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Shifts: 9	Local contact(s): Sergey Nikitenko	
Names and affiliations of applicants (* indicates experimentalists): Prof. Dr. Rik Van deun Dr Tatjana Parac-vogt Drs Els Cartuyvels		

Report:

Hydrolytic Cleavage of an RNA-Model Phosphodiester Catalyzed by a Highly Negatively Charged Polyoxomolybdate $[\text{Mo}_7\text{O}_{24}]^{6-}$ Cluster

Gregory Absillis, Els Cartuyvels, Rik Van Deun, Tatjana N. Parac-Vogt
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Abstract:

Hydrolysis of 2-hydroxypropyl-4-nitrophenyl phosphate (HPNP), a commonly used RNA model substrate, was examined in molybdate solutions by means of ^1H , ^{31}P , and ^{95}Mo NMR, Raman, and Mo K-edge extended X-ray absorption fine structure (EXAFS) spectroscopy. ^1H and ^{31}P NMR spectroscopy indicate that at 50 °C and pD 5.9 the cleavage of the phosphodiester bond in HPNP proceeds with a rate constant of $6.62 \times 10^{-6} \text{ s}^{-1}$, giving a cyclic phosphate ester and *p*-nitrophenol as the only products of hydrolysis. The NMR spectra did not show evidence of any paramagnetic species, excluding the possibility of Mo(VI) reduction to Mo(V), and indicating that the cleavage of the phosphodiester bond is purely hydrolytic. The Mo K-edge XANES region also did not show any sign of Mo(VI) to Mo(V) reduction during the hydrolytic reaction. The pD dependence of k_{obs} exhibits a bell-shaped profile, with the fastest cleavage observed at pD 5.9. Comparison of the rate profile with the concentration profile of polyoxomolybdates shows a striking overlap of the k_{obs} profile with the concentration of heptamolybdate, suggesting that the highly negatively charged $[\text{Mo}_7\text{O}_{24}]^{6-}$ is the hydrolytically active species. Kinetic experiments at pD 5.9 using a fixed amount of $[\text{Mo}_7\text{O}_{24}]^{6-}$ and increasing amounts of HPNP

revealed slight signs of curvature at 25 molar excess of HPNP. The data fit the general Michaelis–Menten reaction scheme, permitting the calculation of the catalytic rate constant k_2 ($3.02 \times 10^{-4} \text{ s}^{-1}$) and K_m (1.06 M). Variable temperature ^{31}P NMR spectra of a reaction mixture revealed broadening of the HPNP ^{31}P resonance upon increase of temperature, implying the dynamic exchange process between free and bound HPNP at higher temperatures. Addition of salts resulted in the inhibition of HPNP hydrolysis, as well as addition of dimethyl phosphate, suggesting competition for the binding to $[\text{Mo}_7\text{O}_{24}]^{6-}$. The hydrolysis of 10 equiv of HPNP could be achieved in the presence of 1 equiv of $[\text{Mo}_7\text{O}_{24}]^{6-}$, and the multiple turnovers demonstrate that the reaction is catalytic. ^{31}P NMR and Mo K-edge EXAFS spectra measured during different stages of the hydrolysis indicated that under catalytic conditions a partial conversion of $[\text{Mo}_7\text{O}_{24}]^{6-}$ into $[\text{P}_2\text{Mo}_5\text{O}_{23}]^{6-}$ occurs.