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단 신

코발트착물 리간드의 구조가 인산 에스테르의 가수분해에 미치는 영향

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Ligands Effects on Phosphate Ester Hydrolysis by Cobalt(III) Complexes

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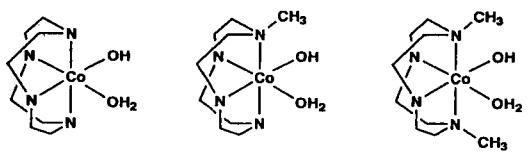
Over the years, there has been enormous interest in developing metal complexes that hydrolyze phosphate backbone of DNA.[1,2] Recently, several metal complexes have been reported with high efficiencies in hydrolyzing phosphate esters such as BNPP. Although some of them could even cleave ss- and ds-DNA, the mechanisms were not clearly elucidated.[3] Those that cleave DNA hydrolytically have yet to be developed. Until now, only a few metal complexes including *cis*-diaquo(tetraamine)Co(III) complexes have been thoroughly studied in their hydrolytic mechanisms. Among them, *cis*-diaquo Co(III)(cyclen) complex (cyclen; 1,4,7,10-tetraazacyclododecane) was highly efficient in hydrolyzing phosphate esters such as BNPP and dimethyl phosphate as well.[4] The proposed mechanism involved a coordination of the substrate, followed by nucleophilic attack of the bound cobalt hydroxide on the bound substrate, forming a four-membered ring chelates. Catalytic efficiency of the metal complexes was largely dependent on the easy formation of the strained four-membered ring chelates and it has been nicely shown with the trpn[tris(aminopropyl)amine], tren[tris(aminoethyl)amine], where their cobalt complexes showed 300 times of rate difference in hydrolyzing BNPP.[1] One way to increase the catalytic efficiency of metal complex is to design ligand system that can force the four-membered ring chelate to be formed easily. Taking this into consideration, we have prepared a series of ligand based on the structure of cyclen. Cyclen was chosen because it was a little less active but much more stable than trpn under the reaction conditions used. The trpn cobalt complex is far the most active catalyst in that series yet the ligand deligation often occurs during the hydrolysis.[4] One (mcyclen) or two(*trans*-dimethyl and *cis*-dimethyl-cyclen: tmcyclen, cmcyclen) methyl groups are introduced into nitrogens of cyclen. Herein we report on the reactivities of cobalt complexes **1, 2, 3** for hydrolyzing BNPP and its implication on structural requirements of efficient catalysts.



1 **2** **3**

EXPERIMENTAL

Instruments. ^{1}H, ^{13}C, ^{31}P NMR were taken on a Varian XL-200 and 300. Kinetic studies were carried out using a Hewlett-Packard 8451 diode array spectrophotometer.

Materials. Bis(*p*-nitrophenyl) phosphate(BNPP) and *p*-nitrophenyl phosphate(NPP) were purchased

from Sigma-Aldrich and bis(2, 4-dinitrophenyl) phosphate(BDNPP) was synthesized by the known method.[5] Most chemicals and solvents were purchased from Sigma-Aldrich and used without further purification.

Ligands. Cyclen was synthesized according to literature procedure.[6a] For the cyclen derivatives, N-tosylaziridine was used as a key reagent. For synthesis of mcyclen and tmcyclen, N-tosylaziridine was reacted with N-methylamine to give N'-methyl-N,N''-ditosyldiethylenetriamine **4**. Disodium salt of **4** was reacted with N,O,O'-tritosylbis(2-hydroxyethyl) amine[6c] or bis (2-chloroethyl) methylamine[6d], yielding 1-methyl-4,7, 10-tritosylcyclen **5** (59%) and 1,7-dimethyl-4,10-ditosylcyclen **6** (35%), respectively. For the synthesis of cmcyclen, N-tosylaziridine was reacted with N,N'-dibenzylethylenediamine and disodium salt of the resulting 4,7-dibenzyl-1,10-ditosyltriethylenetetraamine was reacted with O,O'-ditosylethyleneglycol[6b] **7** to give 1,4-dibenzyl-4,10-ditosylcyclen **8** (12%). 1,4-Dimethyl-4, 10-ditosyl cyclen **9** was obtained after debenzylation (Pd/C)[6e] of **8** followed by methylation (formaldehyde, formic acid)[6f]. Cyclization of 4,7-dimethyl-1,10-ditosyltriethylenetetraamine with **7** was not successful. A detosylation (conc. H_2SO_4) of the cyclized products **5**, **6**, and **9**, and the subsequent base work-up of the ligand-sulfate salts give the desired monomethyl, *cis*-dimethyl, and *trans*-dimethyl cyclens, **2**, **10**, and **3**, in high yields. 1-Methyl-1,4,7,10-tetraazacyclodo decane (mcyclen) **2** : ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.30 (s, CH_3), 2.47 (bs), 2.57 (t, *J*=5.1Hz), 2.66 (t, *J*=5.1 Hz), 2.75-2.78 (m); ^{13}C NMR (75.4 MHz, D_2O) δ43.47 (CH_3), 44.74, 46.30, 48.77, 53.84. 1,4-Dimethyl-7,10-tetraazacyclododecane (cmcyclen) **10** : ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.29 (s, CH_3), 2.41 (s), 2.4-2.45 (m), 2.68-2.74 (m), 2.75 (s); ^{13}C NMR (75.4 MHz, D_2O) δ 43.73(CH_3), 45.29, 47.28, 54.0, 55.56. 1,7-Dimethyl-4,10-tetraazacyclododecane (tmcyclen) **3** : ^{1}H NMR (200 MHz, $CDCl_3$) δ1.22 (m, NH), 2.28 (s, CH_3), 2.46 (bs), 2.63 (t, *J*=4.8 Hz), 3.49 (m, NH); ^{13}C NMR (75.4 MHz, D_2O) δ 43.89 (CH_3), 44.78, 54.24.

[(L)Co(III)CO_3]ClO_4 and [(L)Co(III)$(OH_2)_2$]$(ClO_4)_3$ synthesis. All cobalt complexes [(L)Co(III)CO_3]ClO_4 have been synthesized according to literature procedure (L is tetraamine ligand).[7] [(cyclen)Co (III)CO_3]$ClO_4 \cdot H_2O$[8] : ^{13}C NMR (75.4 MHz, D_2O) δ 167.43(CO_3), 56.47, 54.02, 50.02, 47.91. [(monomethylcyclen)Co(III)CO_3]ClO_4 : ^{13}C NMR (75.4 MHz, D_2O) δ167.77, 66.93, 64.14, 56.42, 53.91, 50.35, 49.32, 48.36, 47.8, 45.7(CH_3). [(*trans*-dimethylcyclen) Co(III)CO_3]ClO_4[9] : ^{13}C NMR (75.4 MHz, D_2O) δ168. 25, 66.85, 64.06, 49.14, 48.09, 46.32 (CH_3). [(*cis*-dimethylcyclen) Co(III)CO_3]ClO_4 : ^{13}C NMR (75.4 MHz, D_2O) δ 167.94, 66.34, 65.93, 60.16, 59.72, 55.89, 49.92, 48.93, 46.77 and 45.71(CH_3). The above cobalt carbonato complexes were converted to [(L)Co(III)$(OH_2)_2$]$(ClO_4)_3$ by adding conc.$HClO_4$ according to published procedure as well.[3] [(cyclen)Co (III)$(OH_2)_2$]$(ClO_4)_3$: ^{13}C NMR (75.4 MHz, D_2O) δ 57.97, 54.6, 50.6, 48.6. [(monomethylcyclen)Co(III) $(OH_2)_2$]$(ClO_4)_3$: ^{13}C NMR (75.4 MHz, D_2O) δ 67.64, 64.55, 57.71, 54.26, 50.07, 49.73 (CH_3), 49.07, 48.54. [(*trans*-dimethylcyclen) Co(III)$(OH_2)_2$]$(ClO_4)_3$: ^{13}C NMR (75.4 MHz, D_2O) δ 67.95, 64.62, 50.93, 50.51, 49.57(CH_3).

Binding studies and kinetics. Cobalt complex promoted hydrolysis of NPP, BNPP and BDNPP was monitored by following the UV change at 400 nm. The anation reactions with inorganic phosphate were monitored by following the absorbance change due to formation of cobalt-phosphate complexes, at wavelengths varied from 550 to 570 nm. All the reactions were carried out under pseudo-first order conditions with a large excess of the cobalt complex over the phosphate ester. The rate constants were obtained by fitting the first 3 half-lives of reaction according to a first order kinetic equation (correlation coefficient > 0.98). Complexation of inorganic phosphate and phenyl phosphonate to the cobalt complexes was monitored by ^{31}P NMR. The NMR spectra were taken after the equilibrium was established (within an hour).

RESULTS AND DISCUSSION

The X-ray structures of the cyclen and the tmcy-

clen complexes were reported.[8,9] Two methyl groups of the tmcyclen complex occupied in axial positions in [(tmcyclen)Co(III)CO_3]ClO_4. According to MMX calculation[10] and the spectral data, the most stable isomer of the mcyclen complex was the one with methyl group in the axial position. Those configurations seemed to be maintained in *cis*-diaqua forms. The two methyl groups in the cmcyclen complex occupied in axial and equatorial positions in its carbonato-complex form, but decomposed during the conversion to its aqua-complex.

The rate constants for the hydrolysis of BDNPP, BNPP, NPP and inorganic phosphate by the cobalt complexes are listed in *Table* 1. The cyclen complex is about two times more active in hydrolyzing BNPP than the mcyclen complex. The tmcyclen complex shows a much lower reactivity compared to the cyclen or mcyclen complexes in hydrolyzing BNPP.

The proposed mechanism for hydrolysis of phosphate diesters involves formation of a four-membered ring intermediate. Tetraamine cobalt complexes with bidentate ligands such as carbonate or acetate are often employed as an analog for this intermediate. There appears to be two major factors involved in chelate formation: a) basicity of the bidentate ligand and b) the tetradentate ligand sructure. A good relationship between the basicity of the bidentate ligand and the equilibrium binding constant for the chelate formation has been found.[11] Strongly basic ligands such as carbonate form chelates with a wide range of the tetraamine cobalt complexes including tren cobalt complex.[1] As the basicity of the bidentate ligand increases, a higher ratio of the chelate over the monodentate is observed. With a weakly basic bidentate ligand, this chelate formation becomes dependent on the tetraamine ligand structure, especially the angle opposite to the four-membered ring intermediate.[1,11] The cobalt complexes of cyclen, mcyclen, and tmcyclen form the chelated acetate suggests that mono- and dimethylation on nitrogen atoms of cyclen do not alter binding ability of their cobalt complexes to acetate. It also indicates that the three cobalt complexes have a similar opposite angle (N-Co-N) to the four-membered ring (O-Co-O) intermediate. The three cobalt complexes also produce the chelated inorganic phosphate (*Fig.* 1). The signal appearing at 20 ppm

Table 1. Observed pseudo first order rate constants (sec^{-1}) for (L)Co(III)$(OH_2)_2$ promoted hydrolysis of phosphate esters at pH 7.0

L	BDNPP[a)]	BNPP[a)]	NPP[a)]	PHOS[b)]
cyclen	9.2×10^{-3}	3.3×10^{-3}	1.2×10^{-2}	7.7×10^{-2}
mcyclen	7.6×10^{-3}	1.4×10^{-3}	2.5×10^{-3}	6.3×10^{-2}
tmcyclen	4.5×10^{-4}	1.2×10^{-6}	3.3×10^{-7}	– [c)]

[a)][Co] 5.0 mM, [substrate] 2.5×10^{-5}M at 50°C, b) 25°C, [Co] 5.0 mM, [PO_4] 5.0×10^{-5}M c) absorbance change is too small to be observed.

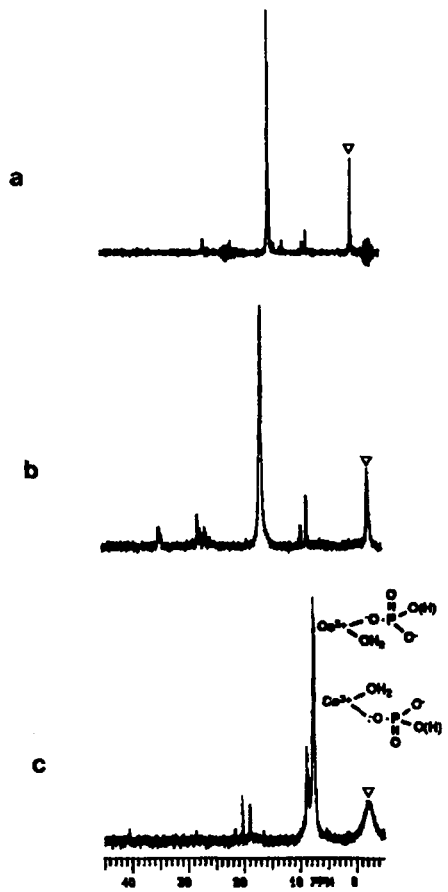


Fig. 1. ^{31}P NMR spectra of (L)Co(III)$(OH_2)_2$ (0.05 M) with one equivalent of inorganic phosphate at pD 6.0, 25°C.; L= a) cyclen, b) mcyclen, c) tmcyclen: ▽=free inorganic phosphate.

corresponds to the chelate.[12] The relatively small ratio of the bidentate over monodentate phosphate with the tmcyclen complex is consistent with the low reactivity in hydrolyzing BNPP. However, a rather significant difference in the reactivity of the tmcyclen complex suggests that there must be another factor than the angle involved.

Binding studies with phenyl phosphonate provide insight into structure and reactivity relationships. The phenyl group in phenyl phosphonate interacts with the axial methyl groups in the complex upon coordination (*Fig.* 2). Both the cyclen and the mcyclen complexes form the chelated phenyl phosphonate, while the tmcyclen complex forms only the monodentate (*Fig.* 3). The siganal at around 30-35 ppm corresponds to the chelate. The two siganls at 20 ppm are attributed to the two configurational isomers of the cyclen complex resulted by two NH protons on the equatorial position of the cyclen complex.[8] Phenyl phosphonate is a better model compound for studying the efficiency of catalysts hydrolyzing diesters. Its structure represents actual phosphate substrate better than inorganic phosphate and it produces a relatively simple spectrum. It became clear that the methyl groups in the tmcyclen complex inhibit the formation of a four-membered ring intermediate during hydrolysis of phosphate diesters. A significant difference towards the hydrolysis of BNPP is observed by placing methyl groups on the macrocyclic nitrogens of cyclen. The mcyclen complex is almost as efficient as the cyclen complex in promoting BNPP hydrolysis, while the tmcyclen complex is 200 times less active than the cyclen complex (*Table* 1). It is clear that the reactivity of the cobalt complexes is highly sensitive to the ligand structure. These complexes are structurally related and have similar pK_as[13] for the metal bound water molecules yet hydrolyze BNPP at different rates. The ligand structure can affect the efficiency of the Co(III) catalysts.

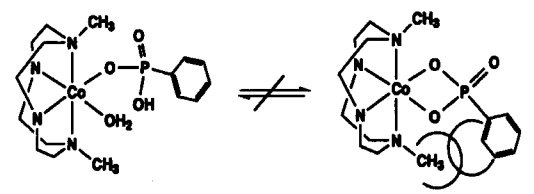


Fig. 2. Binding of phenyl phosphonate to the tmcyclen cobalt complex.

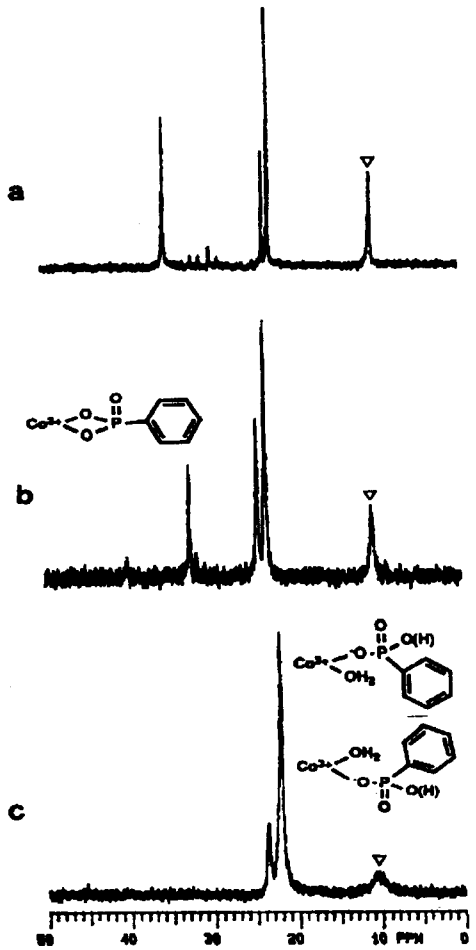


Fig. 3. ^{31}P NMR spectra of (L)Co(III)$(OH_2)_2$ (0.05 M) with one equivalent of phenyl phosphonate at pD 4.6, 25°C.; L: a) cyclen, b) mcyclen, c) tmcyclen: ▽=free phenyl phosphonate.

In addition, the hydrolysis of BNPP with high concentration of the catalysts gives interesting result, leading to the other aspect of structure-reactivity relationship. A plot of hydrolysis rate for BNPP promoted by the three cyclen derivatives vs the catalyst concentration gives a linear slope for both the mcy-

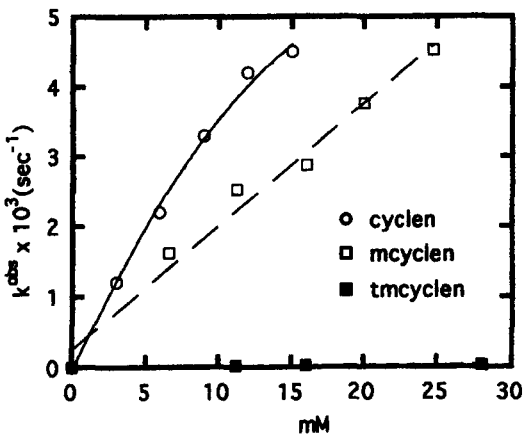


Fig. 4. A plot of observed first order rate constants (sec^{-1}) vs the catalyst concentration [(L)Co(III)(OH)(OH_2)] (mM); L : cyclen, mcyclen, and tmcyclen.

clen and tmcyclen complexes (*Fig. 4*). In contrast, the rate dependence on that of the cyclen complex levels off at the high catalyst concentration. The non-linear plot for the cyclen complex is attributable to the known dimerization reaction for *cis* diaqua cobalt complexes. The dimer form of the complex is not active since there is no free coordination sites available for the substrate binding to the metal complex. The mcyclen and the tmcyclen complexes do not dimerize because of unfavorable steric interaction brought about by the methyl groups. Indeed, the mcyclen complex was two times more active than the cyclen complex in hydrolyzing unactivated esters such as c-AMP under the prolonged reaction time.[14]

In conclusion, **2** hydrolyzes BNPP as efficiently as **1**, and **3** is much less active than **1** and **2**. By placing methyl groups on the nitrogens of cyclen, about 200 times difference in the rate of hydrolysis of BNPP is observed. In addition, the substitution of hydrogen with methyl groups could affect the stability of the cobalt complexes as shown in the case of cmcyclen. These results indicate that it is important to establish the structural requirements of the ligands based on the hydrolysis mechanism and steric hindrance as well. We are in the progress of synthesizing another cyclen derivatives by placing methyl groups on the carbons of cyclen.

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