Enhanced Nucleophilicity and Depressed Electrophilicity of Peroxide by Zinc(II), Aluminum(III) and Lanthanum(III) Ions

Satoshi Nishino[a], Teruyuki Kobayashi[a], Hideaki Matsushima[b], Tadashi Tokii[b], and Yuzo Nishida*,[a]

[a] Department of Chemistry, Faculty of Science, Yamagata University, Yamagata 990–8560, Japan. Fax: +81236284591. E-mail: yuzo@sci.kj.yamagata-u.ac.jp
[b] Department of Chemistry, Faculty of Science and Engineering, Saga University, Saga, 840–8502, Japan

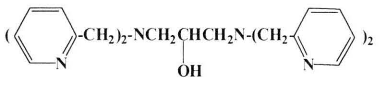
* Author for correspondence and reprints requests

Z. Naturforsch. **56 c,** 138–143 (2001); received August 7/September 11, 2000

Peroxide Ion, Zinc(II), Lanthanum(III)

The binuclear zinc(II) complex, $[Zn_2(HPTP)(CH_3COO)]^{2+}$ was found highly active to cleave DNA (double-strand super-coiled DNA, pBR322 and ϕ×174) in the presence of hydrogen peroxide. However, no TBARS (2-thiobarbituric acid reactive substance) formation was detected in a solution containing 2-deoxyribose (or 2′-deoxyguanosine, etc); where (HPTP) represents N,N,N′-N′-tetrakis(2-pyridylmethyl)-1,3-diamino-2-propanol. These facts imply that DNA cleavage reaction by the binuclear Zn(II)/H_2O_2 system should be due to a hydrolytic mechanism, which may be attributed to the enhanced nucleophilicity but depressed electrophilicity of the peroxide ion coordinated to the zinc(II) ion. DFT (density-functional theory) calculations on the peroxide adduct of monomeric zinc(II) have supported the above consideration. Similar DFT calculations on the peroxide adducts of the Al(III) and La(III) compounds have revealed that electrophilicity of the peroxide ion in these compounds is strongly reduced. This gives an important information to elucidate the fact that La^{3+} can enhance the growth of plants under certain conditions.

The Zn(II) ion is a biologically essential element. (Kimura, 1994) It often constitutes active centers of hydrolytic "zinc enzyme", where zinc atoms are designed by nature to generate nucleophiles (e.g., OH^-, RO^-, or H^-) to attack at the electrophilic centers such as carbonyl $C^{\delta+}$ and phosphate $P^{\delta+}$. Zinc enzymes are classified as (a) DNA and RNA polymerase, (b) alkaline phosphatases, (c) peptidases, such as carboxypeptidase, and many of these enzymes have been intensively characterized. (Kimura, 1994; Kramer, 1999) In this study we have prepared a binuclear zinc(II) complex with H(HPTP), and found that this complex exhibits a high ability to cleave DNA (supercoiled DNA) in the presence of hydrogen peroxide. We concluded that this high activity towards DNA cleavage reaction should be due to a hydrolytic mechanism, which may be attributed to the enhanced nucleophilicity and depressed electrophilicity of the peroxide ion coordinated to the zinc(II) ion; where H(HPTP) (Nishida *et al.*, 1992) represents N,N,N′,N′-tetrakis(2-pyridylmethyl)-1,3-diamino-2-propanol (see structure below).



H(HPTP)

Experimental

Preparation of Zinc(II) complex

The ligand, H(HPTP) was obtained according to Nishida *et al.* (1992). The white precipitation obtained by adding $NaClO_4$ to the methanol solution (20 ml) containing zinc(II) acetate (0.002 mol) and H(HPTP) (0.001 mol), was recrystallized once from an acetonitrile/methanol solution. Found. C, 41.67; H, 3.88; N, 10.51%. Calcd. for $Zn_2(HPTP)(CH_3COO)(ClO_4)_2 \cdot 1/2CH_3CN$: C, 41.76; H, 3.91; N, 10.55%.

Crystal structure determination

A white prism having approximately dimensions of 0.25×0.25×0.30 mm was mounted on a glass fiber. All measurements were made on a Rigaku

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AFC5S diffractometer (Saga University) with graphite monochromated MoKα radiation and a 12 KW rotating anode-generator. Cell constants and an orientation matrix for data collection, obtained from a least-squares refinement using the setting angles of 25 carefully centered reflections in the range 10.0 ° <2θ <25.0 °, corresponds to a triclinic cell dimension with a = 12.565(3), b = 14.227(2), c = 11.072(2) Å, α = 102.27(1), β = 111.58(2), γ = 87.08(2) ° space group P1-(#2), Z = 2, V = 1797.7(7) Å^3, F. W. = 862.80. The calculated density is 1.594 g/cm^3. The structure was solved by direct methods. The non-hydrogen atoms were refined anisotropically. The final cycles of full-matrix least-squares refinement was based on 5229 observed reflections (I>3.00σ(I)), and 478 variable parameters and converged with unweighted and weighted agreement factors of $R = \Sigma||Fo|-|Fc||/\Sigma|Fo| = 0.058$, $R\omega=[(\Sigma||Fo|-|Fc||)^2/\Sigma\omega|Fo|^2]]^{1/2} = 0.071$.

Neutral atom scattering factors were taken from Cromer and Weber (1974) including the values for $\Delta F'$ and $\Delta F''$. Anomalous dispersion effects were included in Fcalc. All calculations were performed using TEXSAN Crystallographic software package of Molecular Structure Corporation (TEXSAN-TEXRAY, 1985).

DNA cleavage by Zinc(II) complex and hydrogen peroxide system

In a typical run, an aqueous solution of zinc(II) complex (4 μl of 0.5 mM solution), DNA (4 μl of 0.1 mg/1 ml solution), tris buffer (2 μl of 0.1 M solution; pH=7.8), and hydrogen peroxide (4 μl of 0.01 – 0.1 M solution) were mixed and allowed to stand for 1 hour at 25 °C. The resulted solution was electrophoresed on a 0.9% agarose gel containing ethidium (3,8-diamino-5-ethyl-6-phenylphenanthridimium) bromide. The bands were photographed with Polaroid 667 film. (Micklos and Freyer, 1996).

Evaluation of TBARS in the solution containing 2′-deoxyribose

The detection of TBARS (2-thiobarbituric acid reactive substance) (Halliwell and Gutteridge, 1985) in the solution containing zinc(II) complex, hydrogen peroxide, and 2′-deoxyribose was performed according to the published method; experimental conditions were essentially the same as those reported for the $Fe_2(HPTP)(OH)(NO_3)_4$ complex. (Akamatsu *et al.*, 1997). In the case of La^{3+} complex, solution of La^{3+} containing diethylenetriamine-pentaacetic acid (DETAPAC) (0.01 M solution, 20 ml; an equimolar amount of $La(NO_3)_3$ and DETAPAC was mixed, and pH of the resulted solution was adjusted to be 7.0 by $NaHCO_3$) was used.

DFT calculations

The DFT (density-functional theory-calculations (Parr and Young, 1989) were performed by the use of Dgauss 4.1 (Oxford Molecular Science Inc., Oxford 1998). Basis set: DZVP; Gradient GGA X B88, GGA C LYP88 for La(III), and B88-PW91, for Zn(II) complex, respectively (see Supplementary data).

ESI-mass spectra

Electro-spray mass spectra (ESI-Mass) were obtained with an API 300 triple quadrupole mass spectrometer (ion-spray interface of PE-Sciex, Thomhill, ON, Canada) at the Institute for Molecular Science, Okazaki, Japan. The solutions of Zn(II) complex (6 μl, 5 mM aqueous solution) and 12-mer (20 μl, 300 mM aqueous solution) were mixed, and ESI-Mass spectra were measured after addition of ammonium acetate (2 μl, 150 mM) and acetonitrile (30 μl) to the above solution (26 μl).

Materials

DNA (supercoiled, pBR322, ϕX174) was purchased from Wako Chemicals (Osaka). Oligomers, 8-mer, d(5′-AAACGTTT)$_2$, and 12-mer, d(5′-CGC TTTAAAGCG)$_2$ were obtained commercially.

Results and Discussion

Crystal structure

The crystal structure of the binuclear zinc(II) complex, $Zn_2(HPTP)(CH_3COO)^{2+}$ is illustrated in Fig. 1, and the selected bond lengths and angles are summarized in Table I. Similar to the corresponding iron(III) complexes, (Nishino *et al.*, 1999) this compound has a binuclear unit with a μ-alkoxo bridge. Both zinc(II) ions are of a five-

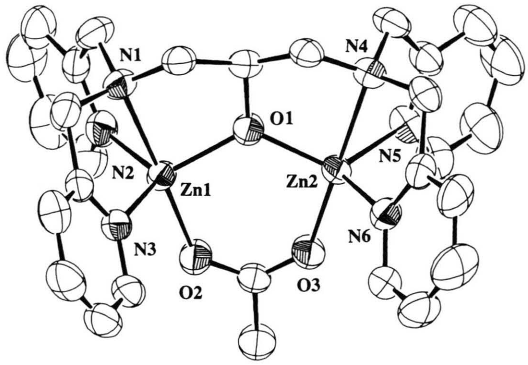


Fig. 1. ORTEP drawing of $Zn_2(HPTP)(CH_3COO)^{2+}$.

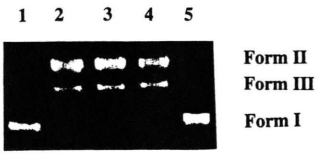


Fig. 2. Relaxation of pBR322 Form I DNA by zinc(II) compound (0.5 mM solution) in the presence of hydrogen peroxide.
Lane 5, DNA alone; lane 1, DNA + zinc(II) complex; lane 2, DNA + zinc(II) complex + H_2O_2 (0.1 M solution); lane 3, DNA + zinc(II) complex + H_2O_2 (0.05 M solution); lane 4, DNA + zinc(II) complex + H_2O_2 (0.01 M solution).

Table I. Selected bond distances (nm) and angles(°) of $Zn_2(HPTP)(CH_3COO)(ClO_4)_2$.

Distance [nm]

ZN1	O1	19.58(4)	ZN1	O2	19.81(4)
ZN1	N1	22.35(5)	ZN1	N2	20.47(5)
ZN1	N3	20.62(5)	ZN2	O1	19.35(4)
ZN2	O3	19.86(4)	ZN2	N4	22.52(5)
ZN2	N5	20.68(5)	ZN2	N6	20.35(5)

Angles [°]

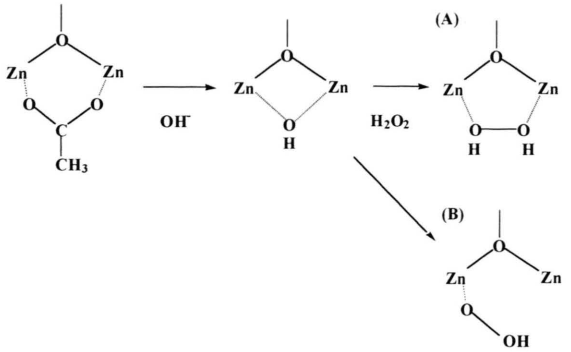
O1	ZN1	O2	100.4(2)	O1	ZN1	N1	81.4(2)
O1	ZN1	N2	116.8(2)	O1	ZN1	N3	113.8(2)
O2	ZN1	N1	178.1(2)	O2	ZN1	N2	100.8(2)
O2	ZN1	N3	99.5(2)	ZN1	O1	ZN2	124.0(2)
N1	ZN1	N2	78.9(2)	N1	ZN1	N3	79.1(2)
N2	ZN1	N3	120.1(2)	O1	ZN2	O3	99.3(2)
O1	ZN2	N4	80.8(2)	O1	ZN2	N5	122.6(2)
O1	ZN2	N6	117.2(2)	O3	ZN2	N4	178.1(2)
O3	ZN2	N5	100.3(2)	O3	ZN2	N6	102.0(2)
N4	ZN2	N5	78.1(2)	N4	ZN2	N6	79.6(2)
N5	ZN2	N6	110.5(2)				

coordinated structure, which can be approximately designed as a trigonal bipyramide.

DNA cleavage

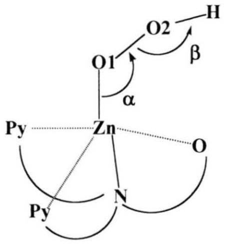
As shown in Fig. 2, the zinc(II) complex did not degrade supercoiled DNA (see lane 1) in the absence of hydrogen peroxide. When hydrogen peroxide was added to the solutions (lanes 2–4), formation of Form II DNA (relaxed circular) and Form III DNA (linear duplex) (Micklos and Freyer, 1996) was detected, indicating that this Zinc(II)/H_2O_2 system can cleave DNA. No formation of Form II and Form III DNA was detected in the absence of zinc(II) complex even in the presence of H_2O_2. The ESI-mass spectra of the solution containing the binuclear zinc(II) complex shows that the original zinc(II) complex exists as a dimer with an acetate bridge in acetonitrile solution (see Supplementary data; m/z=321.8), but in slightly basic aqueous medium (pH=7.5, by $NaHCO_3$), this complex changes to a dimeric one with a μ-hydroxide bridge (m/z=300.8; see Supplementary data). In the ESI-mass spectra (negative pattern: Supplementary data) of the solution containing 12-mer, the peaks at m/z=1821.6, 1214.0, 910.2 and 728.0 are attributed to (-2), (-3), (-4) and (-5) species of the single-stranded 12-mer, based on the calculated isotope patterns. The signal at m/z=1457.2 is due to (-5) species of the double-strand. When the binuclear zinc(II) complex was added, new signals appeared (see Supplementary data). Signals at m/z=1407.6, 1449.8, and 1600.4, may be attributed to the species, $[(12mer\text{-}6H^+)\cdot Zn_2(HPTP)]^{3-}$, $[(12mer\text{-}5H^+)\cdot Zn_2(HPTP)(CH_3COO)]^{3-}$, and $[(12mer\text{-}9H^+)\cdot 2Zn_2(HPTP)]^{3-}$, respectively. When hydrogen peroxide was added to this system, a new signal was found at m/z=1420.4, and this may correspond to the formation of a peroxide adduct, $[(12mer\text{-}6H^+)\cdot(Zn_2(HPTP)(H_2O_2))]^{3-}$. These findings imply that the present binuclear zinc(II) complex can easily bind to the DNA chain, probably at the negatively charged phosphate backbone. All above facts suggest that an active species for DNA cleavage by the $Zn_2(HPTP)/H_2O_2$ system in buffer solution (pH=7.8) should be a peroxide adduct of the zinc(II) compound (see Scheme I), because no DNA cleavage was observed in the absence of hydrogen peroxide.

DNA cleavage occurs in two ways; by hydrolytic and oxidative mechanisms (Stubbe and Kozarich, 1987; Sousa, 1996; Chapman and Breslow, 1995; Yashiro *et al.*, 1997). Because no TBARS was de-



Scheme I

tected in the solution containing $Zn_2(HPTP)(CH_3COO)(ClO_4)_2$, 2′-deoxyribose, and hydrogen peroxide (data not shown), this system exhibits no oxidative activity towards DNA. Thus, it seems reasonable to consider that the DNA cleavage by the present system should proceed by a hydrolytic mechanism. There are two possible structures for a peroxide adduct of the binuclear zinc(II) compound as illustrated in Scheme I. Since the binuclear zinc(II) complex with a μ-hydroxo bridge does not cleave DNA hydrolytically, it seems quite likely that the active species for DNA cleavage in the solution should be (B). To get more information on the electronic property of a metal-peroxide adduct, we have done the DFT calculations on the hydroperoxide adduct of zinc(II) species with η^1-coordination mode, (B) in Scheme I.



Scheme II

DFT calculations on peroxozinc(II) species

DFT calculations were performed for a monomeric hydroperoxide adduct of zinc(II) with N,N-bis(2-pyridylmethyl)-aminoethanolato ion (five-coordinate species, see Scheme II and also Supplementary data), which was constructed using the crystal structure determination of the binuclear species. Special attention was paired for the change of electron densities on oxygen atoms of the peroxide ion. As illustrated in Fig. 3, the electron densities on the oxygen atoms of hydroperoxide ion are highly dependent on the position of the proton (angle β=<O1-O2-H), which is consistent with our previous result (Nishida, 2000). The electron densities on the oxygen atoms are also dependent on the angle α, (angle Zn-O1-O2).

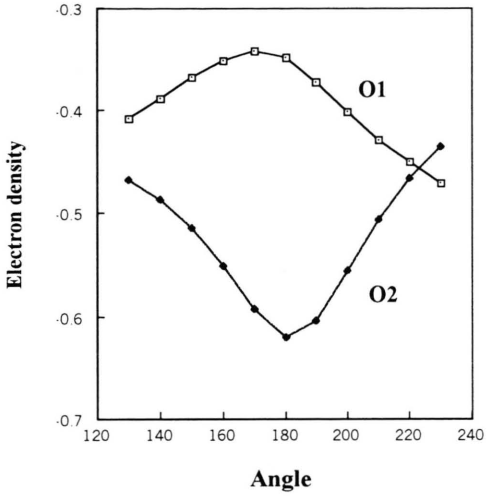
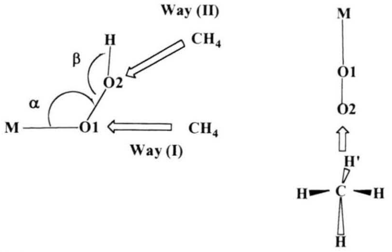


Fig. 3. Change in electron density on oxygen atoms of zinc(II)-peroxide adduct with angle β (=<O1-O2-H).

A methane molecule can approach to this peroxide adduct in two ways; Way (I); methane approaches the O1 atom, and Way (II); approaches the oxygen O2 (see Scheme III, here we have assumed that methane approaches the peroxide adduct in the way where HOMO of methane molecule interacts with the oxygen atom). As shown in Table II, electron density change has occurred when methane approaches the O2 atom, but it should be noted here that in all the cases examined, total electron density on two the oxygen atoms decreases in the zinc(II) compound, which

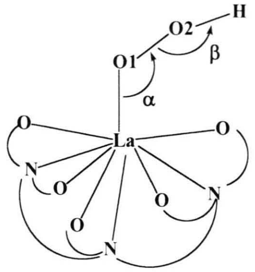


Scheme III

Table II. Electron density on the atoms where methane is approaching the O2 atom in Way (II) (in this time, angle O1-O2-C was set to be 120 °; see Scheme III). *R* = distance between C (methane) and O2 atom/nm. In both Zn(II) and La(III) complexes α was set to be 120 °.

		R[nm] =	18	20	30	40
Zn(II)	[1]*	O1	−0.430	−0.397	−0.369	−0.369
		O2	−0.401	−0.458	−0.513	−0.513
		C(methane)	−0.840	−0.840	−0.837	−0.835
	[2]*	O1	−0.427	−0.396	−0.370	−0.369
		O2	−0.385	−0.458	−0.507	−0.514
		C(methane)	−0.954	−0.912	−0.859	−0.848
La(III)	[3]*	O1	−0.444	−0.422	−0.407	−0.408
		O2	−0.410	−0.482	−0.573	−0.593
		C(methane)	−0.794	−0.813	−0.823	−0.813
	[4]*	O1	−0.495	−0.420	−0.409	−0.409
		O2	−0.275	−0.499	−0.580	−0.595
		C(methane)	−1.393	−0.954	−0.846	−0.828

* [1]: β=150 °, angle O2-C-H′ (see Scheme III)=150 ° [2]: β=150 °, angle O2-C-H′ (see Scheme III)=120 °, [3]: β=160 °, angle O2-C-H′ (see Scheme III)=92 °, [4]: β=160 °, angle O2-C-H′ (see Scheme III)=120 °.



Scheme IV

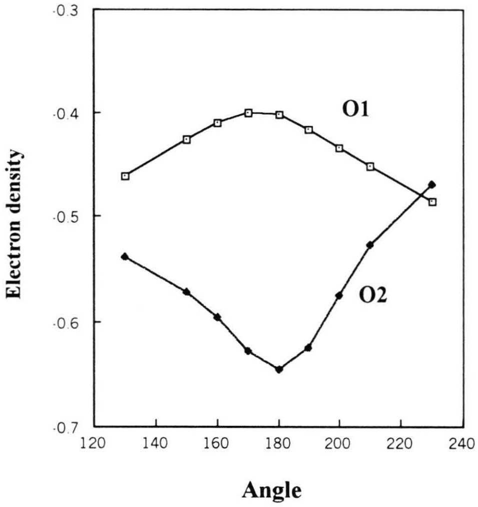


Fig. 4. Change in electron density on oxygen atoms of La(III)-peroxide adduct with angle β (=<O1-O2-H).

is greatly different from those calculated for iron(III) and copper(II) compounds. (Nishida, unpubl.) In the latter cases the increase of electron density on the peroxide oxygen atoms was detected by the approach of methane molecule, which is attributed to the strong electrophilicity of the peroxide adduct of these iron(III) and copper(II) compounds, and this is induced by the presence of unoccupied or half-occupied d-orbital interacting with the peroxide ion. (Nishida, submitted) Above discussion clearly indicates that electrophilicity of the peroxide ion is strongly depressed through the coordination to a zinc(II) ion. On the other hand, the nucleophilicity of the peroxide ion is enhanced in the hydroperoxo-metal complex with a β=170~200 ° region, since the electron density at the O2 atom greatly increases in this conformation (see Fig. 3); this enhanced nucleophilicity should be main origin for the high DNA cleavage activity by hydroperoxozinc(II) species observed in this study.

Calculated results for La(III)-(DETAPAC)-(OOH) complex (nine-coordinate species, see Scheme IV; the structural parameters of this species were based on the crystal structure determination of Th(IV)-(DETAPAC) complex cited in Dgauss 4.1), were essentially the same as those obtained for the zinc(II) and Al(III) compounds (Nishida, submitted) (see Fig. 4 and Table II). These suggest that in the peroxo-metal compounds with Zn(II), Al(III), and La(III) complexes, the nucleophilicity of the peroxide ion is enhanced, whereas the electrophilic nature of the peroxide ion is strongly depressed, and this may explain why Al(III) and La(III) complexes do not give TBARS in the presence of 2′-deoxyribose and hydrogen peroxide. (Nishida and Ito, 1995) This will give important information to elucidate the facts that La^{3+} acts as an efficient agent for phosphate diester cleavage (Takasaki and Chin, 1993), and that La^{3+} can enhance the growth of plants under certain conditions (Evans, 1990). In the latter case it seems quite likely that the oxidative damage by hydrogen peroxide in the cells is strongly depressed by the coordination of hydrogen peroxide to a La^{3+} ion.

Supplementary Data*

1. Crystal data of $Zn_2(HPTP)(CH_3COO)(ClO_4)_2$
2. DFT calculation for Zn(N,N-bis(2-pyridylmethyl)-aminoethanolato)(OOH) complex.
3. DFT calculation for La(DETAPAC)(OOH) complex.
4. ESI-mass spectra of zinc(II) complex solutions.
   A: $Zn_2(HPTP)(CH_3COO)(ClO_4)_2$ in acetonitrile; m/z=321.8 and =741.0 correspond to $[Zn_2(HPTP)(CH_3COO)]^{2+}$ and $[Zn_2(HPTP)(CH_3COO)(ClO_4)]^{+}$ cation, respectively.
   B: $Zn_2(HPTP)(CH_3COO)(ClO_4)_2$ in methanol/water=1/1 (=v/v) solution containing $NaHCO_3$: m/z=300.8 and 601.2 correspond to $[Zn_2(HPTP)(OH)]^{2+}$ and $[Zn_2(HPTP^-)(OH)]^{+}$, respectively. ((HPTP-) represents a double deprotonated form of H(HPTP)).
5. ESI-mass spectra of oligomer solutions.
   A: 12-mer (d(5′-CGCTTTAAAGCG)) in water.
   B: 12-mer + $Zn_2(HPTP)(CH_3COO)(ClO_4)_2$
6. ESI-mass spectra of oligomer, zinc(II) complex, and hydrogen peroxide.(molar ratio of three components are the same as those in Fig. 3)
   A: 12-mer + $Zn_2(HPTP)(CH_3COO)(ClO_4)_2$
   B: 12-mer + $Zn_2(HPTP)(CH_3COO)(ClO_4)_2$ + H_2O_2.

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* Supplementary data on crystal structure determination, DFT calculations and ESI-mass spectra of the compounds are available from the author by request.

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