

## Catalytic Hydrolysis of Phosphate Diesters as DNA Model with Tetranuclear Nickel (II) Complex

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The novel tetranuclear nickel (II) complex is a high rate accelerator in promoting hydrolysis of phosphate diesters. Nickel-bound bis-nitrophenyl phosphate (BNPP) can be  $10^3$  times more reactive than the unbound BNPP. The large rate of enhancements by the complex slightly under basic condition has shown high catalytic activity in phosphate diester cleavage. The bell-shaped pH-rate profile indicated that the nickel-oxide form of the tetranuclear complex or its kinetic equivalent was the active species for cleaving BNPP. The catalytic hydrolysis between tetranuclear nickel (II) complex and phosphate diester proceeds via the formation of bidentate coordination of the anionic phosphate to the Ni (II) atom. This reveals that the complex has the possibility as artificial nuclease.

**Key words:** Catalytic hydrolysis, phosphate diesters as DNA model, tetra-nuclear nickel (II) complex.

Most DNases are activated by metal ions and enhance the rate of hydrolysis of DNA. Transition metals such as Zn (II) and Co (II) are found in various metalloenzymes where they coordinate with amino acids and enhance catalysis at the active site. There has been a rapid development in the understanding of how metalloenzymes function. Specifically, the position and the sequence of reaction at the reactive site, and some clues as to the mechanism, have been forthcoming.<sup>1)</sup> For understanding the catalytic function of enzymes as well as for designing artificial hydrolytic metalloenzymes,<sup>2-8)</sup> it is important to examine the mechanistic pathways available in metal ion (complex) mediated hydrolysis reactions.<sup>9)</sup>

Recently, in the author's previous report, the hydrolytic reactivity for phosphate diesters and biological activities against fungi and weeds of some transition metal complexes were investigated and evaluated.<sup>10)</sup> The roles of metal ions and water molecule in the hydrolysis of bis(*p*-nitrophenyl)-phosphate as DNA model catalyzed by dinuclear Ni (II) complex were studied.<sup>11)</sup> Also, a series of cytotoxic activities *in vitro* against six human cancers and their seventeen cell lines of 3,6-bis(2'-pyridyl)-pyridazine-3,6-bis-(6'-methyl-2'-pyridyl)pyridazine and their transition metals, Ni (II), Cu (II) and Zn (II) complexes,<sup>12-14)</sup> were measured and, later reported that the Cu (II) complex showed higher cytotoxic activities than that of the first generation anticancer agent, Cis-platin.<sup>15)</sup>

In this study, to search the possibility as an artificial nuclease, the kinetics and mechanism on the catalytic hydrolysis of BNPP as DNA model<sup>16)</sup> by a new tetranuclear nickel (II) 3,6-bis(2'-pyridyl)pyridazine complex<sup>17)</sup> were studied and discussed.

### Materials and Methods

**X-ray Crystallography and determination of  $pK_a$ .** The tetranuclear nickel (II) complex of 3,6-bis(2'-pyridyl)pyridazine as catalyst for hydrolysis of phosphodiester ligand was synthesized from the reaction between nickel chloride (M) and ligand (L) (M : L = 4 : 4). Crystal constants (such as axial ratios) of the tetra nickel (II) complex determined generally by X-ray measurements, and based on previously determined X-ray data<sup>18)</sup> crystal morphologic data can be interpreted. The structure was solved by direct method<sup>19)</sup> and the least-squares refinement of the structure was performed by the program SHELXL97.<sup>20)</sup> Also, all the non-hydrogen atoms were refined anisotropically. The ORTEP in NRCVAX was used for molecular graphics.<sup>21,22)</sup> The  $pK_a$  of the tetra-nuclear nickel (II) complex was measured by PCA101 autotitrator (Sirius Co.). The complex was titrated on the pH range of 3-11 at 25°C. In the titration, to protect samples from absorbing atmospheric carbon dioxide, nitrogen gas was used and 0.5 M HCl, 0.5 M KOH, 80% aqueous methanol, and 0.15 M KCl ion adjusted solution were also used. Titration was performed twice and the acid dissociation constant ( $pK_a$ ) values were reproducible within 0.1 pH unit.

**Determination of hydrolysis rate constants.** The hydrolysis rate constants of NPP, BNPP and TNPP as phosphate model compounds were monitored by the absorbance change of hydrolyzed product, *p*-nitrophenol at 400 nm. All the reactions

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**Abbreviations:** NPP, disodium *p*-nitrophenylphosphate; BNPP, sodium bis-(*p*-nitrophenyl)- phosphate; TNPP, tris-(*p*-nitrophenyl)phosphate.

were carried out under pseudo-first-order conditions with a large excess of the tetranuclear nickel (II) complex over the phosphate ester. Each rate constants, the average from at least three kinetic runs, are shown in Table 1. In all cases, rate constants obtained from individual kinetic runs did not deviate from the average value by more than 3%. In a typical UV experiment, the tetra-nuclear nickel (II) complex solution (5 mM) in water would be prepared. The solution was adjusted to pH 7.0. The hydrolysis of NPP and BNPP was initiated by addition of 0.5 mL of a 5 mM NPP and BNPP stock solution in water to 2.5 mL of the freshly prepared 5 mM tetranuclear nickel (II) complex solution at 50°C. No appreciable effect was shown on the rate of hydrolysis reaction by the addition of sodium chloride.

## Results and Discussion

**Tetranuclear Nickel (II) complex.** In titration, quantity of tetranuclear nickel (II) complex used was similar to the quantity of 1 eq. base. From the titration curve, the base (KOH) was required for decomposing with nickel chloride. Therefore, the complex of M (metal) : L (ligand) = 2 : 2 was formed at below pH 7.5 and the tetranuclear nickel (II) complex of M : L = 4 : 4 was formed over the pH (Fig. 1). It was expected that free nickel chloride ( $\text{NiCl}_2$ ) exists at pH conditions higher than pH 9.0, because the  $\text{pK}_a$  of  $\text{NiOH}_2$  is larger than that of the tetranuclear nickel (II) complex. On the basis of these findings, acid dissociation constants of the tetranuclear nickel (II) complex were estimated to be  $\text{pK}_{a1} = 7.50$  and  $\text{pK}_{a2} = 10.4$ , respectively. The crystallographic structure of the tetra-nuclear nickel (II) complex is shown in Fig. 2. The twelve six-member aromatic rings were crooked at the angle within  $17.4(6)^\circ$ . All nickel (II) atoms were octahedrally coordinated by nitrogen and oxygen atoms with bond lengths ranging from  $2.039(11)\text{\AA}$  to  $2.109(8)\text{\AA}$ . The  $\text{O}_1$  and  $\text{O}_2$  atoms were positioned in one direction with  $\text{O}_1\text{-O}_2 = 2.547(10)\text{\AA}$ , and the  $\text{O}_3$  and  $\text{O}_4$  atom in the molecule face the opposite direction  $\text{O}_3\text{-O}_4 = 2.476(11)\text{\AA}$ . Therefore, the neighboring molecules were arranged crosswise. It is believed that the tetranuclear nickel (II) complex<sup>17)</sup> is a new catalyst in the hydrolysis of phosphate esters because the complex is a multi-coordinated metal complex.<sup>23)</sup>

**Kinetics and pH-rate profile.** The pseudo-first-order rate constants for catalyzed reactions were measured by increasing absorbance from 5 mM NPP, BNPP and TNPP solution. The observed hydrolysis rate constants with and without<sup>24)</sup> the tetranuclear nickel (II) complexes are summarized in Table 1. In neutral pH, phosphate diesters are the most stable when compared with esters, nitriles, amides, phosphate monoesters, and phosphate triesters. The half-life for BNPP hydrolysis is about 100 years at neutral pH at 25°C.<sup>25)</sup> However, in this study, there was a high rate of acceleration for the tetranuclear nickel (II) complex promoted hydrolysis of phosphate diester. Nickel-bound BNPP could be  $10^4$  times more reactive than that for the unbound BNPP. The pH-rate profile for the

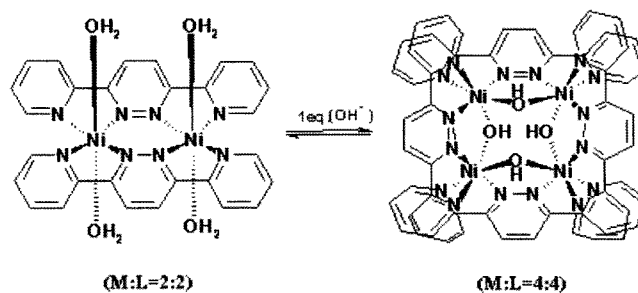


Fig. 1. Formation of tetranuclear nickel (II) complex ( $\text{pK}_{a1} = 7.50$  and  $\text{pK}_{a2} = 10.4$ ) from one equivalent base ( $\text{OH}^-$ ) and M : L = 2 : 2 complex.

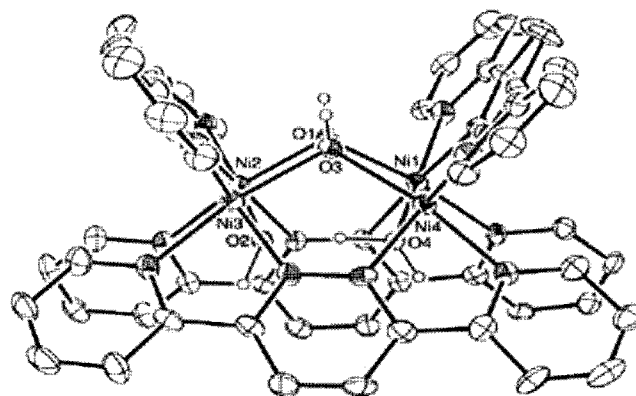


Fig. 2. X-ray crystal structure of tetranuclear nickel (II) complex: An ORTEP view (side) of the tetranuclear  $[\text{Ni}_4(\mu\text{-OH})_2(\mu\text{-dppn})_4(\mu\text{-H}_2\text{O})_2]^{6+}$  cation with the atomic numbering scheme.<sup>14</sup> Thermal ellipsoids are drawn at the 50% probability level. The five perchlorate anions and nine water molecules are omitted for clarity.

complex promoted hydrolysis of BNPP is shown in Fig. 3. The pH-rate profile was fitted according to an equation (1) with a curve-fitting program based on the Newton-Raphson algorithm.<sup>25)</sup>

$$k_{obs} = \frac{k[\text{Ni}]_T}{1 + \left( \frac{[\text{H}^+]}{K_{a1}} + \frac{K_{a2}}{[\text{H}^+]} \right)} \quad (1)$$

Where  $[\text{Ni}]_T$  = The total concentration of the tetranuclear nickel (II) complex,

$[\text{H}^+]$  = The proton concentration,

$K_{a1}$  and  $K_{a2}$  = The first and second acid dissociation constants of the tetranuclear nickel (II) complex,

$k$  = The second-order rate constant.

It was apparent from the bell-shaped pH-rate profile that the nickel-oxide form was the active species that promoted the hydrolysis of BNPP. A bell-shaped curve, having two inflection points, implies two acid/base dissociations in the reactants. One way in which this can produce a bell-shaped curve is if the reactant is a dibasic acid (say  $\text{H}_2\text{A}$ ) whose monoanion of  $\text{HA}^-$  is the most reactive form; then the

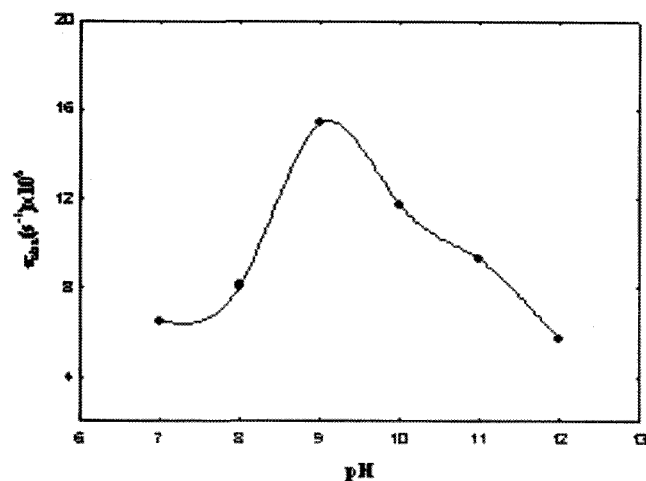


Fig. 3. pH-rate profile for the tetranuclear nickel (II) complex (5 mM) promoted hydrolysis of BNPP (1 mM) at 50°C. Points are experimental and solid line is drawn based on calculations according to the equation (1).

Table 1. Observed first-order rate constants ( $k_{obs}, s^{-1}$ ) for the tetranuclear nickel (II) complex<sup>a</sup> promoted hydrolysis of phosphate esters at 50 and pH 7.0

Catalyst	NPP	BNPP	TNPP
Ni(II)	$6.5 \times 10^{-6}$	$6.5 \times 10^{-6}$	$6.4 \times 10^{-6}$
None	$6.0 \times 10^{-8b}$	$3.0 \times 10^{-10c}$	-

<sup>a</sup>5 mM, <sup>b</sup>Kirby, A. J. and Younas, M. (1970) *J. Chem. Soc. B.* 510-513; extrapolated from rate measured at 100 with  $\Delta S^\ddagger$  value of -25.5 eu., <sup>c</sup>Bunton, C. A. and Farber, S. (1969) *J. Org. Chem.* **34**, 767; rate measured at 50°C.

concentration  $HA^-$ , and hence the rate, rises to a maximum at a pH given by  $pH_{max} = (pK_{a1} + pK_{a2})/2$ . Another type of reaction leading to a bell-shaped curve is in between acid and a base (say  $HA + B$ ). In this case, the ionizable groups are on different molecules. The large rate enhancements by the tetranuclear nickel (II) complex in the cleavage of BNPP under slightly basic condition showed high catalytic activity in phosphate diester cleavage.

**Hydrolysis mechanism of phosphate diester.** The bell-shaped pH-rate profile (Fig. 3) indicated that the nickel-oxide form of the tetra-nuclear nickel (II) complex or its kinetic equivalent was the active species for cleaving BNPP. This behavior can be explained by opposing pH effects on catalyst-BNPP complex formation and conversion of the BNPP within this complex. Therefore, one of the possible mechanisms that can account for the high reactivity involves chelation of the phosphate diester to the nickel (II) complex resulting in double Lewis acid activation.<sup>26)</sup> In general, a phosphate diester that is chelated to metal center should hydrolyze more rapidly than a phosphate diester that is singly coordinates to a metal center. The developing negative charge is stabilized by the cationic metal center.<sup>27)</sup> Moreover, the large rate of enhancements by the tetra-nuclear nickel (II) complex in the cleavage of BNPP showed that it is slightly under basic condition. The

Table 2. Hydrolysis rate constant ( $k_{sec}^{-1}$ ) of the tetranuclear nickel (II) complex

Buffers	pH	Obs.	Calc. <sup>a</sup>
Phosphate <sup>b</sup>	7.0	$6.51 \times 10^{-5}$	$6.50 \times 10^{-5}$
Phosphate <sup>b</sup>	8.0	$8.08 \times 10^{-5}$	$8.08 \times 10^{-5}$
NaOH+H <sub>3</sub> BO <sub>3</sub>	9.0	$1.54 \times 10^{-4}$	$1.54 \times 10^{-4}$
NaOH	10.0	$1.17 \times 10^{-4}$	$1.17 \times 10^{-4}$
NaOH	11.0	$9.72 \times 10^{-5}$	$9.31 \times 10^{-5}$
NaOH	12.0	$5.70 \times 10^{-5}$	$5.71 \times 10^{-5}$

<sup>a</sup>The values were calculated according to the rate equation (1), <sup>b</sup> $KH_2PO_4 + K_2HPO_4$ .

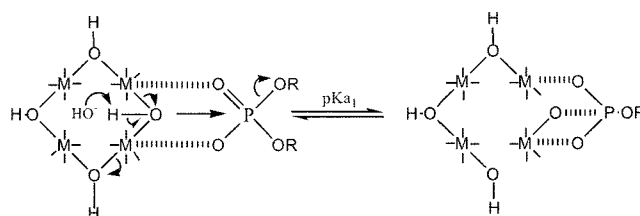


Fig. 4. A proposed mechanism of action of the tetranuclear Ni (II) complex and hydroxide anion in BNPP cleavage (M; nickel (II) atom & OR: p-nitrophenoxy group).

tetranuclear nickel (II) complex promoting cleavage of BNPP suggested that the nickel-oxide form was acted as a nucleophilic catalyst rather than as a general base catalyst. From these findings, a plausible mechanism of the tetranuclear Ni (II) complex's action is depicted in Fig. 4. Generally, high activity of the tetra-nuclear nickel (II) complex in intramolecular phosphate diester hydrolysis proceeds via formation of catalyst-BNPP complexes by bidentate coordination of the anionic phosphate to the nickel (II) atom.<sup>28)</sup> Also, one of four bridged tetrahydroxo species ( $HO_1-HO_4$ ) between two nickel (II) atoms is deprotonated ( $pK_{a1}$ ) by hydroxide anion. After deprotonation, the activated hydroxo group as nucleophile will attack to phosphorous atom in BNPP.<sup>29,30)</sup> From the results, the subsequent cleavage reaction of the P-O bond proceeds by leaving of p-nitrophenoxy (OR) group in BNPP. Therefore, at higher pH, the effective nucleophile concentration increases due to deprotonation of nickel (II) bound hydroxo groups and this increase rate is constant by the tetranickel (II) complex. For higher accuracy, this assumption must be further verified by additional experimental proofs.

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