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Letter

Remarkable phosphodiester hydrolysis activity of a novel Ce^{IV} complex in neutral aqueous solutions

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Abstract

A novel Ce^{IV}-complex of 1,4,7-tris(carbamoylmethyl)-1,4,7-triazacyclononane has been synthesized. The complex is both stable in aqueous solutions at neutral pH and very efficient in promoting the hydrolysis of a phosphodiester model compound and yeast $tRNA^{phe}$. The RNA model compound, 2-hydroxypropyl-*p*-nitrophenylphosphate hydrolysis is accelerated 7400-fold at pH 7.5, as a result of an unprecedented hydrolytic activity.

Keywords: Phosphodiester hydrolysis; Artificial enzymes; Lanthanide complexes; RNA cleavage

1. Introduction

Acceleration of phosphodiester hydrolysis received considerable attention in recent years due to its projected impact in a number of fields, including potential applications in molecular biology [1] and gene therapy [2,3]. Cobalt(III) [4–8] and various lanthanide [9–15] complexes are known to show exceptional activity in phosphodiester hydrolysis. Especially La^{III}, Eu^{III} and Dy^{III} ions have remarkable catalytic activity, both complexed and as simple hydrated ions. The strong Lewis-acid lanthanides can neutralize the negative charge on the phosphate group, and at the same time supply a metal-bound hydroxide as a nucleophile. With larger charge/size ratio Ce^{IV} is expected to cause larger rate accelerations in the phosphodiester hydrolysis, but Ce^{IV} salts at neutral pH, immediately form ceric-hydroxide gel [16], both slowing down the reaction and complicating the kinetic characterization of hydrolysis. The gel formation could be avoided by using nonionic Brij-35 micelles [17] or working at acidic pH values, both approaches considering the potential applications, would not offer optimal solutions. We here report a novel Ce^{IV} complex (**1**) which is remarkably stable in aqueous solutions at pH 7.4.

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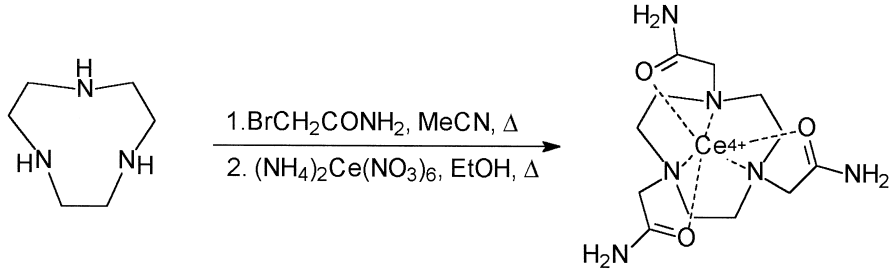
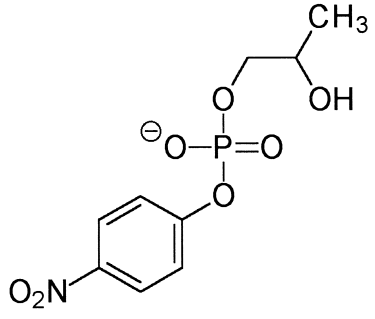
2. Results and discussion

The ligand was synthesized by reacting 1,4,7-triazacyclononane with bromoacetamide in EtOH, the trialkylated azacrown precipitated from the solution (other lanthanide complexes of the ligand were reported in Ref. [18]). Ce^{IV} complex was prepared by simply refluxing a solution of the ligand with $Ce(NH_4)_2(NO_3)_6$ in EtOH. Removal of the solvents, followed by trituration with hexane resulted in compound **1** in the form of a light yellow powder. Satisfactory analytical data were obtained for the nitrate salt of the complex. The hydrolytic activity of the complex was studied using the phosphodiester model compound 2-hydroxypropyl-*p*-nitrophenylphosphate (HPNP, **2**). Deionized, deaerated water was used in all buffers to minimize the possibility of oxidative cleavage.



1

Pseudo-first order rate constant for the transesterification in pH 7.4 HEPES buffer (50 mM) was found to be 0.88 h^{-1}, that represents a remarkable 7400-fold rate increase compared to the uncatalyzed reaction [19]. To the best of our knowledge, this is the largest rate acceleration obtained using a lanthanide complex in an additive-free aqueous solution for the hydrolysis of the RNA-model compound **2**. The complex is stable under the hydrolysis conditions, no precipitation of ceric hydroxides was observed for the duration of hydrolysis.



2

The activity of the Ce^{IV} complex was further studied using yeast $tRNA^{phe}$, as this RNA has been utilized as substrate in a number of previous studies [20–23], where phosphodiester cleaving activity of natural enzymes were mimicked. tRNAs have high degree of secondary structure, and in the earlier studies hydrolytic agents were shown to have differential reactivity at various parts of the RNA structure, mostly attacking the D-loop of the tRNA [23]. Such a result is explained by differing accessibilities of phosphodiester bonds to the metal complex of bulky ligands. Although this may be

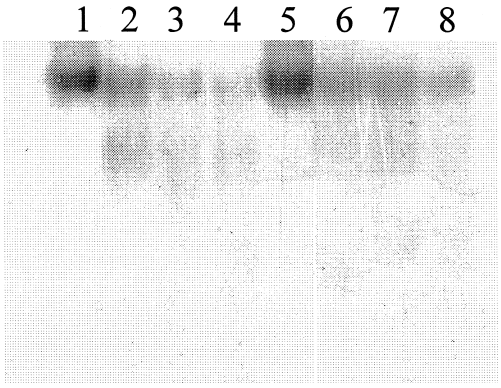


Fig. 1. Silver-stained, 10% non-denaturing polyacrylamide gel showing the extent of RNA degradation in the presence of the Ce^{IV} complex **1**. The reaction was carried out at 37°C in pH 7.4 HEPES buffer (50 mM); yeast $tRNA^{phe}$ (0.13 μg μl^{-1}) was treated with either 5 mM (lanes 1–4) or 0.5 mM (lanes 5–8) Ce^{IV}-complex. Aliquots were taken at 2-h intervals and diluted 10-fold with buffer, and 7 μl of the diluted reaction mixture was applied to the gel. Lanes 1 and 5, 0 h; lanes 2 and 6, 2 h; lanes 3 and 7, 4 h; lanes 4 and 8, 6 h of reaction.

seen as a way to achieve selectivity, in an artificial enzyme construct, the hydrolytic unit ideally be maximally active, regardless of the secondary structure of the intended substrate; in a real-life situation, targeted RNA segment may have a similar protected structure and less than maximal hydrolysis in those regions would not be desirable. Once a highly active complex is developed, better selectivity could achieved by conjugating the hydrolytic unit to an antisense oligonucleotide [11,13].

tRNA hydrolysis was carried out at pH 7.4 HEPES buffer (50 mM). In a typical experiment, yeast $tRNA^{phe}$ (0.13 μg μl^{-1}) was treated with either 5 mM or 0.5 mM Ce^{IV} complex. Aliquots were taken at 2-h intervals (Fig. 1) and the bands were separated in a 0.4 mm 10% non-denaturing polyacrylamide gel. Unlike other works in the field, we chose to visualize the RNA degradation using a non-radioactive method: the bands were made visible by silver staining. The gel was then scanned and analyzed. The cerium complex at 5 mM concentration, results in an essentially complete degradation of the tRNA in 6 h.

It appears even the smaller fragments which are formed are further hydrolyzed to ribonucleoside level. This remarkable hydrolytic activity is not affected by the addition of excess EDTA, further demonstrating that the activity is not due to free Ce^{IV} ions.

3. Conclusion

In conclusion, we have synthesized a complex of high activity towards HPNP and RNA, causing total hydrolysis of yeast $tRNA^{phe}$ at neutral pH. The unprecedented fast hydrolysis of HPNP also demonstrates that the cerium complexes are of significant potential for the phosphodiester hydrolysis, even in neutral pH aqueous solutions. Work towards the development of artificial phosphodiesterases is in progress in our laboratory.

Acknowledgements

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