Actinide (uranyl) hydrolysis of phosphodiesters

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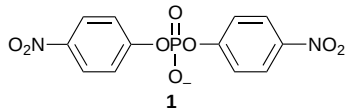
Uranyl (UO_2^{2+}) cations mediate the hydrolysis of aggregated and non-aggregated *p*-nitrophenyl phosphodiesters in mildly acidic aqueous solutions (pH 4.9) with rate enhancements > 1000 at 37 °C.

The past several years have witnessed intensive study of the lanthanide ion catalysis of phosphodiester hydrolysis, stimulated by the drive to develop synthetic nucleases.[1] The activated phosphodiester, bis(*p*-nitrophenyl) phosphate **1** (BNPP), has been a favourite model substrate,[2–4] although lanthanides and their complexes have also cleaved DNA,[5,6] phosphonates[7] and phosphate monoesters.[8] RNA, and the RNA model substrate **2**, have also been catalytically cleaved by lanthanides[1] and other metal cations.[9–11]

Recently, we reported that the Eu^{3+} or La^{3+}–H_2O_2 cleavages of liposomal phosphodiester **3** were accelerated 50–70 times, relative to the non-aggregated model phosphodiester **4** (PNP = *p*-nitrophenyl).[12] Binding of the cationic lanthanide ions to the anionic liposomes afforded additional catalysis above that normally expected from the lanthanides.

Although many lanthanide and transition metal cations have been examined as catalysts for the hydrolysis of phosphodiesters, the actinide uranyl (UO_2^{2+}) cation has not.† This is surprising because UO_2^{2+} is known to bind strongly to various nucleotides,[14] as well as DNA,[15] with P–O–U bonding a key feature.[14] Indeed, UO_2^{2+} bound between the phosphate groups of stacked DNA strands mediates photolytic, oxidative cleavage of the DNA.[15] Moreover, UO_2^{2+} catalyses the oligomerization of nucleotide 5′-phosphoroimidazolides and 5′-thiophosphoroimidazolides *via* U–O–P–Im complexes in which the electrophilic uranium activates the substrate to nucleophilic attack at P by an incoming 2′-OH nucleophile.[16]‡

The clear implication is that UO_2^{2+} should bind activated phosphodiesters and predispose these substrates to hydrolytic cleavage. Here, we report that this inference is correct: substrates **1**–**4**, as well as the micellar phosphodiester **5**, are indeed hydrolysed by UO_2^{2+} in mildly acidic aqueous solutions, with additional catalysis apparent for the aggregated substrates **3** and **5**. These are the initial examples of phosphodiester hydrolysis mediated by uranyl cations, and also feature the first metal ion cataysed hydrolysis of a micellar phosphodiester **5**.



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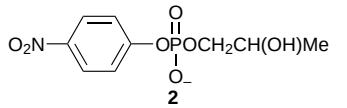

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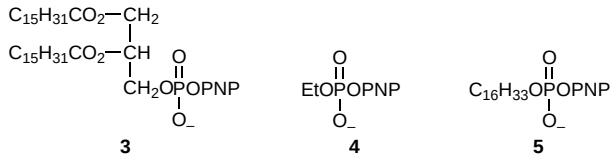

3 **4** **5**

The RNA model, 2-hydroxypropyl *p*-nitrophenyl phosphate **2**,[18] was efficiently hydrolysed in the presence of excess UO_2^{2+} at 37 °C, pH 4.9±0.1.§ The exact conditions appear in Table 1, where kinetic data for comparable hydrolyses mediated by the lanthanide cations, Eu^{3+} and Tm^{3+}, are also collected. Note that these comparisons are under identical conditions in the absence of buffer ions.¶

UO_2^{2+} mediates the quantitative (UV, HPLC) cleavage of substrate **2** with an observed rate constant (2.2×10^{-4} s^{-1}) that is at least 6700 times greater than that for the uncatalysed reaction, where the rate constant for the uncatalysed hydrolysis of **2** is determined at pH 7,[1a] and must be presumed to exceed the value at pH 4.9. (Hydrolysis was not observed over 4 days in the absence of UO_2^{2+} at pH 4.9.) The k_{rel} values in Table 1 are therefore minima. Moreover, the uranyl-catalysed hydrolysis is also 3.5–4.5 times faster than lanthanide cleavage brought about by Eu or Tm cations under these conditions.

Hydrolysis of **2** involves cyclization with displacement of the leaving group by intramolecular OH attack[1] on the metal-bound and activated phosphate. Accordingly, UO_2^{2+} cleavage of ethyl *p*-nitrophenyl phosphate, **4**,[12] which lacks the neighbouring hydroxy is *ca.* 16 times slower ($k_{obs} = 1.4 \times 10^{-5}$ s^{-1}) than the hydrolysis of **2** under the conditions of Table 1. Nevertheless, cleavage of **4** is at least 420 times faster than the uncatalysed hydrolysis of **2** at pH 7.

Interestingly, the UO_2^{2+}-mediated hydrolyses of **2** and **4** are *ca.* 3.7 times faster than analogous Eu^{3+}-catalysed processes (Table 1 and ref. 12). At the low pH used in the present reactions, the lanthanides ($pK_a \sim 8.0$)[19] bear H_2O rather than OH groups and most likely express only electrophilic catalysis due to P–O^- binding; nucleophilic assistance from M–OH would be suppressed. However, UO_2^{2+} has pK_a *ca.* 4.2–6.1 in aqueous solution, depending on ionic strength, added salts and ligand association.[19] Therefore, both electrophilic (M^+, external H_2O) and nucleophilic (M–OH) components may contribute to uranyl cation catalysis at pH 5.

UO_2^{2+} precipitates with BNPP **1** or the aggregated substrates **3**[12] or **5**.‖ However, addition of 0.1 equivalents of *N*-hexadecyl-*N*,*N*′,*N*′-trimethylethylenediamine (HTMED)[12,20] solubilizes the uranyl cations, presumably in a mixed HTMED-substrate aggregate, thus making it possible to obtain stable UO_2^{2+}-substrate solutions. Neither tetramethylethylene diamine nor cetyltrimethylamine solubilizes UO_2^{2+} under our conditions; only a long-chain diamine succeeds, indicating the dependence of solubilization on both cation chelation and HTMED

Table 1 Kinetics of the metal ion catalysed hydrolysis of **2**[a]

Catalyst	k_{obs}/s^{-1}	k_{rel}	% Cleaved at 20 h
None[b]	3.3×10^{-8}	1.0	—
$UO_2(NO_3)_2$	2.2×10^{-4}	6700	100
$EuCl_3$	4.8×10^{-5}	1450	75
$TmCl_3$	6.2×10^{-5}	1900	78

[a] Conditions: [**2**] = 1×10^{-4} mol dm^{-3}, [catalyst] = 1×10^{-3} mol dm^{-3}, 0.01 mol dm^{-3} aq. KCl, pH 4.9 ± 0.1, 37 °C. [b] Data from ref. 1(*a*) at pH 7.0, 37 °C.

Table 2 Kinetics of the uranyl ion catalysed hydrolysis[a]

Substrate	k_{obs}/s^{-1}	k_{rel}	% Cleaved at 20 h
1	9.5×10^{-6}	5.6	84
2	1.5×10^{-5}	8.8	95
3	1.1×10^{-4}	65	95
4	1.7×10^{-6}	1.0	72
5	1.1×10^{-4}	65	92

[a] Conditions: [substrate] = 1×10^{-4} mol dm^{-3}, [HTMED] = 1×10^{-4} mol dm^{-3}, [UO_2^{2+}] = 1×10^{-3} mol dm^{-3}, 2×10^{-3} mol dm^{-3} HEPES buffer, 0.01 mol dm^{-3} KCl, pH 4.9 ± 0.1, 37 °C. Kinetic data were obtained at 317 nm.

coaggregation. We are thus able to measure the UO_2^{2+} hydrolytic rate constants collected in Table 2.**

Note first that the UO_2^{2+} catalysed cleavages of **2** and **4** are slower by factors of *ca.* 15 and 8, respectively, relative to reactions in the absence of HTMED (*cf.* Table 1 and above), presumably because the electrophilic character of the uranyl cation is attenuated by chelation with HTMED. Most importantly, however, Table 2 reveals the additional reactivity inherent in the liposomal **3** and micellar **5** phosphodiester substrates, both of which are hydrolysed 65 times more rapidly than **4** in the presence of UO_2^{2+}. This aggregate catalysis is undoubtedly due to the binding of the metal cations to the anionic aggregates, assisted by the HTMED which probably forms part of a coaggregate. The cleavage of liposomal **3** by the lanthanide, Eu^{3+}, is similarly enhanced by a factor of 56, relative to **4**.[12]

The UO_2^{2+}-mediated hydrolyses of **3** and **5** at pH 5 (Table 2) occur at similar rates to the Eu^{3+} reaction with **3** (k_{obs} = 2×10^{-4} s^{-1}, pH 5.6, 25 °C) at similar reactant concentrations.[12] Relative to substrate **2** in the absence of UO_2^{2+} (Table 1), the actinide plus aggregate catalysis affords a kinetic advantage of > 3300 in the hydrolysis of substrates **3** and **5**.

Finally, we note that both the *exo*- and *endo*-liposomal *p*-nitrophenylphosphate functional groups of **3** are quantitatively cleaved by UO_2^{2+}-HTMED in a uniphasic kinetic process at both 25 and 37 °C (T_c of **3** is 42 °C[12]); there is no evidence of the *exo*-liposomal-specific cleavage observed with 'naked' Eu^{3+} at 25 °C.[12] Presumably, the difference originates in the obligatory presence of HTMED, which can chelate UO_2^{2+} and rapidly ($> k_{hydrol}$) transport it across the liposomal bilayer to mediate *endo*-liposomal cleavage. Alternatively, the HTMED molecules could disrupt the integrity of the liposomal membrane, permitting uranyl cation permeation. Eu^{3+} cleavage of liposomal **3** also becomes complete and uniphasic in the presence of HTMED for related reasons.[12]

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Footnotes

† Martell (ref. 13) reported the rapid cleavage of the fluorophosphonate Sarin by the 1,8-dihydroxynaphthalene-3,6-disodium sulfonate (DNS) complex of UO_2^{2+}. Phosphodiester substrates, however, are much less reactive than fluorophosphonates. Indeed, we find substrates **2** and **3** to be quite unreactive to the UO_2^{2+}–DNS complex at either pH 5 or 7 ($k_{hydrol} \ll 1x10^{-6}$ s^{-1}), with hydrolysis < 50% complete after 5 days at 37 °C.

‡ After our current work had been completed, it was reported that the actinide Th^{4+} accelerates the hydrolyses of various nucleotide phosphomonoester and -diester bonds in acidic aqueous solutions (ref. 17).

§ UO_2^{2+} precipitates as polynuclear metal hydroxide gels at pH ≥ 5.3 (ref. 19) restricting us to pH *ca.* 5.0. Below pH 4.0, no hydrolysis of the phosphodiester substrates was observed over 24 h.

¶ Hydrolyses were followed spectrophotometrically between 200–600 nm; and kinetics were measured at both 290 nm (disappearance of substrate) and 317 nm (appearance of *p*-nitrophenol). Pseudo-first-order rate constants (measured over 20 h) are reported as means of 2 or 3 runs ($r > 0.997$), with reproducibility within ±10%. The pH, adjusted with 0.1 mol dm^{-3} HCl, was buffered by the metal cations and varied no more than 0.2 pH units during the course of reaction. The percentage cleavage was determined from the concentration of liberated *p*-nitrophenoxide ion, measured at pH 12 (400 nm), after 20 h of reaction.

‖ Substrate **5** (mp 165 °C, decomp.) was prepared by phosphorylation of hexadecanol with 4-nitrophenyl phosphorodichloridate (CH_2Cl_2, Et_3N, 0–25 °C, 3.5 h), followed by methanolysis to hexadecyl methyl *p*-nitrophenyl phosphate. The methyl group was removed by reaction with LiBr in refluxing acetone (48 h), affording white crystalline **5** (Li salt), which was characterized by NMR and elemental analysis.

** HEPES buffer (2 mmol dm^{-3}) is present in these runs because it is necessary in the preparation of liposomal **3** (ref. 12). The buffer alone does not solubilize UO_2^{2+} in the presence of substrates **1**, **3** or **5**, nor does it alter the reactivity of the cation toward **2** or **4**. Control experiments also show that HTMED alone does not induce cleavage of the substrates.

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