

## Artificial Metallophosphoesterases Built on Poly(ethylenimine)

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Received November 12, 1993\*

Various derivatives of poly(ethylenimine) (PEI)-containing macrocyclic metal centers were prepared by condensation of PEI with several dicarbonyl compounds in the presence of a series of metal ions involved as templates. In view of the biological functions of transphosphorylation reactions, the PEI-based macrocyclic complexes were tested for their activity as artificial metallophosphoesterases. Hydrolysis of bis(*p*-nitrophenyl) phosphate (BNPP) and *p*-nitrophenyl phosphate (NPP) was catalyzed by the PEI-based macrocyclic complexes. Kinetic data for the hydrolysis of BNPP or NPP revealed complex formation between the substrate and the catalysts, followed by efficient dephosphorylation of the complexed substrate. Depending upon the structure of the catalyst and the reaction conditions, the degree of acceleration observed for BNPP and NPP hydrolysis was up to 6 orders of magnitude. The most effective catalysis was achieved by the macrocyclic complex prepared by condensation of PEI with glyoxal in the presence of Co<sup>II</sup> ion. The PEI-based macrocycles containing other metal ions such as Fe<sup>III</sup>, Ni<sup>II</sup>, Cu<sup>II</sup>, or Zn<sup>II</sup> ion were also quite effective catalysts for the hydrolysis of BNPP or NPP. The catalytic activity of the PEI-based macrocyclic complexes containing divalent metal ions was much greater than that of other synthetic catalysts containing divalent metal ions ever reported. Possible origins of the effective catalysis are discussed, in view of the mechanisms proposed for analogous enzymatic and nonenzymatic hydrolysis of phosphate esters. Characteristics of actions of metalloenzymes such as essential involvement of metal ions, complex formation with the substrates, and efficient catalysis in the conversion of bound substrates were reproduced by the artificial metallophosphoesterases. The metal centers of the artificial metallophosphoesterases are involved both in substrate binding and in catalytic transformation. Catalytic repertoires played by the metal ions are discussed.

Enzymatic catalysis is characterized by complexation with substrates, large rate acceleration, and high selectivity. Efforts to design artificial enzymes are directed toward reproduction of these characteristics of enzymatic actions with synthetic or semisynthetic molecules.

Attempts to design artificial enzymes with purely synthetic molecules have been intensively made by using nonprotein host molecules. A great number of macrocyclic compounds such as cyclodextrins, crown ethers, cyclophanes, and calixarenes as well as noncyclic compounds equipped with convergent functional groups have been prepared and tested for their ability to recognize guest molecules and to perform various molecular functions such as catalysis, transport of materials and energy, or transmission of signals.<sup>1-9</sup> With these host molecules, substrate binding and catalysis (i.e., molecular recognition of the substrate and more effective molecular recognition of the transition state) have been simultaneously achieved in few cases.<sup>10</sup>

In devising artificial enzymes, it is necessary to incor-

porate catalytic principles exploited by enzymes in relatively small spaces on synthetic molecules. Furthermore, in order to achieve cooperative catalytic participation of several functional groups in the chemical transformation of the bound substrate, fine alignment of convergent catalytic groups is needed. Creation of such active sites on molecular skeletons provided by relatively small synthetic hosts would not be easy. For this reason, poly(ethylenimine) (PEI) has been exploited as a molecular backbone in an alternative approach to the design of artificial enzymes.<sup>11-17</sup>

PEI contains ethylamine as the repeating unit and, thus, is highly soluble in water. The molecular weight of the PEI used in construction of artificial enzymes is ca. 60000, corresponding to 1400 monomer residues. Among the 1400 nitrogens present in PEI, 25, 50, and 25% are primary, secondary, and tertiary amines, respectively. The tertiary amines represent branching points on the polymer backbone, and PEI is a highly branched and globular polymer. The amino nitrogens of PEI can be easily modified by alkylation, acylation, or imine formation, allowing incorporation of various catalytic elements to the backbone. Water solubility, easy modification, and highly branched structure are the major advantages of PEI.

One of the basic obstacles to overcome in the design of

\* Abstract published in *Advance ACS Abstracts*, March 1, 1994.

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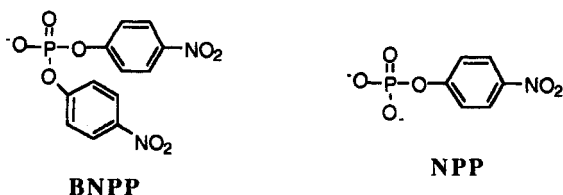
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artificial enzymes by using synthetic polymers is the lack of specific binding sites on the macromolecular backbone. For PEI, we have so far constructed two types of binding sites: one<sup>13</sup> is based on macrocyclic metal centers built on PEI and the other<sup>14</sup> on  $\beta$ -cyclodextrin attached to PEI. The macrocyclic metal centers have been obtained by condensing PEI with dicarbonyl compounds in the presence of metal templates.<sup>13,15</sup> Since metal ions are very tightly bound by the macrocycles built on PEI, the polymeric macrocycles can serve as the skeleton of artificial metalloenzymes.

Several catalytic features such as recognition of anionic substrates, rate enhancement in deacylation of complexed anionic substrates, and selective recognition of transition states have been observed with various types of macrocyclic complexes built on PEI.<sup>13,15,16</sup> However, the ester deacylation resulted in acylation of the amino groups located on the PEI backbone, without achieving catalytic turnover. In addition, the only catalytic role played by metal ions was to anchor anionic centers. In order to improve efficiency as artificial metalloenzymes, it is desirable that catalytic turnover be achieved and the metal centers play various catalytic roles<sup>12,17</sup> available for metal ions, metal-bound water molecules, and metal-bound hydroxide ions.

In the present investigation, catalytic capabilities of various macrocyclic metal centers built on PEI were tested with the hydrolysis of bis(*p*-nitrophenyl) phosphate (BNPP), a phosphate diester, and *p*-nitrophenyl phosphate (NPP), a phosphate monoester. 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.<sup>2</sup> Several metalloenzymes are known to catalyze hydrolysis of phosphodiester and phosphomonoesters. For example, Zn(II) plays essential roles in alkaline phosphatase, 5'-nucleotidase, fructose-1,6-bisphosphatase, phosphodiesterase, cyclic nucleotide phosphodiesterase, and nuclease S<sub>1</sub>.<sup>18,19</sup> In this article, reproduction of characteristics of enzymatic actions such as substrate binding and catalytic turnover by artificial metallophosphoesterases built on PEI is described. In addition, participation of the metal centers of the artificial metalloenzymes both in substrate binding and catalytic transformation is reported.

### Experimental Section

**Materials.** PEI (MW 50000) was purchased from Sigma and purified by ultrafiltration with a PM-30 membrane (Amicon) to remove small (MW <30000) portions and the average MW of the purified PEI was estimated as ca. 60000. Laurylation of PEI was carried out according to the literature<sup>20</sup> to obtain lau<sub>11</sub>-PEI, and

the content of lauryl group in lau<sub>11</sub>-PEI was 11% of the monomer residues. Preparation of Ni<sup>II</sup>[PEI-GO] by the Ni<sup>II</sup>-template condensation of PEI with glyoxal and that of Ni<sup>II</sup>[PEI-GO]H by the NaBH<sub>4</sub> reduction of Ni<sup>II</sup>[PEI-GO] were carried out according to the method reported previously.<sup>15</sup> Content of Ni<sup>II</sup> was 6.6% of the monomer residues for both polymers.

Co<sup>II</sup>[PEI-GO] was prepared according to the general procedure<sup>15</sup> described previously for Ni<sup>II</sup>[PEI-GO] by using glyoxal (40% solution, 1.80 g; 12.5 mmol), CoCl<sub>2</sub>·6H<sub>2</sub>O (9.50 g; 40.0 mmol), and PEI (5.30 g; 123 residue mmol). The product was purified by dialysis against 12 L of water (once), 12 L of 0.1 M NaCl (three times), and 12 L of water (three times). Lyophilization of the polymer produced red powders, and the metal content of the polymer was 7.0% of the monomer residues.

Co<sup>II</sup>[PEI-GO]H was prepared by the reduction of Co<sup>II</sup>-[PEI-GO] (38.6 residue mM, 100 mL; 0.27 mmol Co<sup>II</sup>) with NaBH<sub>4</sub> (5.4 g; 140 mmol) according to the general procedure<sup>15</sup> reported previously for Ni<sup>II</sup>[PEI-GO]H. Lyophilization of the polymer produced pale red powders, and the metal content of the polymer was 6.7% of the monomer residues.

Co<sup>II</sup>[PEI-BD] was prepared by condensation of PEI with butanedione as follows. An ethanolic solution (50 mL) of butanedione (1.15 g; 13.4 mmol) was diluted with 50 mL of hot H<sub>2</sub>O, and the resulting solution was kept at about 60 °C. After a 10% aqueous ethanolic solution of CoCl<sub>2</sub>·6H<sub>2</sub>O (8.00 g; 33.6 mmol) was added, the mixture was stirred for 1 h. Then, a 25% aqueous ethanolic solution of PEI (4.90 g; 114 residue mmol) was added to this mixture, which was refluxed for 72 h and then purified by dialysis as described above. The metal content of the polymer was 7.5% of the monomer residues.

Co<sup>II</sup>[PEI-Bz] was prepared according to the general procedure described above for Co<sup>II</sup>[PEI-GO] by using benzil (0.645 g; 3.65 mmol), CoCl<sub>2</sub>·6H<sub>2</sub>O (5.13 g; 21.6 mmol), and PEI (1.40 g; 32.5 residue mmol) except that 10% (v/v) aqueous ethanol was used instead of water in dialysis due to the limited solubility of the red-pink polymer in water. The metal content of the polymer was 2.0% of the monomer residues.

Co<sup>II</sup>[PEI-GA] was prepared according to the general procedure described above for Co<sup>II</sup>[PEI-GO] by using glutaraldehyde (3.02 g; 29.1 mmol), CoCl<sub>2</sub>·6H<sub>2</sub>O (23.7 g, 100 mmol), and PEI (12.5 g; 291 residue mmol). Lyophilization of the polymer produced red powders and the metal content of the polymer was 9.5% of the monomer residues.

Co<sup>II</sup>[PEI-DAP] was prepared according to the general procedure described above for Co<sup>II</sup>[PEI-GO] by using 2,6-diacetylpyridine (5.76 g; 35.3 mmol), CoCl<sub>2</sub>·6H<sub>2</sub>O (19.0 g; 80.0 mmol), and PEI (15.7 g; 353 monomer residue mmol). Lyophilization of the polymer produced red powders and the metal content of the polymer was 9.5% of the monomer residues.

Co<sup>II</sup>[lau<sub>11</sub>-PEI-GO] was prepared according to the general procedure above for Co<sup>II</sup>[PEI-GO] by using glyoxal (40% solution, 1.13 g; 7.79 mmol), CoCl<sub>2</sub>·6H<sub>2</sub>O (5.57 g; 23.4 mmol), and lau<sub>11</sub>-PEI (0.260 residue mM, 300 mL; 78.0 residue mmol). Lyophilization of the polymer produced red powders and the metal content of the polymer was 3.6% of the monomer residues.

Co<sup>II</sup>[lauroyl<sub>10</sub>-PEI-GO] was obtained by lauroylation of Co<sup>II</sup>-[PEI-GO] as follows. An acetonitrile solution of *N*-(lauroyloxy)succinimide (prepared by coupling *N*-hydroxysuccinimide and lauric acid with the aid of 1,3-dicyclohexylcarbodiimide in tetrahydrofuran, mp 76–77 °C) (2.82 g; 10.4 mmol) was added to a 20% (v/v) aqueous acetonitrile solution of Co<sup>II</sup>[PEI-GO] (0.523 residue M, 200 mL; 105 residue mmol) dropwise over a period of 3 h at room temperature. After the mixture was stirred for 1 h at room temperature and for 3 days at 45–55 °C, the polymer was purified by dialysis leading to dark red product. Contents of Co<sup>II</sup> and lauroyl group was 7.0 and 10%, respectively, of the monomer residues.

Co<sup>II</sup>[imaben<sub>1</sub>-PEI-GO] was obtained by attaching 3-(2-imidazolylazo)benzoyl group to Co<sup>II</sup>[PEI-GO] as follows. An acetonitrile solution (30 mL) of *N*-[[3-(2-imidazolylazo)benzoyl]oxy]succinimide<sup>21</sup> (1.68 g; 5.36 mmol) was added to a 20% (v/v) aqueous acetonitrile solution of Co<sup>II</sup>[PEI-GO] (0.523 residue M, 100 mL; 52.3 residue mmol) dropwise over a period of 3 h at

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room temperature. The mixture was stirred for further 1 h and then stirred at 45–55 °C for 24 h and purified by dialysis. The content of the (imidazolylazo)benzoyl group introduced to the polymer was estimated as 0.9% of the monomer residues by <sup>1</sup>H NMR. Lyophilization of the polymer produced red-brown powders and the metal content of the polymer was 7.2% of the monomer residues. The polymer exhibited λ<sub>max</sub> at 450 nm. This peak is attributable to the (imidazolylazo)benzene moiety as the absorbance at this wavelength was much greater than that of Co<sup>II</sup><sub>7</sub>[PEI-GO]. For imabeng-PEI,<sup>21</sup> λ<sub>max</sub> was 344 nm.

Co<sup>III</sup><sub>7</sub>[PEI-GO] was prepared by oxidation of Co<sup>II</sup><sub>7</sub>[PEI-GO] with PbO<sub>2</sub> following the procedure<sup>22</sup> reported in the literature. This reagent oxidizes the Co<sup>II</sup> center without oxidizing the amine moieties of the ligand.<sup>22</sup> To Co<sup>II</sup><sub>7</sub>[PEI-GO] (355 residue mM, 140 mL; 3.5 mmol Co<sup>II</sup>) was added the mixture of NaHCO<sub>3</sub> (0.455 g; 5.42 mmol) and PbO<sub>2</sub> (2.55 g; 10.6 mmol) in 100 mL of water. After 60% HClO<sub>4</sub> (16 mL) was added over 1 h, the solution was refluxed for 48 h with stirring and then purified by dialysis. Lyophilization of the polymer produced purple-red powders and the metal content of the polymer was 7.0% of the monomer residues.

Co<sup>III</sup><sub>7</sub>[PEI-GO]H was prepared by oxidation of Co<sup>II</sup><sub>7</sub>[PEI-GO]H as described above for Co<sup>III</sup><sub>7</sub>[PEI-GO] by using Co<sup>II</sup><sub>7</sub>[PEI-GO]H (237 residue mM, 210 mL; 3.5 mmol Co<sup>II</sup>), NaHCO<sub>3</sub> (0.455 g; 5.42 mmol), and PbO<sub>2</sub> (2.55 g; 10.6 mmol) in 100 mL of water. Lyophilization of the polymer produced pink powders and the metal content of the polymer was 7.2% of the monomer residues. Co<sup>III</sup><sub>7</sub>[PEI-GO] or Co<sup>III</sup><sub>7</sub>[PEI-GO]H was much less soluble than the corresponding Co<sup>II</sup> complexes at pH > 7, presumably due to the ionization of the Co<sup>III</sup>-bound water molecules.

Fe<sup>III</sup><sub>9</sub>[PEI-GO] was prepared according to the general procedure described above for Co<sup>II</sup><sub>7</sub>[PEI-GO] by using glyoxal (40% solution, 1.82 g; 31.3 mmol), FeCl<sub>3</sub>·H<sub>2</sub>O (16.0 g; 59.2 mmol), and PEI (13.5 g; 314 residue mmol). Lyophilization of the polymer produced yellow powders and the metal content of the polymer was 8.9% of the monomer residues.

Cu<sup>II</sup><sub>8</sub>[PEI-GO] was prepared according to the general procedure described above for Co<sup>II</sup><sub>7</sub>[PEI-GO] by using glyoxal (40% solution, 2.83 g; 19.7 mmol), CuCl<sub>2</sub>·2H<sub>2</sub>O (12.4 g; 72.8 mmol), and PEI (8.27 g; 192 residue mmol). Lyophilization of the polymer produced green powders and the metal content of the polymer was 8.2% of the monomer residues.

Zn<sup>II</sup><sub>2</sub>[PEI-GO] was prepared according to the general procedure described above for Co<sup>II</sup><sub>7</sub>[PEI-GO] by using glyoxal (40% solution, 1.82 g; 12.5 mmol), ZnCl<sub>2</sub>·6H<sub>2</sub>O (11.4 g; 83.6 mmol), and PEI (5.56 g; 129 residue mmol). Lyophilization of the polymer produced yellow powders and the metal content of the polymer was 2.2% of the monomer residues.

Zn<sup>II</sup><sub>2</sub>[PEI-GO]H was prepared by the reduction of Zn<sup>II</sup>[PEI-GO] (21.9 residue mM, 100 mL; 0.048 mmol Zn<sup>II</sup>) with NaBH<sub>4</sub> (6.0 g; 160 mmol) according to the general procedure described above for Co<sup>II</sup><sub>7</sub>[PEI-GO]H. Lyophilization of the polymer produced yellow powders and the metal content of the polymer was 2.2% of the monomer residues.

The IR spectra of the macrocyclic complexes containing C=N bonds formed by the metal-template condensation of PEI with various dicarbonyl compounds showed peaks at 1620–1640 cm<sup>-1</sup> for the C=N bonds, which disappeared upon reduction of the macrocyclic complexes with NaBH<sub>4</sub>.

BNPP purchased from Aldrich was used after recrystallization from acetonitrile, and NPP obtained from Sigma was used without further purification. Water was distilled and deionized prior to use in the preparation of the polymers and in kinetic studies.

**Measurements.** Metal contents of the macrocyclic complexes built on PEI were determined by ICP (inductively coupled plasma spectroscopy), which was performed by Korea Basic Science Center. XPS (X-ray photoelectron spectroscopy) measurements were carried out with a VG ESCALAB 200X (Ar gas etching, 10<sup>-9</sup> Torr high vacuum, and Al K<sub>α</sub> radiation source), which was performed by Samsung Electronics.

Kinetic measurements were performed spectrophotometrically by following the release of *p*-nitrophenol (NP) with a Beckman 5260 spectrophotometer. Temperature was kept at 50 ± 0.1 °C

(controlled with a Haake E12 or a Lauda Model T-2 circulator). Buffers (0.05 M) used were (4-morpholineethanesulfonate (pH 5.5–6.5) and *N*-(2-hydroxyethyl)-1-piperazineethanesulfonate (pH 7–8). pH was measured with a Dongwoo Medical system pH/Ion meter DP-125M. Hydrolysis of BNPP was carried out in the presence of 1.2% (v/v) acetonitrile which was added as the solvent for the stock solution of substrate. For NPP, stock solutions were made in water. For Co<sup>II</sup><sub>2</sub>[PEI-Bz], buffer solutions for kinetic measurements contained 10% (v/v) ethanol due to the limited solubility of the polymer in water.

Quantitation of inorganic phosphate ion formed during the hydrolysis or NPP or BNPP catalyzed by the PEI-based macrocyclic complexes was performed according to the literature.<sup>28</sup>

## Results

**Polymeric Macrocyclic Complexes Prepared.** The macrocyclic complexes prepared by the condensation of PEI with dicarbonyl compounds in the presence of various metal ions in the present study are schematically presented in Figure 1. Each of the schematic structures listed in Figure 1 indicates the nature of the metal ion and the dicarbonyl compound used in the condensation, instead of the exact molecular structure of the complex.<sup>24</sup> The subscripts included in the abbreviated names of the PEI-based macrocyclic complexes represent the % molar contents of inorganic or organic modifiers relative to monomer residues. For example, Co<sup>II</sup><sub>7</sub>[PEI-GO] was prepared by the Co<sup>II</sup>-template condensation of PEI with glyoxal and the content of Co<sup>II</sup> macrocyclic metal center is 7% of the monomer residues.

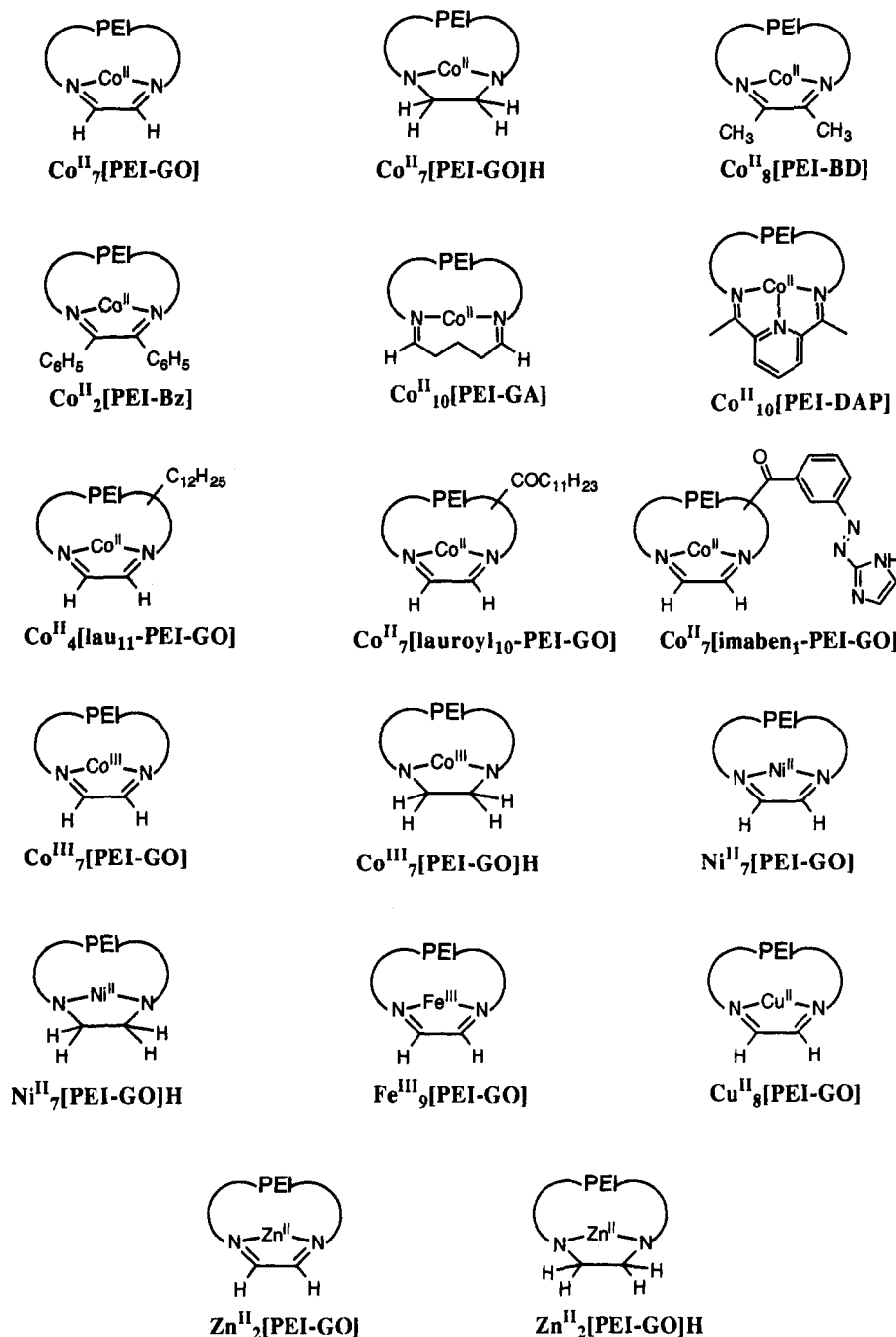
It is known that Co<sup>II</sup> ions bound to polyaza macrocyclic ligands are not spontaneously oxidized to Co<sup>III</sup> ions under the conditions of the present studies.<sup>25</sup> Amine forms Co<sup>II</sup><sub>7</sub>[PEI-GO]H, Ni<sup>II</sup><sub>7</sub>[PEI-GO]H, and Zn<sup>II</sup><sub>2</sub>[PEI-GO]H were prepared by reduction of the corresponding imine forms with NaBH<sub>4</sub>, whereas Co<sup>III</sup><sub>7</sub>[PEI-GO] and Co<sup>III</sup><sub>7</sub>[PEI-GO]H were prepared by oxidation of the corresponding Co<sup>II</sup> species with PbO<sub>2</sub>. As the metal content was not altered during the reduction or oxidation process, it is very likely that identical metal binding sites are involved in Co<sup>III</sup><sub>7</sub>[PEI-GO]H, Co<sup>II</sup><sub>7</sub>[PEI-GO]H, Co<sup>III</sup><sub>7</sub>[PEI-GO], and Co<sup>II</sup><sub>7</sub>[PEI-GO] and in Ni<sup>II</sup><sub>7</sub>[PEI-GO]H and Ni<sup>II</sup><sub>7</sub>[PEI-GO] as well as in Zn<sup>II</sup><sub>2</sub>[PEI-GO]H and Zn<sup>II</sup><sub>2</sub>[PEI-GO]. Chemical yields for the reduction of the imine bonds with NaBH<sub>4</sub> and for the oxidation of the Co<sup>II</sup> centers with PbO<sub>2</sub> are not known. It is possible that Co<sup>II</sup><sub>7</sub>[PEI-GO]H, Co<sup>III</sup><sub>7</sub>[PEI-GO]H, Ni<sup>II</sup><sub>7</sub>[PEI-GO]H, or Zn<sup>II</sup><sub>2</sub>[PEI-GO]H contains unreduced imine groups although C=N peaks were not detected in the IR spectra. In addition, Co<sup>III</sup><sub>7</sub>[PEI-GO] or Co<sup>III</sup><sub>7</sub>[PEI-GO]H may contain some Co<sup>II</sup> centers.

XPS analysis of Co<sup>II</sup><sub>7</sub>[PEI-GO] and Co<sup>III</sup><sub>7</sub>[PEI-GO] revealed 2p<sub>3/2</sub> binding energies of 779.49 eV (satellite band energy 6.25 eV) and 780.46 eV (satellite band energy 6.21 eV), respectively. The satellite band was much stronger

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(24) As indicated previously,<sup>13,15</sup> the exact structure of the macrocycles formed on PEI (such as the number of nitrogen atoms interacting with the metal ions, the size of the chelate ring on the side of the PEI backbone, the extent of hydration of the imine bonds leading to the formation of carbinolamines, etc.) is not known. Metal ions of the macrocyclic complexes are bound to the polymer very tightly and the metal-binding imine linkages of the PEI-based macrocycles are not hydrolyzed appreciably over extended periods. These indicate that macrocyclic complexes are formed with both of the imine nitrogen atoms, originating from condensation with dicarbonyl compounds, being coordinated to the same metal ion.

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**Figure 1.** Schematic structures of the PEI-based macrocyclic complexes prepared in the present study.

for the  $\text{Co}^{\text{II}}$  derivative. The higher binding energy and weaker satellite observed for  $\text{Co}^{\text{III}}_7[\text{PEI-GO}]$  is in agreement with the data reported in the literature.<sup>26</sup>

The hydrophobic groups in  $\text{Co}^{\text{II}}_7[\text{lauroyl}_{10}\text{-PEI-GO}]$  or  $\text{Co}^{\text{II}}_7[\text{imaben}_1\text{-PEI-GO}]$  were introduced by acylation of  $\text{Co}^{\text{II}}_7[\text{PEI-GO}]$ , and, therefore, the metal-binding sites in these macrocyclic complexes are the same. On the other hand,  $\text{Co}^{\text{II}}_4[\text{lau}_{11}\text{-PEI-GO}]$  was prepared by  $\text{Co}^{\text{II}}$ -template condensation of  $\text{lau}_{11}\text{-PEI}$  with glyoxal, and, thus, the metal binding sites of  $\text{Co}^{\text{II}}_4[\text{lau}_{11}\text{-PEI-GO}]$  are not identical with those of  $\text{Co}^{\text{II}}_7[\text{lauroyl}_{10}\text{-PEI-GO}]$ ,  $\text{Co}^{\text{II}}_7[\text{imaben}_1\text{-PEI-GO}]$ , or  $\text{Co}^{\text{II}}_7[\text{PEI-GO}]$ .

**Kinetic Data.** The absorbance increase during the

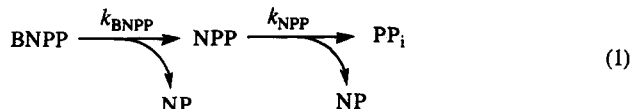
hydrolysis of BNPP and NPP always corresponded to the release of 2 equiv and 1 equiv, respectively, of NP from BNPP and NPP. In addition, the amount of inorganic phosphate ion released from the hydrolysis of BNPP or NPP was measured for selected catalysts ( $\text{Co}^{\text{II}}_7[\text{PEI-GO}]$ ,  $\text{Zn}^{\text{II}}_2[\text{PEI-GO}]$ , and  $\text{Ni}^{\text{II}}_7[\text{PEI-GO}]$ ) and was found to be almost quantitative ( $100 \pm 5\%$ ). The release of NP was followed for  $\text{Co}^{\text{II}}_7[\text{PEI-GO}]$  under the conditions of  $C_0$  (initially added concentration of the catalyst; calculated in terms of the total concentration of metal centers)  $< S_0$  (initially added concentration of the substrate) in order to ensure the regeneration of the polymeric catalysts during the hydrolysis of BNPP or NPP.<sup>27</sup> The PEI-based

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(27)  $C_0$  was not lowered below 0.4 mM due to the reaction rates and  $S_0$  was not raised above 2 mM because of the large absorption by the product.

macrocyclic complexes, therefore, act as catalysts in the hydrolysis of BNPP and NPP.

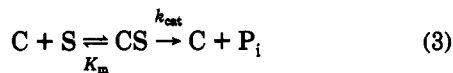
The hydrolyses of BNPP to produce NPP and NP and that of NPP to yield NP and  $PP_i$  proceed in a consecutive manner (eq 1). The pseudo-first-order rate constant ( $k_{NPP}$ ) for the hydrolysis of NPP was obtained by using NPP as the substrate in the presence of various catalysts. When NPP hydrolysis is much faster than BNPP hydrolysis,  $k_{\phi}$  (pseudo-first-order rate constant) measured with BNPP represents  $k_{BNPP}$ . When the rates for NPP hydrolysis and BNPP hydrolysis are comparable, the dependence of [NP] on time was analyzed according to eq 2,<sup>28</sup> an expression



$$[\text{NP}] = [\text{BNPP}]_0 \{ 2 - 2e^{-k_{BNPP}t} [k_{BNPP}/(k_{NPP} - k_{BNPP})] (e^{-k_{BNPP}t} - e^{-k_{NPP}t}) \} \quad (2)$$

derived from eq 1, with a nonlinear regression program. The value of  $k_{NPP}$  obtained directly with NPP under the identical conditions was used in the analysis of the rate data with eq 2.

If the hydrolysis of BNPP or NPP catalyzed by the PEI-based macrocycles proceeds through the formation of complex between the substrate and the catalyst (eq 3), pseudo-first-order kinetic behavior is obtained under the conditions of  $C_0 \gg S_0$  with  $k_{BNPP}$  or  $k_{NPP}$  being related to  $C_0$  by eq 4.



$$k_{\phi} (= k_{BNPP} \text{ OR } k_{NPP}) = k_{cat} C_0 / (K_m + C_0) \quad (4)$$

When  $K_m \gg C_0$ , eq 4 leads to eqs 5 and 6.

$$k_{\phi} (= k_{BNPP} \text{ OR } k_{NPP}) = k_{cat} C_0 / K_m \quad (5)$$

$$k_{bi} = k_{\phi} / C_0 = k_{cat} / K_m \quad (6)$$

When  $K_m \ll C_0$ , eq 5 leads to eq 7.

$$k_{\phi} (= k_{BNPP} \text{ OR } k_{NPP}) = k_{cat} \quad (7)$$

For the reactions catalyzed by  $\text{Co}^{II}_7[\text{PEI-GO}]$ ,  $k_{BNPP}$  was calculated by analysis of time-dependent release of [NP] according to eq 2. Some of the values of  $k_{BNPP}$  and  $k_{NPP}$  obtained for  $\text{Co}^{II}_7[\text{PEI-GO}]$  are illustrated in Figures 2 and 3, respectively. As revealed by Figure 2,  $k_{BNPP}$  for  $\text{Co}^{II}_7[\text{PEI-GO}]$  is proportional to  $C_0$ . On the other hand,  $k_{NPP}$  for  $\text{Co}^{II}_7[\text{PEI-GO}]$  exhibits saturation behavior with respect to  $C_0$  at  $\text{pH} \leq 6.5$  (Figure 3), which is consistent with the complex formation between the catalyst and NPP.

For the hydrolysis of BNPP and NPP catalyzed by other PEI-based macrocycles,  $k_{BNPP}$  and  $k_{NPP}$  were measured over relatively wide range of  $C_0$  concentrations<sup>29</sup> except for very slow reactions. When the reactions were too slow for accurate rate measurements, approximate values of  $k_{BNPP}$  or  $k_{NPP}$  were obtained. When  $k_{BNPP}$  or  $k_{NPP}$  was

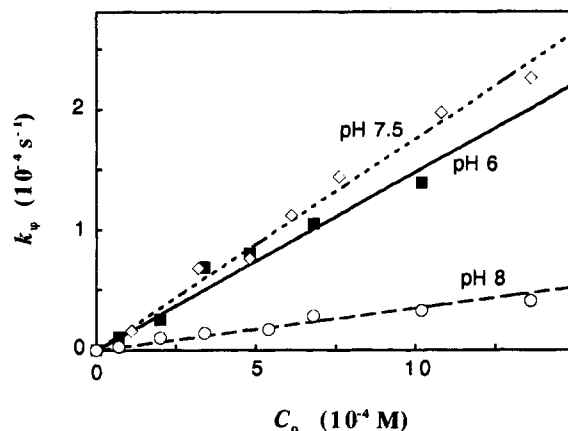


Figure 2. Kinetic data measured for the hydrolysis of BNPP catalyzed by  $\text{Co}^{II}_7[\text{PEI-GO}]$  at pH 6–8.

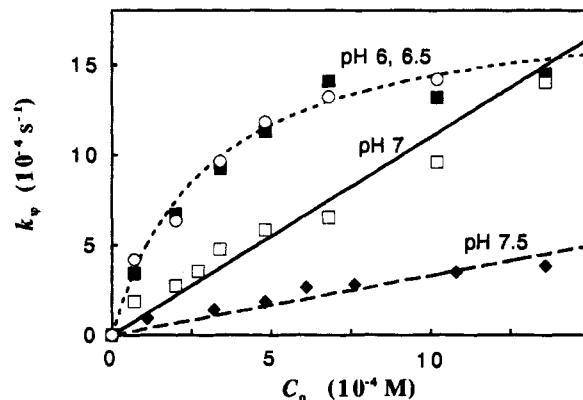


Figure 3. Kinetic data measured for the hydrolysis of NPP catalyzed by  $\text{Co}^{II}_7[\text{PEI-GO}]$  at pH 6 (■), 6.5 (○), 7 (□), and 7.5 (◆).

proportional to  $C_0$ , the proportionality constant was taken as  $k_{bi}$  ( $= k_{cat}/K_m$ ). When the approximate rate was measured for BNPP at one  $C_0$  concentration due to very slow rates,  $k_{BNPP}$  was assumed to be proportional to  $C_0$  and  $k_{BNPP}/C_0$  was taken as  $k_{bi}$ . When saturation kinetic behavior was observed,  $k_{cat}$  and  $K_m$  were estimated by analysis of the dependence of  $k_{\phi}$  ( $k_{BNPP}$  or  $k_{NPP}$ ) on  $C_0$  with a nonlinear regression program. The values of kinetic parameters thus obtained for the hydrolyses of BNPP and NPP according to eqs 4–7 are summarized in Tables 1 and 2, respectively.

## Discussion

**Kinetic Features.** Phosphate diesters are very stable against hydrolysis. Half-lives estimated for the hydrolysis at pH 7 and 25 °C are  $10^{11}$  yr,  $2 \times 10^8$  yr, and  $2 \times 10^3$  yr for dimethyl phosphate, DNA, and BNPP, respectively.<sup>22,30</sup> Phosphate monoesters are more reactive than the diesters, but they are still quite stable, with the half-life of NPP at neutral pHs and 25 °C being 4 yr.<sup>31</sup> The half-life of NPP is comparable to that (7 yr) of unactivated amides under identical conditions.<sup>32</sup>

The most effective catalytic systems artificially designed so far for the hydrolysis of phosphate diesters and

(28) Equation 2 was derived by following the general method described in Frost, A. A.; Pearson, R. G. *Kinetics and Mechanism*, 2nd ed.; Wiley: New York, 1956; Chapter 8.

(29) The upper limit of the  $C_0$  concentration employed in the kinetic study was restricted by the solubility of the polymeric catalysts or by absorbance of the catalysts at the wavelengths employed for the measurement of the release of NP.

(30) Chin, J.; Banaszczyk, M.; Jubian, V.; Zou, X. *J. Am. Chem. Soc.* 1989, 111, 186.

(31) Calculated with the data reported in Kirby, A. J.; Jencks, W. P. *J. Am. Chem. Soc.* 1965, 87, 3209.

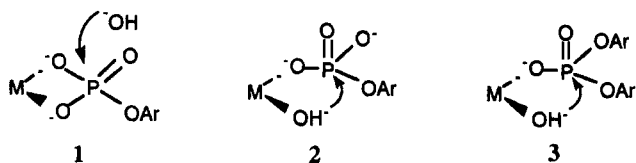
(32) Kahne, D.; Still, W. C. *J. Am. Chem. Soc.* 1988, 110, 7529.

Table 1. Values of Kinetic Parameters Obtained for the Hydrolysis of BNPP Catalyzed by Various Macrocyclic-Containing PEI Derivatives at 50 °C

catalyst	pH	$C_0$ (M) <sup>a</sup>	parameter value
Co <sup>II</sup> <sub>7</sub> [PEI-GO]	6.0	$(0.7-10) \times 10^{-4}$	$k_{bi} (k_{cat}/K_m) = 0.15 \pm 0.01 \text{ M}^{-1} \text{ s}^{-1}$
	6.5	$(0.7-10) \times 10^{-4}$	$k_{bi} (k_{cat}/K_m) = 0.17 \pm 0.01 \text{ M}^{-1} \text{ s}^{-1}$
	7.0	$(0.7-14) \times 10^{-4}$	$k_{bi} (k_{cat}/K_m) = 0.14 \pm 0.01 \text{ M}^{-1} \text{ s}^{-1}$
	7.5	$(1-14) \times 10^{-4}$	$k_{bi} (k_{cat}/K_m) = 0.18 \pm 0.01 \text{ M}^{-1} \text{ s}^{-1}$
	8.0	$(0.7-14) \times 10^{-4}$	$k_{bi} (k_{cat}/K_m) = 0.035 \pm 0.003 \text{ M}^{-1} \text{ s}^{-1}$
Co <sup>II</sup> <sub>7</sub> [PEI-GO]H	6.5	$1.3 \times 10^{-2}$	$k_{bi} (k_{cat}/K_m) = 6 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$
	7.0	$1.3 \times 10^{-2}$	$k_{bi} (k_{cat}/K_m) = 7 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$
	7.5	$1.3 \times 10^{-2}$	$k_{bi} (k_{cat}/K_m) = 9 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$
Co <sup>II</sup> <sub>8</sub> [PEI-BD]	7.5	$1 \times 10^{-2}$	$k_{bi} (k_{cat}/K_m) < 3 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$
Co <sup>II</sup> <sub>2</sub> [PEI-Bz]	7-8	$1.6 \times 10^{-3}$	$k_{bi} (k_{cat}/K_m) < 2 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$
Co <sup>II</sup> <sub>10</sub> [PEI-GA]	7.5	$9 \times 10^{-3}$	$k_{bi} (k_{cat}/K_m) < 1 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$
Co <sup>II</sup> <sub>10</sub> [PEI-DAP]	7.0	$(2-7) \times 10^{-4}$	$k_{bi} (k_{cat}/K_m) = 0.0023 \pm 0.0001 \text{ M}^{-1} \text{ s}^{-1}$
	7.5	$(1-10) \times 10^{-4}$	$k_{bi} (k_{cat}/K_m) = 0.0070 \pm 0.0007 \text{ M}^{-1} \text{ s}^{-1}$
Co <sup>II</sup> <sub>4</sub> [lau <sub>11</sub> -PEI-GO] <sup>b</sup>	7.0	$(4-20) \times 10^{-3}$	$k_{cat} = \text{ca. } 6 \times 10^{-7} \text{ s}^{-1}$ $K_m < 4 \times 10^{-3} \text{ M}$
	7.5	$(4-20) \times 10^{-3}$	$k_{cat} = \text{ca. } 4 \times 10^{-7} \text{ s}^{-1}$ $K_m < 4 \times 10^{-3} \text{ M}$
Co <sup>II</sup> <sub>7</sub> [lauroyl <sub>10</sub> -PEI-GO]	7.0	$6.8 \times 10^{-4}$	$k_{bi} (k_{cat}/K_m) = \text{ca. } 2 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$
Co <sup>II</sup> <sub>7</sub> [imaben <sub>1</sub> -PEI-GO]	7.0	$2.6 \times 10^{-4}$	$k_{bi} (k_{cat}/K_m) < 4 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$
Co <sup>III</sup> <sub>7</sub> [PEI-GO]	5.5	$7.6 \times 10^{-3}$	$k_{bi} (k_{cat}/K_m) = \text{ca. } 1 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$
	6.0	$7.6 \times 10^{-3}$	$k_{bi} (k_{cat}/K_m) = \text{ca. } 1 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$
Co <sup>III</sup> <sub>7</sub> [PEI-GO]H	6.0	$7.6 \times 10^{-3}$	$k_{bi} (k_{cat}/K_m) = \text{ca. } 1 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$
	7.5	$(4-40) \times 10^{-3}$	$k_{cat} = (1.7 \pm 0.3) \times 10^{-4} \text{ s}^{-1}$ $K_m = (1.4 \pm 0.6) \times 10^{-2} \text{ M}$ $k_{cat}/K_m = (1.2 \pm 0.5) \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$
Fe <sup>III</sup> <sub>9</sub> [PEI-GO]	4.0	$(4-60) \times 10^{-4}$	$k_{bi} (k_{cat}/K_m) = (4.8 \pm 0.1) \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$
	5.0	$(4-60) \times 10^{-4}$	$k_{bi} (k_{cat}/K_m) = (7.9 \pm 0.1) \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$
	6.0	$(4-110) \times 10^{-4}$	$k_{bi} (k_{cat}/K_m) = (1.3 \pm 0.1) \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$
	7.0	$(4-110) \times 10^{-4}$	$k_{bi} (k_{cat}/K_m) = (1.1 \pm 0.1) \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$
Ni <sup>II</sup> <sub>7</sub> [PEI-GO]	6.5	$5 \times 10^{-2}$	$k_{bi} (k_{cat}/K_m) = \text{ca. } 1 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$
Ni <sup>II</sup> <sub>7</sub> [PEI-GO]H	6.0	$1.6 \times 10^{-2}$	$k_{bi} (k_{cat}/K_m) = \text{ca. } 1 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$
	6.5	$1.6 \times 10^{-2}$	$k_{bi} (k_{cat}/K_m) = \text{ca. } 5 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$
	8.0	$1.6 \times 10^{-2}$	$k_{bi} (k_{cat}/K_m) = \text{ca. } 2 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$
	6.5	$1.2 \times 10^{-2}$	$k_{bi} (k_{cat}/K_m) < 1 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$
Cu <sup>II</sup> <sub>9</sub> [PEI-GO]	7.0	$(1-35) \times 10^{-4}$	$k_{bi} (k_{cat}/K_m) = (4.0 \pm 0.1) \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$
Zn <sup>II</sup> <sub>2</sub> [PEI-GO]	7.5	$(1-14) \times 10^{-4}$	$k_{bi} (k_{cat}/K_m) = (7.4 \pm 0.3) \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$
	7.0	$1.8 \times 10^{-3}$	$k_{bi} (k_{cat}/K_m) = \text{ca. } 2 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$
Zn <sup>II</sup> <sub>2</sub> [PEI-GO]H	7.0	$1.8 \times 10^{-3}$	$k_{bi} (k_{cat}/K_m) = \text{ca. } 2 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$
spontaneous <sup>c</sup>	7.0	-	$k_{BNPP} = 3 \times 10^{-10} \text{ s}^{-1}$
OH <sup>-</sup> (35 °C) <sup>d</sup>	-	-	$k_{bi} = 2.4 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$

<sup>a</sup> The ranges of  $C_0$  used for the collection of rate data which were used in the calculation of the values of the kinetic parameters are indicated. When the reaction was too slow to measure the rate data correctly, the approximate value of  $k_p$  measured at a  $C_0$  concentration was used to calculate  $k_{bi} (= k_p/C_0)$ . <sup>b</sup>  $k_{BNPP}$  for the Co<sup>II</sup><sub>4</sub>[lau<sub>11</sub>-PEI-GO]-catalyzed hydrolysis was independent of  $C_0$  when  $C_0$  was  $(4-20) \times 10^{-3} \text{ M}$ , indicating the complete complexation of BNPP to the catalyst. The  $k_{BNPP}$  value thus obtained represents  $k_{cat}$  (eq 7). <sup>c</sup> Reference 30. <sup>d</sup> Reference 38.

monoesters are metal complexes. Kinetics and mechanisms of metal-catalyzed hydrolysis of phosphate monoesters such as NPP and phosphate diesters such as BNPP have been extensively investigated.<sup>22,30,33-40</sup> The mechanisms most widely proposed are indicated by 1-3.



For the hydrolysis of BNPP catalyzed by the PEI-based macrocyclic complexes, saturation kinetic behavior was manifested by Co<sup>III</sup><sub>7</sub>[PEI-GO]H and Co<sup>II</sup><sub>4</sub>[lau<sub>11</sub>-PEI-GO]. For Co<sup>II</sup><sub>7</sub>[PEI-GO], Co<sup>II</sup><sub>7</sub>[PEI-GO]H, Co<sup>II</sup><sub>10</sub>[PEI-DAP], Fe<sup>III</sup><sub>9</sub>[PEI-GO], or Zn<sup>II</sup><sub>2</sub>[PEI-GO], saturation kinetic behavior was not observed over the  $C_0$  range indicated in

(33) Chin, J. *Acc. Chem. Res.* 1991, 24, 145.

(34) Chin, J.; Zou, X. *J. Am. Chem. Soc.* 1988, 110, 223.

(35) Anderson, B.; Milburn, R. M.; Harrowfield, J. MacB., Robertson, G. B.; Sargeson, A. M. *J. Am. Chem. Soc.* 1977, 99, 2652.

(36) Jones, D. R.; Lindoy, L. F.; Sargeson, A. M. *J. Am. Chem. Soc.* 1983, 105, 7327.

(37) Hendry, P.; Sargeson, A. M. *J. Am. Chem. Soc.* 1989, 111, 2521.

(38) Koike, T.; Kimura, E. *J. Am. Chem. Soc.* 1991, 113, 8935.

(39) Hendry, P.; Sargeson, A. M. *Inorg. Chem.* 1990, 29, 97.

(40) De Rosch, M. A.; Troglor, W. C. *Inorg. Chem.* 1990, 29, 2409.

Table 1. For the rest of the PEI-based macrocyclic complexes, attempts were not made to analyze dependence of  $k_{BNPP}$  on  $C_0$  due to slow rates. Strong complex formation of Co<sup>II</sup><sub>4</sub>[lau<sub>11</sub>-PEI-GO] with BNPP may be related to the hydrophobic environments created on the surface of the macrocyclic-containing PEI by lauryl group. Electrostatic interaction between anionic BNPP and the macrocyclic metal centers would be strengthened in hydrophobic microenvironments.<sup>16</sup> In addition, binding of BNPP containing two aromatic rings could be facilitated in the hydrophobic microdomain. Complex formation with BNPP observed for Co<sup>III</sup><sub>7</sub>[PEI-GO]H suggests that electrostatic interactions with BNPP of the trivalent Co<sup>III</sup> centers is considerably greater than that of the divalent Co<sup>II</sup> centers.<sup>41</sup>

The rate of BNPP hydrolysis can be accelerated up to the  $k_{cat}$  values at sufficiently large  $C_0$  concentrations. For many catalysts examined in this study, however, saturation kinetic behavior was not observed, and the largest  $k_{BNPP}$  actually observed corresponds to  $k_{bi}$  multiplied by the largest  $C_0$  value. For example, the  $k_{BNPP}$  observed with

(41) Due to the spectral properties of Co<sup>II</sup><sub>7</sub>[PEI-GO], its kinetic data were not extended above 1.4 mM. The proportionality between  $k_{BNPP}$  and  $C_0$  indicates  $K_m \gg 1.4 \text{ mM}$ . It is not certain at present, therefore, whether  $K_m$  for Co<sup>II</sup><sub>7</sub>[PEI-GO] is greater than that (14 mM) for Co<sup>III</sup><sub>7</sub>[PEI-GO]H. A previous study suggested that the Co<sup>II</sup> center of Co<sup>II</sup><sub>7</sub>[PEI-GO] may possess enhanced Lewis acidity for a Co<sup>II</sup> ion due to the  $d\pi-p\pi$  back-bonding involving the diimine bond.<sup>15</sup>

Table 2. Values of Kinetic Parameters Obtained for the Hydrolysis of NPP Catalyzed by Various Macrocyclic-Containing PEI Derivatives at 50 °C

catalyst	pH	$C_0$ (M) <sup>a</sup>	parameter value
Co <sup>II</sup> <sub>7</sub> [PEI-GO]	6.0	(0.7–10) × 10 <sup>-4</sup>	$k_{cat} = (1.8 \pm 0.2) \times 10^{-3} \text{ s}^{-1}$ $K_m = (3.0 \pm 0.9) \times 10^{-4} \text{ M}$ $k_{cat}/K_m = 6.0 \pm 1.9 \text{ M}^{-1} \text{ s}^{-1}$
	6.5	(0.7–10) × 10 <sup>-4</sup>	$k_{cat} = (1.9 \pm 0.2) \times 10^{-3} \text{ s}^{-1}$ $K_m = (3.3 \pm 0.8) \times 10^{-4} \text{ M}$ $k_{cat}/K_m = 5.8 \pm 1.4 \text{ M}^{-1} \text{ s}^{-1}$
	7.0	(0.7–14) × 10 <sup>-4</sup>	$k_{bi} (k_{cat}/K_m) = 1.0 \pm 0.1 \text{ M}^{-1} \text{ s}^{-1}$
	7.5	(1–14) × 10 <sup>-4</sup>	$k_{bi} (k_{cat}/K_m) = 0.33 \pm 0.02 \text{ M}^{-1} \text{ s}^{-1}$
	8.0	(0.7–14) × 10 <sup>-4</sup>	$k_{bi} (k_{cat}/K_m) = 0.27 \pm 0.03 \text{ M}^{-1} \text{ s}^{-1}$
Co <sup>II</sup> <sub>7</sub> [PEI-GO]H	7.5	(1–13) × 10 <sup>-3</sup>	$k_{cat} = (2.2 \pm 0.2) \times 10^{-6} \text{ s}^{-1}$ $K_m = (1.7 \pm 0.5) \times 10^{-3} \text{ M}$ $k_{cat}/K_m = (1.3 \pm 0.4) \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$
Co <sup>II</sup> <sub>8</sub> [PEI-BD]	6.5	(1–17) × 10 <sup>-4</sup>	$k_{cat} = (1.2 \pm 0.1) \times 10^{-5} \text{ s}^{-1}$ $K_m < 1 \times 10^{-4} \text{ M}$ $k_{cat}/K_m > 0.12 \text{ M}^{-1} \text{ s}^{-1}$
Co <sup>II</sup> <sub>2</sub> [PEI-Bz]	7.5	5.9 × 10 <sup>-4</sup>	$k_{NPP} = \text{ca. } 1 \times 10^{-5} \text{ s}^{-1}$
Co <sup>II</sup> <sub>10</sub> [PEI-GA]	7.5	4.4 × 10 <sup>-3</sup>	$k_{NPP} = \text{ca. } 1 \times 10^{-6} \text{ s}^{-1}$
Co <sup>II</sup> <sub>10</sub> [PEI-DAP]	7.5	(1–10) × 10 <sup>-4</sup>	$k_{cat} = (4.6 \pm 0.4) \times 10^{-5} \text{ s}^{-1}$ $K_m = (1.7 \pm 0.6) \times 10^{-4} \text{ M}$ $k_{cat}/K_m = 0.27 \pm 0.09 \text{ M}^{-1} \text{ s}^{-1}$
Co <sup>II</sup> <sub>4</sub> [lau <sub>11</sub> -PEI-GO]	7.0	2.2 × 10 <sup>-2</sup>	$k_{NPP} = \text{ca. } 7 \times 10^{-5} \text{ s}^{-1}$
Co <sup>II</sup> <sub>7</sub> [lauroyl <sub>10</sub> -PEI-GO]	7.0	(0.2–6.8) × 10 <sup>-4</sup>	$k_{cat} = (1.1 \pm 0.1) \times 10^{-5} \text{ s}^{-1}$ $K_m < 2.0 \times 10^{-5} \text{ M}$
Co <sup>II</sup> <sub>7</sub> [imaben <sub>1</sub> -PEI-GO]	6.5	(7–13) × 10 <sup>-4</sup>	$k_{bi} (k_{cat}/K_m) = 0.18 \pm 0.01 \text{ M}^{-1} \text{ s}^{-1}$
Co <sup>III</sup> <sub>7</sub> [PEI-GO]	6.0	(2–80) × 10 <sup>-4</sup>	$k_{cat} = (5.5 \pm 0.4) \times 10^{-4} \text{ s}^{-1}$ $K_m = (4.8 \pm 1.5) \times 10^{-4} \text{ M}$ $k_{cat}/K_m = 1.2 \pm 0.3 \text{ M}^{-1} \text{ s}^{-1}$
Co <sup>III</sup> <sub>7</sub> [PEI-GO]H	6.5	(2–80) × 10 <sup>-4</sup>	$k_{cat} = (7.0 \pm 0.5) \times 10^{-5} \text{ s}^{-1}$ $K_m = (1.8 \pm 0.1) \times 10^{-3} \text{ M}$ $k_{cat}/K_m = (4.0 \pm 0.4) \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$
	7.0	(5–80) × 10 <sup>-4</sup>	$k_{cat} = (1.7 \pm 0.2) \times 10^{-5} \text{ s}^{-1}$ $K_m = \text{ca. } 1 \times 10^{-3} \text{ M}$ $k_{cat}/K_m = \text{ca. } 1.7 \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$
	6.5	(4–110) × 10 <sup>-4</sup>	$k_{cat} = (4.7 \pm 0.3) \times 10^{-3} \text{ s}^{-1}$ $K_m = (6.3 \pm 0.7) \times 10^{-3} \text{ M}$ $k_{cat}/K_m = (7.4 \pm 0.9) \times 10^{-1} \text{ M}^{-1} \text{ s}^{-1}$
Ni <sup>II</sup> <sub>7</sub> [PEI-GO]	7.0	(4–110) × 10 <sup>-4</sup>	$k_{bi} (k_{cat}/K_m) = 0.32 \pm 0.03 \text{ M}^{-1} \text{ s}^{-1}$
	7.0	(7–200) × 10 <sup>-4</sup>	$k_{cat} = (1.3 \pm 0.1) \times 10^{-5} \text{ s}^{-1}$ $K_m = (5.8 \pm 1.1) \times 10^{-3} \text{ M}$ $k_{cat}/K_m = (2.3 \pm 0.4) \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$
	8.0	(7–200) × 10 <sup>-4</sup>	$k_{cat} = (1.0 \pm 0.1) \times 10^{-4} \text{ s}^{-1}$ $K_m = (3.0 \pm 0.8) \times 10^{-3} \text{ M}$ $k_{cat}/K_m = (3.4 \pm 1.1) \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$
Ni <sup>II</sup> <sub>7</sub> [PEI-GO]H	6.5	(3–130) × 10 <sup>-4</sup>	$k_{cat} = (2.4 \pm 0.1) \times 10^{-4} \text{ s}^{-1}$ $K_m < 3 \times 10^{-4} \text{ M}$ $k_{cat}/K_m > 0.8 \text{ M}^{-1} \text{ s}^{-1}$
	7.0	(7–130) × 10 <sup>-4</sup>	$k_{cat} = (8.1 \pm 1.0) \times 10^{-5} \text{ s}^{-1}$ $K_m = (5.8 \pm 0.6) \times 10^{-3} \text{ M}$ $k_{cat}/K_m = 0.014 \pm 0.002 \text{ M}^{-1} \text{ s}^{-1}$
Cu <sup>II</sup> <sub>8</sub> [PEI-GO]	6.5	(2–60) × 10 <sup>-3</sup>	$k_{cat} = (9.1 \pm 0.9) \times 10^{-5} \text{ s}^{-1}$ $K_m = \text{ca. } 5 \times 10^{-3} \text{ M}$ $k_{cat}/K_m = \text{ca. } 0.02 \text{ M}^{-1} \text{ s}^{-1}$
	7.5	(2–60) × 10 <sup>-3</sup>	$k_{cat} = (1.6 \pm 0.2) \times 10^{-5} \text{ s}^{-1}$ $K_m = (9.8 \pm 1.4) \times 10^{-3} \text{ M}$ $k_{cat}/K_m = (1.6 \pm 0.3) \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$
Zn <sup>II</sup> <sub>2</sub> [PEI-GO]	7.0	(1–35) × 10 <sup>-4</sup>	$k_{cat} = (2.6 \pm 0.3) \times 10^{-4} \text{ s}^{-1}$ $K_m = (1.1 \pm 0.2) \times 10^{-4} \text{ M}$ $k_{cat}/K_m = 2.4 \pm 0.5 \text{ M}^{-1} \text{ s}^{-1}$
	7.5	(0.4–14) × 10 <sup>-4</sup>	$k_{cat} = (3.9 \pm 0.1) \times 10^{-4} \text{ s}^{-1}$ $K_m = (1.1 \pm 0.1) \times 10^{-4} \text{ M}$ $k_{cat}/K_m = 3.7 \pm 0.3 \text{ M}^{-1} \text{ s}^{-1}$
Zn <sup>II</sup> <sub>2</sub> [PEI-GO]H	7.0	(0.6–18) × 10 <sup>-4</sup>	$k_{cat} = (2.1 \pm 0.1) \times 10^{-5} \text{ s}^{-1}$ $K_m = \text{ca. } 7 \times 10^{-5} \text{ M}$ $k_{cat}/K_m = \text{ca. } 0.3 \text{ M}^{-1} \text{ s}^{-1}$
spontaneous <sup>b</sup>			$k_{NPP} = 6 \times 10^{-8} \text{ s}^{-1}$

<sup>a</sup> The ranges of  $C_0$  used for the collection of rate data which were used in the calculation of the values of the kinetic parameters are indicated.

<sup>b</sup> Reference 34.

Co<sup>II</sup>[PEI-GO] at pH 7.5 and  $C_0 = 1.4 \text{ mM}$  is  $2.5 \times 10^{-4} \text{ s}^{-1}$ , corresponding to ca.  $10^6$ -fold acceleration. The  $k_{BNPP}$  observed with Fe<sup>III</sup>[PEI-GO] at pH 6 and  $C_0 = 11 \text{ mM}$  is  $1.4 \times 10^{-4} \text{ s}^{-1}$ , whereas that observed with Zn<sup>II</sup>[PEI-GO] at pH 7 and  $C_0 = 3.5 \text{ mM}$  is  $1.4 \times 10^{-5} \text{ s}^{-1}$ . For these catalysts, greater acceleration would be achieved when  $C_0$  is increased. On the other hand, saturation kinetic

behavior was observed for Co<sup>III</sup><sub>7</sub>[PEI-GO]H and Co<sup>II</sup><sub>4</sub>[lau<sub>11</sub>-PEI-GO] with the limiting  $k_{BNPP}$  being  $1.7 \times 10^{-4} \text{ s}^{-1}$  and  $4 \times 10^{-7} \text{ s}^{-1}$ , respectively, at pH 7.5.

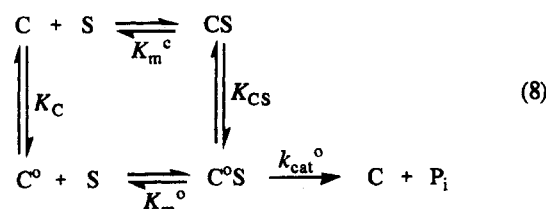
Although saturation kinetic behavior was observed for only two of the PEI-based macrocyclic complexes for the hydrolysis of BNPP, complex formation with NPP was observed for most of the PEI-based macrocyclic complexes

examined in this study. The stronger complexation of NPP to the macrocyclic complexes built on PEI compared with BNPP is attributable to the greater electrostatic interaction of the cationic catalysts with dianionic NPP compared with monoanionic BNPP.

The largest  $k_{\text{NPP}}$  actually achieved by the PEI-based macrocyclic complexes in NPP hydrolysis at sufficiently large  $C_0$  concentrations are given by  $k_{\text{cat}}$  values. For example, the maximum  $k_{\text{NPP}}$  value for  $\text{Co}^{\text{II}}_7[\text{PEI-GO}]$  is  $1.9 \times 10^{-3} \text{ s}^{-1}$ , corresponding to ca.  $3 \times 10^4$ -fold acceleration. Similarly,  $(1-8) \times 10^3$ -fold acceleration was achieved by the PEI-based macrocyclic complexes containing  $\text{Fe}^{\text{III}}$ ,  $\text{Ni}^{\text{II}}$ ,  $\text{Zn}^{\text{II}}$ , or  $\text{Cu}^{\text{II}}$  centers. The smaller degree of rate acceleration for NPP compared with BNPP may be partly ascribed to the availability of the metaphosphate mechanism for the uncatalyzed hydrolysis of NPP.<sup>34</sup>

The number of macrocyclic centers created on the PEI backbone is quite large. For example, 140 sites are present when the content of the metal center is 10% of the monomer residues. Individual macrocyclic sites built on the same PEI molecule would possess various structures and, therefore, exhibit widely different catalytic activities. It is possible that only small fractions of the macrocyclic metal centers are catalytically active. On the other hand, substrate binding involves electrostatic and hydrophobic interactions between the substrate and the catalyst. Even catalytically inactive metal centers would be able to form a complex with the anionic substrates. The  $C_0$  concentrations used in the analysis of kinetic data were based on the total concentration of the metal centers.

The scheme of eq 3 can be modified as eq 8 to take  $\text{C}^{\circ}\text{S}$  (the complex formed between S and  $\text{C}^{\circ}$ , the catalytically active metal center) into consideration. Here,  $K_{\text{CS}}$  and  $K_{\text{C}}$  represent fractions  $[\text{C}^{\circ}\text{S}]/[\text{CS}]$  and  $[\text{C}^{\circ}]/[\text{C}]$ , respectively, and  $K_{\text{m}}^{\circ}$  and  $K_{\text{m}}^{\circ}$  are the dissociation constants for CS and  $\text{C}^{\circ}\text{S}$ , respectively. Under the conditions of  $C_0 \gg S_0$ ,  $K_{\text{CS}} \ll 1$ , and  $K_{\text{C}} \ll 1$ , the rate expression for eq 8 is derived as eqs 9–11.



$$k_p = k_{\text{cat}}^{\circ} K_{\text{CS}} C_0 / (K_{\text{m}}^{\circ} + C_0) = k_{\text{cat}}^{\circ} K_{\text{CS}} C_0 / (K_{\text{m}}^{\circ} K_{\text{CS}} / K_{\text{C}} + C_0) \quad (9)$$

$$k_{\text{cat}}^{\circ} = k_{\text{cat}} / K_{\text{CS}} \quad (10)$$

$$k_{\text{cat}}^{\circ} / K_{\text{m}}^{\circ} = k_{\text{cat}} / K_{\text{m}} K_{\text{C}} \quad (11)$$

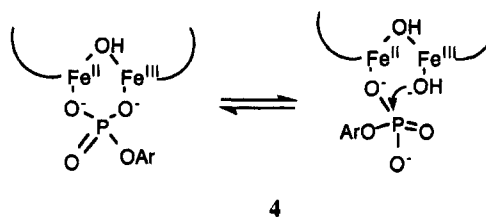
The actual catalytic activity of the active sites of the PEI-based macrocyclic complexes is represented by  $k_{\text{cat}}^{\circ}$  or  $k_{\text{cat}}^{\circ} / K_{\text{m}}^{\circ}$  and, therefore, is considerably greater than that estimated by  $k_{\text{cat}}$  or  $k_{\text{cat}} / K_{\text{m}}$  listed in Tables 1 and 2 if only very small fractions of the metal centers created on PEI are catalytically active. Substrates can be bound to the PEI-based macrocyclic complexes at both catalytically active and inactive metal centers, and  $K_{\text{m}}$  represents the overall dissociation constants ( $K_{\text{m}}^{\circ}$ ) for the mixture of various complexes.

Since  $\text{Co}^{\text{II}}_7[\text{PEI-GO}]$  catalyzes the hydrolysis of both BNPP and NPP effectively, kinetics of its catalytic action

was examined at several pHs. For BNPP,  $k_{\text{bi}} (= k_{\text{cat}} / K_{\text{m}})$  is independent of pH at pH 6–7.5 and decreases rapidly at pH > 7.5. For NPP,  $k_{\text{cat}} / K_{\text{m}}$  is identical at pH 6 and 6.5, and decreases rapidly at pH > 6.5. The pH profiles for the reactivity of metal-catalyzed hydrolysis of BNPP or NPP have been related to the ionization of water molecules bound to the metal centers.<sup>30,33</sup> For the mechanism of 1–3, ionization of the metal-bound water would inhibit coordination of oxyanions of BNPP or NPP to the metal center, whereas the nucleophilic attack by the metal-bound hydroxide ion requires the production of the hydroxo ion.

The pH profiles for the hydrolysis of BNPP and NPP catalyzed by  $\text{Co}^{\text{II}}_7[\text{PEI-GO}]$  can be accounted for in terms of the ionization of the  $\text{Co}^{\text{II}}$ -bound water molecules indicated in Scheme 1. If A is the reactive species for NPP, the identical values of  $k_{\text{cat}} / K_{\text{m}}$  measured at pH 6 and 6.5 as well as the decreased values at pH  $\geq 7$  are expected. Complexation of NPP to A would produce D–F, which are in the same ionization state with their relative concentrations being independent of pH. If A and B are the reactive forms for BNPP, the decrease in  $k_{\text{cat}} / K_{\text{m}}$  at pH  $\geq 8$  is anticipated. Mechanisms indicated by E–H are analogous to those of 1–3. Much stronger complexation to the PEI-based complexes of NPP than BNPP is consistent with the binding mode of D–H where NPP acts as a bidentate ligand whereas BNPP acts as a monodentate ligand.<sup>42</sup>

Di- or trinuclear metal ion centers with Zn, Mg, Mn, Fe, or Ni ions are involved in several metalloproteinases and metallophosphatases, and cooperation among the metal centers plays crucial catalytic roles.<sup>43–53</sup> For example, the mechanism of 4 has been proposed for the action of purple acid phosphatases.<sup>45</sup> The PEI derivatives prepared in the present study contain 30–100 macrocyclic metal centers in a molecular frame work. It is possible that some adjacent pairs of metal centers in the PEI derivatives cooperate with each other by analogy with 4.



**Comparison with Other Catalytic Systems.** The best catalysts synthesized so far for the hydrolysis of BNPP are *cis*-diaquotetraza  $\text{Co}^{\text{III}}$  complexes containing ligands such as 5–7.<sup>30</sup> The catalytic activity of the  $\text{Co}^{\text{III}}$  complexes

(42) Since the active sites of  $\text{Co}^{\text{II}}[\text{PEI-GO}]$  may be different for BNPP and for NPP, it is difficult to analyze the pH dependence more rigorously.

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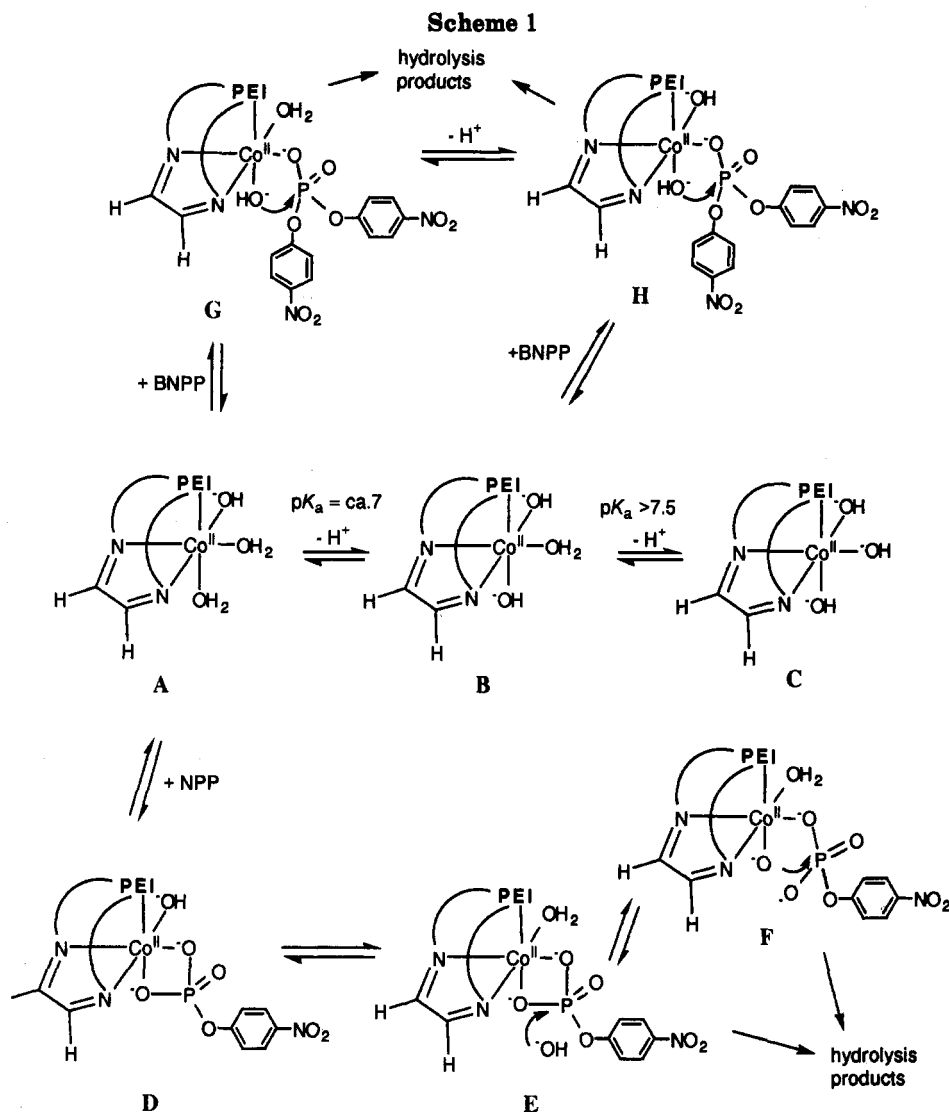
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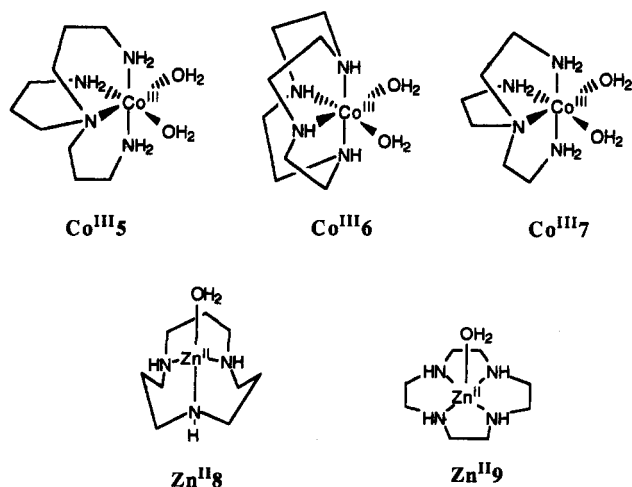
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depends sensitively on the tetramine ligand structure as summarized in Table 3. The catalytic reaction was proposed to proceed through mechanism 3, and the effect of ligand structure on the catalytic efficiency was explained in terms of bond angles in the transition state containing a four-membered ring.



For the hydrolysis of BNPP, the best synthetic catalyst containing divalent metal ions ever reported is Zn<sup>II</sup>8.<sup>38</sup>

**Table 3. Values of Kinetic Parameters for the Hydrolysis of BNPP Catalyzed by Metal Complexes Reported in the Literature**

catalyst	temp, °C	pH	parameter value <sup>a</sup>	ref
Co <sup>III</sup> 5	50	6.5–7.5	$k_{bi} = 2.5 \text{ M}^{-1} \text{ s}^{-1}$	30
Co <sup>III</sup> 6	50	6.5–7.5	$k_{bi} = 0.46 \text{ M}^{-1} \text{ s}^{-1}$	30
Co <sup>III</sup> 7	50	6.5–7.5	$k_{bi} = 8.1 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$	30
Co <sup>III</sup> (en) <sub>2</sub>	50	7	$k_{bi} = 2.7 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$	34
Co <sup>III</sup> (tren)	50	7	$k_{bi} = 4.8 \times 10^{-2} \text{ M}^{-1} \text{ s}^{-1}$	34
Co <sup>III</sup> (dien)	50	7	$k_{bi} < 1 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$	34
Zn <sup>II</sup> 8	35	7	$k_{bi} = 2 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$	38
	35	7.5	$k_{bi} = 7 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$	38
	35	>8.5	$k_{bi} = 8.5 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$	38
Zn <sup>II</sup> 9	35	7	$k_{bi} = 2 \times 10^{-6} \text{ M}^{-1} \text{ s}^{-1}$	38
	35	>10	$k_{bi} = 2.1 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$	38
Ir <sup>III</sup> (en) <sub>2</sub>	25	8.5	$k_{cat} = 3 \times 10^{-4} \text{ s}^{-1}$	37
Ir <sup>III</sup> (NH <sub>3</sub> ) <sub>5</sub>	25	14	$k_{cat} = 5 \times 10^{-4} \text{ s}^{-1}$	39
Ni(tren) <sup>2+</sup>	75	8.6	8-fold acceleration, C <sub>0</sub> = 0.1 mM	40
Ni(bpy) <sub>3</sub> <sup>2+</sup>	75	8.6	7-fold acceleration, C <sub>0</sub> = 0.1 mM	40
Cu(bpy) <sub>2</sub> <sup>2+</sup>	75	8.6	20-fold acceleration, C <sub>0</sub> = 0.1 mM	40
Zn(bpy) <sub>2</sub> <sup>2+</sup>	75	8.6	2-fold acceleration, C <sub>0</sub> = 0.1 mM	40

<sup>a</sup> Except for Ir<sup>III</sup> catalysts, the pseudo-first-order rate constant ( $k_p$ ) was proportional to C<sub>0</sub>, and  $k_{bi}$  stands for the bimolecular rate constant ( $k_p/C_0$ ). For the Ir<sup>III</sup> catalysts, BNPP coordinated to the catalyst was isolated, which corresponds to the CS complex (eq 3). The  $k_p$  measured therein represents  $k_{cat}$ .

The pK<sub>a</sub> values of Zn<sup>II</sup>-bound water molecules of Zn<sup>II</sup>8 and Zn<sup>II</sup>9 are 7.3 and 8.0, being considerably smaller than

**Table 4.** Values of Kinetic Parameters for the Catalysis in the Hydrolysis of NPP Reported in the Literature

catalyst	temp, °C	pH	rate data	remark
Co <sup>III</sup> 5 <sup>a</sup>	50	6.5–7.5	$k_{\text{NPP}} > 2.5 \times 10^{-2} \text{ s}^{-1}$	$C_0 = 0.01 \text{ M}$
Co <sup>III</sup> 6 <sup>a</sup>	50	6.5–7.5	$k_{\text{NPP}} > 4.6 \times 10^{-3} \text{ s}^{-1}$	$C_0 = 0.01 \text{ M}$
Co <sup>III</sup> 7 <sup>a</sup>	50	6.5–7.5	$k_{\text{NPP}} > 8.1 \times 10^{-6} \text{ s}^{-1}$	$C_0 = 0.01 \text{ M}$
Co <sup>III</sup> (en) <sub>2</sub> <sup>b</sup>	50	7	$k_{\text{NPP}} = 3.0 \times 10^{-4} \text{ s}^{-1}$	$C_0 = 0.01 \text{ M}$
Co <sup>III</sup> (en) <sub>2</sub> <sup>b,c</sup>	25	9–12	$k_{\text{cat}} = 8 \times 10^{-4} \text{ s}^{-1}$	
Co <sup>III</sup> (tn) <sub>2</sub> <sup>d,e</sup>	25	7.5	$k_{\text{cat}} = 3 \times 10^{-6} \text{ s}^{-1}$	
Co <sup>III</sup> (trien) <sub>2</sub> <sup>b</sup>	50	7	$k_{\text{NPP}} = 5.6 \times 10^{-3} \text{ s}^{-1}$	$C_0 = 0.01 \text{ M}$
Co <sup>III</sup> (dien) <sub>2</sub> <sup>b</sup>	50	7	$k_{\text{NPP}} < 1 \times 10^{-6} \text{ s}^{-1}$	$C_0 = 0.01 \text{ M}$
Ir <sup>III</sup> (en) <sub>2</sub> <sup>d,e</sup>	25	8.7	$k_{\text{cat}} = 6 \times 10^{-6} \text{ s}^{-1}$	
Zn <sup>II</sup> 8 and Zn <sup>II</sup> 9 <sup>f</sup>	35	7–9	no catalysis	
catalytic antibody <sup>g</sup>	30	9	$k_{\text{cat}} = 6 \times 10^{-6} \text{ s}^{-1}$	

<sup>a</sup> Reference 30. <sup>b</sup> Reference 34. <sup>c</sup> Reference 35. <sup>d</sup> NPP coordinated to the catalyst was isolated, which corresponds to the CS complex (eq 3). The  $k_p$  measured therein represents  $k_{\text{cat}}$ . <sup>e</sup> Reference 36. <sup>f</sup> Reference 38. <sup>g</sup> Reference 54.

that ( $\text{p}K_a = 9.0$ ) of (aquo)Zn<sup>II</sup> ion. In the nucleophilic attack at BNPP, the Zn<sup>II</sup>-bound hydroxide ion of Zn<sup>II</sup>8 and Zn<sup>II</sup>9 manifested reactivity similar to that of hydroxide ion, in spite of very large differences in basicity. At neutral pHs, therefore, large rate acceleration was achieved. The study with Zn<sup>II</sup>8 also indicates the sensitive dependence of the catalytic activity of metal complexes on the ligand structure.

For the hydrolysis of NPP, *cis*-diaquo(tetraza)Co<sup>III</sup> complexes are also the best synthetic catalysts reported so far. Again, the catalytic activity is heavily affected by the nature of the tetraza ligand as indicated by the data summarized in Table 4. In contrast to the hydrolysis of BNPP, the hydrolysis of NPP is not catalyzed by Zn<sup>II</sup>8 or Zn<sup>II</sup>9.

The degree of rate acceleration achieved by Co<sup>II</sup><sub>7</sub>[PEI-GO] for the hydrolysis of BNPP and NPP is much greater than that ever achieved by complexes of divalent metal ions and is comparable to some of the best known catalysts involving trivalent metal ions. Compared with Zn<sup>II</sup>8 or Zn<sup>II</sup>9, Zn<sup>II</sup><sub>2</sub>[PEI-GO] manifests greater catalytic activity toward BNPP ( $k_{\text{bi}} = k_{\text{cat}}/K_m$ ) and NPP.

For the PEI-based macrocyclic complexes, Co<sup>III</sup> complexes are much less active than the corresponding Co<sup>II</sup> complexes.<sup>55</sup> This stands in sharp contrast with non-polymeric Co<sup>III</sup> complexes which are far better catalysts than the corresponding Co<sup>II</sup> complexes. Greater catalytic activity of Co<sup>II</sup> complexes built on PEI may be taken to indicate that the structures of Co<sup>II</sup> centers are particularly suitable for the catalytic activity. In Co<sup>II</sup><sub>7</sub>[PEI-GO], the possible  $d\pi$ - $p\pi$  back-bonding between the metal and the imine bonds may enhance the Lewis acidity of the Co<sup>II</sup> ion.<sup>15</sup> In addition to these electronic effects, the geometry<sup>15</sup> around the Co<sup>II</sup> such as bond lengths of C=N and N(imine)-Co<sup>II</sup>, which are smaller than those of C-N and N(amine)-Co<sup>II</sup>, can lead to steric effects which might be responsible for the high catalytic activity of Co<sup>II</sup><sub>7</sub>[PEI-GO].

Some catalysts containing divalent metal centers have been previously reported for BNPP hydrolysis (Table 3), although their reactivity is very low. For NPP hydrolysis, however, no catalysts containing divalent metal ions have

ever been reported. The catalytic activity of the PEI-based macrocyclic complexes containing various divalent metal ions suggests that the geometry around the metal centers is well suited for the catalysis. It is also possible that the high catalytic activity toward NPP is due to the cooperation between two neighboring metal centers located on the PEI backbone, by analogy with the mechanism of 4.

To date, effective catalysis in BNPP or NPP hydrolysis has been mainly achieved with complexes of Co<sup>III</sup> or Ir<sup>III</sup>. These trivalent metal ions are useful for mechanistic analysis due to their slow rates of ligand exchange. Sometimes, BNPP or NPP coordinated to these metal ions were isolated and kinetics of the dephosphorylation of the resulting complexes were measured.<sup>34–39</sup> In designing effective catalysts, however, it is rather impractical to use these trivalent metal ions since complexation of the substrate by the metal center or release of the products from the metal center can limit the overall reaction rate.<sup>34,56</sup> On the other hand, the PEI-based catalysts contain divalent metal ions and Fe<sup>III</sup> ion, and, thus, are free from possible complications related to the slow rates of ligand exchange processes.

**Effectiveness as Artificial Metallophosphoesterases.** Major characteristics of actions of metalloenzymes reproduced by the artificial metallophosphoesterases developed in the present investigation include essential involvement of metal ions, complex formation with the substrates, and fast catalytic transformation within the resultant supramolecular complexes. Moreover, the metal ions of the artificial metalloenzymes play several catalytic roles both in the complexation process and the catalytic transformation step. It is also noteworthy that a series of catalysts have been designed to optimize the catalytic efficiency in the present study.

Recently, we have summarized repertoires of metal ions acting as Lewis acid catalysts in organic reactions.<sup>12,17</sup> One of the major characteristics of the Lewis acid catalysis by metal ions is the operation of a multiple number of catalytic features in one reaction. Similarly, metal ions play several catalytic roles in the action of the PEI-based artificial metallophosphoesterases as follows. First, recognition of anions by the metal centers is involved in complexation of BNPP or NPP. Second, the metal centers mask anionic character of the bound substrates, which otherwise electrostatically inhibits the approach of hydroxide nucleophile.<sup>57</sup> Third, if complexes similar to 1 are involved, strain is induced upon complexation of the substrate to the metal ion, which is relieved in the transition state containing trigonal bipyramidal phosphorus atom.<sup>35,57</sup> Fourth, ionization of a water molecule is enhanced upon coordination to the metal centers. Fifth, the resulting metal-bound hydroxide ion can make nucleophile attack at phosphorus atoms. Sixth, the template effect of the metal ion converts the attack of metal-bound hydroxide ion at the substrate into an intramolecular process. Seventh, negative charge developed on the phosphoryl oxygen atom in the transition state can be stabilized by the metal ion.

In the design of PEI-based artificial metallophosphoesterases, various attempts were made to alter catalytic properties. In particular, the structure of the active site has been systematically varied for the Co<sup>II</sup>-macrocycles.

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(55) It is possible that the catalytic activity of the Co<sup>III</sup> complexes built on PEI is due to the contaminating Co<sup>II</sup> centers which remained unoxidized during treatment with PbO<sub>2</sub>. Even if the catalytic conversion is due to the residual Co<sup>II</sup> centers, the enhanced binding ability is attributable to the Co<sup>III</sup> centers.

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The catalytic reactivities of  $\text{Co}^{\text{II}}$ [PEI-BD],  $\text{Co}^{\text{II}}$ [PEI-Bz],  $\text{Co}^{\text{II}}$ [PEI-GA],  $\text{Co}^{\text{II}}$ [PEI-DAP], and  $\text{Co}^{\text{II}}$ [PEI-GO] are widely different from one another. Depending on the structure of the dicarbonyl compounds used in the  $\text{Co}^{\text{II}}$ -template condensation with PEI, therefore, the electronic, the structural, and, consequently, the catalytic properties of the  $\text{Co}^{\text{II}}$  center are greatly affected, although the mechanistic basis for the structure-activity relationship is not known at present. The importance of steric effects is also reflected by a much smaller catalytic activity of  $\text{Co}^{\text{II}}$ [lauroyl<sub>10</sub>-PEI-GO] which was prepared by acylation of  $\text{Co}^{\text{II}}$ [PEI-GO]. It was reported that the lauroyl chains introduced to PEI form aggregates within the polymeric molecular framework.<sup>58</sup> The aggregation of lauroyl moieties can distort the molecular geometry around the  $\text{Co}^{\text{II}}$  centers, possibly leading to the large decrease in the catalytic activity. The small reactivity of  $\text{Co}^{\text{II}}$ [imaben<sub>1</sub>-PEI-GO] may be related to the coordination<sup>59</sup> of the (imidazolylazo)benzene moiety to the metal center, blocking the catalytic sites. The coordination is evidenced by the  $\lambda_{\text{max}}$  (450 nm) of the  $\text{Co}^{\text{II}}$ [imaben<sub>1</sub>-PEI-GO] which is considerably greater than that (344 nm) of imabeng-

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PEI. Although a major portion of the  $\text{Co}^{\text{II}}$  centers remain uncoordinated to the (imidazolylazo)benzene moieties in view of the molecular composition of the polymer, the coordination of the (imidazolylazo)benzene moiety appears to alter the conformation of these  $\text{Co}^{\text{II}}$  centers leading to considerable reduction of the catalytic activity.

Several catalytic systems have been designed for a variety of organic reactions by using metal ions acting as Lewis acid catalysts.<sup>12,17,33,60-64</sup> The PEI-based macrocyclic complexes prepared in the present investigation may manifest catalytic activity toward other organic reactions. In addition, several catalytic elements can be additionally introduced to the PEI-based macrocyclic complexes leading to more versatile artificial metalloenzymes.

**Acknowledgment.** This work was supported by Basic Science Research Institute Program (1993), R.O.K. Ministry of Education, Lucky Ltd., and the Organic Chemistry Research Center.

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