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Hydrolysis of bis(*p*-Nitrophenyl) Phosphate Promoted by Ni^{II} Complex of Pyrazine-2,5-dicarboxylate

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Abstract: The Ni^{II} complex of pyrazine-2,5-dicarboxylate is found to be the best catalyst containing a divalent metal center ever discovered for the hydrolysis of bis(*p*-nitrophenyl) phosphate

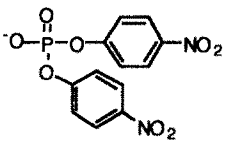
Currently, there is much interest in catalytic hydrolysis of phosphate esters in connection with the biological functions of transphosphorylation reactions such as information storage and processing, energy storage, regulation, signal transduction, and cofactor chemistry.[1] Several metalloenzymes are known to catalyze hydrolysis of phosphodiesters and phosphomonoesters. For example, Zn(II) plays essential roles in alkaline phosphatase, 5'-nucleotidase, fructose-1,6-biphosphatase, phosphodiesterase, cyclic nucleotide phosphodiesterase, and nuclease S_1.[2,3]

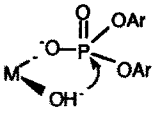
Phosphate diesters are very stable against hydrolysis. Half-lives estimated for the hydrolysis at pH 7 and 25 °C are 10^{11} yr, 2 X 10^{8} yr, and 2 X 10^{3} yr for dimethyl phosphate, DNA, and bis(*p*-nitrophenyl) phosphate (BNPP), respectively.[4,5] The best catalysts synthesized so far for the hydrolysis of BNPP are metal complexes, and it is widely proposed that the catalytic reactions proceed through the mechanism of **1** which involves formation of intermediates containing four-membered rings.[6-13]

In attempts to design effective artificial enzymes for phosphate diester hydrolysis,[14] we have searched for the structural units to be incorporated into the active sites of the artificial enzymes. Although the Co^{III} derivatives such as Co^{III}**2**, Co^{III}**3**, and Co^{III}**4** are among the best catalysts for BNPP hydrolysis, we tried to build artificial metalloenzymes using metal ions exploited by biological catalysts. In addition, Co^{III}-based artificial enzymes may suffer from rate-limiting substrate binding or product dissociation due to the exchange-inertness generally observed with Co^{III} complexes. In search of catalytic centers for artificial metallophosphoesterases, we have tested a variety of metal complexes for their catalytic activity in the hydrolysis of BNPP. In this Letter, effective catalysis by the Ni^{II} complex (Ni^{II}**5**) of pyrazine-2,5-dicarboxylate (**5**) is reported.

The dissociation constant (K_d) of Ni^{II}**5** was estimated as 1.2 X 10^{-6} at 25 °C by the polarographic method.[15,16] Kinetics of the hydrolysis of BNPP were studied under the conditions of $[NiCl_2]_o = [\mathbf{5}]_o =$ (1-10) X 10^{-4} M^{-1} and $[BNPP]_o =$ (0.5-1) X 10^{-5} M at pH 6.5-7.5 (0.05 M 4-morpholineethanesulfonate or *N*-(2-hydroxyethyl)-1-piperazineethanesulfonate) and 50 °C. At a given concentration of **5**, the pseudo-first-order rate constant (k_o) was proportional to $[NiCl_2]_o$ when $[NiCl_2]_o$ was not greater than $[\mathbf{5}]_o$. The K_d value indicates that binding of Ni$^{\rm II}$ to **5** is almost quantitative under these conditions. When [NiCl$_2$]$_o$ was raised above [**5**]$_o$, however, k_o did not increase. The catalytically active species, therefore, is the 1:1-type complex Ni$^{\rm II}$**5**. The values of k_o were proportional to [Ni$^{\rm II}$**5**]$_o$ at a constant pH. The proportionality constants (k_{b1}), which represent the bimolecular rate constants between the Ni$^{\rm II}$**5** and BNPP, were measured at pH 6.5-7.5 are summarized in Table 1. The pH dependence of k_{b1} for Ni$^{\rm II}$**5** is consistent with the mechanism of **1**.[17] Under these catalytic conditions, hydrolysis of *p*-nitrophenyl phosphate, the monoester analogue of BNPP, was found to be much slower than that of BNPP.

Catalytic efficiency of Ni$^{\rm II}$**5** is compared in Table 1 with that of other metal complexes reported in the literature. The best catalyst containing divalent metal center reported prior to this work is Co$^{\rm II}{}_7$[PEI-GO] recently prepared in this laboratory.[14] This catalyst was prepared by the Co$^{\rm II}$-template condensation of poly(ethylenimine) (PEI) with glyoxal and the content of the metal center is 7 residue molar % of the polymer. Using Ni$^{\rm II}$, Cu$^{\rm II}$, or Zn$^{\rm II}$ ion as the template, Co$^{\rm II}{}_7$[PEI-GO], Cu$^{\rm II}{}_8$[PEI-GO] or Zn$^{\rm II}{}_2$[PEI-GO] was prepared. By the reduction of Ni$^{\rm II}{}_7$[PEI-GO] with NaBH$_4$, NI$^{\rm II}{}_7$[PEI-GO]H was obtained. Except the macrocyclic complexes built on PEI, Zn$^{\rm II}$**6** and Zn$^{\rm II}$**7** are the most effective catalysts containing divalent metal centers reported to date.


BNPP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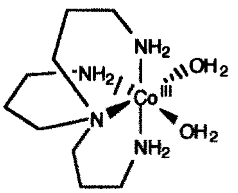
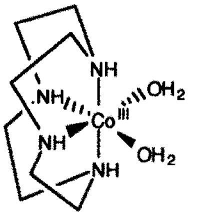

Co$^{\rm III}$2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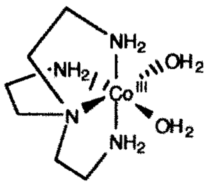
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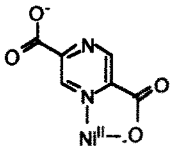
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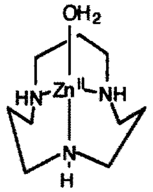
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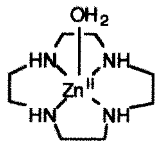
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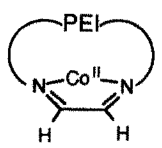
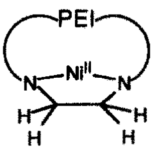
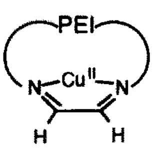

Co$^{\rm II}{}_7$[PEI-GO]


Ni$^{\rm II}{}_7$[PEI-GO]H


Cu$^{\rm II}{}_8$[PEI-GO]

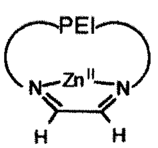

Zn$^{\rm II}{}_2$[PEI-GO]

Table 1. Values of Kinetic Parameters for BNPP Hydrolysis Catalyzed by Various Complexes

catalyst	temp	pH	parameter value[a]	ref
Ni^{II}**5**	50	6.5	$k_{bi} = 0.036\ M^{-1}s^{-1}$	this study
	50	7.0	$k_{bi} = 0.29\ M^{-1}s^{-1}$	this study
	50	7.5	$k_{bi} = 0.084\ M^{-1}s^{-1}$	this study
Co^{III}**2**	50	6.5-7.5	$k_{bi} = 2.5\ M^{-1}s^{-1}$	5
Co^{III}**3**	50	6.5-7.5	$k_{bi} = 0.46\ M^{-1}s^{-1}$	5
Co^{III}**4**	50	6.5-7.5	$k_{bi} = 8.1 \times 10^{-3}\ M^{-1}s^{-1}$	5
$Co^{III}(en)_2$	50	7	$k_{bi} = 2.7 \times 10^{-3}\ M^{-1}s^{-1}$	7
Co^{III}(trien)	50	7	$k_{bi} = 4.8 \times 10^{-2}\ M^{-1}s^{-1}$	7
Co^{III}(dien)	50	7	$k_{bi} < 1 \times 10^{-4}\ M^{-1}s^{-1}$	7
Zn^{II}**6**	35	> 8.5	$k_{bi} = 8.5 \times 10^{-5}\ M^{-1}s^{-1}$	11
Zn^{II}**7**	35	> 10	$k_{bi} = 2.1 \times 10^{-5}\ M^{-1}s^{-1}$	11
$Ni(tren)^{2+}$	75	8.6	8-fold acceleration, $C_o = 0.1$ mM	13
$Ni(bpy)_3^{2+}$	75	8.6	7-fold acceleration, $C_o = 0.1$ mM	13
$Cu(bpy)^{2+}$	75	8.6	20-fold acceleration, $C_o = 0.1$ mM	13
$Zn(bpy)^{2+}$	75	8.6	2-fold acceleration, $C_o = 0.1$ mM	13
Co^{II}_7[PEI-GO]	50	7.5	$k_{bi} = 0.18\ M^{-1}s^{-1}$	14
Ni^{II}_7[PEI-GO]H	50	6.5	$k_{bi} =$ ca. $5 \times 10^{-4}\ M^{-1}s^{-1}$	14
Cu^{II}_8[PEI-GO]	50	6.5	$k_{bi} = <1 \times 10^{-5}\ M^{-1}s^{-1}$	14
Zn^{II}_2[PEI-GO]	50	7.5	$k_{bi} = 7.4 \times 10^{-3}\ M^{-1}s^{-1}$	14
spontaneous	50	7.0	$k_o = 3 \times 10^{-10}\ s^{-1}$	5
OH^-	35	-	$k_{bi} = 2.4 \times 10^{-5}\ M^{-1}s^{-1}$	11

[a] Parameters k_o and k_{bi} stand for the first-order and the second-order rate constants, respectively.

When judged with k_{bi}, Ni^{II}**5** is the best catalyst containing a divalent metal center ever discovered for BNPP hydrolysis and is comparable to Co^{III}**3**, the second best Co^{III}-catalyst. Other Ni^{II} complexes such as $Ni(tren)^{2+}$ or $Ni(bpy)_3^{2+}$ are practically ineffective in BNPP hydrolysis (Table 1). Our study indicated that the Ni^{II} complex of pyrazine-2-carboxylate did not catalyze BNPP hydrolysis. The reason why the Ni^{II} complex of **5** is much more effective than other Ni^{II} complexes is not clear. As revealed by the data summarized in Table 1, the catalytic efficiency of the Co^{III} complexes is greatly affected by the ligand structure. This has been attributed to bond angles in the transition state containing a four-membered ring.[6] The remarkable difference in catalytic efficiency between the Ni^{II} complexes of **5** and pyrazine-2-carboxylate suggests that both electronic and steric effects exerted by the ligands affect the catalytic power of metal complexes.

In order to design artificial enzymes which reproduce important features of enzymatic actions, it is necessary to incorporate effective catalytic centers into the backbone of the artificial enzymes.[14,18-23] In the previously reported artificial metallophosphoesterases built on PEI, the macrocyclic centers were constructed by random condensation of PEI with dicarbonyl compounds in the presence of metal ions.[14] The structures of catalytic centers of the PEI-based macrocycles such as Co^{II}_7[PEI-GO] are not clearly known. On the other hand, the catalytic site on Ni^{II}**5** is well defined and Ni^{II}**5** may be exploited as catalytic centers of artificial metallophosphoesterases.

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