Inorg. Chem. **2008**, *47*, 2919−2921

New Gadolinium Complex with Efficient Hydrolase-like Activity: A 100-Million-Fold Rate Enhancement in Diester Hydrolysis

Maryene A. Camargo,[†] Ademir Neves,[*,†] Adailton J. Bortoluzzi,[†] Bruno Szpoganicz,[†] Adriano Martendal,[‡] Michael Murgu,[§] Franciele L. Fischer,[⊥] Hernán Terenzi,[⊥] and Patricia Cardoso Severino[⊥]

Laboratório de Bioinorgânica e Cristalografia (LABINC), Departamento de Química, Universidade Federal de Santa Catarina, 88040-900 Florianópolis, Santa Catarina, Brazil, Laboratório de Expressão Gênica, Departamento de Bioquímica, CCB, Universidade Federal de Santa Catarina, 88040-900 Florianópolis, Santa Catarina, Brazil, and Waters Technologies do Brazil, Alameda Tocantins 125, 27° andar, 06455-020, Barueri, São Paulo, Brazil

Received November 3, 2007

The synthesis, structure, and hydrolase-like catalytic activity of a new mononuclear gadolinium complex $[Gd(L)(NO_3)(H_2O)_3](NO_3)_2$ (**1**) are reported. A clean two-stage kinetic reaction for hydrolysis of the diester 2,4-bis(dinitrophenyl)phosphate by **1** was followed, and the rate constants were determined. A high DNA cleavage activity was also demonstrated. The active species in the hydrolytic process is proposed based on the X-ray structure, electrospray ionization mass spectrometry analysis, and kinetic and potentiometric equilibrium studies of **1**.

Lanthanide salts have proven to be extraordinarily effective in accelerating the rate of phosphate ester hydrolysis by several orders of magnitude.[1] This efficiency results from the combination of a high oxidation state and charge density, the absence of redox chemistry, and high ligand exchange rates. These characteristics make the Ln^{III} ions potential centers in the development of artificial nucleases.[2]

Although hydrated Ln^{III} ions have been shown to be effective as hydrolases, the free ions become unstable at slightly above pH 7, tending to precipitate from solution as hydroxides at around pH 9, and they are toxic to biological systems. Thus, considerable efforts are being made to develop lanthanide complexes that efficiently hydrolyze phosphate diester bonds.[3] Gómez-Tagle and Yatsimirsky have reported that a system consisting of Ln^{III} and bis-Tris propane (BTP) protected the lanthanide cation from precipitation besides promoting high hydrolytic activity in a basic solution.[4]

In this Communication, we report the synthesis and structure of a new mononuclear $[Gd(L)(NO_3)(H_2O)_3](NO_3)_2$ (**1**) complex, which displays high efficiency toward the hydrolysis of the activated substrate 2,4-bis(dinitrophenyl)phosphate (BDNPP). Through potentiometric studies and electrospray ionization mass spectrometry (ESI-MS) analysis of **1** and its kinetic behavior in a CH_3CN/water solution, we propose the active catalytic species as well as its mechanism of action. Interestingly, complex **1** displays a high proficiency in the cleavage of plasmid DNA under physiological conditions and at very low concentrations of **1**, indicating its activity as a chemical nuclease.

Complex **1**[5] was prepared by adding $Gd(NO_3)_3 \cdot 6H_2O$ to a methanolic solution containing the ligand L[6] (1:1 stoichiometry), under magnetic stirring at 40 °C for 40 min. The solid obtained after total evaporation of the solvent was recrystallized in an acetonitrile/acetone solution, yielding colorless monocrystals suitable for X-ray analysis.

Complex **1** crystallizes[7] in a monoclinic cell, space group $P2_1/n$ (Figure 1).

Despite the unsymmetrical ligand L containing two potentially distinct coordination moieties, which facilitates

* To whom correspondence should be addressed. E-mail: ademir@qmc.ufsc.br.

† Laboratório de Bioinorgânica e Cristalografia (LABINC), Departamento de Química.

‡ Present address: Departamento de Química, Universidade do Sul de Santa Catarina, 88704-900 Tubarão, Santa Catarina, Brazil.

§ Waters Technologies do Brazil.

⊥ Laboratório de Expressão Gênica, Departamento de Bioquímica.

(1) (a) Roigk, A.; Hettich, R.; Schneider, H.-J. *Inorg. Chem.* **1998**, *37*, 751–756. (b) Kuzuya, A.; Machida, K.; Sasayama, T.; Shi, Y.; Mizoguchi, R.; Komiyama, M. *J. Alloys Compd.* **2006**, *408−412*, 396–399.

(2) Franklin, S. J. *Curr. Opin. Chem. Biol.* **2001**, *5*, 201–208.

(3) (a) Peluffo, F.; Torres, J.; Kremer, C.; Domínguez, S.; Mederos, A.; Kremer, E. *Inorg. Chim. Acta* **2006**, *359*, 2107–2114. (b) Guoqiang, S.; Xiaogang, Q. *Chin. J. Anal. Chem.* **2006**, *34*, 10–15.

(4) (a) Gómez-Tagle, P.; Yatsimirsky, A. K. *J. Chem. Soc., Dalton Trans.* **1998**, *2957*, 2959. (b) Gómez-Tagle, P.; Yatsimirsky, A. K. *Inorg. Chem.* **2001**, *40*, 3786–3796.

(5) Yield: 76%. Found: C, 43.26; H, 4.51; N, 11.87. Calcd for $[Gd(C_{34}H_{35}N_5O_2)(NO_3)(H_2O)_3](NO_3)_2$: C, 43.31; H, 4.38; N, 11.88. Selected IR data (KBr): 3200, 1607, 1573, 1480, 1446, 1384, 1313, 1265, 1192, 1157, 1100, 1011, 777, 759, 636, 492 cm^{-1}.

(6) (a) L = 2-bis[{(2-pyridylmethyl)aminomethyl}-6-{(2-hydroxybenzyl)-(2-pyridylmethyl)}aminomethyl]-4-methylphenol. (b) Mitic, N.; Smith, S. J.; Neves, A.; Guddat, L. W.; Gahan, L. R.; Schenk, G. *Chem. Rev.* **2006**, *106*, 3338–3363.

(7) X-ray analysis: $C_{34}H_{41}GdN_8O_{14}$, fw 943.00, $a = 19.269(4)$ Å, $b = 10.267(2)$ Å, $c = 19.662(4)$ Å, $\beta = 102.80(1)°$, $V = 3793.2(13)$ Å^3, $Z = 4$, $\mu = 1.827$ mm^{-1}, unique 6723 [R(int) = 0.0364], parameters 532, GOF(F^2) = 1.033, R1 [$I > 2\sigma(I)$] = 0.0357, wR2 (all data) = 0.0928.

10.1021/ic702167p CCC: $40.75
Published on Web 03/26/2008

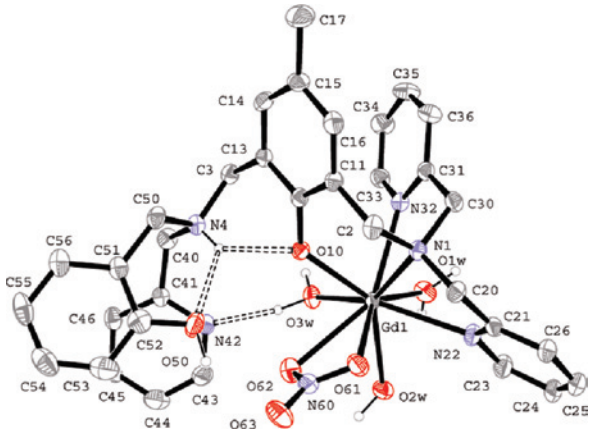

Figure 1. ORTEP plot of the cation $[Gd(C_{34}H_{41}N_6O_8)]^{2+}$. Ellipsoid at the 40% probability level. Selected bond lengths and angles (Å and deg): Gd1–O10, 2.288(3); Gd1–O3W, 2.412(4); Gd1–O1W, 2.412(4); Gd1–O2W, 2.484(4); Gd1–O61, 2.492(4); Gd1–N32, 2.536(4); Gd1–N22, 2.623(4); Gd1–N1, 2.638(4); Gd1–O62, 2.656(4); O10–Gd1–O61, 84.33(12); O10–Gd1–N32, 76.04(12); O61–Gd1–N32, 136.53(13); O10–Gd1–N22, 137.44(13); O61–Gd1–N22, 73.34(13); N32–Gd1–N22, 95.74(13); O10–Gd1–N1, 75.56(12); O61–Gd1–N1, 71.44(12); N32–Gd1–N1, 66.28(12); N22–Gd1–N1, 63.24(13); O10–Gd1–O62, 71.55(12); O61–Gd1–O62, 49.03(13), N32–Gd1–O62, 146.27(13); N22–Gd1–O62, 114.40(13); N1–Gd1–O62, 113.09(12).

the generation of homo- and heterodinuclear mixed-valence $M^{III}M^{II}$ complexes,[8] we present the formation of the mononuclear gadolinium complex. In this complex, the Gd metal engages the available chelate in the soft side of the ligand, that is, one tertiary amine nitrogen atom N1, two pyridine nitrogen atoms N22 and N32, and one phenolate oxygen O10, in addition to the two oxygens of the bidentate nitrate O61 and O62 and three water molecules coordinated by the Gd atom. The tertiary amine nitrogen atom N4, a component group of the hard site of L, is protonated, which could be evidenced by the intramolecular hydrogen bonds (N4−H4···O10 and N4−H4···O50) and by the bond length Gd−O10, typical of Gd−phenolate bonds.[9]

Kinetic experiments for the hydrolysis of the activated aryl diester BDNPP were followed spectrophotometrically for the absorbance increase at 400 nm due to the formation of 2,4-dinitrophenolate over time, under conditions of excess complex **1** at 25 °C. This system showed high catalytic activity, and its study was only possible through the stopped-flow technique.

An unusual and interesting kinetic behavior was observed from the absorbance versus time profile (Figure S1 in the Supporting Information). Because of the significant difference between the two hydrolysis rates, we could clearly follow the two saturation exponential curves, referring to the

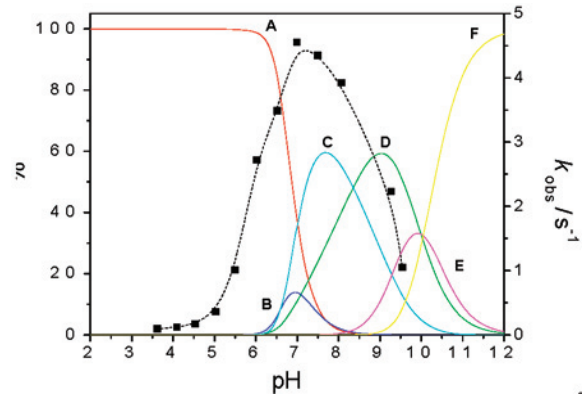

Figure 2. Solid lines representing the species distribution curves of the Gd^{III}−L system for dissolution of 0.05 mmol of complex **1** in an acetonitrile/water solution, $\mu = 0.1$ mol L^{-1} (KCl) at 25 °C. The dashed line corresponds to a variation in the observed rate constants for the first hydrolysis of BDNPP as a function of the pH in an acetonitrile/water solution. Conditions: [**1**] = 2.0×10^{-3} mol L^{-1}; [BDNPP] = 4×10^{-5} mol L^{-1}; at 25 °C. (A) Gd(III). (B) $[Gd_2H_{-1}L]^{4+}$. (C) $[Gd_2(OH)H_{-1}L]^{3+}$. (D) $[GdH_{-1}L]^{+}$. (E) $[H_{-1}LGd(OH)GdH_{-1}L]^{+}$. (F) $[H_{-1}LGd(OH)_2GdH_{-1}L]$.

hydrolysis of, first, BDNPP to 2,4-dinitrophenylphosphate (DNPP) and, second, DNPP to inorganic phosphate.

The pH dependence of the catalytic activity for the first hydrolysis shows a bell-shaped profile (superimposed curve in Figure 2) with optimum activity conditions at around pH 7. Thus, complete kinetic studies were performed at this pH, where the rate of hydrolysis of BDNPP to DNPP shows a nonlinear dependence on the complex concentration (Figure S2 in the Supporting Information). Considering a system of consecutive reactions and the rate law for reactions under conditions of excess complex,[10] the kinetic parameters $k_1 = 17$ s^{-1} and $K_1 = 158$ M^{-1} for the first hydrolysis were obtained from a nonlinear least-squares fit of k_{obs} versus [**1**] (Figure S2 in the Supporting Information). The first-order rate constant corresponds to an enhancement in the reaction rate of 100 million times in comparison to the spontaneous hydrolysis.[11] Moreover, this value is around 600 times higher compared with the value obtained for the hydrolysis reaction of the same substrate using different Ln^{III} complexes with BTP as the catalyst.[12]

The second BDNPP reaction, corresponding to the hydrolysis of the monoester DNPP and the formation of inorganic phosphate, was found to be independent of the concentration of **1**, with a rate constant $k_2 = 1 \times 10^{-2}$ s^{-1} at pH 7. Indeed, this is an interesting and rare two-step hydrolysis reaction in the sense that both the diesterase and monoesterase activities of **1** can be monitored independently.

In order to determine the active catalytic species of **1** in solution, ESI-MS analysis and potentiometric titration experiments were carried out. The equilibrium constants were determined (Table T1 in the Supporting Information) using the *BEST7* program,[13] and the values obtained were used to calculate the species distribution curves (Figure 2) and the species involved in the catalysis.

(8) (a) Batista, S. C.; Neves, A.; Bortoluzzi, A. J.; Vencato, I.; Peralta, R. A.; Szpoganicz, B.; Aires, V. V. E.; Terenzi, H.; Severino, P. C. *Inorg. Chem. Commun.* **2003**, *6*, 1161–1165. (b) Lanznaster, M.; Neves, A.; Bortoluzzi, A. J.; Aires, V. V. E.; Szpoganicz, B.; Terenzi, H.; Severino, P. C.; Fuller, J. M.; Drew, S. C.; Gahan, L. R.; Hanson, G. R.; Riley, M. J.; Schenk, G. *J. Biol. Inorg. Chem.* **2005**, *10*, 319–332. (c) Neves, A.; Lanznaster, M.; Bortoluzzi, A. J.; Peralta, R. A.; Casellato, A.; Castellano, E. E.; Herrald, P.; Riley, M. J.; Schenk, G. *J. Am. Chem. Soc.* **2007**, *129*, 7486–7487.
(9) Liu, S.; Yang, L.; Rettig, S. J.; Orvig, C. *Inorg. Chem.* **1993**, *32*, 2773–2778.
(10) Wilkins, R. G. *Kinetics and Mechanism of Reactions of Transition Metal Complexes*, 2nd ed.; VCH Verlagsgesellschaft mbH: Weinheim, Germany, 1991; p 24.
(11) Bunton, C. A.; Farber, S. *J. Org. Chem.* **1969**, *34*, 767–772.
(12) Longhinotti, E.; Domingos, J. B.; da Silva, P. L. F.; Szpoganicz, B.; Nome, F. *J. Phys. Org. Chem.* **2005**, *18*, 167–172.
(13) Martell, A. E.; Motekaitis, R. J. *Determination and Use of Stability Constants*, 2nd ed.; VCH: New York, 1992.

Although the X-ray crystal structure showed a mononuclear gadolinium complex in which Gd is bound to the soft site of the ligand, it is believed that in a H_2O/CH_3CN solution **1** generates a mononuclear complex in which the Gd^{III} ion resides at the hard side of L with two coordinated phenolate groups. The peak at *m*/*z* 701 in the ESI-MS spectrum (Figure S3-a in the Supporting Information) strongly corroborates this hypothesis. In addition, it is proposed that this mononuclear complex disproportionates and that a dinuclear Gd species and free ligand are formed under these experimental conditions, with the dinuclear complex being the active species in diester hydrolysis. This is in agreement with the strong tendency of the lanthanides to form dinuclear complexes in solution.[14] Such an assumption is supported by the ESI-MS spectra, which revealed peaks at *m*/*z* 332 and 347 corresponding to fragmentation of the free ligand (Figure S3-b in the Supporting Information) and a peak at *m*/*z* 993 attributed to the single-charged dinuclear $[(L)Gd_2(OH)_3(CH_3CN)_2]^+$ species (Figure S3-c in the Supporting Information). All peaks displayed the appropriate isotopic patterns. It should also be noted that the catalytic activity is twice as fast when the catalyst is generated in solution in a 2:1 $(Gd(NO_3)_3/L)$ ratio and that the electronic spectrum of this solution is similar to that obtained for **1** under identical experimental conditions, thus confirming the dinuclear species as the prominent catalyst.

Potentiometric titration experiments of **1** revealed that the coordination compounds are formed only at pH > 6. The lack of any dissociated water molecule within the dinuclear $[Gd_2H_{-1}L]^{4+}$ (B) species most probably explains its low catalytic activity. Dissociation of one bound water molecule in B results in the formation of the $[Gd_2(OH)H_{-1}L]^{3+}$ (C) species with a pK_a value of 6.59. This value is in reasonable agreement with the pK_a obtained from the sigmoidal fit of the curve pH versus k_{obs} (superimposed curve in Figure 2). Thus, the dinuclear $[Gd_2(OH)H_{-1}L]^{3+}$ (C) species is likely the most prominent catalyst in hydrolysis of the diester BDNPP. Finally, the decrease in reactivity at pH > 7 most probably arises because of the formation of the mononuclear $[GdH_{-1}L]^+$ (D) species (ESI-MS *m*/*z* 701), in which there are no dissociated water molecules and, consequently, no nucleophile to attack the diester bond. At higher pH values, dinuclear (2Gd:2L) species are formed (E and F). However, their contribution to the hydrolysis of BDNPP is not significant. The presence of relatively poor μ-hydroxo nucleophiles[8c] and steric hindrances in these dinuclear species are the factors most likely to be responsible for their low catalytic activity.

The second step of the hydrolysis reaction (monoester DNPP to inorganic phosphate) is also influenced by the pH of the reaction. A nonsymmetric bell-shaped pH versus rate profile (Figure S4 in the Supporting Information) was obtained with an optimum at about pH 8.0, a value which is one pH unit higher than that found in the hydrolysis of the diester. In fact, this result is in agreement with a lower Lewis acidity of the Gd^{III} center within the intermediate in which the monoester DNPP is most probably bound to the dinuclear catalyst in a bidentate fashion. On the other hand, the rate of hydrolysis of DNPP is only slightly affected by the concentration of **1** at a given pH, which strongly suggests an intramolecular nucleophilic attack by a Gd^{III}-bound hydroxide.

From the combined experimental data, we propose the following mechanism: monodentate binding of the diester with displacement of a Gd-bound water molecule (K_1) followed by a nucleophilic attack on the phosphorus atom by a conveniently oriented Gd-bound hydroxide and concomitant release of 2,4-dinitrophenolate. The hydrolysis of the intermediate to inorganic phosphate is proposed to occur through intramolecular attack of a second Gd-bound OH^- group (probably a μ-OH^- group generated within the intermediate). Indeed, the 1700 times slower hydrolysis reaction observed for the monoester can be tentatively explained in terms of a poorer μ-OH^- nucleophile[8c] or alternatively through the fact that the monoester is not adequately oriented for a specific nucleophilic attack of a Gd-bound hydroxide.

In order to assess the efficiency of complex **1** in the catalytic degradation of DNA, kinetic experiments were carried out under pseudo-first-order conditions using the complex in excess over the substrate (DNA) (Figure S5 in the Supporting Information). Complex **1** efficiently promotes the hydrolytic cleavage of plasmid DNA where a clear degradation of the supercoiled form I to the circular relaxed form II was observed after 6 h of incubation at pH 7.0 and 50 °C (Figure S6 in the Supporting Information), with a rate constant of 0.47 h^{-1}. This represents around a 1.7×10^7-fold rate increase compared with the estimated[15] uncatalyzed DNA hydrolysis rate. In addition, the catalytic activity of (**1**) is comparable with values reported in the literature for some dicerium complexes.[16]

In summary, complex **1** shows a high efficiency toward the hydrolysis of the activated substrate BDNPP and cleavage of plasmid DNA. Solution studies indicate the formation of mono- and dinuclear complexes, a pH-dependent process, and the active dinuclear $[Gd_2(OH)H_{-1}L]^{3+}$ species as a highly efficient catalyst under mild conditions. The synthesis of the corresponding Tb^{III} and Eu^{III} complexes and their kinetic and theoretical studies are in progress to unequivocally identify the active species and to elucidate the mechanisms involved. Groove binding molecules (distamycin and ethidium bromide), as well as specific inhibitors of radicalar species, are being used to fully understand the specific contact surfaces between the catalytic species and the DNA helix and the molecular mechanism used to promote cleavage of the phosphodiester bond. The results obtained will be the subject of a full paper.

Acknowledgment. Financial support was received from CNPq, FINEP, FAPESC, and PRONEX. M.A.C. is grateful to CNPq for a doctoral grant.

Supporting Information Available: Figures S1−S6 and Table T1. This material is available free of charge via the Internet at http://pubs.acs.org. X-ray crystallographic data is available free of charge upon request at www.ccdc.cam.ac.uk (CCDC 665738).

IC702167P

(14) Jurek, P. E.; Jurek, A. M.; Martell, A. E. *Inorg. Chem.* **2000**, *39*, 1016–1020.
(15) Radzicka, A.; Wolfenden, R. *Science* **1995**, *267*, 90–93.
(16) Branum, M. E.; Tipton, A. K.; Zhu, S.; Que, L. *J. Am. Chem. Soc.* **2001**, *123*, 1898–1904.