Catalytic Activity of Ni(II)−Terpyridine Complex in Phosphodiester Transesterification Remarkably Enhanced by Self-Assembly of Terpyridines on Poly(ethylenimine)

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Abstract: To enhance the catalytic activity of metal−terpyridyl complexes toward transesterification of an RNA model [(2-hydroxypropyl)-*p*-nitrophenyl phosphate, HPNPP], the conformation of terpyridyl moieties assembled on poly(ethylenimine) (PEI) is optimized by attaching the Ni(II) complex of a terpyridyl derivative ($Ni^{II}TP$) and lauryl (Lau) group to PEI in random combinations. The catalytic activity per Ni(II) center for transesterification of HPNPP varies by up to several thousand times depending on pH as the content of $Ni^{II}TP$ or Lau is changed. The best catalyst obtained is $[Ni^{II}TP]_5Lau_{12}PEI$, in which the contents of $Ni^{II}TP$ and Lau are 5 and 12 residue mol %, respectively. The catalytic activity of $[Ni^{II}TP]_5Lau_{12}PEI$ expressed in terms of k_{cat} is much higher than those of previously reported binuclear metal complexes whose structures were designed through deliberate planning. In the PEI derivatives containing $Ni^{II}TP$ and Lau, several $Ni^{II}TP$ groups can be positioned in proximity in the hydrophobic clusters formed on the polymer. When the $Ni^{II}TP$ groups take productive positions, the two phosphoryl−oxygen bonds and the hydroxyl group of HPNPP can be effectively activated. Thus, artificial active sites comprising multiple catalytic groups are obtained by self-assembly of the catalytic groups. Moreover, cooperative action among the catalytic groups is optimized through combinatorial approach.

Introduction

The drive to develop synthetic nucleases has stimulated intensive studies in catalysis of phosphoester hydrolysis in recent years. The artificial nucleases designed to date include nonmetallic compounds[1−5] as well as transition metal complexes[6−30] and lanthanide complexes.[31−36] Many enzymes hydrolyzing or synthesizing phosphate esters use synergistic action of two or more metal centers.[37−41] Thus, several research groups have challenged to mimic those metalloenzymes with model compounds containing two metal centers.[15−25]

To reproduce characteristics of enzymatic action such as complex formation with substrates, large degrees of rate acceleration, and high selectivity, it is necessary to place multiple

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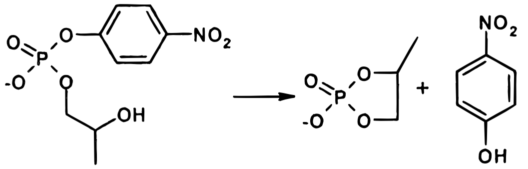
catalytic elements in proximity on the catalyst and align them in a productive way. Just as polypeptides form backbones of the active sites of natural enzymes comprising several catalytic elements, macromolecules have been employed as the skeleton of artificial enzymes. For example, immunoglobulins are used as backbones for catalytic antibodies[42−45] and synthetic macromolecules[46−50] are used as skeletons in the design of synzymes[46] (synthetic polymers with enzyme-like activities). At present, in the area of artificial enzymes, major efforts are being made to develop new strategies for designing the active site. Whether the strategy is successful is judged by the activity of the artificial enzymes produced, although the structure of the active site may not be well-characterized and the mechanism of catalysis may not be understood on the molecular level.

For designing active sites of artificial enzymes on synthetic macromolecules, various methodologies have been developed. For example, several catalytic elements were assembled around a template and then cross-linked with a macromolecular spacer. After removal of the template, an effective artificial active site was produced.[51] A functional group was introduced in proximity to cyclodextrin attached to a polymer by site-directed functionalization.[52] In molecular imprinting, monomers with functional groups are bound by a template and then copolymerized under conditions leading to the formation of highly cross-linked polymers with chains in a fixed arrangement.[49] After removal of the template, an active site that recognizes structures related to the template can be generated.

The simplest way to design active sites containing several catalytic groups is self-assembly of the catalytic groups.[53] If the self-assembled groups take highly productive positions, cooperation among them can result in effective catalysis. In water, hydrophobic interaction leads to assembly of amphiphilic compounds as exemplified by formation of micelles or vesicles. To overcome the basic instability element of micelles or vesicles, amphiphilic structures are built on synthetic polymers. In the present study, we have tested the idea of self-assembled catalytic groups by attaching pendants to a synthetic polymer in random combinations.

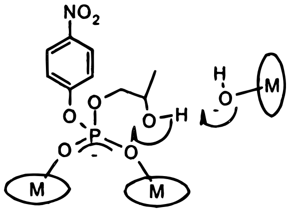
Transesterification (eq 1) of (2-hydroxypropyl)-*p*-nitrophenyl phosphate (HPNPP), an RNA model, is chosen as the target reaction, as it undergoes catalysis by participation of several metal ions. Since the leaving phenol of HPNPP does not require activation, catalysis of transesterification of HPNPP has been explained in terms of the activation of the two phosphoryl−oxygen bonds and the general base assistance for the intramolecular attack of the hydroxyl group as indicated by **1**.[17,24,30]

Catalysts are designed in the present study by attaching a terpyridine derivative to branched poly(ethylenimine) (PEI).[54]



HPNPP

(1)



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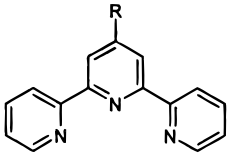
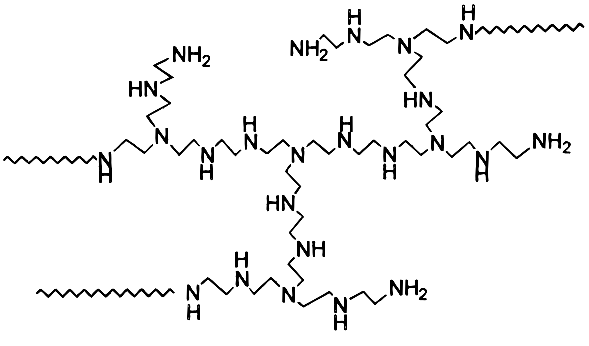
PEI has been frequently used as the skeleton of biomimetic functional molecules in view of branched structure, high solubility in water, and easy modification of its amino groups.[46−48,51,52,55−61] Terpyridine (**2a**) is chosen as the ligand to anchor metal ions since its Cu(II) complex is among the most active transition metal complexes that have been reported to catalyze hydrolysis of RNA or transesterification of HPNPP.[26−30] If several moieties of a metal−terpyridine complex assemble to form clusters on the PEI backbone to take positions suitable for stabilization of the transitions state for transesterification of HPNPP as illustrated by **1**, cooperation among them can lead to effective catalysis. In this paper, we report a remarkable increase in the catalytic activity of Ni(II)−terpyridine complex for cleavage of HPNPP achieved simply by variation of the contents of a terpyridyl (TP, **2b**) derivative and lauryl (Lau) group attached to PEI.



2a: R = H
2b (TP-H): R = C_6H_4-CH_3
2c (TP-Br): R = C_6H_4-CH_2-Br



PEI

Experimental Section

Preparation of PEI Derivatives. PEI (MW 50 000−60 000, purchased from Aldrich) was purified by dialysis (cutoff MW 12 000). Laurylation of PEI was carried out with Lau bromide according to the

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[54] The molecular weight of the PEI used in the construction of artificial enzymes is ca. 60 000, corresponding to 1400 monomer residues. Among the 1400 nitrogens present in PEI, ca. 25, ca. 50, and ca. 25% are primary, secondary, and tertiary amines, respectively. The tertiary amines represent branching points on the polymer backbone, and PEI is highly branched.

procedure reported in the literature[62] to obtain Lau_1PEI, Lau_6PEI, $Lau_{12}PEI$, and $Lau_{18}PEI$. The contents of Lau groups in Lau_1PEI, Lau_6PEI, $Lau_{12}PEI$, and $Lau_{18}PEI$ were 1.2, 5.9, 12, and 18 residue mol %, respectively, as measured by NMR spectrometry. TP residue was attached to PEI derivatives by reacting the polymers with 4′-(*p*-bromomethylphenyl)-2,2′:6′,2″-terpyridine (**2c**)[14,63,64] (mp 160−161 °C; lit.[63] 162−163 °C) in dimethyl sulfoxide at 50 °C for 3 days. The TP-containing PEI derivatives prepared were $[TP]_1Lau_{12}PEI$, $[TP]_5Lau_1PEI$, $[TP]_5Lau_6PEI$, $[TP]_5Lau_{12}PEI$, $[TP]_5Lau_{18}PEI$, $[TP]_6PEI$, and $[TP]_{10}Lau_{12}PEI$, whose contents of TP residues were 0.73, 4.8, 5.1, 4.7, 5.1, 5.7, and 9.7 residue mol %, respectively, as measured by NMR spectrometry. The amounts of TP residues attached to these PEI derivatives were 80−90% of **2c** used in the synthetic step. The PEI derivatives were purified by repetitive dialysis against 30% (v/v) aqueous ethanol (3 times), water (2 times), 0.1 M NaCl solution (3 times), and water (5 times) in succession. By adding $NiCl_2$ or $CuCl_2$ (0.95 equiv of the TP sites determined by the spectral titration) to the PEI derivatives, Ni(II) or Cu(II) complexes of the PEI derivatives containing TP and Lau groups were obtained. The formation constant for the Ni(II) complex of the TP group in $[Ni^{II}TP]_5Lau_{12}PEI$ was measured by using iminodiacetic acid (IDA) as the competing ligand.

Measurements. Rates of transesterification of HPNPP[65] were measured spectrophotometrically with a Beckman 68 UV−vis spectrophotometer. For reactions with half-lives of 1−7 days, the absorbance changes were followed for up to 1−3 half-lives at 25 °C and then the temperature was raised to 50 °C to obtain the absorbance of the product. The absorbance of the product agreed well with that of *p*-nitrophenol. By using the absorbance values at 400 nm of *p*-nitrophenol released during the reaction, pseudo-first-order rate constants were calculated. For the background reactions at pH 7−8.5, absorbance changes were followed for up to 5−10% reaction and the pseudo-first-order rate constants were calculated with the absorbance of *p*-nitrophenol separately measured under identical conditions. Kinetic measurements were repeated 2−3 times under identical conditions. Distilled and deionized water was used for preparation of buffer solutions. Buffers (0.05 M) used for the kinetic measurements were 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (pH 7−8) and boric acid (pH 8.5−10). Nickel and cupric chlorides were prepared from NiO (99.99%) and CuO (99.99+%), respectively, as described previously.[66] pH measurements were carried out with a Dongwoo Medical DP-880 pH/ion meter. All of the thermodynamic and the kinetic measurements were carried out at 25 ± 0.1 °C. NMR spectra were obtained with a Bruker DPX 300 FT-NMR spectrometer. For ^{31}P NMR measurements, 4.3 mM HPNPP was incubated at 25 °C in the presence (1.9 mM) or absence of PEI derivatives until the transesterification of HPNPP was almost complete as judged by visible spectra (1.5 h in the presence of $[Ni^{II}TP]_5Lau_{12}PEI$ at pH 9.50, 3 h in the presence of $[Cu^{II}TP]_5Lau_{12}PEI$ at pH 9.50, 12 h in the presence of $Ni^{II}Lau_{12}PEI$ at pH 9.50, and 24 h in the absence of any catalyst at pH 10.00). After separation of the PEI derivatives by ultrafiltration with a centrifugal concentrator (cutoff MW 3000), the product solution was lyophilized. The residue was dissolved in D_2O and subjected to the NMR measurement. HPLC analysis was carried out with a Waters 600 HPLC system.

Results

PEI derivatives containing TP and/or Lau groups were prepared by alkylation of the amino groups of PEI with **2c** and/or Lau bromide. They include $[TP]_1Lau_{12}PEI$, $[TP]_5Lau_1PEI$, $[TP]_5Lau_6PEI$, $[TP]_5Lau_{12}PEI$, $[TP]_5Lau_{18}PEI$, $[TP]_6PEI$, and $[TP]_{10}Lau_{12}PEI$. Here, the contents (expressed in terms of residue mol %) of TP and Lau groups measured by NMR spectrometry are indicated as the respective subscripts.

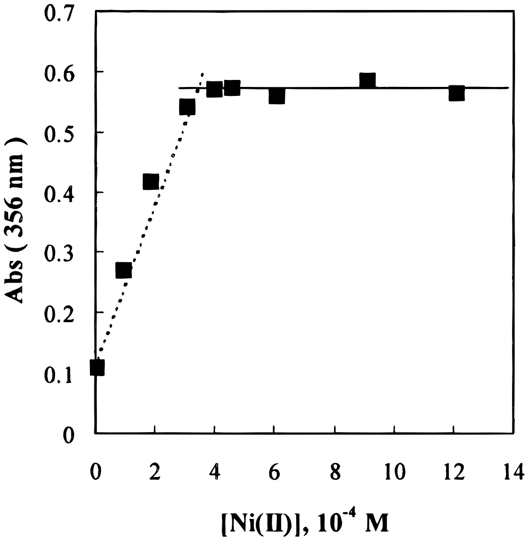

Figure 1. Plot of absorbance at 356 nm against the concentration of Ni(II) ion added to a solution of $[TP]_5Lau_{12}PEI$ (3.88×10^{-4} M in terms of concentration of TP residue determined by NMR measurement) at pH 7.50 and 25 °C.

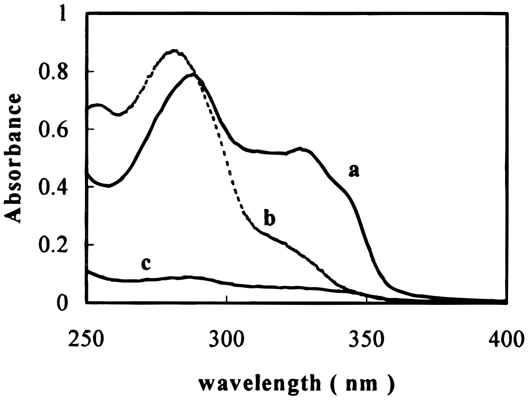

Figure 2. UV−vis spectra of $[Ni^{II}TP]_5Lau_{12}PEI$ (2.11×10^{-5} M in terms of the TP concentration: (a) $[TP]_5Lau_{12}PEI$ (2.11×10^{-5} M in terms of TP concentration); (b) and $Ni^{II}Lau_{12}PEI$ (prepared by adding 1.91×10^{-5} M Ni(II) ion to 4.52×10^{-4} residue M $Lau_{12}PEI$); (c) at pH 7.50 and 25 °C.

The content of TP residue was also estimated by spectral titration as illustrated in Figure 1 by using Ni(II) ion as the titrant. The content of TP residue estimated by the spectral titration agreed well (within ±10%) with that by NMR spectrometry. This indicates that each TP residue forms a 1:1-type complex with Ni(II) ion. As indicated by an example illustrated in Figure 2, addition of Ni(II) or Cu(II) ion to the TP-containing PEI derivatives affected the chromophoric property of the TP residue significantly. Addition of Ni(II) or Cu(II) ion to PEI derivatives without TP pendants did not affect the spectra of the polymer at >300 nm appreciably. The result of Figure 1, therefore, demonstrates that the metal ion is selectively complexed to the TP residue instead of the PEI backbone as far as the total concentration of the meal ion does not exceed that of the TP residue.

By adding $NiCl_2$ or $CuCl_2$ (0.95 equiv of the TP sites determined by the spectral titration) to the polymer, Ni(II) or

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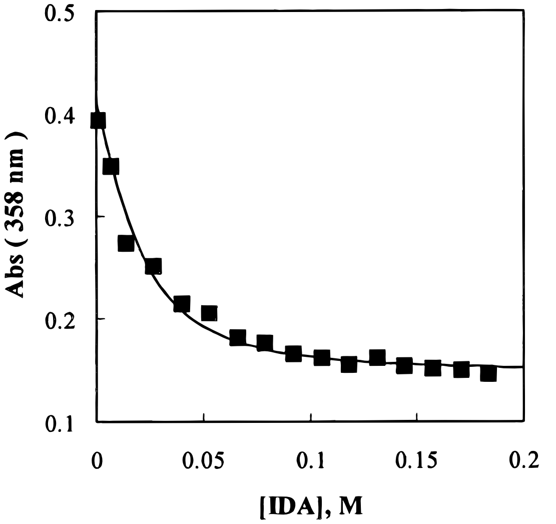

Figure 3. Plot of absorbance at 358 nm against the initially added concentration of IDA for abstraction of Ni(II) ion from $[\mathrm{Ni^{II}TP}]_5\mathrm{Lau}_{12}$PEI ($3.73 \times 10^{-4}$ M in terms of the TP concentration) at pH 7.50 and 25 °C. The curve is obtained by fitting the data to eq 2.

Cu(II) complexes of the PEI derivatives containing TP and Lau groups (designated as $[\mathrm{M^{II}TP}]_n\mathrm{Lau_m}$PEI) were obtained. By mixing $\mathrm{NiCl_2}$ or $\mathrm{CuCl_2}$ with **2b** in 1:1 mole ratios, the metal complexes of TP ($\mathrm{Ni^{II}TP}$ and $\mathrm{Cu^{II}TP}$) were obtained.

The formation constant for the Ni(II) complex of TP group in $[\mathrm{Ni^{II}TP}]_5\mathrm{Lau}_{12}$PEI at pH 7.50 and 25 °C was measured by using IDA as the competing ligand, according to the method[60] described previously. The degree of abstraction of Ni(II) ion from $[\mathrm{Ni^{II}TP}]_5\mathrm{Lau}_{12}$PEI by IDA is summarized in Figure 3. The data illustrated in Figure 3 were analyzed in terms of the scheme of eq 2 . In eq 2, BS stands for the binding site of Ni(II) ion

$$\mathrm{Ni(II){-}BS} + 2\mathrm{IDA} \overset{K_{\mathrm{ex}}}{\rightleftharpoons} \mathrm{BS} + \mathrm{Ni^{II}(IDA)_2} \qquad (2)$$

on $[\mathrm{TP}]_5\mathrm{Lau}_{12}$PEI and it is assumed that complexation of Ni(II) ion to a TP residue does not affect the succeeding bindings. Fitting the data of Figure 3 to an expression derived from eq 2 led to K_{ex} of 0.650 ± 0.081. By using the K_{f} value for the Ni(II) complex of IDA reported[67] in the literature, log K_{f} for the Ni(II) complex of TP residues in $[\mathrm{Ni^{II}TP}]_5\mathrm{Lau}_{12}$PEI was calculated as 10.60 ± 0.05.[68]

The rate for transesterification of HPNPP catalyzed by various complexes of TP was measured by following the release of *p*-nitrophenol at 400 nm spectrophotometrically. Under the conditions of C_0 (initially added concentration of the catalyst expressed in terms of the concentration of the metal center) $\gg$ S_0 (initially added concentration of HPNPP), pseudo-first-order kinetic behavior was observed. Pseudo-first-order rate constants (k_0) for the catalyzed reactions were measured at $C_0 = 1.9$ mM for various complexes of TP at several pHs. The pseudo-first-order rate constants (k_{bg}) for the background reaction (i.e., transesterification of HPNPP by the buffer solution) were also measured under identical conditions. In Figures 4 and 5, the logarithmic values of the catalytic rate constant (k_{c}: defined as $k_0 - k_{\mathrm{bg}}$ measured at $C_0 = 1.9$ mM) are summarized for various derivatives of $\mathrm{Ni^{II}TP}$. The corresponding data for $\mathrm{Cu^{II}TP}$ derivatives are summarized in Figure S1. Among the $\mathrm{Cu^{II}TP}$-containing PEI derivatives, $[\mathrm{Cu^{II}TP}]_5\mathrm{Lau}_{12}$PEI shows the highest catalytic activity. The data for $\mathrm{Cu^{II}TP}$ and $[\mathrm{Cu^{II}TP}]_5\mathrm{Lau}_{12}$PEI are included in Figure 5 to compare their activity with that of the corresponding Ni(II) catalysts. Also included in Figure 4 are log k_{bg} values measured at several pHs.

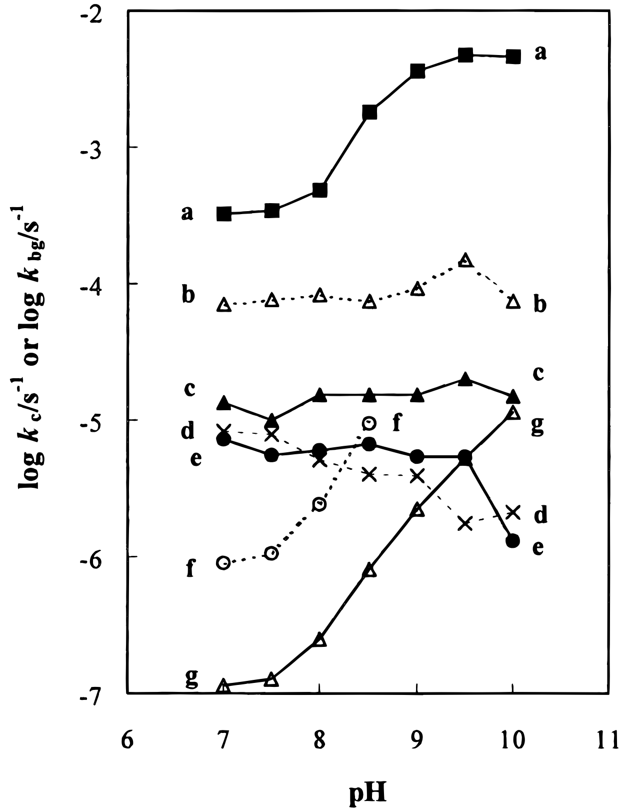

Figure 4. Plot of log k_{c} measured at various pHs and 25 °C for PEI derivatives containing 5−6 residue mol % $\mathrm{Ni^{II}TP}$ pendant: $[\mathrm{Ni^{II}TP}]_5\mathrm{Lau}_{12}$PEI (a, ■); $[\mathrm{Ni^{II}TP}]_5\mathrm{Lau}_{18}$PEI (b, △); $[\mathrm{Ni^{II}TP}]_5\mathrm{Lau}_6$PEI (c, ▲); $[\mathrm{Ni^{II}TP}]_6$PEI (d, ×); $[\mathrm{Ni^{II}TP}]_5\mathrm{Lau}_1$PEI (e, ●). Also included are log k_{c} for $\mathrm{Ni^{II}TP}$ (f, ○) and log k_{bg} for the background reaction (g, △).

PEI derivatives without $\mathrm{Ni^{II}TP}$ or $\mathrm{Cu^{II}TP}$ residues such as PEI, $\mathrm{Lau_1}$PEI, $\mathrm{Lau_{12}}$PEI, $\mathrm{Ni^{II}}$PEI, $\mathrm{Ni^{II}Lau_1}$PEI, $\mathrm{Ni^{II}Lau_{12}}$PEI, $[\mathrm{TP}]_2\mathrm{Lau}_{12}$PEI, $[\mathrm{TP}]_5$PEI, $[\mathrm{TP}]_5\mathrm{Lau}_1$PEI, $[\mathrm{TP}]_5\mathrm{Lau}_{12}$PEI, $[\mathrm{TP}]_5\mathrm{Lau}_{18}$PEI, and $[\mathrm{TP}]_{10}\mathrm{Lau}_{12}$PEI also manifested catalytic activity for transesterification of HPNPP. For these PEI derivatives, k_{c} values were measured under the conditions comparable to those for the PEI derivatives containing $\mathrm{Ni^{II}TP}$ or $\mathrm{Cu^{II}TP}$ moieties. The values of log k_{c} as well as the polymer concentrations used in kinetic measurement for these PEI derivatives are included in Figure S2. The control catalysts manifest activity comparable to that of $[\mathrm{Ni^{II}TP}]_1\mathrm{Lau}_{12}$PEI, $[\mathrm{Ni^{II}TP}]_5\mathrm{Lau}_1$PEI, or $[\mathrm{Ni^{II}TP}]_5\mathrm{Lau}_6$PEI at pH 7−9. To compare the rates of the control catalysts with those of the $\mathrm{Ni^{II}TP}$ derivatives, log k_{c} values for $\mathrm{Ni^{II}Lau_{12}}$PEI are also included in Figure 5.

In Figure 6, the logarithmic values of relative catalytic constant (k_{rel}) for the $\mathrm{Ni^{II}TP}$-containing catalysts are plotted against pH. Here, k_{rel} is defined as k_{c} for each catalyst divided by k_{c} for $[\mathrm{Ni^{II}TP}]_5\mathrm{Lau}_{12}$PEI measured at the same pH. The values of log k_{rel} plotted at optimum pH in Figure 6 are calculated by using k_{c} measured at the optimum pH value for each catalyst. For example, log k_{rel} at optimum pH for $[\mathrm{Ni^{II}TP}]_5\mathrm{Lau}_1$PEI is calculated by dividing k_{c} for $[\mathrm{Ni^{II}TP}]_5\mathrm{Lau}_1$PEI measured at pH 8.50 with k_{c} for $[\mathrm{Ni^{II}TP}]_5\mathrm{Lau}_{12}$PEI measured at pH 9.50. The k_{rel} values of the $\mathrm{Cu^{II}TP}$-containing catalysts were calculated by the same method except that the reference

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[67] Martell, A. E.; Smith, R. M. In *Critical Stability Constants*; Plenum: New York, 1975; Vol. 1, pp 116−119.

[68] Although the K_{f} value was not measured with the corresponding Cu(II) derivative, a greater K_{f} is expected for the Cu(II) derivative according to the Irving−Williams order (Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*, 3rd ed.; Wiley: New York, 1972; p 596).

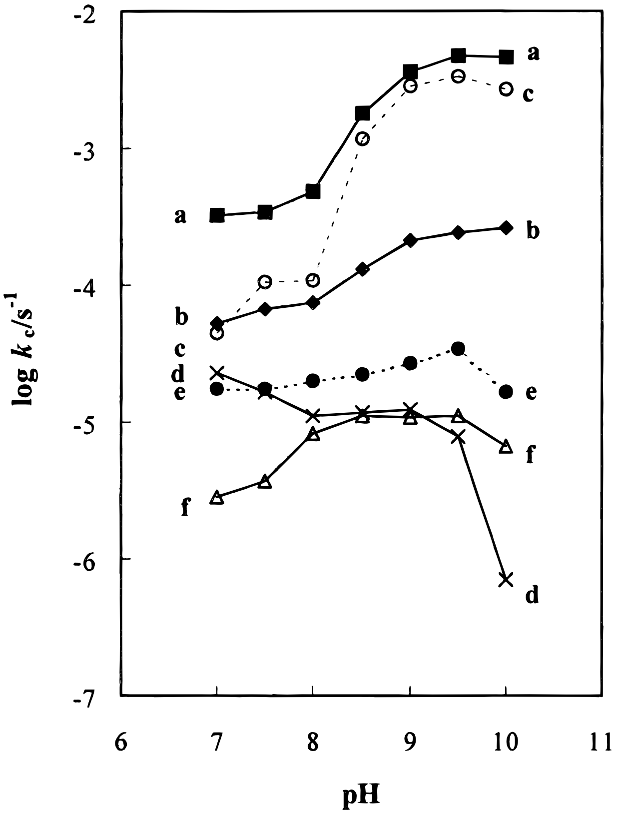

Figure 5. Plot of log k_c measured at various pHs and 25 °C for the PEI derivatives containing 12 mol % Lau pendant: $[Ni^{II}TP]_5Lau_{12}PEI$ (a, ■); $[Cu^{II}TP]_5Lau_{12}PEI$ (b, ◆); $[Ni^{II}TP]_{10}Lau_{12}PEI$ (c, ○); $[Ni^{II}TP]_1Lau_{12}PEI$ (d, ×); $Ni^{II}Lau_{12}PEI$ (e, ●). Also included are log k_c values for $Cu^{II}TP$ (f, △).

catalyst is $[Cu^{II}TP]_5Lau_{12}PEI$ and the plot of k_{rel} against pH is illustrated in Figure S3.

For the reaction catalyzed by $[Ni^{II}TP]_5Lau_{12}PEI$, the dependence of k_0 on C_0 was measured at pHs 7.40 and 9.50, and the results are summarized in Figure 7. The proportionality constants for the data are 0.111 ± 0.003 and 2.47 ± 0.07 M^{-1} s^{-1} at pHs 7.40 and 9.50, respectively.

Quantitative formation of *p*-nitrophenol during the transesterification of HPNPP investigated in the present study was confirmed by UV−vis spectra and HPLC analysis of the products. It is known that HPNPP undergoes spontaneous transesterification in water to produce propane-1,2-diol cyclic phosphate.[65] Formation of the cyclic phosphate by the reaction of HPNPP in the presence of PEI derivatives such as $Ni^{II}Lau_{12}PEI$, $[Ni^{II}TP]_5Lau_{12}PEI$, and $[Cu^{II}TP]_5Lau_{12}PEI$ was confirmed by measuring ^{31}P NMR spectra of the products. The PEI derivatives were separated from the product solutions by ultrafiltration with a centrifugal concentrator (cutoff MW 3000) prior to the NMR measurement. Chemical shifts (δ 18.7 ppm relative to 85% phosphoric acid used as the external reference) observed for the products obtained in the presence of the PEI derivatives agreed with that for the product (δ 18.7 ppm) of spontaneous reaction as well as that reported[69] for the cyclic phosphate (δ 18.8 ppm) in the literature. In the present study, the cyclic phosphate was formed as the sole product that can be detected by ^{31}P NMR spectrometry. The chemical shift measured in this study for HPNPP (δ −4.2 ppm) was identical with that reported[69] in the literature.

Kinetic data were also obtained under the conditions of S_0 (21 mM) > C_0 (1.9 mM) for the transesterification of HPNPP in the presence of $[Ni^{II}TP]_5Lau_{12}PEI$. The results illustrated in Figure S4 demonstrated catalytic turnover. This result in combination with quantitative formation of *p*-nitrophenol and propane-1,2-diol cyclic phosphate as the reaction products

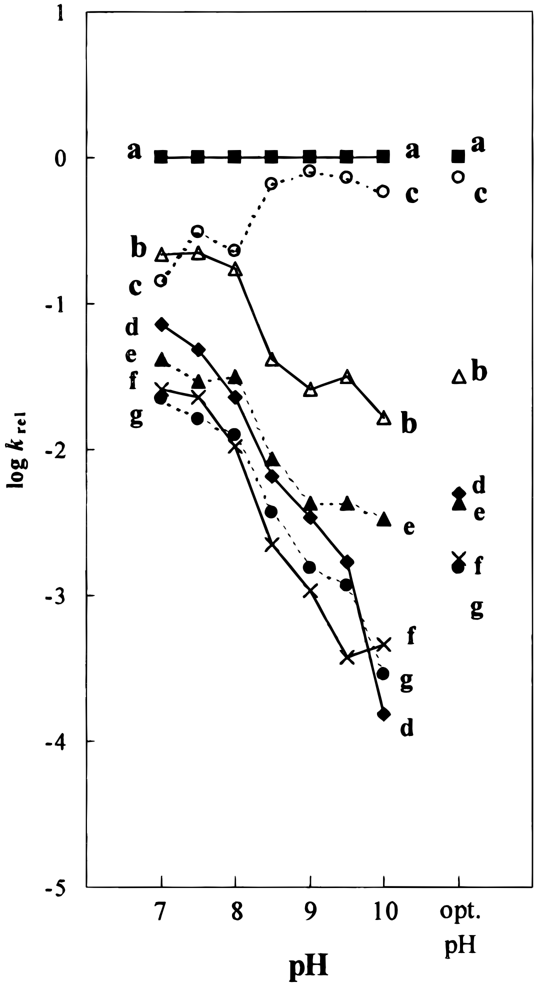

Figure 6. Plot of log k_{rel} measured at various pHs and 25 °C for TP derivatives such as $[Ni^{II}TP]_5Lau_{12}PEI$ (a, ■); $[Ni^{II}TP]_5Lau_{18}PEI$ (b, △); $[Ni^{II}TP]_{10}Lau_{12}PEI$ (c, ○); $[Ni^{II}TP]_1Lau_{12}PEI$ (d, ◆); $[Ni^{II}TP]_5Lau_6PEI$ (e, ▲); $[Ni^{II}TP]_6PEI$ (f, ×); $[Ni^{II}TP]_5Lau_1PEI$ (g, ●).

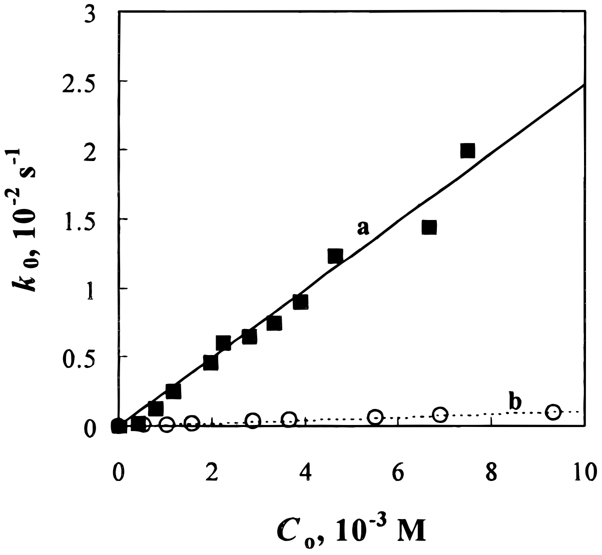

Figure 7. Plot of k_0 against C_0 for $[Ni^{II}TP]_5Lau_{12}PEI$ at 25 °C and pH 9.50 (a, ■) or 7.40 (b, ○).

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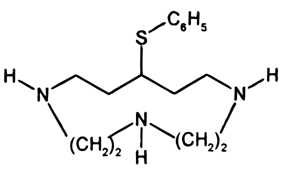
(69) Oost, T.; Filippazzi, A.; Kalesse, M. *Liebigs Ann. Chem.* **1997**, 1005.

indicate that the PEI derivatives act as catalysts in transesterification of HPNPP.

Discussion

Several metal complexes have been designed recently as catalysts for the transesterification of HPNPP. They include mononuclear complexes such as the Cu(II) complex[29,30] of terpyridine (**2a**) or *o,o′*-bipyridine, the Zn(II) complex of macrocyclic polyamines such as **3**,[20] and Eu(III), Y(III), or La(III) complexes[36] of 1,4,7,10-tetraazacyclododecane derivatives. Much better catalysis has been achieved by using binuclear metal complexes indicated by **4**−**6**.[17,24,25] Ligands for bimetallic catalysts have been also prepared by linking two molecules of **3** or **2a** with spacers as indicated by **7** or **8**.[20,30]



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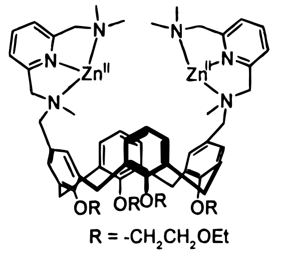

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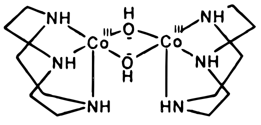

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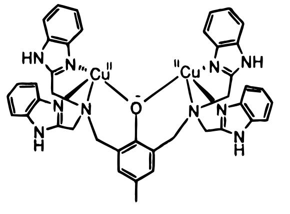

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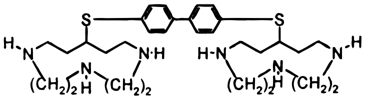

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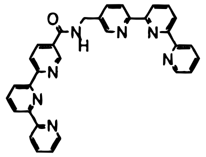

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In the present study, catalysts containing several metal centers are designed by attaching TP and Lau groups randomly to PEI. Changes in the contents of TP and Lau groups of PEI would affect conformation of the polymer considerably. Long alkyl chains such as Lau group attached to PEI form clusters to minimize exposure to water.[70] It is also reported that bulky aromatic residues such as azobenzene derivatives form clusters when they are attached to PEI.[71] Thus, clusters containing both TP and Lau groups would be formed on the backbone of PEI containing both TP and Lau groups. Although PEI derivatives have high conformational flexibility,[72] formation of the clusters by TP and Lau groups would impose constraints on the conformation. Thus, PEI derivatives containing Lau and TP groups are expected to possess unique conformations depending on the content of the pendants. When the contents of TP and Lau groups are varied, relative positions of TP moieties in the clusters are to be affected, leading to changes in the catalytic activity toward transesterification of HPNPP.

The rate constant measured at pH 7.0 for Cu$^{\rm II}$TP, the Cu(II) complex of **2b**, is about 2 times smaller than that measured[29] with the Cu(II) complex of **2a** under almost identical conditions. Thus, introduction of tolyl group to the terpyridine ring results in small rate reduction. Ni$^{\rm II}$TP shows about 3 times smaller rate constant at pH 7.0 compared with Cu$^{\rm II}$TP. The difference in reactivity between Ni$^{\rm II}$TP (curve f of Figure 4) and Cu$^{\rm II}$TP (curve f of Figure 5) is reduced as pH is raised. For Ni$^{\rm II}$TP, precipitation of the complex hampered kinetic measurement at pH ≥ 9.

The value of k_c for [Ni$^{\rm II}$TP]$_6$PEI (curve d of Figure 4) is about 9 times greater than that for Ni$^{\rm II}$TP (curve f of Figure 4) and about 70 times greater than $k_{\rm bg}$ (curve g of Figure 4) at pH 7. [Ni$^{\rm II}$TP]$_6$PEI becomes less effective than Ni$^{\rm II}$TP as pH is raised and almost ineffective at pH 10. Thus, attachment of Ni$^{\rm II}$TP to unmodified PEI does not improve the catalytic property of Ni$^{\rm II}$TP considerably. The reactivity of Ni$^{\rm II}$TP moiety attached to PEI is, however, affected remarkably by the variation in the content of TP and Lau residues. With the content of Ni$^{\rm II}$TP moiety fixed at 5.2 ± 0.5 residue mol %, the residue mol content of Lau residue is varied as 0% ([Ni$^{\rm II}$TP]$_6$PEI; curve d of Figure 4), 1% ([Ni$^{\rm II}$TP]$_5$Lau$_1$PEI; curve e of Figure 4), 6% ([Ni$^{\rm II}$TP]$_5$Lau$_6$PEI; curve c of Figure 4), 12% ([Ni$^{\rm II}$TP]$_5$Lau$_{12}$PEI; curve a of Figure 4), and 18% ([Ni$^{\rm II}$TP]$_5$Lau$_{18}$PEI; curve b of Figure 4). Introduction of Lau group up to 6 residue mol % results in small changes in the catalytic effects of the Ni$^{\rm II}$TP moieties. Remarkable enhancement of the reactivity of the Ni$^{\rm II}$TP moiety is achieved, however, when the content of Lau group is 12 residue mol %.

With the content of Lau group being fixed at 12 residue mol %, the residue mole content of the Ni$^{\rm II}$TP moiety is varied as 1% ([Ni$^{\rm II}$TP]$_1$Lau$_{12}$PEI; curve d of Figure 5), 5% ([Ni$^{\rm II}$TP]$_5$Lau$_{12}$PEI; curve a of Figure 5), or 10% ([Ni$^{\rm II}$TP]$_{10}$Lau$_{12}$PEI; curve c of Figure 5). Although [Ni$^{\rm II}$TP]$_1$Lau$_{12}$PEI manifested catalytic activity better than Ni$^{\rm II}$TP at pH 7, it becomes almost inactive at pH 10. On the other hand, [Ni$^{\rm II}$TP]$_5$Lau$_{12}$PEI and [Ni$^{\rm II}$TP]$_{10}$Lau$_{12}$PEI show high catalytic activity at pH 7−10.

The data summarized in Figure 6 reveal that up to 7000-fold differences are manifested depending on pH by the Ni$^{\rm II}$TP-containing catalysts prepared in this study. The data points with small values of log $k_{\rm rel}$ are associated with large standard deviations since k_0 values for the catalyzed reactions are not much greater than $k_{\rm bg}$ values for these data points. When the catalytic activities of the Ni$^{\rm II}$TP-containing catalysts measured at their respective optimum pHs are compared, up to 700-fold difference is observed.

As control catalysts, PEI, Lau$_1$PEI, Lau$_{12}$PEI, Ni$^{\rm II}$PEI, Ni$^{\rm II}$Lau$_1$PEI, Ni$^{\rm II}$Lau$_{12}$PEI, [TP]$_2$Lau$_{12}$PEI, [TP]$_5$PEI, [TP]$_5$Lau$_1$PEI, [TP]$_5$Lau$_{12}$PEI, [TP]$_5$Lau$_{18}$PEI, and [TP]$_{10}$Lau$_{12}$PEI were tested. A previous study indicates that the added Ni(II) ion is completely bound to Lau$_{12}$PEI or PEI under the experimental conditions.[72] Addition of Ni(II) ion to PEI or laurylated PEIs does not increase the catalytic activity at pH 7−9 (Figure S2), suggesting that the amino groups of PEI makes major contribution to catalysis by these PEI derivatives.[73] These control catalysts manifest activity comparable to that of [Ni$^{\rm II}$TP]$_1$Lau$_{12}$PEI, [Ni$^{\rm II}$TP]$_5$Lau$_1$PEI, or [Ni$^{\rm II}$TP]$_5$Lau$_6$PEI at pH 7−9. Thus, the PEI backbone contributes significantly to the activity manifested by the PEI derivatives containing Ni$^{\rm II}$TP except for [Ni$^{\rm II}$TP]$_5$Lau$_{12}$PEI or [Ni$^{\rm II}$TP]$_{10}$Lau$_{12}$PEI. Then, the difference

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(70) Johnson, T. W.; Klotz, I. M. *Macromolecules* **1974**, *7*, 618.

(71) Suh, J.; Kim, M.-J. *Bioorg. Chem.* **1992**, *20*, 366.

(72) Suh, J.; Lee, S. H.; Kim, S. M.; Hah, S. S. *Bioorg. Chem.* **1997**, *25*, 221.

(73) Amino groups of the PEI derivatives may act as general base catalysts to assist the nucleophilic attack by the hydroxyl group of HPNPP. Ammonium ions of the polymers may also contribute to catalysis by promoting complexation of HPNPP to the polymers through electrostatic interaction.

in the activity of the $Ni^{II}TP$ moiety between $[Ni^{II}TP]_5Lau_{12}PEI$ and another $Ni^{II}TP$-containing PEI derivative should be greater than that (7000-fold) estimated above with k_{rel}.

Among the $Cu^{II}TP$-containing PEI derivatives ($[Cu^{II}TP]_2Lau_{12}PEI$, $[Cu^{II}TP]_5PEI$, $[Cu^{II}TP]_5Lau_1PEI$, $[Cu^{II}TP]_5Lau_6PEI$, $[Cu^{II}TP]_5Lau_{12}PEI$, $[Cu^{II}TP]_5Lau_{18}PEI$, and $[Cu^{II}TP]_{10}Lau_{12}PEI$), variation in k_c at the same pH upon changing contents of $Cu^{II}TP$ and Lau residues is less than 300-fold (Figures S1 and S3). This can be compared with the up to 7000-fold variation in k_c observed with the corresponding $Ni^{II}TP$ derivatives.

The dependence of k_0 on C_0 was examined for $[Ni^{II}TP]_5Lau_{12}PEI$, the best catalyst prepared in this study. For this catalyst, $k_0 \gg k_{bg}$ and thus $k_0 = k_c$. Kinetic data observed for reactions catalyzed by PEI derivatives are analyzed by analogy with Michaelis−Menten scheme (eq 3).[46−48,55−57] Under the conditions of C_0 (the initially added concentration of catalyst) $\gg S_0$ (the initially added concentration of substrate), expression of eq 4 is derived for k_0 from eq 3. When C_0 is not much different from K_m, eq 4 predicts saturation kinetic behavior for the dependence of k_0 on C_0. When $C_0 \ll K_m$, k_0 is proportional to C_0 with the proportionality constant of k_{cat}/K_m. Moreover, k_{cat} is much greater than the k_0 value measured under the conditions of $C_0 \ll K_m$.

$$C + S \underset{K_m}{\rightleftharpoons} CS \xrightarrow{k_{cat}} C + P_i \qquad (3)$$

$$k_0 = k_{cat}C_0/(K_m + C_0) \qquad (4)$$

The data illustrated in Figure 7 reveal that $k_{cat}/K_m = 2.47$ M^{-1} s^{-1}, $k_{cat} \gg 2 \times 10^{-2}$ s^{-1}, and $K_m \gg 1 \times 10^{-2}$ M at pH 9.50 and $k_{cat}/K_m = 0.111$ M^{-1} s^{-1}, $k_{cat} \gg 1 \times 10^{-3}$ s^{-1}, and $K_m \gg 1 \times 10^{-2}$ M at pH 7.40. Thus, complexation of HPNPP to $[Ni^{II}TP]_5Lau_{12}PEI$ is weak and HPNPP is bound to $[Ni^{II}TP]_5Lau_{12}PEI$ only partially under the experimental conditions. If HPNPP is fully complexed to $[Ni^{II}TP]_5Lau_{12}PEI$, it should undergo transesterification with k_0 much greater than 2×10^{-2} s^{-1} at pH 9.50 and 1×10^{-3} s^{-1} at pH 7.40.

Among the metal complexes designed so far as catalysts for transesterification of HPNPP, the calix[4]arene-based binuclear Zn(II) complex (**4**) has been claimed to be the most effective.[25] This catalyst manifests optimum activity at pH 7.4. Due to the presence of the two metal centers, complexation between **4** and HPNPP is quite strong ($K_{assn} = 1/K_m = 1.7 \times 10^4$ M^{-1} at pH 7.4 and 25 °C). Thus, HPNPP is to be fully bound to **4** at $C_0 > 1$ mM under the conditions of $C_0 \gg S_0$. The value of k_{cat} observed at pH 7.4 is 1.0×10^{-3} s^{-1}. Thus, the maximum value of k_0 attainable by **4** is 1.0×10^{-3} s^{-1} regardless of pH or C_0.[74]

Due to the exchange inertness of Co(III) complexes, HPNPP is initially complexed to the metal centers in **5**.[24] Thus, the rate constant measured for cleavage of HPNPP bound to **5** corresponds to k_{cat}. At pH 6−9.5 and 25 °C, k_{cat} for HPNPP bound to **5** was proportional to $[OH^-]$ with the proportionality constant of 430 M^{-1} s^{-1}. The k_{cat} for HPNPP bound to **5** is 4.3×10^{-5} s^{-1} at pH 7 and 1.4×10^{-2} s^{-1} at pH 9.5. A recent report of detailed mechanistic studies on **5** revealed that HPNPP bound to **5** is cleaved by the nucleophilic attack by the oxide ion bridging the two Co(III) ions.[75] On the other hand, the analogue of HPNPP without the nitro substituent undergoes transesterification upon binding to **5**. The rates for transesterification of HPNPP catalyzed by **5** are, therefore, considerably slower than the kinetic data reported for cleavage of HPNPP by **5**.

The highest k_0 values attained with $[Ni^{II}TP]_5Lau_{12}PEI$ at pHs 7.4 and 9.5 (Figure 7) are greater than the maximal rate constants obtained with **4** or **5**. Moreover, the k_{cat} value for $[Ni^{II}TP]_5Lau_{12}PEI$ is much greater than those of **4** and **5**. This indicates that the intrinsic catalytic activity of $Ni^{II}TP$ moieties of $[Ni^{II}TP]_5Lau_{12}PEI$ is much higher than the binuclear metal centers of **4** and **5**.

Binuclear metal complexes prepared by connecting two mononuclear complexes with organic spacers have achieved some improvements in catalytic power. For example, the activity at optimum pH of Cu(II)−**8** complex was about 20 times greater than that of Cu(II)−**2a** complex.[30] The activity at pH 8.36 of Zn(II)−**7** complex was about 6 times greater than that of Zn(II)−**3** complex.[20] PEI derivatives are used in this study as macromolecular spacers to connect $Ni^{II}TP$ moieties, leading to enhancement in the reactivity of $Ni^{II}TP$ at optimum pH by several hundred times as indicated by the data points for $[Ni^{II}TP]_5Lau_{12}PEI$ and $Ni^{II}TP$ in Figure 6. The degree of rate enhancement achieved in the present study by using PEI as the spacer far exceeds that achieved by connecting two molecules of **2a** or **3** with the spacers.

Among the PEI derivatives containing $M^{II}TP$ moieties, $[M^{II}TP]_5Lau_{12}PEI$ manifests the highest catalytic activity for both the Ni(II) and the Cu(II) series. Although detailed information on the structure of the microdomains of $[M^{II}TP]_5Lau_{12}PEI$ is not available at present, the kinetic data suggest that catalytic groups take optimal positions. Although $Ni^{II}TP$ is less active compared with $Cu^{II}TP$, $[Ni^{II}TP]_5Lau_{12}PEI$ is more active than $[Cu^{II}TP]_5Lau_{12}PEI$ by 5−18 times depending on pH. Moreover, the degree of variation in the catalytic ability caused by changes in the content of $M^{II}TP$ and Lau residues is much greater for the Ni(II) derivatives compared with the Cu(II) derivatives. Thus, activity of the Ni(II) derivative is optimized better than that of the Cu(II) derivatives by changing the contents of the pendants attached to PEI.

A mechanism (**9**) similar to that of **1** may be proposed for the transesterification of HPNPP by $[Ni^{II}TP]_5Lau_{12}PEI$. Aggregation of hydrophobic pendants attached to PEI results in cluster formation, leading to a compact conformation of the polymer such as **10** rather than the extended conformation such as **11**.[72] In $[Ni^{II}TP]_5Lau_{12}PEI$, $Ni^{II}TP$ moieties and Lau groups would form hydrophobic clusters. Although the $Ni^{II}TP$ groups are attached to PEI randomly, several $Ni^{II}TP$ groups can be positioned in proximity in the hydrophobic clusters. Cluster formation would impose constraints on the conformation of the polymer. Thus, the positions of the $Ni^{II}TP$ groups in a cluster should depend on the content of Lau and TP groups, although the exact geometry of the active site comprising several $Ni^{II}TP$ moieties is not known. The two phosphoryl oxygen atoms of HPNPP may interact with two Ni(II) centers in the cluster, and the nucleophilic hydroxyl group may be subjected to general base assistance by hydroxide ion attached to another metal center. An amino group on the PEI backbone may play the role of general base catalyst instead of the Ni(II)-bound hydroxide ion, in view of some catalytic activity manifested by PEI derivatives devoid of $M^{II}TP$ residues. The medium properties of the reaction sites on PEI derivatives would be also affected by the contents of $M^{II}TP$ and Lau groups, although medium effects on the metal-catalyzed transesterification of HPNPP have not been investigated systematically yet.

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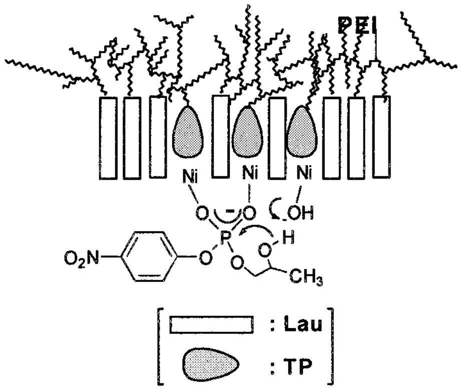
(74) After submission of this paper, it was reported that the Cu(II) complex of a calix[4]arene derivative related to **4** catalyzes transesterification of HPNPP with k_{cat} of 2.1×10^{-3} s^{-1} at the optimum pH and 25 °C (Molenveld, P.; Engbersen, J. F. J.; Kooijman, H.; Speck, A. L.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1998**, *120*, 6726).

(75) Williams, N. H.; Cheung, W.; Chin, J. *J. Am. Chem. Soc.* **1998**, *120*, 8079.



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Catalytic ability of the PEI derivatives is remarkably affected by variation in the contents of not only M^{II}TP residue but also Lau group. The Lau group may contribute to the catalysis through adjustment of both the geometry and the hydrophobicity of active site. As implicated by **9**, contents of both Lau and TP groups would determine the positions of the catalytic groups especially when Lau and TP groups form clusters together. By changing the contents of Lau and TP groups, therefore, the geometry of the active sites comprising the metal complexes of TP would be optimized. In addition, the hydrophobicity of the active site would be tuned by varying the content of Lau group.

In summary, an effective catalyst for the RNA model reaction is prepared by changing the contents of TP and Lau groups randomly attached to PEI. Although the catalyst has been obtained by a simple combinatorial approach,[76] its catalytic activity surpasses those of previously reported binuclear metal complexes whose structures were designed through deliberate planning. The present study reports a novel methodology for designing artificial active sites: self-assembly of catalytic groups to form clusters on macromolecular backbones and optimization of cooperative action among the catalytic groups through combinatorial attachment of pendants.

Acknowledgment. This work was supported by grants from Lotte Fellowship (1997) and from Korea Science and Engineering Foundation through Center for Molecular Catalysis at Seoul National University. Publication cost was supported by Research Institute of Molecular Science, Seoul National University.

Supporting Information Available: Figure S1 illustrating plot of log k_c measured at various pHs and 25 °C for PEI derivatives containing Cu^{II}TP residues, Figure S2 illustrating plot of log k_c measured at various pHs and 25 °C for PEI derivatives without Ni^{II}TP or Cu^{II}TP residues, Figure S3 illustrating plot of log k_{rel} measured at various pHs and 25 °C for PEI derivatives containing Cu^{II}TP residues, and Figure S4 illustrating release of *p*-nitrophenol from transesterification of HPNPP ($S_0 = 21$ mM) in the presence ($C_0 = 1.9$ mM) or absence of $[Ni^{II}TP]_5Lau_{12}PEI$ at pHs 9.00 and 25 °C (4 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA982705V

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[76] Examples of previous studies to optimize activity of synzymes through combinatorial approach are given in the following: Menger, F. M.; Eliseev, A. V.; Migulin, V. A. *J. Org. Chem.* **1995**, *60*, 6666. See also ref 55.