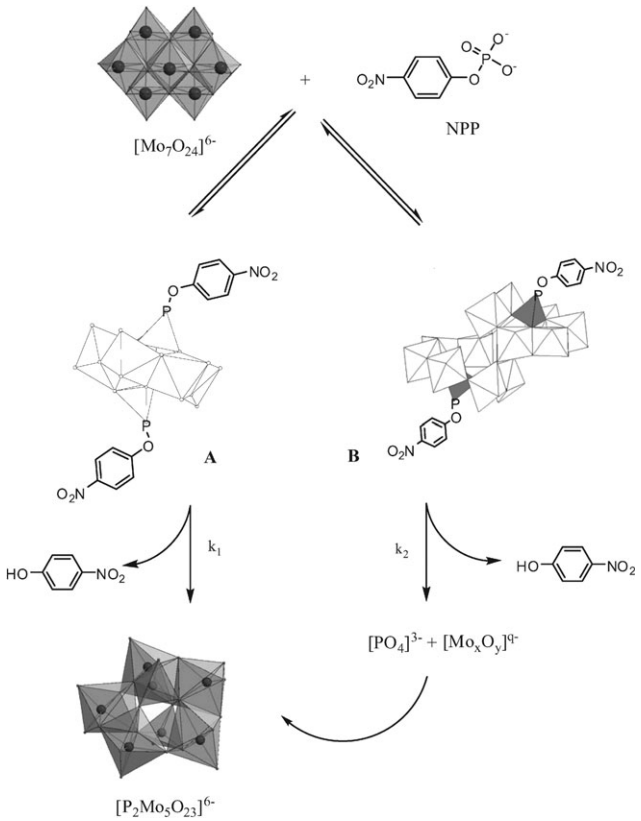

Fig. 2 Schematic presentation of NPP hydrolysis promoted by $[Mo_7O_{24}]^{6-}$ cluster.

structure, one other minor complex with Mo_6PhP type structure can also be formed.[10b] Calculated diffusion coefficient of analogous NPP complexes, namely $(NPP)Mo_6O_{18}(H_2O)_3$ (4.9×10^{-10} m^2 s^{-1}) did not match the diffusion coefficient observed for complex **B**. However, the diffusion coefficient of complex **B** agreed very well with the diffusion coefficient calculated for a dimeric $(NPP)_2Mo_{12}O_{36}(H_2O)_6^{4-}$ complex (2.8×10^{-10} m^2 s^{-1} *vs.* 3.0×10^{-10} m^2 s^{-1}, oblate ellipsoid). An analogous $(PhP)_2Mo_{12}O_{36}(H_2O)_6^{4-}$ complex, which consists of two weakly binding $(PhP)Mo_6O_{18}(H_2O)_3$ entities has been isolated and characterized by X-ray crystallography.[12] Although it has been proposed that this complex forms in strongly acidic solutions[10b,12] it is possible that slight changes in ligand structure may cause its formation also in mildly acidic media.

Based on our ^{31}P and DOSY NMR data, the hydrolysis of NPP in the presence of $[Mo_7O_{24}]^{6-}$ can be schematically represented as shown in Fig. 2. Formation of complexes **A** and **B** results from incorporation of the phosphoester group into the polyxomolybdate skeleton, and sharing of oxygen atoms with Mo(VI) centres. This may lead to bond strain and cause polarization of the P–O ester bond and its activation toward external attack by water.

In conclusion, the model for the cleavage of a phosphoester bond promoted by a highly negatively charged polyoxomolybdate cluster presented in this work was based on the differentiated diffusion behavior of hydrolytically active complexes. DOSY NMR appears as a tool *in situ*, complementary to X-ray scattering, which enables monitoring of the catalytic reaction progress and obtaining structural information on the reactive transient complexes. Considering the biological activity and wide application of polyoxometalates in catalysis, one can envision this technique as a powerful tool for studying the reaction processes involving this important class of compounds.

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