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Study on kinetics and mechanism of mononuclear rare earth metal complexes in promoting the hydrolysis of p-nitrophenyl phosphate (NPP)

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Abstract

Two novel tripodal ligands, N,N',N''-tri-(3′-phenylpropionic acid-2′-yl-)-1,3,5-triaminomethylbenzene (L1), N,N',N''-tri-(4′-methylvaleric acid-2′-yl-)-1,3,5-triaminomethylbenzene (L2) have been synthesized. The hydrolytic kinetics of p-nitrophenyl phosphate (NPP) catalyzed by complexes of L1, and L2 with La(III) and Gd(III) have been studied in aqueous solution at 298 K, $I = 0.10\,\text{mol}\,\text{dm}^{-3}$ KNO$_3$, at pH 6.7–8.2, respectively. In the study it is found that the catalytic effect of GdL2 was the best in the four complexes for hydrolysis of HPNP. Its $k_{LnLH_{-1}}$, k_{LnL} and pK_a are $0.0198\,\text{mol}^{-1}\,\text{dm}^3\,\text{s}^{-1}$, $0.0000187\,\text{mol}^{-1}\,\text{dm}^3\,\text{s}^{-1}$ and 7.76, respectively. This paper expounds the result from the structure of the ligands and the properties of the metal ions, and deduces the catalysis mechanism.
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1. Introduction

Mimic enzyme is a kind of compound artificially synthesized based on the understanding of the structure of the enzymes and the reaction mechanism and having specific attribute for hydrolysis biological macromolecular The characteristic of the mimic enzymes is that the small molecular, the simple structure. However, they have the active groups and spacial structure similar to the groups that nature enzymes have.

There are many hydrolytic enzymes in the process of organic evolution, for example carbonic anhydras, carboxypeptidase, alkalline phosphatase and so on. They take part in the hydrolysis of some very important organic molecular and take an important role in the process of life [1].

The action mechanism of hydrolase and the effect of the metal ions in the active center are always important in bioinorganic chemistry field [2]. Recently there have been many research efforts to design and synthesize model complexes for promoting the hydrolysis of p-nitrophenyl phosphate (NPP) [3] and 2-hydroxypropyl-p-nitrophenyl phosphate (HPNP) [4]. Ligands containing more amino groups and more carboxyl groups or ligands bearing multi-ions are potentially useful to achieve more active molecular catalysts. In this paper, it is studied that the property of hydrolysis of NPP for the complexes of N,N',N''-tri-(3′-phenylpropionic acid-2′-yl-)-1,3,5-triaminomethylbenzene (L1), N,N',N''-tri-(4′-methylvaleric acid-2′-yl-)-1,3,5-triaminomethylbenzene (L2) with La(III) and Gd(III), respectively. Our interest was to understand functions of metal ions in biological hydrolysis process.

2. Experimental

2.1. Materials

All reagents and solvents were of analytical reagent grade and were used without further purification, unless otherwise noted. All aqueous solution were prepared using redistilled water. Metal ion stock solutions were prepared from their respective salts and were titrated against EDTA following standard procedures. The buffer component tris-(hydroxymethyl)-aminomethane (Tris) was used as supplied by the manufacturer. The p-nitrophenyl phosphate (NPP) were prepared and purified as a sodium salt following literature methods [5]. N,N',N''-tri-(3′-phenylpropionic

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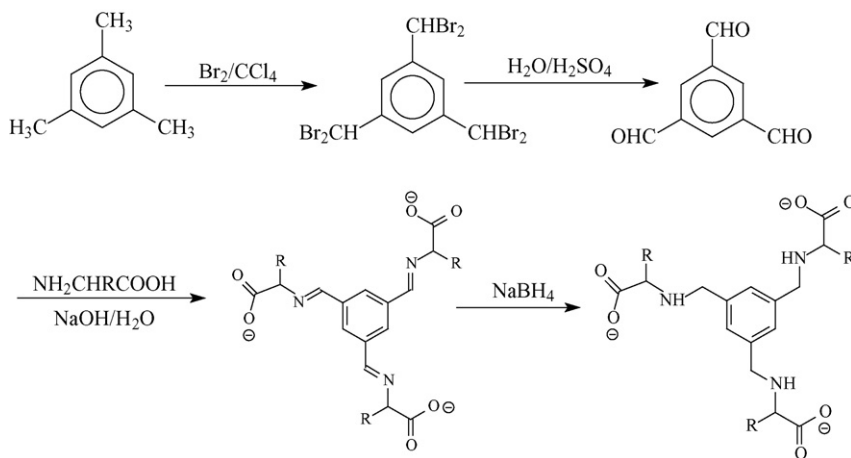*E-mail address:* hklin@nankai.edu.cn (H. Lin).

Fig. 1. The processes of the preparation of ligands. L1 = C$_6$H$_5$CH$_2$-, L2 = (CH$_3$)CHCH$_2$-.

acid-2′-yl-)-1,3,5-triaminomethylbenzene (L1), N,N',N''-tri-(4′-methylvaleric acid-2′-yl-)-1,3,5-triaminomethylbenzene (L2) were synthesized following literature methods [6] as Fig. 1.

2.2. Potentiometric determination

Potentiometric determination was measured in a 50 cm^3 jacketed cell thermostated at 298.2 ± 0.1 K by a refrigerated circulating water bath. Anaerobic condition were maintained using pre-purified N$_2$ as an inert atmosphere, and the ionic strengths were maintained by adding KNO$_3$ to achieve $I = 0.1$ mol dm^{-3}. The calibration of the glass electrode was the same as described in the literature [7]. In a typical experiment, the ligand was dissolved in an adequate amount of dilute HNO$_3$ and then titrated with 0.1 mol dm^{-3} KOH. The values of $k_w = 1.008\ 10^{-14}$, $\gamma_{H^+} = 0.825$ of water were used for the calculations. The calculations were carried out by SCMAR program [8] based on the improved TITFIT technique [9]. The final results were the averages of three independent titrations, each titration containing about 70 experimental points.

2.3. Kinetics of NPP hydrolysis

A kinetic study was carried out by the UV–vis spectra method using a Beckman DU-8B spectrophotometer equipped with a thermostated cell holder. The hydrolysis rate of NPP in aqueous solution was measured by an initial slope method following the increase in absorption at 400 nm due to the release of 4-nitrophenolate. The reaction solution was maintained at 298 K and the ionic strength was adjusted to 0.10 mol dm^{-3} with KNO$_3$. The buffer component tris-(hydroxymethyl)-aminomethane (Tris) was used to maintain pH, and it do not coordinate with Ln(III) ions under this condition. For the initial rate determination, the following typical procedure was employed.

After NPP and the Ln(III) complexes solution at the appropriate pH were mixed, the UV absorption increase was recorded immediately (the reference experiment did not contain the cat-

alyst).The increase in concentration of p-nitrophenolate was measured every 60 s. The initial slope (<5% conversion) of a plot of the measured absorbance versus time was determined (correlation coefficient > 0.99). All the experiments were in triplicate and the tabulated data represent the average of these experiments.

3. Results and discussion

3.1. Active nucleophile in 1:1 Ln/L systems

The stepwise protonation constants of L1 and L2 are shown in Table 1, respectively. Potentiometric titration indicated that the complexes formed by La(III) and Gd(III) with L1 and L2 are performed at 1:1 metal–ligand molar ratios, respectively. The stability constants of LnLH$_m$ ($m = 2$ to -1) complexes in the 1:1 ratio are in Table 2. From the species percentage distribution diagrams of Ln–L binary system (Fig. 2) it is find that the modes LnLH$_{-1}$ (1,1,-1) and LnL (1 1 0) having ability to catalyze hydrolysis of NPP are formed in the solution when pH > 6.5.Therefore the kinetic experament studied would be carried out in pH 6.7–8.2.

The deprotonation constants of coordination water on LnL (1 1 0) can be obtained according to pK_a = log β_{LnL} − log $\beta_{LnLH_{-1}}$ shown in Table 4. LnL (1 1 0) can release a proton to give LnLH$_{-1}$ at weak basic (pH > 7.0) solution which is a good nucleophilic metal-bond hydroxide specie and they can be used as enzyme models for catalyzing the hydrolysis of phosphate monoester NPP because of their nucleophilic group OH$^-$.

Table 1
The protonation constants of ligands L1 and L2 (pH range 2.5–10.8) (25.0 ± 0.1 °C, $I = 0.1$ mol dm^{-3} KNO$_3$, $C_L = 5 \times 10^{-4}$ mol dm^{-3})

	log β_1	log β_2	log β_3	log K_1	log K_2	log K_3
L1	9.23	17.71	25.36	9.23	8.48	7.65
L2	9.82	19.04	26.95	9.82	9.22	7.91

Table 2
The stability constants of binary complexes of ligands L1, L2 with La(III), Gd(III) ($C_L = C_{Ln} = 5 \times 10^{-4}$ mol/L, $I = 0.1$ mol dm^{-3} KNO$_3$, $25 \pm 0.1\,^\circ$C)

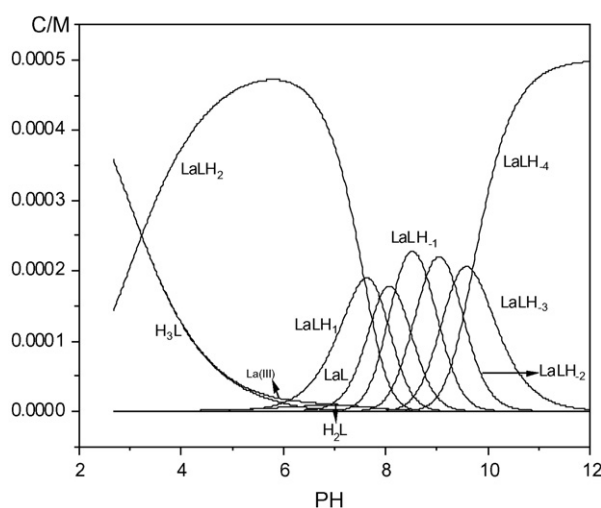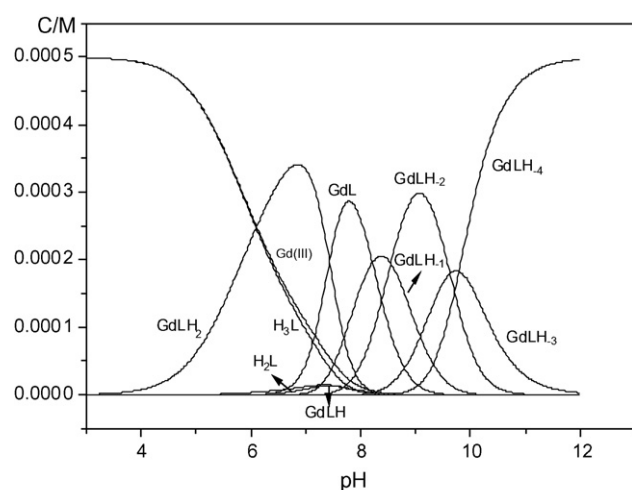| Complexes	La(III)	Gd(III)
L1		
log β_{112}	22.48	23.83
log β_{111}	14.39	16.71
log β_{110}	7.14	8.98
log $\beta_{1,1,-1}$	−0.76	1.32
log $\beta_{1,1,-2}$	−9.57	−7.35
log $\beta_{1,1,-3}$	−18.85	−16.62
L2		
log $\beta_{1,1,-4}$	−28.80	−26.54
log β_{112}	24.14	26.48
log β_{111}	16.34	19.30
log β_{110}	9.54	12.41
log $\beta_{1,1,-1}$	1.68	4.56
log $\beta_{1,1,-2}$	−6.90	−3.48
log $\beta_{1,1,-3}$	−16.50	−12.81
log $\beta_{1,1,-4}$	−26.27	−22.41

Fig. 2. The species percentage distribution diagrams of Gd(III)L1, La(III)L1 binary system (298.2 K, $I = 0.1$ mol dm^{-3} KNO$_3$, $C_{L1} = C_{Gd} = 5 \times 10^{-4}$ mol dm^{-3}).

3.2. Molar extinction coefficient of NP$^-$

The kinetic study of NPP hydrolysis was carried out by monitoring the amount of p-nitrophenolate ion (NP$^-$) produced in solution at 400 nm. The use of buffer for the studies of hydrolysis kinetics of NPP was required since the change of pH value of the solution would cause change of the concentration of the NP$^-$. To determine the rate constants one should know the molar extinction coefficient of NP$^-$, which varies considerably with pH values of the solution. In solution p-nitrophenol dissociate as following:

$$HNP = H^+ + NP^- \tag{1}$$

$$K_a = [NP^-][H^+]/[HNP] \tag{2}$$

According to Beer's law,

$$A = \varepsilon_{obs} b[HNP]_T = \varepsilon_{NP} b[NP] \tag{3}$$

where ε_{obs} is the observed extinction coefficient of HNP, ε_{NP} the extinction coefficient of the NP$^-$ anion, b the cell length (1 cm) and A is the absorption of the samples. From Eqs. (2) and (3), Eqs. (4) and (5) are obtained:

$$\varepsilon_{obs} = \varepsilon_{NP} K_a/(K_a + [H^+]) \tag{4}$$

$$1/\varepsilon_{obs} = 1/\varepsilon_{NP} + [H^+]/\varepsilon_{NP} K_a \tag{5}$$

With the plot of $1/\varepsilon_{obs}$ versus [H$^+$], the moler extinction coefficient ε_{NP} and the dissociation constant of p-nitrophenol K_a at 400 nm in 0.020 mol dm^{-3}. 'Good's buffers were obtained with the values of 17,900 mol^{-1} dm^3 cm^{-1} and 7.66×10^{-8} mol dm^{-3}, respectively, which is consistent with the reported value [10].

3.3. Kinetic studies of hydrolysis of NPP

The following typical procedure was employed for the initial rate detemination: after p-nitrophenyl phosphate (NPP) and complexes in aqueous solution at an appropriate pH (the reference experiment did not contain the catalyst-complexes) were mixed. The hydrolysis of the phosphate monoester NPP catalyzed by Ln–L system binary complexes was monitored by following the increase in absorbance at 400 nm due to the release of p-nitrophenolate ion. The UV absorption increased was recorded immediately; the increase in concentration of p-nitrophenylate was measured every 60 s; the initial slope (<5% conversion) of a plot of the measured absorbance versus time was determined (correlation coefficient > 0.99). All experiments were run in triplicate and the tabulated data represent the average of these experiments. Under the experimental conditions, the hydrolysis rate increases linearly with the increase of NPP concentration, which indicates that the hydrolysis is first-order with respect to NPP, and can be written in the following form:

$$V = dC_{NPP}/dt = dA/b\varepsilon \, dt = k_{obs}[NPP]$$
$$= (k_{cat}^{obs}[complex]^T + k_{OH}[OH] + k_0)[NPP] \tag{6}$$

Table 3
The apparent rate constants catalyzed hydrolysis of NPP by Ln–L complexes, k_{cat}^{obs}, at different pH values

pH	[H⁺] (10^{-8} mol dm⁻³)	k_{cat}^{obs} (mol dm⁻³ s⁻¹)			
		LaL1	LaL2	GdL1	GdL2
6.721	23.15	0.000281	0.000437	0.000914	0.001466
7.074	10.22	0.000594	0.000915	0.001584	0.002693
7.285	6.29	0.000873	0.001377	0.002789	0.003894
7.564	3.31	0.001462	0.002234	0.004125	0.005981
7.851	1.71	0.002334	0.003346	0.006362	0.008368
8.138	0.88	0.003278	0.004521	0.009231	0.001083

where V is the hydrolysis rate, k_{obs} the observed rate constant, and k_{obs} includes all the catalytic spesies, such as the Ln–L complexes, base (OH⁻) and other spesies (solvent H₂O). Therefore, k_{obs} can be written:

$$k_{obs} = k_{cat}^{obs}[complex]^T + k_{OH}[OH^-] + k_0 \quad (7)$$

where k_{cat}^{obs} and k_{OH} are the apparent catalytic rate constants of Ln–L complexes and OH⁻, respectively, and k_0 is constant probably due to the solvolysis of NPP (i.e. water attack on the monoester). At a given pH, when the observed hydrolysis rate constant, k_{obs}, was plotted against total concentration of complexes Ln–L, the apparent rate constant of complexes, k_{cat}^{obs}, can be gotten. Table 3 shows the apparent hydrolysis rate constant, k_{cat}^{obs}, at different pH. As shown in Table 3, the apparent hydrolysis rate constant of complexes Ln–L, k_{cat}^{obs}, increases with the increase of the pH value of reaction solution. When the total apparent hydrolysis rate constant of complexes Ln–L, k_{cat}^{obs}, is plotted against pH, resulting curve indicate the characteristics of a kinetic process controlled by acid–base equilibrium (due to deprotonation of the coordination water on complex LnL) (see Fig. 3). In Eq. (7) [complex]^T is the total concentration of Ln–L complexes. As discussed earlier, there are two types of nucleophile in the 1:1 system: LnL and LnLH$_{-1}$, with the equilibrium constant pK_a^2 (pK_a^2 = log β_{110} − log $\beta_{1,1,-1}$). The concentrations of LnL and LnLH$_{-1}$ have been controlled by pH value of
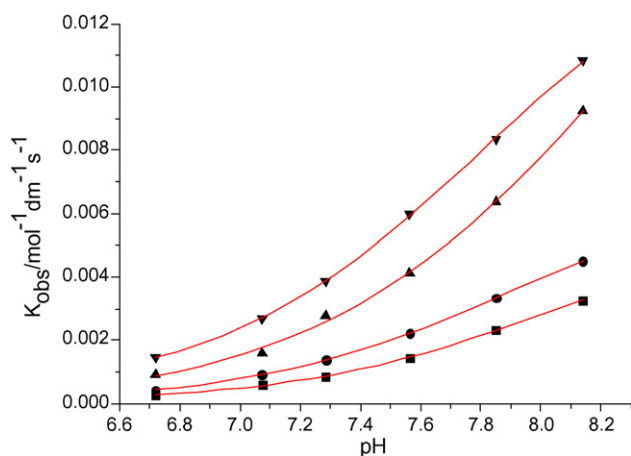

Fig. 3. The curve of relation of apparent hydrolysis rate constant of NPP, k_{cat}^{obs} vs. pH catalyzed by complexes Ln–L. (■) LaL1; (●) LaL2; (▲) GaL1; (▼) GaL2.

Table 4
The second-order rate constants k_{LnL}, $k_{LnLH_{-1}}$ and the deprotonation constant of water coordinated on LnL pK_a^2 in the hydrolysis of NPP

Complexes	$10^3 k_{LnL}$ (mol⁻¹ dm³ s⁻¹)	$k_{LnLH_{-1}}$ (mol⁻¹ dm³ s⁻¹)	pK_a (titration)	pK_a
LaL1	0.0063	0.0057	7.90	7.93
LaL2	0.0065	0.0071	7.86	7.82
GdL1	0.0172	0.0152	7.66	7.68
GdL2	0.0187	0.0198	7.76	7.71

the reaction solution and can be represented by Eqs. (8) and (9):

$$[LnL] = [complex]^T[H]/(k_a^2 + [H]) \quad (8)$$

$$[LnLH_{-1}] = [complex]^T K_a^2/(K_a^2 + [H]^+) \quad (9)$$

According to kinetics theory of enzumatic catalysis, Eq. (10) is then obtained:

$$k_{cat}^{obs}[complex]^T = k_{LnL}[LnL] + k_{LnLH_{-1}}[LnLH_{-1}] \quad (10)$$

where k_{LnL} and $k_{LnLH_{-1}}$ stand for the second-order hydrolysis rate constants of NPP catalyzed by LnL and LnLH$_{-1}$, respectively. Eq. (11) could be obtained from Eqs. (8)–(10):

$$k_{cat}^{obs} = (k_{LnL}[H] + k_{LnLH_{-1}}k_a^2)/([H] + k_a^2)$$
$$= k_{LnL} + (k_{LnLH_{-1}} - k_{LnL})/(1 + [H^+]/k_a^2) \quad (11)$$

Thus, by means of non-linear least-squares fit of k_{cat}^{obs} versus [H⁺], the values of k_{LnL}, $k_{LnLH_{-1}}$ and sK_a^2 can be obtained according to Eq. (11). From Table 4 it could be found that dissociation constants of coordination water on complex LnL (pK_a^2) got from the kinetical experiment is consistent with the result got from the titration experiment.

As a rule, in the species LnL the coordinated water is the nucleophilic group, while the hydroxyl group, OH⁻ is the nucleophilic group in the species LnLH$_{-1}$.

From the data in Tables 3 and 4, the mechanism of the catalysis of hydrolysis of NPP can be deduced (Fig. 4). From the data in Table 3 it could be seen that the total apparent rate constant (k_{cat}^{obs}) of hydrolysis of NPP catalyzed by Ln–L binary system complexes increase with the increase of pH value of reaction solution. It is interesting that ionization of mononuclear rare earth complex LnL at the solution of weak basic pH is due to loss of a proton from a water bound on Ln(III) of complex LnL, because the pH-rate profiles show that the ionization state of the water bound on Ln(III) of complex LnL is critical for catalytic activity. The pH-rate profiles of the second-order rate constants k_{cat}^{obs} for catalysis of transesterification of NPP by Ln–L provide the following important insights into catalytic reaction mechanism.

There is a downward break in the pH-profile for the hydrolysis of NPP catalyzed by Ln–L complex system that is centered at the pK_a^2 for deprotonation of a bound water molecule to form the monohydroxyl complex LnLH$_{-1}$. These pH-rate profile show that the complex LnL is less active species catalyzed hydrolysis of NPP than LnLH$_{-1}$ and is converted to a more active form LnLH$_{-1}$ upon loss of a proton.

Fig. 4. The mechanism of the catalyzed the hydrolysis of HPNP.

We consider that the lost proton related with a downward break in the pH-rate profile come from the water molecule coordinated on Ln(III) of the complex LnL, because the pK_a of free water at 298.2 K is about 14. When water molecule coordinated with Ln(III) of complex LnL, at action of Ln(III) of complex LnL the pK_a of coordinated water markedly decrease. The complex LnL lost the proton of coordination water on itself and changed to the complex LnLH$_{-1}$ then the hydroxyl group of the complex LnLH$_{-1}$ attack P atom of substrate NPP. The proton releasing from the coordination water on complex LnL decreased the pH value of reaction solution. From Table 4 it is could be found that dissociation constants of coordination water on the complex LnL (pK_a^2) got from the kinetics experiment was consistent

with the result got from the titration experiment. This is a powerful evidence of our viewpoint.

HO$^-$ coordinated on Ln(III) of complex LnLH$_{-1}$ attacked to P atom of NPP. This result lead p-nitrophenate ion leave from NPP. And that H$_2$O coordinated on Ln(III) of complex LnL directly attacked P atom of NPP, this result lead p-nitrophenate ion leave from NPP. Because the electron cloud density on oxyanion of hydroxy coordinated on Ln(III) of complex LnLH$_{-1}$ is large much than one on oxygen atom of H$_2$O coordinated on Ln(III) of complex LnL, therefore, $k_{LnLH_{-1}}$ is large much than k_{LnL}.

Many metals provide acceleration in phosphate ester hydrolysis, but cleavage by Co(III) bearing substitutable

cis-coordination sites is amongst the fastest. While phosphodiesters require only one Co(III) to effect hydrolysis, phosphomonoesters require two: one for charge neutralization and one to deliver a metal hydroxide [10]. Because Ln(III) ion has three positive charges, 8–12 coordination number, big volume and can bind two oxyanion, one hydroxyl group. In this paper the hydrolysis of phosphomonoester NPP had been catalyzed by mononuclear Ln(III) complexes and obtained the result showed second-order rate constants and deprotonation constant are $k_{LnLH_{-1}} = 0.0198 \, mol^{-1} \, dm^3 \, s^{-1}$, $k_{LnL} = 0.0000187 \, mol^{-1} \, dm^3 \, s^{-1}$ and $pK_a = 7.76$ for complexes Gd(III)L2. The comparison of the activity catalyzed the hydrolysis of phosphmonoester NPP for Gd(III)L2 in this paper with the rate constant $k = 0.0133 \, mol^{-1} \, dm^3 \, s^{-1}$ for Co(III)–dinuclear complex reported Vance and Czarnik [10] indicated that the complex Gd(III)L2 designed and synthesized by us is very excellent mononuclear catalyst and can effectivety catalyzed the hydrolysis of NPP.

Important problem is found in Chin's paper that the rate constant, $k_{Co_2} = 0.051 \, s^{-1}$ for hydrolysis of NPP catalyzed by $[Co_2(bpmp)(O_3P)(OPh)(OH_2)(OH)](ClO_4)$ at 298 K is first-order rate constant and the dissociation constant of water coordinated on Co(III) of dinuclear complex is 9.8 [3]. This fact that the second-order rate constants and deprotonation constant of coordination water of complexes $GaL2H_{-1}$, GaL2 designed and synthesized in this work are $0.0198 \, mol^{-1} \, dm^3 \, s^{-1}$, $0.0000187 \, mol^{-1} \, dm^3 \, s^{-1}$ and 7.76 also sufficiently indicated that the complex Ga–L2 designed and synthesized in this work can catalyze the hydrolysis of monophosphoester NPP at physiological condition (almost neutral solution $pH = 6.7$–8.1, $pK_a = 7.76$), but the complex $[Co_2(bpmp)(O_3POPh)(H_2O)(OH)](ClO_4)$ designed and synthesized in Jik Chin's work [3] can catalyze hydrolysis of NPP at base solution ($pK_a = 9.8$). Therefore, The complexes Gd(III)L2 designed and synthesized in this work are the excellent catalyst which can catalyze hydrolysis of monophosphoester NPP at almost neutral solution.

3.4. The influencing factors

From the data in Table 4, it is shown for the hydrolysis rate constants of complexes LnL and $LnLH_{-1}$, L1 < L2. So we think the steric hindrance is a very important influencing factor. The substituent group of L1 is big rigid benzyl group, and that of L2 is isobutyl. The bigger steric hindrance of L1 makes the coordination of complexes LnL and $LnLH_{-1}$ with substrate harder that counteract the catalysis of complexes to hydrolysis of NPP.

And meanwhile, for the ligands L1, L2, the complexes of Gd(III) has better catalysis effect than the complexes of La(III). Due to Gd(III), La(III) have the same electron charge. But Gd(III) has the smaller radius and has the bigger electron density. Therefore, coordination water on Gd(III) of complexes Gd(III)L is easier deprotonate to form hydroxyl group, OH^-, than La(III) complexes. Finally, the conclusion has been gotten that the catalysis rate constants of the complexes of Gd(III) is bigger than that of the complexes of La(III).

Acknowledgment

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