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Remarkable Hydrolysis of Phosphodiester by Neutral Lanthanide(III) DO3A complexes

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Abstract: The hydrolysis of the 4-nitrophenyl phosphate ester of propylene glycol (HPNP) is catalyzed by *neutral* lanthanide(III) DO3A complexes. Hydrolysis experiments with complexes of modified ligands where the metal complex was positively charged did not yield in a significant increase of the rate constant. Copyright © 1996 Elsevier Science Ltd

The hydrolysis of the phosphate backbone of nucleic acids is one area where charged metal ion complexes,[1] especially those containing lanthanides(III) ions, have been employed. A wide variety of systems has been reported in which lanthanides and especially europium(III) was used as the catalytic lanthanide ion.[2] Bruice et al. demonstrated the great potential of lanthanides in the hydrolysis of phosphodiesters by using a phosphodiester with two intramolecular lanthanide binding sites. They observed a 10^{13} fold rate enhancement in the hydrolysis of phosphodiesters. Other systems have been described, in which stable complexes significantly increase the hydrolysis of phosphodiesters. It seems that two vacant coordination sites on the metal are necessary for efficient hydrolysis. Therefore complexes with late lanthanides and hosts possessing eight ligands are inactive species.

Since most of these systems exhibit the active species as a positively charged lanthanide(III) complex, it is generally accepted that the hydrolytic behavior of those systems benefits from the ionic attraction of the phosphate anion. On the other hand, non-ionic molecules are believed to benefit from their lower osmolality due to their absence of charge. Therefore the pharmaceutical requirements these lanthanide(III) complexes have to meet are low osmolality, thermodynamic and/or kinetic stability.

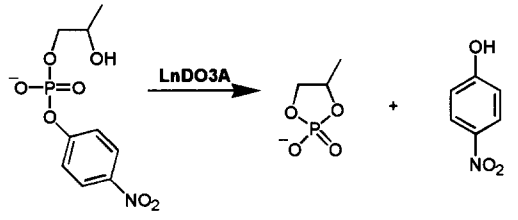
Here we report on the hydrolytic capability of lanthanide(III) DO3A (**1 a, b, c**) complexes. Recent studies have demonstrated that lanthanide complexes with polyamino carboxylates exhibit good complex stability.[3] 1,4,7,10-Tetraazacyclo-dodecane-1,4,7-triacetic acid (DO3A) was chosen as the most promising molecule. In an effort to compare neutral lanthanide(III) complexes with positively charged complexes of basically the same geometry we synthesized a tri-substituted cyclene derivative that forms positively charged complexes and can be attached to peptides or antisense oligonucleotides for selective recognition of targeted RNAs.

Since DO3A provides only seven donor atoms, it was envisioned that two coordination sites on the metal ion could be used for phosphate ester hydrolysis.

The questions to be answered are:

1) Is a neutral lanthanide(III)DO3A complex capable of hydrolyzing phosphate diesters?

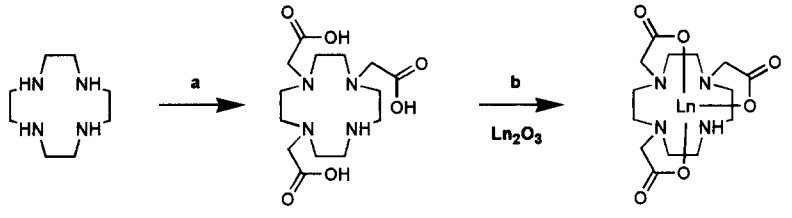
2) Does a positive charge at the metal center increase the hydrolytic behavior of such complexes?



HPNP

Scheme1

In order to investigate the hydrolysis of phosphate diesters, the 4-nitrophenyl phosphate ester of propylene glycol (HPNP) was used as an RNA model compound.[4] The rate of transesterification of HPNP (Scheme 1) was monitored by the production of nitrophenolate at 400 nm by UV-spectroscopy.



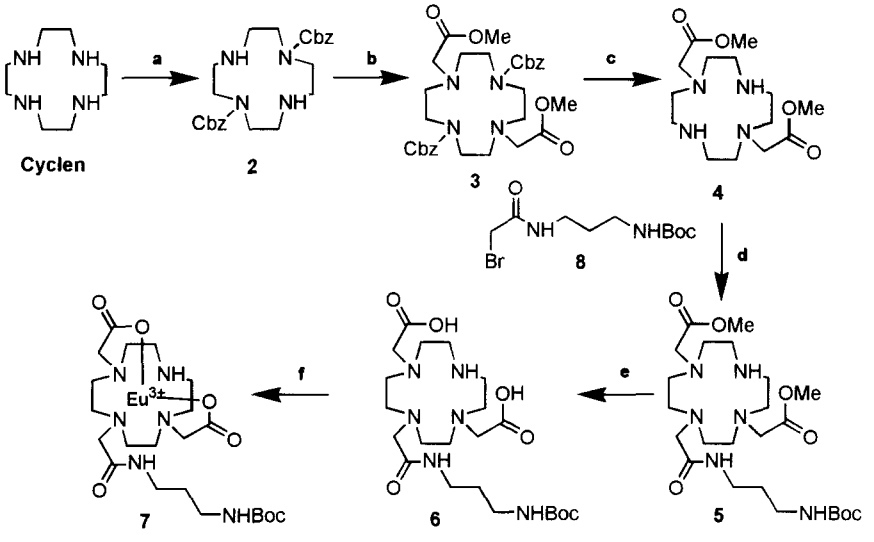
Cyclen 1

a) Chloroacetic acid, H_2O, pH = 8.0, 50° C; b) H_2O, reflux 2h.

Scheme 2

Three different neutral DO3A Ln(III) complexes (**1a** EuDO3A, **1b** YDO3A, **1c** LaDO3A) were prepared (Scheme 2) and tested for hydrolytic activity; the Eu(III)DO3A complex was the most active hydrolytic species (Figure 1). In order to compare neutral with charged complexes we synthesized a ligand that could form stable, charged complexes. The cyclen derivative **7** used to form positively charged complexes was synthesized by

double Cbz-protection of cyclen followed by double alkylation with methyl bromoacetate. Hydrogenolytic cleavage of the protecting groups yielded the bis-substituted cyclen derivative **4**. Mono alkylation with side chain **8**[5] was achieved in CH_3CN with K_2CO_3 as the base.



a) CbzCl, EtOH, dimethoxymethane, 24 h, 98 %; b) methyl bromoacetate K_2CO_3, MeCN, 66 %; c) H_2, Pd/C, EtOH, 56 %; d) side chain **8**, MeCN, 36 %; e) LiOH, MeOH/H_2O, 78 %; f) Eu_2O_3, H_2O, reflux 60 %.

Scheme 3

Compound **5** was purified by flash chromatography and the carboxylates were liberated with LiOH in MeOH/H_2O. The europium complex was generated by refluxing excess ligand with Eu_2O_3. The free ligand was removed by treating the solution with basic ion exchange resin. After filtration and evaporation of water complex **7** was obtained in 60 % yield. The DO3A-ligand was prepared as described[6] and confirmed by NMR spectroscopy and elemental analysis. The metal complexes[7] were used as 0.02 M solutions (μ = 0.1 $NaNO_3$; HEPES[8] buffer 0.05 M). In order to be certain that indeed the metal complex and not traces of free metal ions were the hydrolytic species, an additional amount of 25% ligand was added to the complex isolated from the ion-free (1μS) solution. The ligand and the metal complex were again refluxed for five hours and the cold solution was filtered through a 2μm Spartan filter. The difference in the initial rates obtained from these samples was within experimental error, when compared to initial rate data obtained from the stoichiometrically-prepared complexes.

All cleavage experiments were performed under pseudo first order conditions (DO3A 0.01M; 0.05 M HEPES/NaHEPES, pH= 7.3-8.4, μ = 0.1 $NaNO_3$). The initial rate constants were determined at 10% conversion of the starting material.[9]

Scheme 1 shows the pH-dependence of the rate constants ($k_{obs.}$) of four different neutral complexes, the positively charged complex and the spontaneous (background) hydrolysis under our conditions. Approximately 300-500fold rate enhancement was observed in the case of the uncharged europium complex. Pseudo first order hydrolysis experiments with the charged europium complex gave hydrolysis constants in about the same range as with the uncharged complexes. The positive charge on the metal does not significantly contribute to the rate of hydrolysis even though at pH 8 a 1.5 times higher rate constant was observed.

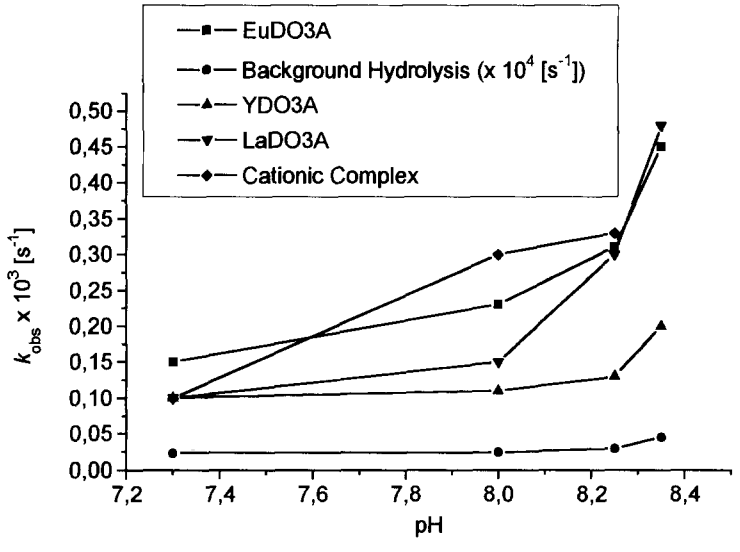


Figure 1

These results demonstrate the remarkable hydrolytic behavior of neutral EuDO3A and of ligand 7. Ligand 7 will be used as a building block in the design of artificial ribonucleases. Further application of these hydrolytic complexes will be reported in due course.

pH	k_{obs} [s^{-1}] **1a** (0.01 M)	background hydrolysis [s^{-1}]	k_{obs} [s^{-1}] **1b** (0.01 M)	k_{obs} [s^{-1}] **1c** (0.01M)	k_{obs} [s^{-1}] **7** (0.01 M)
7.3	1.5×10^{-4}	2.3×10^{-6}	1.0×10^{-4}	1.0×10^{-4}	1.0×10^{-4} [s^{-1}]
8	2.3×10^{-4}	2.4×10^{-6}	1.1×10^{-4}	1.6×10^{-4}	3.0×10^{-4} [s^{-1}]
8.25	3.1×10^{-4}	2.9×10^{-6}	1.3×10^{-4}	3.0×10^{-4}	$3.3x\ 10^{-4}$ [s^{-1}]
8.31	4.5×10^{-4}	4.5×10^{-6}	2.0×10^{-4}	4.5×10^{-4}	4.0×10^{-4} [s^{-1}]

Table 1: k_{obs} for the metal complexes and the background hydrolysis

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7. DO3A is the abbreviation of 1,4,7,10-tetraazacyclododecane 1,4,7-triacetic acid. The lanthanide DO3A complexes were prepared by refluxing stoichiometric amounts of the ligand and Ln_2O_3 for five hours. To remove any trace of free lanthanide ions the cold solution was filtered through a 0.2 μm Spartan filter and titrated first with a strongly acidic ion exchange resin (IRA 120) and consecutively with a strongly basic ion exchange resin (IRA 400) (1 μS; pH = 7.5). Complexes were obtained as white solids after evaporation of water. Spartan 30/A (0.2μm, Brown Rim D) filter are purchased from Schleicher & Schuell, Germany. Elemental analysis and FAB mass spectra confirmed the composition of the products. All kinetic experiments were performed in Millipore water and the pH was checked after every kinetic run.
8. HEPES is the commercial name of N-2-Hydroxyethylpiperazine-N'-2-ethane sulfonic acid and was purchased from Biomol.
9. Kinetic studies: HEPES is the commercial name for *N*-(2-hydroxyethyl)piperazine-*N*-2-ethanesulfonic acid and was purchased from Biomol. The pH value of HEPES buffer solutions was adjusted with Metrohm titrator/pH meter (Metrohm 716 DMS Titrino) at room temperature. Molar extinction coefficients of *p*-nitrophenol were determined under hydrolysis conditions over a pH range from 5.2 to 10.2. Hydrolysis experiments were performed in teflon sealed quarz cuvettes (1 cm path length) which were incubated in an water bath at 50°C ± 0.2 °C. Additionally, in every run a reference cuvette was incubated which only contained buffer and HPNP. Absorbance of *p*-nitrophenolate was measured at 400 nm against background hydrolysis with a Shimadzu UV-visible UV-1601 dual-beam spectrophotometer fitted with a thermostated cuvette holder. After each hydrolysis experiment the pH of the sample and reference solution was checked (differences ≤ 0.03). Rate constants were reproducible to ± 10 %. Every kinetic run was repeated in the presence of stoichiometric amounts of EDTA and no change of hydrolytic activity was observed.

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