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# Cooperation of Lanthanum Ion and Non-lanthanide Metal Ions for the Hydrolysis of Bis(4-nitrophenyl)phosphate

**Naoya Takeda, Makoto Irisawa and Makoto Komiyama***

*Department of Chemistry and Biotechnology, Faculty of Engineering, The University of Tokyo, Hongo, Tokyo 113, Japan*

Lanthanum(III) ion and non-lanthanide metal ions [iron(III), manganese(III), tin(IV), indium(III) and gallium(III)] show a cooperative catalysis for the hydrolysis of bis(4-nitrophenyl)phosphate.

Phosphoesters play a number of essential roles *in vivo*, and thus their non-enzymatic hydrolysis has attracting interest from various viewpoints.[1] Efficient catalysts involving lanthanide ions and complexes,[2-10] transition metal complexes[11-16] and organic compounds[17,18] have been reported. However, still greater catalytic activities are desirable for more versatile applications.

In most phosphodiesterases and phosphomonoesterases, two metal ions, *e.g.* $Zn^{II}$, $Mg^{II}$ and $Mn^{II}$, show a synergetic catalysis.[19-22] However, cooperative catalysis of this kind has not yet been satisfactorily mimicked in nonenzymatic systems.† Here we report that bis(4-nitrophenyl)phosphate (BNPP) is efficiently hydrolysed by the cooperation of lanthanum(III) ion and various non-lanthanide metal ions. Although each of the metal ions is only slightly active when used independently, the combination of them gives rise to a significant increase in catalytic activity.

The hydrolysis of BNPP at pH 7 (50 mmol $dm^{-3}$ hepes buffer) and 50 °C was followed by reversed-phase HPLC. When $LaCl_3$ (10 mmol $dm^{-3}$) is used as catalyst, the pseudo-first-order rate constant is only 0.03 $h^{-1}$. However, the addition of $FeCl_3$ to the mixture greatly accelerates the hydrolysis. The rate constant with a 1 : 1 $La^{III}/Fe^{III}$ combination (both 10 mmol $dm^{-3}$) is 1.0 $h^{-1}$, which is 33 times as large as that for $La^{III}$ alone (Table 1). $FeCl_3$ itself is a poor catalyst (the rate constant is 0.02 $h^{-1}$ when $[FeCl_3]_0$ = 10 mmol $dm^{-3}$). Thus, the cooperation of $LaCl_3$ and $FeCl_3$ is conclusive. The product is 4-nitrophenolate (two moles per BNPP), and no byproducts, expected for the oxidative cleavage of BNPP, are formed. The hydrolytic character of the scission is clearly evidenced. The intermediate (4-nitrophenyl)phosphate is hydrolysed more rapidly than BNPP, and is not significantly accumulated in the mixture.

As depicted in Fig. 1(*a*), the rate constant for the BNPP hydrolysis increases linearly with $[Fe^{III}]_0$, when $[La^{III}]_0$ is kept constant at 10 mmol $dm^{-3}$. The plot of the rate constant *vs.* $[La^{III}]_0$, with $[Fe^{III}]_0$ kept constant at 10 mmol $dm^{-3}$, is also a fairly straight line [Fig. 1(*b*)]. Apparently, one $La^{III}$ ion and one $Fe^{III}$ ion participate in the cooperative catalysis.‡ This fact is further confirmed by the continuous variation plot in Fig. 2, which is symmetrical and reaches a maximum at a molar fraction of 0.5 for $La^{III}$.

**Table 1** The pseudo-first-order rate constants for the hydrolysis of BNPP by the combinations of $La^{III}$ (10 mmol $dm^{-3}$) and non-lanthanide metal ion (10 mmol $dm^{-3}$) at pH 7 and 50 °C[a,b]

| Non-lanthanide metal ion | Rate constant/$h^{-1}$ |
|---|---|
| $Fe^{III}$ | 1.0 |
| $Mn^{III}$ | 0.61 |
| $Sn^{IV}$ | 0.56 |
| $In^{III}$ | 0.26 |
| $Ga^{III}$ | 0.13 |
| None | 0.03 |

[a] The rate constant with $Fe^{III}$ alone is 0.02 $h^{-1}$, and the other non-lanthanide metal ions are virtually inactive for the hydrolysis in the absence of $La^{III}$. [b] The reaction mixture for the combination with $Fe^{III}$ is homogeneous, whereas those with $Mn^{III}$, $Sn^{IV}$, $In^{III}$ and $Ga^{III}$ involve some precipitates.

When molecular oxygen was removed by repeated freeze–thaw cycles, the rate of BNPP hydrolysis by the $La^{III}/Fe^{III}$ combination was identical with the value under air, within experimental error. Molecular oxygen is therefore not required for the cooperative catalysis.§ Redox reaction between $La^{III}$ and $Fe^{III}$ is energetically unable to occur, since the redox potentials for $La^{III} \rightarrow La^0$ and for $Fe^{III} \rightarrow Fe^{II}$ are −2.37 and +0.77 V, respectively [$La^{II}$ and $La^{I}$ ions are too unstable).[23]

The cooperative catalysis probably involves a mixed hydroxide cluster of $La^{III}$ and $Fe^{III}$, since the formation of a hydroxide gel of $FeCl_3$ is greatly suppressed by the addition of $LaCl_3$. The mixtures are totally homogeneous when the $La^{III}/Fe^{III}$ ratio is $\geqslant 1$. Otherwise, gel formation prevails at pH 7. Clearly, the interaction between $La^{III}$ and $Fe^{III}$ prevents the formation of polymeric aggregates of $Fe^{III}$ hydroxide (the detailed structure of the mixed cluster has not yet been clarified). The round shape of the continuous variation plot in Fig. 2 indicates that the formation constant of the mixed cluster is rather small (the value estimated by the least-squares fitting is 4.9 $dm^3\ mol^{-1}$).

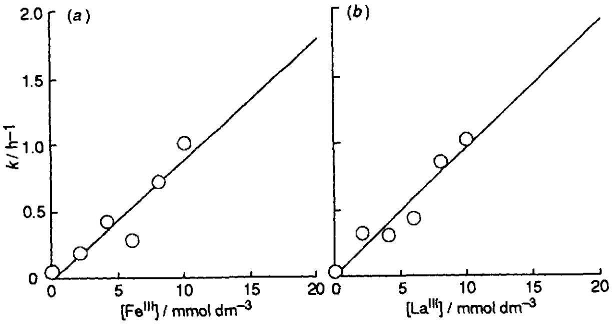

**Fig. 1** Plots of the rate constant *vs.* (*a*) $[Fe^{III}]_0$ (with $[La^{III}]_0$ constant at 10 mmol $dm^{-3}$) and (*b*) $[La^{III}]_0$ (with $[Fe^{III}]_0$ constant at 10 mmol $dm^{-3}$) for the hydrolysis of BNPP at pH 7 and 50 °C

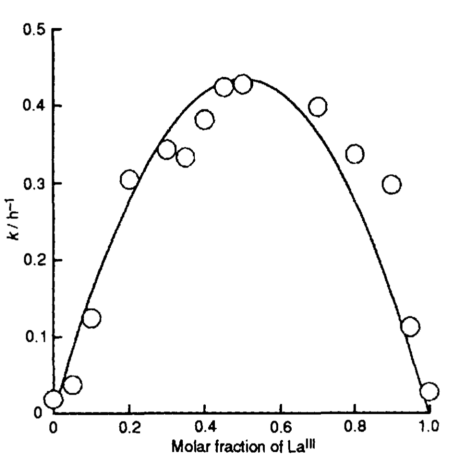

**Fig. 2** Continuous variation plot for the hydrolysis of BNPP at pH 7 and 50 °C: ($[La^{III}]_0$ + $[Fe^{III}]_0$) is kept constant at 10 mmol $dm^{-3}$. The solid line is the theoretical one calculated under the assumptions that $La^{III}$ and $Fe^{III}$ form a 1 : 1 mixed cluster with a formation constant 4.9 $dm^3\ mol^{-1}$, and that its catalytic rate constant is $3.7 \times 10^3$ $dm^3\ mol^{-1}\ h^{-1}$.

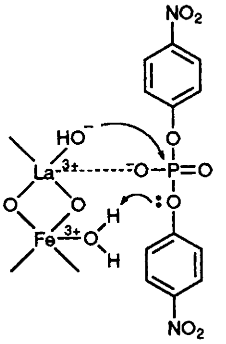

**Fig. 3** Proposed mechanism for the hydrolysis of BNPP by the combination of La$^{III}$ and Fe$^{III}$

One of the possible mechanisms for the observed catalytic behaviour is schematically depicted in Fig. 3. The phosphate residue of BNPP coordinates to the La$^{III}$ ion in the mixed cluster, and the hydroxide ion bound to the La$^{III}$ ion intramolecularly attacks the phosphorus atom. The La$^{III}$-bound hydroxide is an eminent intramolecular nucleophile (all the lanthanide ion-promoted hydrolyses of phosphate esters previously reported proceed *via* a similar mechanism).[2–10] Furthermore, the Fe$^{III}$-bound water, which is located nearby, functions as an acid catalyst as it is more acidic than the La$^{III}$-bound water (the p$K_a$'s are 2.5 and 7.4, respectively)[24] and thus is a more potent acid catalyst.¶ The two metal ions share the roles in the cooperative catalysis (depending on the acid–base properties), resulting in the prompt hydrolysis of the phosphodiester linkage. The proposed mechanism is parallel to the cooperation of two metal ions in enzymatic reactions.[19–22]

Mn$^{III}$(acac)$_3$, Sn$^{IV}$Cl$_4$, In$^{III}$Cl$_3$ and Ga$^{III}$Cl$_3$ also showed a cooperation with La$^{III}$ for the BNPP hydrolysis (Table 1). The possibility of redox reactions between these metal ions and La$^{III}$ ion is ruled out in terms of the corresponding redox potentials.[23,25] In contrast, no significant cooperative acceleration was observed when Mn$^{II}$, Ni$^{II}$, Zn$^{II}$, Ru$^{III}$, Rh$^{III}$, Pd$^{II}$, Pt$^{II}$, Ir$^{III}$ and Bi$^{III}$ (all as chlorides) were used. Much more effective cooperation by Mn$^{III}$ than by Mn$^{II}$ is consistent with the proposed mechanism, since the acidity of the Mn$^{III}$-bound water (p$K_a$ −0.6) is much greater than that of the Mn$^{II}$-bound water (p$K_a$ 10.5).[24] With the La$^{III}$/Fe$^{II}$ and the La$^{III}$/Sn$^{II}$ combinations, oxidative cleavage of BNPP took place, and several unidentified products were formed.

In conclusion, cooperative catalyses of La$^{III}$ ion and non-lanthanide metal ions for BNPP have been clearly proven. This finding sheds light on the reaction mechanisms for the natural phosphodiesterases and phosphomonoesterases. A study on binuclear complexes of these two metal ions is currently under way in our laboratory.

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## Footnotes

† Recently, a supercoiled plasmid DNA was hydrolysed by a diiron complex, although the detailed mechanism has not been clarified (ref. 16).

‡ The theoretical lines obtained by use of the kinetic parameters (presented in the legend for Fig. 2) virtually superimposed the straight lines depicted in Fig. 1. Neither the equilibrium for the mixed cluster formation nor that for the coordination of BNPP to the cluster causes any significant deviation of the plot from linearity under the conditions employed.

§ It was reported that the addition of hydrogen peroxide accelerates the La$^{III}$-catalysed hydrolysis of BNPP, where La$^{III}$ promotes nucleophilic attack of the hydrogen peroxide (ref. 8). However, the mechanism is not applicable to the present hydrolysis, since *in situ* formation of hydrogen peroxide in the absence of molecular oxygen is quite unlikely.

¶ At pH 7, the coordination water molecules of Fe$^{III}$ exist in the form of Fe–OH$_2$, except for the one which is dissociating under the conditions. When the metal-bound hydroxide is protonated in a rapid proton-exchange process, the other metal-bound water molecules should release a proton in turn and thus can function as highly active acid catalysts.

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