X-Ray structure and solvolytic activity towards phosphate diesters of a zirconium(IV) complex

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The X-ray structure of the complex $Zr^{IV}(acac)_2$(salicylideneaminoethanol) [$Zr(acac)_2$(sae)] could be solved, and the reactivity of the complex towards phosphate diester solvolysis was investigated showing substantial rate acceleration over background in benzene–methanol solution (*ca.* 10^8) and in aqueous medium (*ca.* 10^5).

The development of artificial nucleases is an attractive feature in the design of new compounds for application in antisense therapy, or as new tools in gene technology.[1] Predominantly metal complexes as activators for phosphate ester hydrolysis are under intense investigation, since metal ions are known to promote phosphoester transesterification reactions with high rate accelerations.[2] Especially with dinuclear Co(II) complexes[3] and with lanthanide ions,[4] including high-valent Ce(IV)[5] and Th(IV),[6] remarkable activities were observed.

We have investigated complexes of Zr(IV)[7] as activators for phosphate ester solvolysis, because of (i): its high stable oxidation state +IV (ideally suited for electrostatic activation); (ii): its hard Lewis acid character (promising strong interactions with phosphates);† and (iii): its maximum coordination number of eight. In general, tetravalent metal ions are known to hydrolyse phosphate esters more efficiently than tervalent ions.[8] The hydrolytic activity of Zr^{4+} towards phosphate diesters has first been described 60 years ago.[9] Only recently, while our investigations were underway, two reports addressing the hydrolytic activity of $ZrCl_4$ with bis(*p*-nitrophenyl) phosphate (BNPP) **1**[10] appeared. However, neither catalytic behaviour nor a detailed structure of the complexes used were disclosed. Here we report, for the first time, the catalytic activity of the Zr(IV) complex $Zr(acac)_2$(sae) **2** (sae = salicylideneaminoethanol) in phosphate diester solvolysis.

The Zr(IV) complex **2** was prepared from $Zr(acac)_4$ and the ligand H_2sae according to a known procedure.[11] Recrystallisation of **2** from hot toluene produced crystals suitable for X-ray analysis. Complex **2** crystallises in a centrosymmetric dimeric form, **2**–**2**, with the inversion centre in between the two zirconium atoms held together *via* two oxygens of the aminoethanolato-sidechains.‡ The geometry of the zirconium ligand sphere is dodecahedral. Extension to the maximum coordination number eight around zirconium can be considered to be the driving force for dimerisation. Fig. 1 shows the refined structure of **2**–**2**.§ Considerable changes in the UV–VIS spectrum of **2** were observed upon changing from aprotic to protic solvents. This indicates a weak acid–base interaction between zirconium and the protic solvents, and is in accordance with **2** existing predominantly in the monomeric, solvated form under these conditions.

The reactivity of **2** towards phosphate diester cleavage was investigated in C_6D_6–methanol (1 : 1) as solvent owing to the low solubility of **2** in pure protic solvents. The progress of the solvolysis reaction was monitored by ^{31}P NMR spectroscopy.

Upon mixing of **2** (4 mM) with dimethylphosphate (DMP) **3** (16 mM) in C_6D_6–MeOH (1 : 1) the ^{31}P NMR spectra showed a small, broad signal (δ −6; 4.2%) and a large, sharp signal (δ 2.8; 95.8%). The former signal was assigned to zirconium-complexed DMP, the latter refers to free DMP **3**. From this a dissociation constant of 76.2 mM was calculated. This value is significantly higher than the previously reported dissociation constant of $ZrCl_4$ with **1** in water (K_M = 1.3 mmol dm^{-3}).[10b]

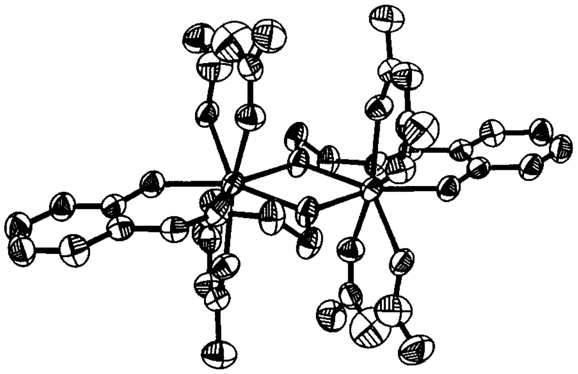
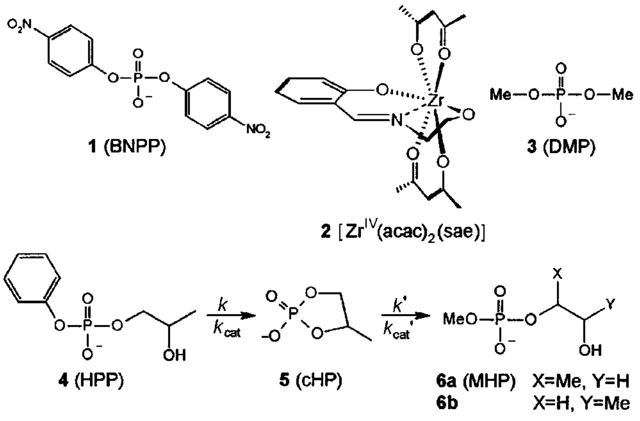


Fig. 1: ORTEP plot of **2**–**2**. Thermal ellipsoids are drawn at the 50% probability level; hydrogens are omitted for clarity.

Analysis by ^{31}P NMR spectroscopy [C_6D_6–MeOH (1 : 1)] revealed that the 'RNA-model' 2-hydroxypropyl phenyl phosphate (HPP) **4** (δ −5.2) reacts in the presence of catalytic amounts of **2** to initially give the cyclic phosphate (cHP) **5** (δ 15.5), which further reacts with methanol to give a mixture of the two isomeric methyl hydroxypropyl phosphates (MHP) **6a** (δ 2.3) and **6b** (δ 1.1) in comparable ratios (Scheme 1). The reactions were performed in unbuffered¶ solutions at 28 °C with either variable concentrations in complex **2** ([**2**] = 1, 2, 3, 4 mM) at constant phosphate concentration ([**4**] = 24 mM) or *vice versa* ([**4**] = 8, 12, 24, 28 mM; [**2**] = 2 mM). The variations of phosphate concentrations *vs.* reaction time were fitted to the model for a consecutive irreversible first-order reaction[12] and gave the pseudo first-order rate constants k_1 and k_1' (Fig. 2).

At constant zirconium concentration (2 mM) the rate constants k_1 (2.77×10^{-4} s^{-1}) and k_1' (1.18×10^{-4} s^{-1}) were independent of phosphate concentration thus proving the catalytic reactivity of **2**. At constant phosphate concentrations (24 mM) a linear dependence on the concentration of complex



Scheme 1

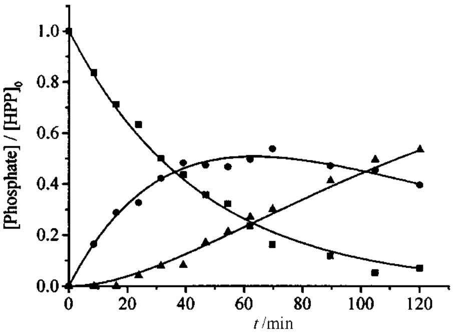


Fig. 2 Time dependent variation of the concentrations of HPP **4** (■), cHP **5** (●), MHP **6** (▲) with curve fitting according to the equations for a consecutive irreversible first-order reaction.[12] [**2**] = 2 mM, [**4**] = 8 mM.

2 is observed. From this linear correlation, the second-order rate constants were determined to be $k_2 = 0.14$ dm^3 mol^{-1} s^{-1} and $k_2' = 0.058$ dm^3 mol^{-1} s^{-1}. These values are slightly lower than the reported values for the hydrolysis of 2-hydroxypropyl *p*-nitrophenyl phosphate with Zr^{4+} (2.2 dm^3 mol^{-1} s^{-1}),[10a] but indicate a reactivity that is comparable to that of lanthanide complexes.[13]

From the kinetic measurements the initial rates v_0 for the cyclisation reaction **4**⟶**5** were also evaluated. The plots of log (v_0) *vs.* log ([**2**]) (with [**4**] = const. = 24 mM) or *vs.* log ([**4**]) (with [**2**] = const. = 2 mM) revealed that the reaction is first order in both reactants giving a clean overall second-order reaction. This indicates that the active species is a distinct 1 : 1 complex between **2** and phosphate diester. The initial rates (for [**2**] = const.) allowed an analysis according to Michaelis–Menten kinetics. From the Eadie–Hofstee plot, the values K_M = 68.6 mmol dm^{-3} and k_{cat} = 0.013 s^{-1} could be obtained. The K_M value is almost identical to the dissociation constant of **3** with **2** (76.2 mmol dm^{-3}), obtained by direct ^{31}P NMR measurement, and therefore represents a true dissociation constant for a 1 : 1 Zr(IV)–phosphate complex. The discrepancy between this K_M value and that for the system ZrCl$_4$ and **1** (1.3 mmol dm^{-3})[10b] indicates activity of higher order Zr(IV) complexes in the latter case formed upon mixing of ZrCl$_4$ with water.

Compared to the uncatalysed reaction ($k_{1,0} = 1.9 \times 10^{-11}$ s^{-1}; $k_{1,0}' = 1.24 \times 10^{-11}$ s^{-1})¶ the reaction rates k_{cat} and k_{cat}' (determined from the pseudo first-order reaction rates k_1' K_M/[**2**]; $k_{cat}' = 0.0043$ s^{-1}) represent a rate acceleration of 6.8×10^8 for the cyclisation of **4**⟶**5** and 3.5×10^8 for the re-esterification **5**⟶**6**. These rate accelerations agree well with the reported reactivity of Zr^{4+}.[10] In contrast to earlier reports, however, the transesterification reactions described here were catalytic in zirconium and proceed *via* a well defined 1 : 1 Zr(IV) : phosphate stoichiometry.

We investigated also the reactivity of **2** towards **4** in the presence of water (C$_6$D$_6$–MeOH–H$_2$O 5 : 5 : 1; T = 40 °C). The best conditions for solubility of **2** were found to be in buffered solution (100 mM Tris-HCl) at pH 9.0 (**2** dissociates under acidic conditions and is barely soluble at higher pH values; no pH rate profile could therefore be measured). In this system the activity of **2** ([**2**] = 1.5 mM, [**4**] = 12 mM) dropped significantly to a rate acceleration of only 75 over background ($k_1 = 9.7 \times 10^{-7}$ s^{-1}; $k_{1,0} = 1.3 \times 10^{-8}$ s^{-1}), but catalytic turnover was still observed. This loss of reactivity may be due to the deactivation of the zirconium in the presence of water or hydroxide.

In aqueous medium without organic cosolvents (H$_2$O; pH 7.0; 10 mM Tris-HCl; 10 mM KCl and MgCl$_2$; 5 mM CaCl$_2$. T = 37 °C), catalyst **2** ([**2**] = 4.5×10^{-5} M, nearly sat. soln.) hydrolysed **1** ([**1**] = 1.6×10^{-5} M) with an observed pseudo first-order rate constant $k_{1,obs} = 1.5 \times 10^{-5}$ s^{-1}. This leads to a rate acceleration of 2.9×10^5, compared to the uncatalysed reaction (5.1×10^{-11} s^{-1}).[14] No significant background reaction was observed under these conditions, therefore hydrolysis of **1** is not due to the salts added to mimic physiological conditions. Although this reaction was performed with excess **2** over **1**, it shows that **2** is still highly active towards **1** even in water.

In conclusion, we have shown that the Zr(IV) complex **2** exhibits substantial activity towards phosphate diesters in transesterification or hydrolysis, despite the fact that **2** is charge-neutral and cannot compensate for the negative charge of the substrates.

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Notes and references

† A preliminary ^{1}H NMR experiment in D$_2$O using Zr(OAc)$_4$ (68 mM), which should give polymeric structures to a lesser extent than ZrCl$_4$ (S. Hannane, F. Bertin and J. Bouix, *Bull. Soc. Chim. Fr.*, 1994, **131**, 262) and dimethyl phosphate (DMP **3**, 270 mM) showed rapid formation (< 2 min) of a Zr(IV)–DMP complex (δ 3.55, broad signal; free DMP: δ 3.43). The signals were not enough resolved to allow determination of an association constant, but approximately 2 equiv. DMP were complexed. This agrees with other observations.[10b]

‡ *Crystallographic data*: C$_{38}$H$_{46}$N$_2$O$_{12}$Zr$_2$; M = 905.21; triclinic, $P\bar{1}$, a = 11.6425(2), b = 12.0019(2), c = 15.7179(3) Å, α = 89.321(3), β = 70.008(3), γ = 86.675(3)°, V = 2060.4(7) Å^3, T = 223 K, Z = 2 (dimers), μ = 0.566 mm^{-1}; 16331 total reflections; 7494 independent reflections; R_{int} = 0.0711, R_1 = 0.0889; $R(\sigma)$ = 0.1133, wR_2 = 0.1252. CCDC 182/1123. See http://www.rsc.org/suppdata/cc/1999/239/ for crystallographic files in .cif format.

§ In the unit cell, **2**–**2** appears as two symmetrically independent molecules. The structural differences however are rather small and mainly occur in the conformation of the acac-ligands along the O···O-edges. This is a known structural feature of Zr(IV)–acac complexes (R. B. VonDreele, J. J. Stezowski and R. C. Fay, *J. Am. Chem. Soc.*, 1971, **93**, 2887; J. V. Silverton and J. L. Hoard, *Inorg. Chem.*, 1963, **2**, 243). For simplicity reasons, Fig. 1 only shows one of the two conformers.

¶ The background rate constants were measured at [NaOMe] = 1 mM to be $k_{2,0} = 2.93 \times 10^{-3}$ dm^3 mol^{-1} s^{-1} and $k_{2,0}' = 1.97 \times 10^{-3}$ dm^3 mol^{-1} s^{-1}. The pH of C$_6$D$_6$–MeOH (1 : 1) was 8.2 corresponding to [MeO$^-$] = 6.3×10^{-9} M and did not change during the catalytic and background reactions. With $k_{1,0} = k_{2,0}$ [MeO$^-$] the first order background rate constants were extrapolated to the conditions used in the catalysed reactions.

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