Rapid Communication J. Biochem. **120**, 1067-1069 (1996)

Dinuclear Lanthanum(III) Complex for Efficient Hydrolysis of RNA[1]

Morio Yashiro, Akira Ishikubo, and Makoto Komiyama[2]

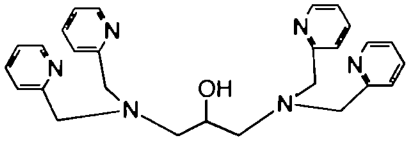
Department of Chemistry and Biotechnology, Graduate School of Engineering, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113

Received for publication, September 24, 1996

A dinuclear La^{3+} complex hydrolyzes the phosphodiester bond in diribonucleotides efficiently under mild conditions. The rate of hydrolysis is remarkably accelerated on the complex formation between La^{3+} and TPHP (TPHP = *N,N,N′,N′*-tetrakis[(2-pyridyl)methyl]-2-hydroxy-1,3-diaminopropane), and the highest activity is attained when the dinuclear structure is formed. The half-life of ApA hydrolysis by the dinuclear La^{3+} complex (1 mM) is 350 s, whereas that by the free La^{3+} ion (1 mM) is 23,000 s at pH 7.2 and 50°C. The dinuclear La^{3+} complex is promising as a catalytic center of artificial nucleases.

Key words: artificial metalloenzyme, dinuclear metal complex, hydrolysis, ribonucleotide dimer.

The molecular design of artificial nucleases has been attracting much attention, since it will provide novel tools for biotechnology. Site-specific scission of nucleic acids will be achieved when a catalyst for hydrolysis is activated only at the target site of nucleic acids. From this standpoint, many catalysts for the hydrolysis of RNA have been studied (*1-18*). Recently, the remarkable cooperation of two La^{3+} ions was demonstrated on the hydrolysis of a specially designed phosphonate monoester or model compounds of nucleic acids (*19, 20*). Therefore, a dinuclear La^{3+} complex is expected to be useful as the catalytic center of artificial nucleases. However, no dinuclear La^{3+} complex that hydrolyzes RNA itself has been found. Here we report the preparation of a dinuclear La^{3+} complex using a non-macrocyclic tetrapyridine ligand, TPHP (TPHP = *N,N,N′,N′*-tetrakis[(2-pyridyl)methyl]-2-hydroxy-1,3-diaminopropane) (*21-23*),[3] and that the catalytic activity for the RNA hydrolysis is remarkably enhanced on the formation of the dinuclear structure.



TPHP

The complex formation between $LaCl_3$ and TPHP in aqueous solutions was directly observed on ^1H NMR spectroscopy in D_2O (pD 7) (Fig. 1).[4] The formation of two types of complexes was observed. When the $[LaCl_3]:[TPHP]$ ratio was 1.0 (Fig. 1b), each of the protons of TPHP gave a corresponding broad signal, indicating the formation of a complex.[5] On the further addition of $LaCl_3$ (Fig. 1, c, d, and e), however, a new set of signals appeared, which increased in intensity with the increase in the ratio. These observations indicate the stepwise formation of 1:1 and 2:1 La^{3+}-TPHP complexes (**1** and **2**) depending on the $[LaCl_3]:[TPHP]$ ratio.[6]

$$La^{3+} + TPHP \overset{K_1}{\rightleftharpoons} La^{3+}\text{-}TPHP \quad (1)$$

$$La^{3+} + \mathbf{1} \overset{K_2}{\rightleftharpoons} (La^{3+})_2\text{-}TPHP \quad (2)$$

The formation constant, K_2, was directly determined by ^1H NMR spectroscopy to be 160 M^{-1} at 25°C (80 M^{-1} at 50°C). The value of K_1 (10,000 M^{-1} at 50°C) could be obtained by means of the following kinetic analysis.

The hydrolysis of a diribonucleotide, ApA, by the $LaCl_3$-TPHP complex system was conducted under the following conditions: $[ApA]_0 = 0.1$ mM, $[LaCl_3]_0 = 5$ mM, $[TPHP]_0 = 0\text{-}10$ mM, pH = 7.2 (Hepes buffer, 50 mM), and 50°C. The reactions were followed by reversed-phase ODS HPLC. The products were adenosine, its 2′- and 3′-phosphates, and its 2′,3′-cyclic phosphate. The reactions consistently exhibited pseudo-first-order kinetics.

Figure 2 shows the rate constants for the ApA hydrolysis

---

[1] This work was partially supported by a Grant-in-Aid for Priority Areas from the Ministry of Education, Science, Sports and Culture of Japan. The support by the Nagase Foundation and the Mitsubishi Foundation is also acknowledged.

[2] To whom correspondence should be addressed.

[3] The choice of TPHP as the ligand is based on our recent finding that the mononuclear La^{3+} complex with *N,N,N′,N′*-tetrakis[(2-pyridyl)-methyl]ethylenediamine (TPEN) is stable in an aqueous solution (*10*). Although TPHP has been shown to form dinuclear complexes with Mn^{2+}/Mn^{3+} (*21*), Co^{2+} (*22*), Fe^{3+} (*23*), and Zn^{2+} (*18*), complexes with "hard" lathanide ions have never been explored.

[4] ^1H NMR spectral data (270 MHz, r.t., pD 7). TPHP: 2.46(2H,dd,14Hz,8Hz), 2.60(2H,dd,14Hz,5Hz), 3.63(1H,m), 3.79(8H,s), 7.30(4H,t,6Hz), 7.38(4H,d,8Hz), 7.76(4H,t,8Hz), 8.37(4H,d,5Hz). **2**: 2.45(2H,dd,11Hz,11Hz), 2.66(2H,dd,13Hz,1Hz), 3.58(1H,m), 4.09(4H,AB pattern, J_{AB} 14Hz, $\delta\nu$ 17Hz), 4.12(4H,AB pattern, J_{AB} 16Hz, $\delta\nu$ 46Hz), 7.22(2H,d,8Hz), 7.35(2H,t,7Hz), 7.39(2H,d,9Hz), 7.43(2H,t,7Hz), 7.74(2H,t,8Hz), 7.86(2H,t,7Hz), 8.54(2H,d,5Hz), 8.74(2H,d,5Hz).

[5] The broad signals of **1** can be ascribed to a relatively slow equilibrium process in a 1:1 complex, presumably due to slow exchange of donor atoms of TPHP. Consistently, the line widths of signals decreased with increasing temperature.

[6] $[S_{total}] = [S] + [La^{3+}\text{-}S] + [1\text{-}S] + [2\text{-}S]$, $[La^{3+}\text{-}S] = [La^{3+}][S]/K_{m,La\text{-}S}$, $[1\text{-}S] = [1][S]/K_{m,1\text{-}S}$, $[2\text{-}S] = [2][S]/K_{m,2\text{-}S}$.

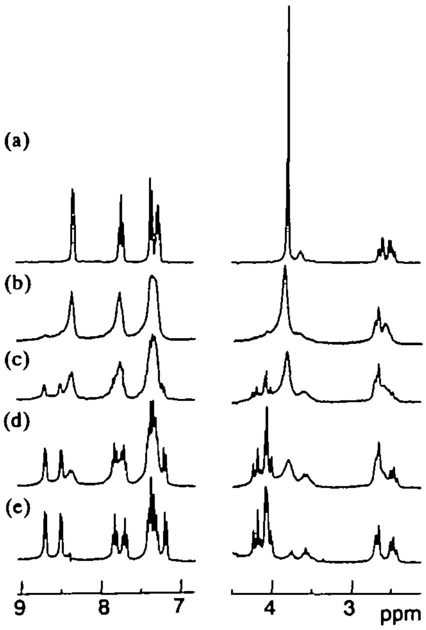


Fig. 1. **^{1}H NMR spectra (270 MHz) of mixtures of TPHP (5 mM) and various concentrations of LaCl$_3$ in D$_2$O (pD=7, r.t.).** (a) $[LaCl_3]_0=0$ mM ($r=[LaCl_3]_0/[TPHP]_0=0$); (b) $[LaCl_3]_0=5$ mM ($r=1$); (c) $[LaCl_3]_0=10$ mM ($r=2$); (d) $[LaCl_3]_0=20$ mM ($r=4$); (e) $[LaCl_3]_0=100$ mM ($r=20$).

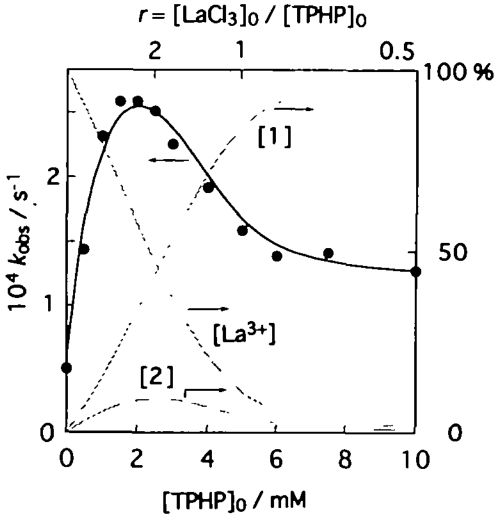


Fig. 2. **Dependence of the hydrolysis rate on the concentration of TPHP.** $[ApA]_0=0.1$ mM, $[LaCl_3]_0=5$ mM, pH =7.2 (Hepes buffer, 50 mM), 50°C. The solid line is the theoretical one (see text), and the dotted lines represent the mole fractions of the free La^{3+} ion, **1**, and **2** in the reaction mixtures.

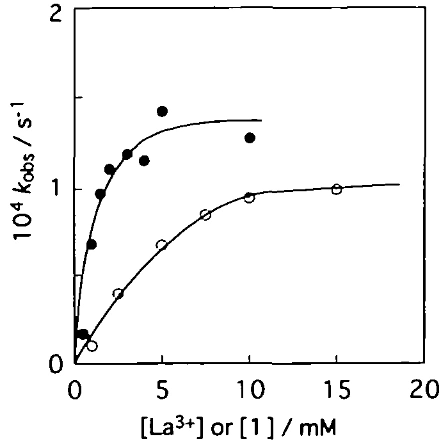


Fig. 3. **Concentration dependence of the rate constants for ApA hydrolysis by La^{3+} (○) and 1 (●) (footnote 7).** pH =7.2 (Hepes buffer, 50 mM), 50°C.

TABLE I. **Michaelis constants and rate constants for ApA hydrolysis by the free La^{3+} ion, 1:1 complex (1), and 2:1 complex (2).**

	K_m (M)	k (s^{-1})	k/K_m ($M^{-1}\cdot s^{-1}$)	k^* (s^{-1})[b]	$t^*_{1/2}$ (s)[b]
La^{3+}-S	0.004	0.00012	0.03	0.00003[b]	23,000[b]
1-S	0.0007	0.00014	0.2	0.0002[b]	3,500[b]
2-S	—[a]	—[a]	2	0.002[b]	350[b]

[a] See footnote 8. [b] k^* and $t^*_{1/2}$ are the estimated rate constants and half-lives, respectively, under the condition that 1 mM of each species is present in the solution.

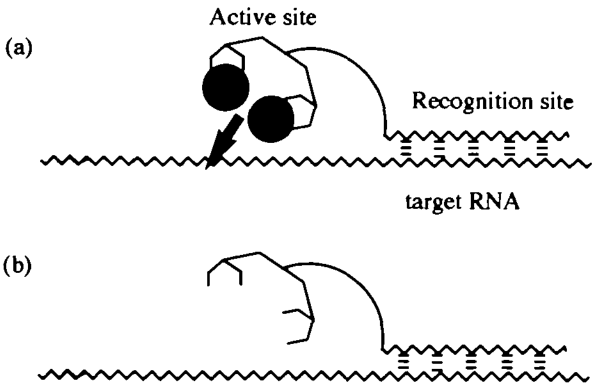


Fig. 4. **A conceptive drawing of an artificial RNase involving a dinuclear metal complex as its catalytic center.** (a) The activated artificial metalloenzyme, and (b) the inactive apo-enzyme.

with various concentrations of TPHP and a constant LaCl$_3$ concentration ($[LaCl_3]=5$ mM). The rate of ApA hydrolysis by the LaCl$_3$-TPHP complex system is very sensitive to the $[LaCl_3]_0$: $[TPHP]_0$ ratio (r). With increasing $[TPHP]_0$, the rate constant first increases monotonously, showing a maximum around the ratio, $r\approx2$. With still higher ligand concentrations ($[TPHP]_0>2.5$ mM, *i.e.* $r<2$), however, the rate constant decreases with an increase in $[TPHP]_0$, and gradually reaches a constant value. Apparently, several complex species with different activities are generated depending on the $[LaCl_3]_0$: $[TPHP]_0$ ratio, consistent with the results of the above ^{1}H NMR spectroscopy.

The plots in Fig. 2 were analyzed in terms of the following scheme. Here, competitive binding of the free La^{3+} ion, **1**, and **2** to the substrate (S) was considered, since the La^{3+} species is in excess as over the substrate, ApA.

$$La^{3+}+S \rightleftharpoons La^{3+}\text{-}S \xrightarrow{k_{La\text{-}S}} P+(La^{3+})$$

$$1+S \rightleftharpoons 1\text{-}S \xrightarrow{k_{1\text{-}S}} P+(1)$$

$$2+S \rightleftharpoons 2\text{-}S \xrightarrow{k_{2\text{-}S}} P+(2)$$

Thus, the rate of hydrolysis is expressed as sum of the terms due to La^{3+}-S, **1**-S, and **2**-S complexes (Eq. a).

$$v = k_{\mathrm{La\text{-}S}}[\mathrm{La^{3+}\text{-}S}] + k_{\mathrm{1\text{-}S}}[\mathbf{1}\text{-}\mathrm{S}] + k_{\mathrm{2\text{-}S}}[\mathbf{2}\text{-}\mathrm{S}] = k_{\mathrm{obs}}[\mathrm{S_{total}}] \tag{a}$$

On the basis of regular treatment for Michaelis-Menten type kinetics, Eq. a is transformed into Eq. b.[6]

$$k_{\mathrm{obs}} = (k_{\mathrm{La\text{-}S}}[\mathrm{La^{3+}}]/K_{\mathrm{m,La\text{-}S}} + k_{\mathrm{1\text{-}S}}[\mathbf{1}]/K_{\mathrm{m,1\text{-}S}} + k_{\mathrm{2\text{-}S}}[\mathbf{2}]/K_{\mathrm{m,2\text{-}S}})/(1 + [\mathrm{La^{3+}}]/K_{\mathrm{m,La\text{-}S}} + [\mathbf{1}]/K_{\mathrm{m,1\text{-}S}} + [\mathbf{2}]/K_{\mathrm{m,2\text{-}S}}) \tag{b}$$

The parameters for the free La^{3+} ion and **1** were determined independently by analysis of the plots in Fig. 3.[7] By curve analysis of the data in Fig. 2 using Eq. b, the parameters for the **2**-S complex ($k_{\mathrm{2\text{-}S}}/K_{\mathrm{m,2\text{-}S}}$)[8] and the formation constant of **1** (K_1) were obtained. The solid line in Fig. 2 is the theoretical one, and the dotted lines represent the mole fractions of the free La^{3+} ion, **1**, and **2** in the reaction mixtures.

The kinetic parameters are summarized in Table I.[9] The rate constants for the hydrolysis in the presence of 1 mM of each species (k^*) are also listed. The rate of hydrolysis by **2** is 70 times greater than that by the free La^{3+} ion. The results clearly show that the cooperation of two La^{3+} ions is very effective for RNA hydrolysis.

It has been shown that a number of enzymes hydrolyze phosphoesters through a two-metal ion assisted mechanism (*24*). The cooperation effects of two metal ions for RNA hydrolysis have recently been shown by using dinuclear Zn^{2+} or Cu^{2+} complexes (*18, 16*). The present results indicate that the cooperation of two La^{3+} ions gives much greater RNA hydrolysis activity. It is significant that the activity of the dinuclear La^{3+} complex (**2**) is comparable to those of the most eminent lanthanide chlorides ($LuCl_3$ or $TmCl_3$) (*4*).

In conclusion, a dinuclear La^{3+} complex with TPHP efficiently hydrolyzes the phosphodiester bond in a diribonucleotide. This dinuclear complex is very promising as a catalytic center of artificial nucleases (*25*), because high activity can be exerted only at the target site on the formation of the dinuclear structure (Fig. 4).

REFERENCES

1. Yoshinari, K., Yamazaki, K., and Komiyama, M. (1991) Oligoamines as simple and efficient catalysts for RNA hydrolysis. *J. Am. Chem. Soc.* **113**, 5899-5901
2. Smith, J., Ariga, K., and Anslyn, E.V. (1993) Enhanced imidazole-catalyzed RNA cleavage induced by a bis-alkylguanidinium receptor. *J. Am. Chem. Soc.* **115**, 362-364
3. Kuusela, S., Azhayev, A., Guzaev, A., and Lönnberg, H. (1995) The effect of the 3′-terminal monophosphate group on the metal ion-promoted hydrolysis of the phosphodiester bonds of short oligonucleotides. *J. Chem. Soc. Perkin Trans. 2* **1995**, 1197-1202
4. Komiyama, M., Matsumura, K., and Matsumoto, Y. (1992) Unprecedentedly fast hydrolysis of the RNA dinucleoside monophosphates, ApA and UpU, by rare earth metal ions. *J. Chem. Soc. Chem. Commun.* **1992**, 640-641
5. Ciesiolka, J., Marciniec, T., and Krzyzosiak, W.J. (1989) Probing the environment of lanthanide binding sites in yeast $tRNA^{Phe}$ by specific metal ion-promoted cleavage. *Eur. J. Biochem.* **182**, 445-450
6. Hayashi, N., Takeda, N., Shiiba, T., Yashiro, M., Watanabe, K., and Komiyama, M. (1993) Site-selective hydrolysis of tRNA by lanthanide metal complexes. *Inorg. Chem.* **32**, 5899-5900
7. Morrow, J.R., Buttrey, L.A., Shelton, V.M., and Berback, K.A. (1992) Efficient catalytic cleavage of RNA by lanthanide(III) macrocyclic complexes: Toward synthetic nucleases for in vivo applications. *J. Am. Chem. Soc.* **114**, 1903-1905
8. Magda, D., Miller, R.A., Sessler, J.L., and Iverson, B.L. (1994) Site-specific hydrolysis of RNA by europium(III) texaphyrin conjugated to a synthetic oligodeoxyribonucleotide. *J. Am. Chem. Soc.* **116**, 7439-7440
9. Chin, K.O.A. and Morrow, J.R. (1994) RNA cleavage and phosphate diester transesterification by encapsulated lanthanide ions: Traversing the lanthanide series with lanthanum(III), europium(III), and lutetium(III) complexes of 1,4,7,10-tetrakis-(2-hydroxyalkyl)-1,4,7,10-tetraazacyclododecane. *Inorg. Chem.* **33**, 5036-5041
10. Yashiro, M., Ishikubo, A., Takarada, T., and Komiyama, M. (1995) Lanthanide complexes with high stability and efficiency for the hydrolysis of a ribonucleotide dimer. *Chem. Lett.* **1995**, 665-666
11. Stern, M.K., Bashkin, J.K., and Sall, E.D. (1990) Hydrolysis of RNA by transition metal complexes. *J. Am. Chem. Soc.* **112**, 5357-5359
12. Modak, A.S., Gard, J.K., Merriman, M.C., Winkeler, K.A., Bashkin, J.K., and Stern, M.K. (1991) Toward chemical ribonucleases. 2. Synthesis and characterization of nucleoside-bipyridine conjugates. Hydrolytic cleavage of RNA by their copper(II) complexes. *J. Am. Chem. Soc.* **113**, 283-291
13. Bashkin, J.K., Frolova, E.I., and Sampath, U.S. (1994) Sequence-specific cleavage of HIV mRNA by a ribozyme mimic. *J. Am. Chem. Soc.* **116**, 5981-5982
14. Irisawa, M., Takeda, N., and Komiyama, M. (1995) Synergetic catalysis by two non-lanthanide metal ions for hydrolysis of diribonucleotides. *J. Chem. Soc. Chem. Commun.* **1995**, 1221-1222
15. Linkletter, B. and Chin, J. (1995) Rapid hydrolysis of RNA with a Cu^{II} complex. *Angew. Chem. Int. Ed. Engl.* **34**, 472-474
16. Young, M.J. and Chin, J. (1995) Dinuclear copper(II) complex that hydrolyzes RNA. *J. Am. Chem. Soc.* **117**, 10577-10578
17. Chapman, W.H., Jr. and Breslow, R. (1995) Selective hydrolysis of phosphate esters, nitrophenyl phosphates and UpU, by dimeric zinc complexes depends on the spacer length. *J. Am. Chem. Soc.* **117**, 5462-5469
18. Yashiro, M., Ishikubo, A., and Komiyama, M. (1995) Preparation and study of a dinuclear Zn(II) complex for the efficient hydrolysis of the phosphodiester linkage in a diribonucleotide. *J. Chem. Soc. Chem. Commun.* **1995**, 1793-1794
19. Tsubouchi, A. and Bruice, T.C. (1994) Remarkable ($\sim 10^{13}$) rate enhancement in phosphonate ester hydrolysis catalyzed by two metal ions. *J. Am. Chem. Soc.* **116**, 11614-11615
20. Takasaki, B.K. and Chin, J. (1993) Synergistic effect between La(III) and hydrogen peroxide in phosphate diester cleavage. *J. Am. Chem. Soc.* **115**, 9337-9338
21. Suzuki, M., Sugisawa, T., Senda, H., Oshio, H., and Uehara, A. (1989) Synthesis and characterization of a novel tetranuclear manganese(II,III,III,II) mixed valence complex. *Chem. Lett.* **1989**, 1091-1094
22. Suzuki, M., Sugisawa, T., and Uehara, A. (1990) Dinuclear cobalt(II) complexes containing 1,3-(or 1,5-)bis[bis(2-pyridylmethyl)amino]-2-propanolato (or 3-pentanolato): Preparation and reaction with molecular oxygen. *Bull. Chem. Soc. Jpn.* **63**, 1115-1120
23. Dong, Y., Ménage, S., Brennan, B.A., Elgren, T.E., Jang, H.G., Pearce, L.L., and Que, L., Jr. (1993) Dioxygen binding to diferrous centers. Models for diiron-oxo proteins. *J. Am. Chem. Soc.* **115**, 1851-1859
24. Steitz, T.A. and Steitz, J.A. (1993) A general two-metal ion mechanism for catalytic RNA. *Proc. Natl. Acad. Sci. USA* **90**, 6498-6502
25. Komiyama, M. (1995) Sequence-specific and hydrolytic scission of DNA and RNA by lanthanide complex-oligoDNA hybrids. *J. Biochem.* **118**, 665-670

---

[7] Hydrolyses by 1 were carried out at the ratio, $[\mathrm{LaCl_3}]_0/[\mathrm{TPHP}]_0 = 1/2$. Under the conditions, 1 is dominantly formed, and hydrolyses due to the free La^{3+} and 2 are negligible.

[8] The values of $k_{\mathrm{2\text{-}S}}$ and $K_{\mathrm{m,2\text{-}S}}$ can not be determined independently by means of the present data analysis, because one of the two terms involving these parameters in Eq. b, $[2]/K_{\mathrm{m,2\text{-}S}}$, does not significantly affect the value of k_{obs} due to the small mole fraction of 2 (<9%).

[9] The parameters satisfactorily fit the experimental results under various conditions, *i.e.* 2.5 or 10 mM $LaCl_3$ with 0-10 mM TPHP, at pH 7.2 and 50°C (data not shown).