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Review

DNA hydrolysis promoted by di- and multi-nuclear metal complexes

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Contents

Abstract

DNA hydrolysis mediated by di- and multi-nuclear metal complexes is of increasing importance in biotechnology and medicine. Many systems for enzyme-mediated nucleic acid hydrolysis, including type II restriction endonucleases and phosphatases, contain di- and multi-nuclear metal active sites. Recent progress in the design of di- and multi-nuclear metal artificial nucleases has included Fe^{3+}, Zn^{2+}, Cu^{2+}, Co^{3+}

Abbreviations: BDBPH, 3,6,9,17,20,23-hexaaza-29,39-dihydroxy-13,27-dimethyl-tricyclo[23,3,1,1]triaconta-1(28),11, 13,15(30),25,25-hexene; BDNPP, bis(2,4-dinitrophenyl) phosphate; BPAN, 2,7-bis[2-(2-pyridylethyl)-aminomethyl]-1,8-naphthyridin; BNPP, bis(*p*-nitrophenyl) phosphate; BPClNOL, *N*-(2-hydroxylbenzyl)-*N*-(pyridylmethyl)[(3-chloro)(2-hydroxyl)]propylamine; BPTA, *N*,*N*-bis(2-pyridylmethyl)-*tert*-butylamine; bpy′, 4-(butyric acid)-4′-methyl-2,2′-bipyridine; BTP, 1,3-bis-tris(hydroxymethyl)aminomethane propane; D^1, dinucleating ligand with two tris(2-pyridylmethyl)-amine units covalently linked in their 5-pyridyl positions by a $–CH_2CH_2–$ bridge; dien, diethylenetriamine; DIP, diphenyl-1,10-phenanthroline; DTPB, 1,1,4,7,7-penta (2′-benzimidazol-2-yl-methyl)-triazaheptane; EDTB, *N*,*N*′-bis(2′-benzimidazol-2-yl-methyl)-ethylenediamine; EGTB, *N*,*N*,*N*′,*N*′-tetrakis(2′-benzimidazol-2-yl-methyl)-1,4-bis(ethylamino)-bis(ether); H_2bbppnol, *N*,*N*′,*N*,*N*′-bis[(2-hydroxylbenzyl)(2-pyridylmethyl)]-2-ol-1,3-propanediamine; HP, 3_{10}-helical synthetic heptapeptides modified with two triazacyclononane rings; HPNP, 2-hydroxypropyl-*p*-nitrophenyl phosphate; HPTA, 2-hydroxyl- propenylene-1,3-diamine-*N*,*N*,*N*′,*N*′-tetraacetic acid; HPTB, *N*,*N*,*N*′,*N*′-tetrakis(2-benzimidazolylmethyl)-2-hydroxyl-1,3-diaminopropane; HXTA, 5-methyl-2-hydroxyl-1,3-xylene-α,α-diamine-*N*,*N*,*N*′,*N*′-tetraacetic acid; HXTP, 5-methyl-2-hydroxyl-1,3-xylene-α,α-diamine-*N*,*N*,*N*′,*N*′-tetrapropanoic acid; IDB, *N*,*N*-bis(2′-benzimidazol-2-yl-methyl)amine; Im, imidazole; Im^-, imidazole anion; L1, an optically active hexaaza triphenolic macrocycle; L2, 3,13-dihydroxyl-1,5,8,11,15,18-hexaazacyclicamine; Macro, 4,7-$((NH_2CH_2CH_2)_2NCH_2CH_2NHSO_2C_6H_4)$-1,10-phenanthroline; NPA, 4-nitrophenyl acetate; NPP, nitrophenyl phosphate; N_4S_4, 1,2,4,5-tetrakis(1′-amino-3′-thiobutyl)benzene; NTB, tris(2-benzimidazolylmethyl)amine; phen, 1,10-phenanthroline; phi, 9,10-phenanthrenequinone diimine; PNP, *p*-nitrophenyl phosphate; Suc, succinate; tach-Me_3, *N*,*N*′,*N*″-trimethyl-*cis*-1,3,5- triaminocyclohexane; Tdci, 1,3,5- trideoxy-1,3,5-tris(dimenthylamino)-*cis*-inositol; TPNP, tris(*p*)nitrophenyl phosphate; $TrenP_3$, Tren + 3P, Tren: tris(2-aminoethyl)amine, P: H-Iva-Api-ATANP-Iva-Api-Iva- $NHCH_3$, Iva: (*S*)-isovaline, Api: 4-amino-4-carboxypiperidine, ATANP: (*S*)-2-amino-3-[1-(1,4,7- triazacyclononane)]propanoic acid; TTHA, triethylene tetraaminehexaacetic acid

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and $Ln^{3+/4+}$-azamacrocyclic, aminocarboxylic and pyridyl- or benzimidazolyl-based organic ligands complexes and their conjugates to biomacromolecules. The focus in this article is on di- and multi-nuclear metal complexes that promote DNA cleavage via hydrolytic rather than oxidative pathway. Our purpose is to highlight the relationships between the structures of di- and multi-nuclear metal complexes and their functions, the cooperativities between metal and ligands and between metal sites in the course of DNA hydrolysis and the problems that are faced toward the development of di- and multi-nuclear metal-based artificial restriction enzymes by applying the principles of coordination chemistry and enzymatic chemistry. In order to be able to conveniently compare kinetic data that have been reported for many di- and multi-nuclear metal complexes, we propose two parameters α and β that are associated with the kinetic studies on DNA hydrolysis. The former α is defined as the ratio of the hydrolytic rate constant mediated by a di- or multi-nuclear metal complex to that by the analogous mononuclear complex under identical or similar conditions, indicating the degree of cooperativity between metal sites. The latter β is recognized as a rate enhancement over unhydrolyzed double-stranded DNA.

Keywords: DNA hydrolysis; Phosphate diester bond; Di- and multi-nuclear metal complexes; Catalytic cooperativity; Structure–function relationships; Type II restriction endonucleases; Artificial nucleases

1. Introduction

DNA and RNA hydrolysis promoted by mono-, di- and multi-nuclear metal complexes, in a nondegradative manner and with high levels of selectivity for a site, sequence or structure, is of increasing importance in biotechnology and medicine, because this will offer many applications for the manipulation of genes, the design of structural probes and the development of novel therapeutics [1–5]. In addition, the metal complex-mediated hydrolysis of phosphate esters is providing valuable information for modeling and elucidating the reactivity of metal-containing nuclease molecule [1–11]. The focus in this article is on di- and multi-nuclear metal complexes that promote DNA cleavage via hydrolytic rather than oxidative pathway. Many excellent reviews on mono-, di- and multi-nuclear metal complexes that selectively recognize DNA and catalyze DNA hydrolysis have appeared recently [1–10]. Our purpose is to highlight the current progress, the relationship between the structures of di- and multi-nuclear metal complexes and their functions, the cooperativities between metal and ligands and between metal sites in the course of DNA hydrolysis and the problems that are faced toward the development of di- and multi-nuclear metal-based artificial restriction enzymes by applying the principles of coordination chemistry and enzymatic chemistry.

The half-life of a typical phosphate diester bond in DNA in neutral water solutions at 25 °C was estimated to be on the order of tens to hundreds of billions of years assuming that the hydroxide rate dominates the water rate under physiological conditions as in RNA cleavage [6,12]. However, hydrolysis of phosphate diester bond is of critical importance in most basic cellular functions, including DNA repair, excision, transcription, integration and metabolism, signal transduction. Although the second-order rate constant for hydroxide-catalyzed hydrolysis of the phosphate diester bond of DNA has not been directly measured to date, it has been estimated that a catalyst would have to provide over 10^{17}-fold rate acceleration to hydrolyze this bond within a few minutes [6]. As a result, it is necessary to develop such reactive catalysts to efficiently hydrolyze DNA. Nature has already found ways to hydrolyze DNA within 1 s using metal-containing enzymes. The main hindrance in DNA hydrolysis is the large negative charge that inhibits attack of nucleophile at the DNA backbone, and so charge neutralization is one of several mechanisms used by natural nuclease molecules.

Metal-containing enzyme-mediated nucleic acid hydrolysis and phosphoryl transfer shares a common catalytic pathway in nucleic acid biochemistry [13]. Although magnesium is the first choice of nuclease enzymes, mainly as a result of its high natural abundance and availability of appropriate hydration states, ligand exchange rates, redox inertness and charge density, other multinuclear metal active sites are also well found in hydrolase molecules, with Zn^{2+}, Fe^{2+} and Mn^{2+} as the most frequent cases [3,14]. Alkaline and purple acid phosphatases utilize dinuclear metal active sites for removal of a phosphate group from phosphomonoesters. The former possess a dinuclear Zn^{2+} and Mg^{2+} centers (Fig. 1a) [15], while purple acid phosphatase employs one Fe^{3+} center that is coupled to a second divalent Fe^{2+} or Zn^{2+} center (Fig. 1b) [16]. P1 nuclease, catalyzing hydrolysis of single-stranded DNA and RNA, contains a trinuclear Zn^{2+} active site (Fig. 1c) [17]. The structure of *Eco*RV has been determined in a variety of different states with many divalent cations (Mg^{2+}, Mn^{2+}, Co^{2+}, Ca^{2+}, Mg^{2+}/Ca^{2+} and Mn^{2+}/Ca^{2+}, Fig. 1d) [18]. The data available indicate that *Eco*RV uses one to three metal ions to cleave DNA, but the number of metal ion involved in the catalytic mechanism is still in question. A proposed model is illustrated in Fig. 4a. Moreover, Tn5 transposase-mediated transposition is also catalyzed by two-metal ion active site [19,20].

The hydrolysis of deoxynucleotide phosphates proceeds by the nucleophilic attack at phosphorus by a water oxygen, to give a five-coordinate phosphate intermediate. Subsequent cleavage of either the P-O3′ or P-O5′ (dependent on the catalytic system) causes a strand break yielding R-OH and R-O-PO_3H_2 termini. The most important progress in the studies on the enzyme-mediated nucleic acid hydrolysis and phosphoryl transfer is that a pentacoordinate

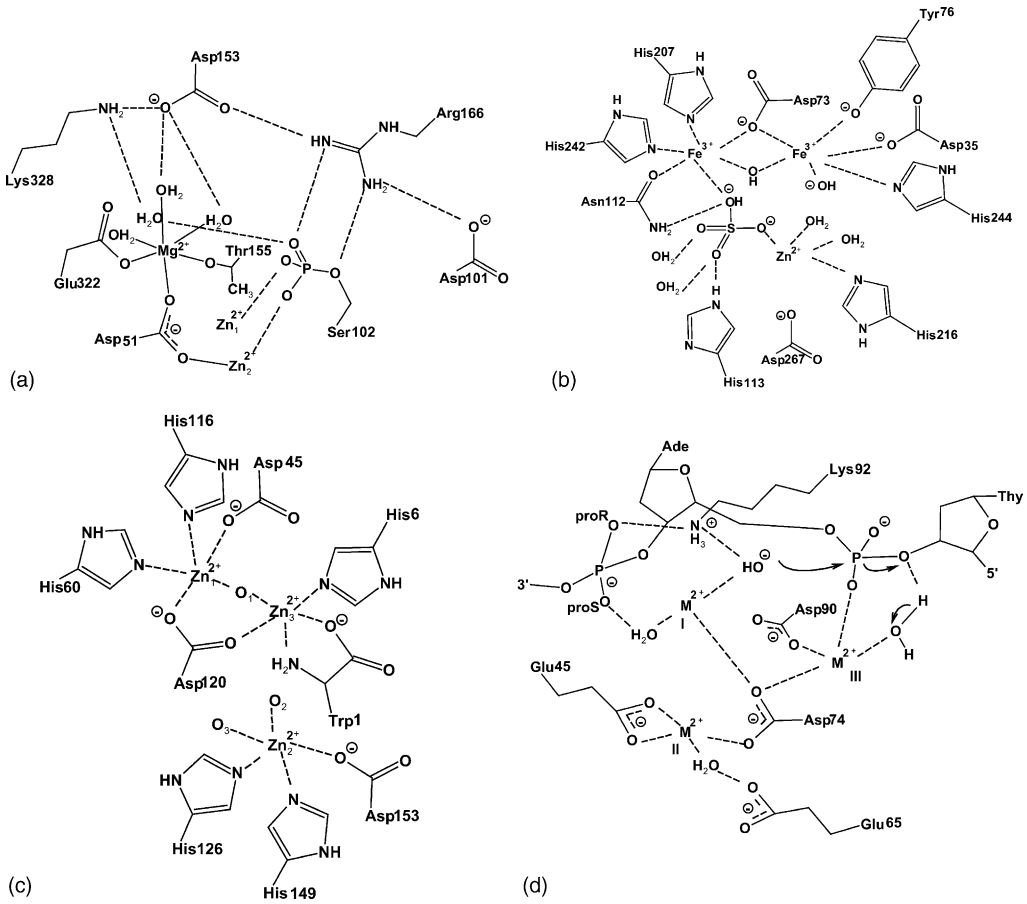


Fig. 1. Structures of dinuclear and multinuclear metal active sites in nucleic acid hydrolases: (a), alkaline phosphatase; (b) mammalian purple acid phosphatase; (c) P1 nuclease; (d) *Eco*RV-substrate complex.

oxyphosphorane intermediate of the enzyme-Mg^{2+}-glucose 1,6-(bis)phosphate complex formed in the isomerization of β-glucose 1-phosphate to β-glucose 6-phosphate catalyzed by β-phosphoglucomutase has just been so gratifyingly confirmed by X-ray crystal diffraction, as shown Fig. 2 [21].

Currently, there are more and more frequent examples of catalytic systems based on two and even more metal ions [2,6,7,9,22], due to the fact that some enzymes, catalyzing the hydrolysis of phosphate ester, are activated by two or more metal ions [23]. These di- and multi-nuclear metal-based catalytic systems are considerably helpful in developing more reactive and efficient chemical nucleases with sequence or structure specificity. However, one of the challenging problems faced by the elegant di- and multi-nuclear metal complexes is to elucidate the Structure–function relationships for the di- and multi-nuclear complexes and to identify the roles of the metal ions.

Hydrolysis requires a metal cofactor capable of binding hard oxygen atoms, polarizing bonds (Lewis acidity), and rapid ligand exchange for catalytic turnover. In general, there are three direct (inner sphere) modes of activation that a metal ion can cooperatively provide for accelerating the rate of phosphate ester hydrolysis [6]. The first is the Lewis acid activation that correlates with metal ionization potential, in which the p*Ka* of a metal-bound water nucleophile is lowered and a phosphoryl oxygen atom coordinates to a metal, for example, the substitutionally inert binuclear Co^{III} and Cu^{II} complexes [24–26]. The second is the nucleophile activation in which a nucleophile such as hydroxide coordinates to the metals, for example, Cu^{II} hydroxides and alkoxides. The third is leaving group activation in which a leaving group oxygen atom coordinates to the metals. DNA hydrolysis requires nucleophile activation in addition to double Lewis acid activation, while double Lewis acid activation alone is enough to rapidly hydrolyze RNA. Additionally, three indirect (outer sphere) modes of activation mediated by the metal ions are also included in DNA hydrolysis [6].

2. Learning from type II restriction enzymes

Enzymes that hydrolyze nucleic acids evolve to neutralize the strongly anionic charge of the substrate and subsequently mediate catalysis. Divalent metal ions are uniquely suited for participation in these mechanistic duties. Perhaps due to the universal nature of nuclease activity, it is significant to understand exactly how divalent metal ions support this common hydrolytic activity. Among the best studied

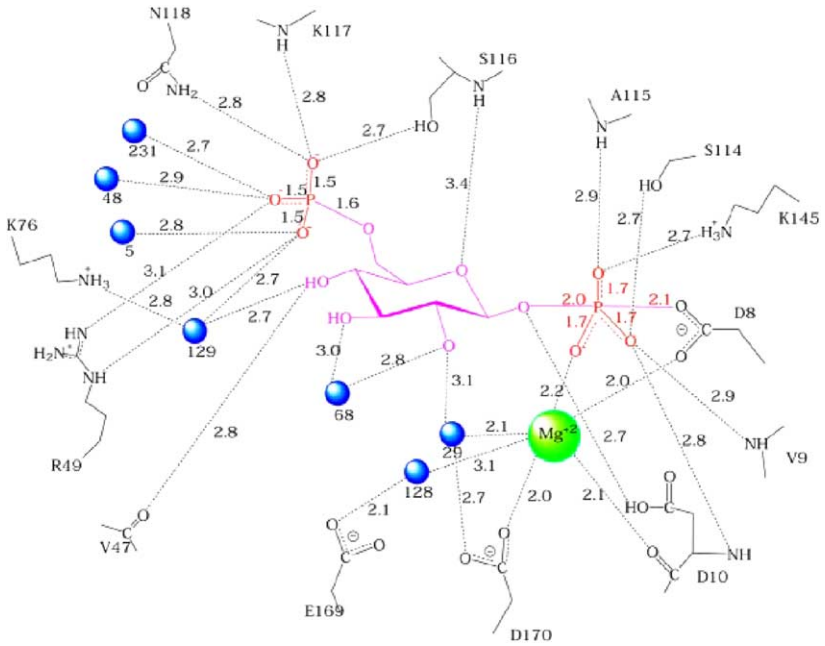


Fig. 2. Schematic illustration of interactions between the β-G 1,6-pentacoordinate phosphorous intermediate and β-PGM with all hydrogen bonds (<4 Å) depicted as dashed lines and labeled with bond length. Water molecules are shown as blue sphere [21]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

nuclease are restriction endonucleases, mono-, di- and even multi-nuclear metal-dependent enzymes which recognize and cleave 4-6 base pair sequences of DNA with a high degree of specificity [27]. However, the focus of study has long been on the fundamental biochemical, structural, kinetic and catalytic features of restriction endonuclease-DNA interactions [28]. Here we will briefly outline the structures of and catalytic mechanisms of two-metal active sites in type II restriction endonucleases, in order to explain and compare the DNA hydrolysis mechanisms promoted by di- and multi-nuclear metal complexes.

There are two main varieties of active metal sites in type II restriction endonucleases [29]. The enzymes *Bgl*II and *Eco*RI have been observed to contain a single metal binding site per subunit [30]. In contrast, the enzymes *Bam*HI, *Bgl*I, *Pvu*II, and *Ngo*MIV have been observed to contain two metal binding sites per subunit (Fig. 3) [31]. The structure of *Bam*HI has been determined in both substrate and product complexes, indicating that a two-metal mechanism is responsible for endonuclease activity. One of the two metal-binding sites is homologous to the single site observed in *Bgl*II and *Eco*RI (M1, Fig. 3) [29]. This site is formed by the two conserved acidic residues, the backbone carbonyl of the hydrophobic residue, and a nonbridging oxygen from the scissile phosphate. The second metal site (M2) is created by the first conserved aspartic acid, a substrate nonbridging oxygen, and a complement of other acidic residues depending on the specific enzyme. In *Pvu*II, the backbone carbonyl of G56 completes the second metal site, while in *Bam*HI, it is the side chain E77.

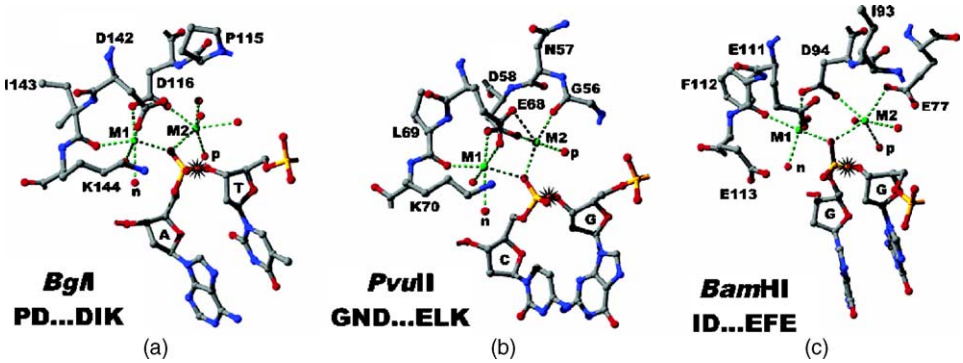


Fig. 3. Ball and stick representations of dinuclear metal active sites in type II restriction endonuclease-substrate complexes: *Bgl*I (a), *Pvu*II (b), *Bam*HI (c). Each active site is labeled along with its sequence motif and is shown in the same orientation for ease of comparison. The metal ion site that is conserved in all four structures is labeled M1, and the second site is labeled M2. Water molecules are labeled according to their proposed function: nucleophile (n) or proton donor (p). The scissile bond is denoted with an asterisk.

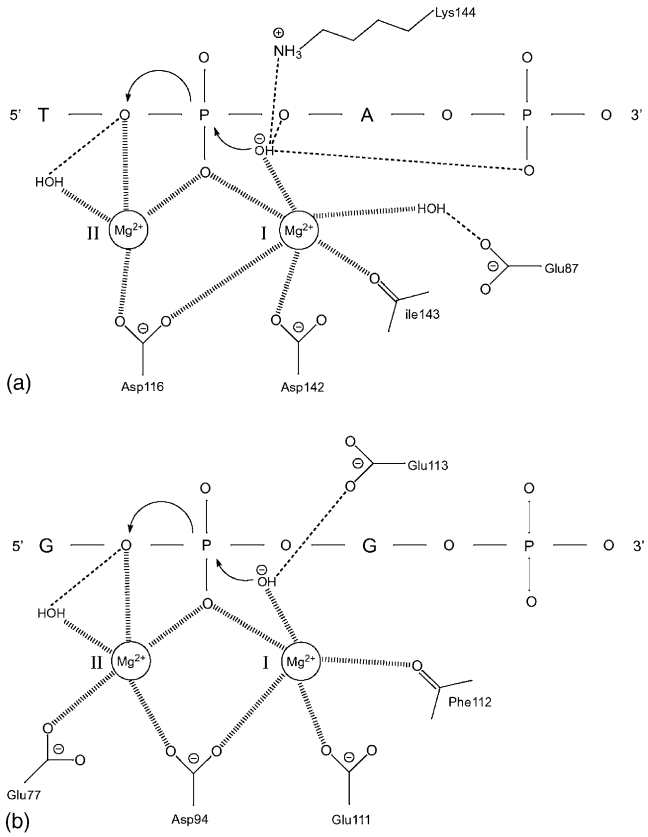


Fig. 4. Schemetic illustration of catalytic mechanisms proposed for type II restriction endonucleases. (a), Two-metal mechanism of cleavage proposed for *Bgl*I. A water molecule activated by metal I and hydrogen bonded to Lys144, the O5′ oxygen of adenosine, and the O1P oxygen of the guanosine performs an in-line attack of the scissile phosphate. The transition state is stabilized by the metals I and II. A water molecule bound to metal II acts as a general acid protonating the leaving group. (b) Two-metal mechanism of catalysis proposed for *Bam*HI. A water molecule bound to metal I is activated by Glu113 and attacks the scissile phosphate. Both metals act to stabilize the negatively charged pentavalent transition state. A water molecule bound to metal II donates a proton to the leaving group.

The precise mechanism by which type II restriction endonucleases catalyze DNA cleavage depends on the minimal number and positions of metal ions in the active site (Fig. 4). One of the two metal ions in the two-metal mechanism is responsible for positioning the nucleophilic water that is coordinated to the metal for in-line nucleophilic attack of the electrophilic phosphorus atom. The nucleophilic water molecule with a *p*Ka that is lowered makes hydrogen bonds to some protein residues, both bridging and nonbridging oxygen atoms from the scissile phosphate, in general [30]. Another metal-coordinated water molecule also makes hydrogen bonds to the leaving group oxygen. Here, it is possible that a bulk solvent-supplied metal-bound hydroxide ion that does not need to be deprotonated plays an important role in the DNA hydrolytic course [28].

The second metal is located on the other side of the scissile phosphate from the nucleophilic water molecule and is well positioned to activate a water molecule for protonation of the leaving group oxygen and to help stabilization of the negative charge that develops in an associative transition state (Fig. 3). It is interesting that E113 in the Ca^{2+} derivative of *Bam*HI makes a hydrogen bond to the nucleophilic water molecule (2.9 Å) and represents a possible general base [32]. However, neither *Pvu*II nor *Bgl*I (Fig. 4a) has a potential general base positioned closed to the nucleophilic water, which results in mechanistic issues similar to those in the single-metal mechanism. Furthermore, a mutation of E113K in *Bam*HI inactivates the enzyme despite the fact that E113 is located in the lysine position of the conserved active site motif [33]. This divergence of enzyme active sites even between enzymes that belong to a putative two-metal subgroup of type II enzymes illustrates a crucial point [29]. Therefore, based on the current level of understanding, it seems completely possible for type II enzymes to use different hydrolytic mechanisms. Even the enzymes that have been observed to bind dinuclear metal ions might differ in their reaction mechanisms and reaction coordinates. For example, *Bam*HI (with a putative general base, Fig. 4b) might proceed along a fully associative path, while *Pvu*II that lacks a putative general base could catalyze the reaction through a more dissociative path [28].

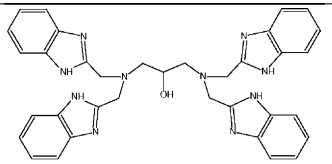
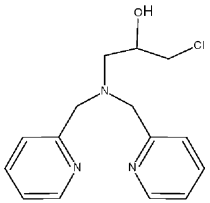
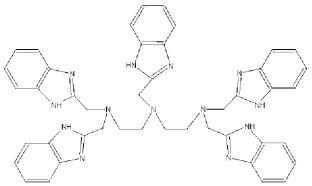
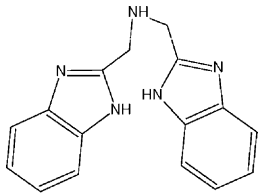
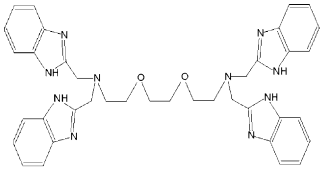
3. DNA hydrolysis promoted by di- and multi-nuclear metal complexes

There are two main reasons for studying simple di- and multi-nuclear metal complexes as artificial phosphoesterases. First, the relationship between the structure and reactivity is much easier to understand in simple model complexes than those of the enzymes themselves. Fundamental knowledge from such studies may then provide valuable insights into how the enzymes themselves function. Then, the metal complexes that can hydrolyze DNA sequence specifically are significant as tools for nucleic acid manipulation and as therapeutic agents. Therefore, over the past decade, a lot of elegant di- and multi-nuclear metal complexes have been developed as structural functional models of the phosphoesterases.

First-row transition metals are generally used as an agent that can mediate cleavage of phosphate diester backbone, due to virtue of their high electron density and Lewis acid activity. It is notable that almost all of the catalysts that have been designed are used to excess over nucleic acid substrate to obtain readily measurable activities, and so few have been tested for multi-turnover behavior. Therefore, there is much room for improvement relative to enzyme-mediated reactions.

Kinetic studies of the formation of both nicked and linear DNA have been reported for many di- and multi-nuclear metal complexes, summarized in Table 1, when the supercoiled DNA has been used as a substrate. It is not straightforward to compare these data with each other, since the studies were not necessarily carried out under analogous

Table 1
Ligand structures and kinetic parameters for DNA or model substrate hydrolysis catalyzed by dinuclear and multi-nuclear metal complexes

Complex	Structure	Substrate	r[a]	k (s^{-1})[b]	K_m (M)	k_{cat} (s^{-1})	α[c]	β[d]	Reference
$Fe_2(HPTB)(OH)(NO_3)_2$	 HPTB	pBR322 DNA	<0.22						[34]
$[Fe_2(BPClNOL)_2(H_2O)_2]^{2+}$	 BPClNOL	pBSKII/genomic DNA	<4.87						[37]
$Fe_2(DTPB)(\mu\text{-}O)(\mu\text{-}OAc)Cl(BF_4)_2$	 DTPB	pBR322 DNA	1.30	1.2×10^{-3}[e] 2.1×10^{-3}[f]				1.2×10^{8}[e] 2.1×10^{8}[f]	[38]
$Zn_2(DTPB)Cl_4$		pBR322 DNA	0.63	5.3×10^{-5}[e] 2.4×10^{-5}[f]				5.3×10^{6}[e] 2.4×10^{6}[f]	[57]
$Fe_2(IDB)_2(\mu\text{-}O)(\mu\text{-}OAc)_2Cl_2$	 IDB	pBR322 DNA	0.65						[38]
$Fe_2(EGTB)(Im^-)Cl_5$	 EGTB	pBR322 DNA	0.65						[38]

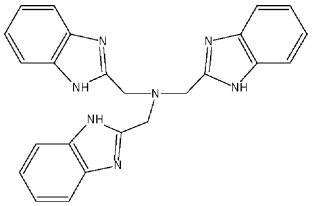
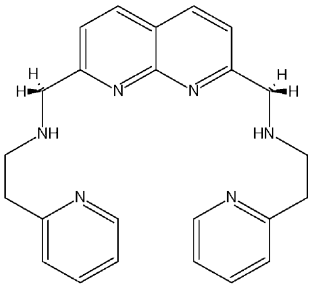
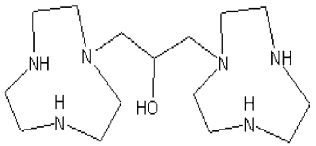
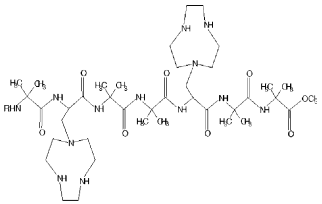
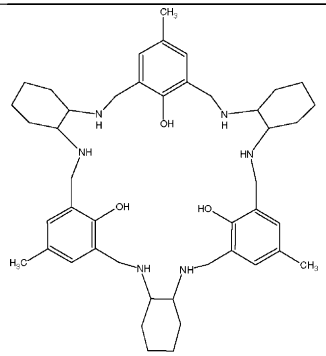
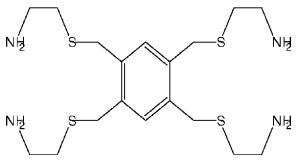
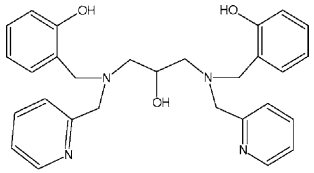
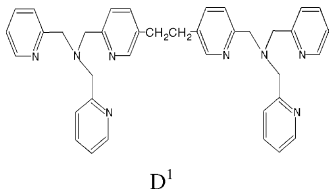
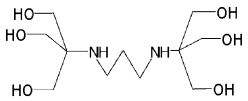
$[Fe_4(NTB)_4(\mu\text{-}O)_2(\mu_4\text{-}Suc)]^{6+}$	 NTB	pBR322 DNA	0.04 ∼ 0.65	2.2×10^{-5} ∼ 1.4×10^{-4}	7.4×10^{-6}	2.4×10^{-4}		2.4×10^{7}	[41]
$[Zn_2(BPAN)(\mu\text{-}OH)(\mu\text{-}O_2PPh_2)]^{2+}$	 BPAN	BNPP	0.1	$(6.5 \pm 0.7) \times 10^{-7}$	$(1.2 \pm 3.1) \times 10^{-2}$	$(4.1 \pm 1.2) \times 10^{-6}$	1.8	1.8×10^{5}	[50]
Zn_2(L2O)	 L2O	HPNP		$0.71\,M^{-1}\,s^{-1}$[g]			11.6		[52]
Zn_2HP	 HP	pBR322 DNA	0.3	1.0×10^{-5}				1.0×10^{6}	[53]

Table 1 (*Continued*)

Complex	Structure	Substrate	r[a]	k (s^{-1})[b]	K_m (M)	k_{cat} (s^{-1})	α[c]	β[d]	Reference
$Zn_3L1(\mu\text{-OAc})(H_2O)_2(ClO_4)_2$	 L1	CTDNA							[59]
$Cu_2(N_4S_4)$	 N_4S_4	pBluescript DNA	0.41	3.3×10^{-5}				3.3×10^{6}	[64]
$Cu_2(H_2bbppnol)(\mu\text{-Oac})(H_2O)_2Cl_2$	 $H_2bbppnol$	BNPP pBSKII/genomic DNA	10 ∼ 100 <48.70	$5 \times 10^{-5} \sim 4.5 \times 10^{-5}$ 6.7×10^{-5}	2×10^{-3}	5.4×10^{-5}		26 6.7×10^{6}	[65]
$Cu_2(D^1)(H_2O)_2(ClO_4)_2$	 D^1	Oligodeoxynucleotides	1 ∼ 10						[67]
$Ln_2(BTP)_2(OH)_n^{6-n}$	 BTP	BNPP	>50	$\sim 10^{-4}$				$\sim 2.0 \times 10^{7}$	[72]

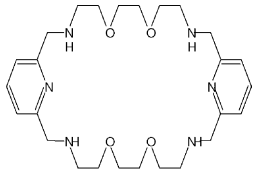
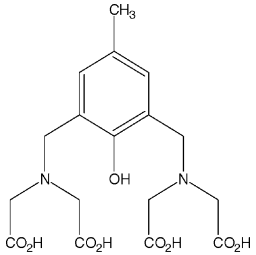
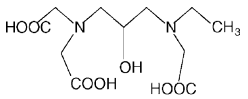
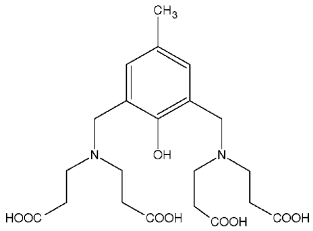
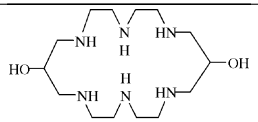
Pr_2(Macro)	 Macro	pBR322 DNA	51.28		3.0×10^{-4}	2.5×10^{-4}	2.5×10^{7}	[75]
Ce_2(HXTA)	 HXTA	Litmus 29 DNA	0.06 ~ 0.65	$5(4) \times 10^{-4}$ 5.8×10^{-5}			~1×10^{7}	[78]
Ce_2(HPTA)	 HPTA	Litmus 29/pBR322 DNA	~0.65					[79]
La_2(HPTA)	 HXTP	Litmus 29/pBR322 DNA	~0.65					
Ce_2(HXTP)		Litmus 29 DNA	~0.65					[78]

Table 1 (*Continued*)

Complex	Structure	Substrate	r[a]	k (s^{-1})[b]	K_m (M)	k_{cat} (s^{-1})	α[c]	β[d]	Reference
M_2-L2 M: Co^{2+}, Ni^{2+}, Cu^{2+}, and Ca^{2+}	 L2	pBR322 DNA	16.4	8.0×10^{-4} for M: Co^{2+}			1.6-10	8.0×10^{7} for M: Co^{2+}	[86]
BamHI		oligodeoxynucleotides		2.6×10^{5} [h]	2.5×10^{-6}	0.66		6.6×10^{10}	[108]
Mg-EcoRV		oligodeoxynucleotides		1.4×10^{6} [h]	5.5×10^{-7}	0.75		7.5×10^{10}	[109]
Alkaline phosphatase		p-Nitrophenyl phosphate		4.5×10^{6} [h]	1.0×10^{-5}	44.8			[110]
Purple acid phosphatase		ATP		7.3×10^{5} [h]	5.3×10^{-4}	390			[111]

[a] The catalyst/substrate concentration ratio.
[b] The pseudo-first-order rate constant at a given concentration.
[c] The ratio of the hydrolytic rate constant mediated by a dinuclear or multinuclear complex to that by the analogous mononuclear complex under identical or similar conditions.
[d] Rate enhancement over unhydrolyzed double-stranded DNA where $k = 1 \times 10^{-11}$.
[e] The pseudo-first-order rate constant at a given concentration for the disappearance of supercoiled DNA.
[f] The pseudo-first-order rate constant at a given concentration for formation of linear DNA from supercoiled DNA.
[g] The value is a second-order rate constant.
[h] The k_{cat}/K_m ($M^{-1}s^{-1}$) ratio.

conditions and the catalyst/substrate concentration ratios differ over a large range of values. However, in order to be able to conveniently compare these kinetic data, we define two parameters α and β that are associated with the kinetic studies. The first one α is defined as the ratio of the hydrolytic rate constant mediated by a di- or multi-nuclear complex to that by the analogous mononuclear complex under identical or similar conditions, indicating the degree of cooperativity between metal sites. The cooperativity plays important roles in the activation of substrates, deprotonation of water molecule, stabilization of transition states and recognition and selection of substrates. The α values that are more or less than, or equal to 2 correspond to positive, negative or non cooperativity between metal sites, respectively. The α values have been rarely reported, because of difficulty in determinations. The second parameter β is recognized as a rate enhancement over unhydrolyzed double-stranded DNA. It is not difficult to obtain a β value for the DNA substrate.

3.1. Di- and multi-nuclear iron complexes

Di- and multi-nuclear ferric complexes are one of such DNA hydrolysis systems frequently reported. The dinuclear ferric complex Fe_2(HPTB)(OH)$(NO_3)_4$ (see Table 1, HPTB = *N*,*N*,*N*′,*N*′-tetrakis (2-benzimidazolylmethyl)-2-hydroxyl-1,3-diaminopropane) reported early by Que promoted plasmid DNA cleavage via "hydrolytic fashion" in the presence of hydrogen peroxide or O_2 and reductant [34]. However, this situation is similar to the DNA cleavage mediated by the Fe-bleomycin complex and its mimetic compounds [35] in a sense.

In the catalytic hydrolysis of bis(2,4-dinitrophenyl) phosphate (BDNPP) as a model of DNA by Fe_2(μ-O)$(phen)_4(H_2O)_2$ (phen: 1,10-phenanthroline), the two Fe^{3+}-bound water molecules were deprotonated with pK_a = 5.0 and 6.5. One of the ferric ions acts as a Lewis acid activator of the substrate, whereas another provides the nucleophilic hydroxide ion [36]. DNA hydrolysis promoted by the dinuclear trivalent iron complex $[Fe_2(BPClNOL)_2(H_2O)_2]^{2+}$ (indeed a dimer, see Table 1, BPClNOL: *N*-(2-hydroxylbenzyl)-*N*-(pyridylmethyl)[(3-chloro)(2-hydroxyl)]propylamine) has been reported [37]. This complex is only active for pBSKII plasmid DNA with a bound hydroxide, but is not for BDNPP under mild conditions (pH 6.1–8.0). This result has implied that the conclusions from model substrate must be carefully extended to the double-stranded DNA, because the hydrolysis degree varies with the removal easiness of leaving groups.

Supercoiled DNA hydrolytic cleavage promoted by the dinuclear trivalent iron complexes $[Fe_2$(DTPB)(μ-O)(μ-OAc)$]Cl(BF_4)_2$, $[Fe_2(IDB)_2$(μ-O)(μ-OAc)$_2]Cl_2$ (structure shown in Fig. 5a), and Fe_2(EGTB)$(Im^-)Cl_5$ (DTPB: 1,1,4,7,7-penta (2′-benzimidazol-2-yl-methyl)-triazaheptane, IDB: *N*,*N*-bis(2′-benzimidazol-2-yl-methyl) amine, EGTB: *N*,*N*,*N*′,*N*′-tetrakis(2′-benzimidazol-2-yl-methyl)- 1,4-bis-(ethylamino)-bis(ether), Im^-: imidazole anion and OAc = acetate) is supported by the evidence from anaerobic reactions, free radical quenching, high performance liquid chromatography experiments and enzymatic manipulation such as T4 ligase ligation, 5′-^{32}P end-labeling and footprinting analysis [38]. The competence that these dinuclear iron complexes degrade DNA under the nonoxidative conditions decreases in the order of appearance of linearized DNA: Fe_2(DTPB)(μ-O)(μ-Ac)$Cl(BF_4)_2$ > Fe_2(EGTB)Cl_6 > $Fe_2(IDB)_2$(μ-O)(μ-Ac)$_2Cl_2$. It is not difficult to understand this activity order according to the composition of ligand spheres and structures of these dinuclear metal complexes (vide infra). The estimation of rate for the supercoiled DNA double strand cleavage by $[Fe_2$(DTPB)(μ-O)(μ-OAc)Cl$](BF_4)_2$ shows one of the largest known rate enhancement factor of $\sim 10^8$ against supercoiled DNA. Moreover, the DNA hydrolysis chemistry needs no co-reactant such as hydrogen peroxide. In addition, Fe_2(DTPB)(μ-O)(μ-Ac)$Cl(BF_4)_2$ can also catalyze the hydrolysis of the linear DNA, such as calf thymus DNA and linearized pBR322 DNA. However, the poor sequence-specific DNA cleavage indicated by the restriction analysis of the pBR322 DNA linearized by this dinuclear iron complex might be due to its interaction with DNA by a coordination of its two ferric ions to the DNA phosphate oxygens, as suggested by spectral characterizations. Concerning the hydrolytic mechanisms, this diiron complex might share many points in common with the native purple acid phosphatases [39].

We find in a current study that a tetranuclear trivalent iron complex $[Fe_4(NTB)_4$(μ-O)$_2$(μ$_4$-Suc)$]^{6+}$ [40] (structure shown in Fig. 5b, Suc: succinate, NTB: tris(2-benzimidazolylmethyl)amine) catalyzes DNA hydrolysis in pH < 7 acetate buffer, confirmed by anaerobic reactions, high performance liquid chromatography experiments and enzymatic manipulation such as T4 ligase ligation, 5′-^{32}P end-labeling and footprinting analysis [41]. Like $[Fe_2$(DTPB)(μ-O)(μ-OAc)Cl$](BF_4)_2$, the addition of phosphate can inhibit this reactivity. The reason may be that the phosphate ligation to the ferric ions in this tetra-iron complex can interfere the coordination binding between the ferric ions and the phosphate groups from DNA. The catalytic rate constant (k_{cat}) and Michaelis–Menten constant (K_m) are $0.85\,s^{-1}$ and 7.4×10^{-6} M, respectively, in the presence of plasmid DNA as a substrate. These values are more than k_{cat} values, but less than K_m values by $[Zn_2$(BPAN)(μ-OH)(μ-$O_2PPh_2)](ClO_4)_2$ [19] and the analogous mononuclear complex, respectively, where the substrate is a DNA model compound of bis(*p*-nitrophenyl) phosphate (BNPP) [42]. Although this is the first well-characterized tetranuclear metal complex that can mediate DNA hydrolysis with the high rate constant, its kinetic parameters are far less than those by natural enzymes.

3.2. Di- and multi-nuclear zinc complexes

Zn-binding sites in proteins can be divided into two categories: (1) sites that play predominantly a catalytic role, and

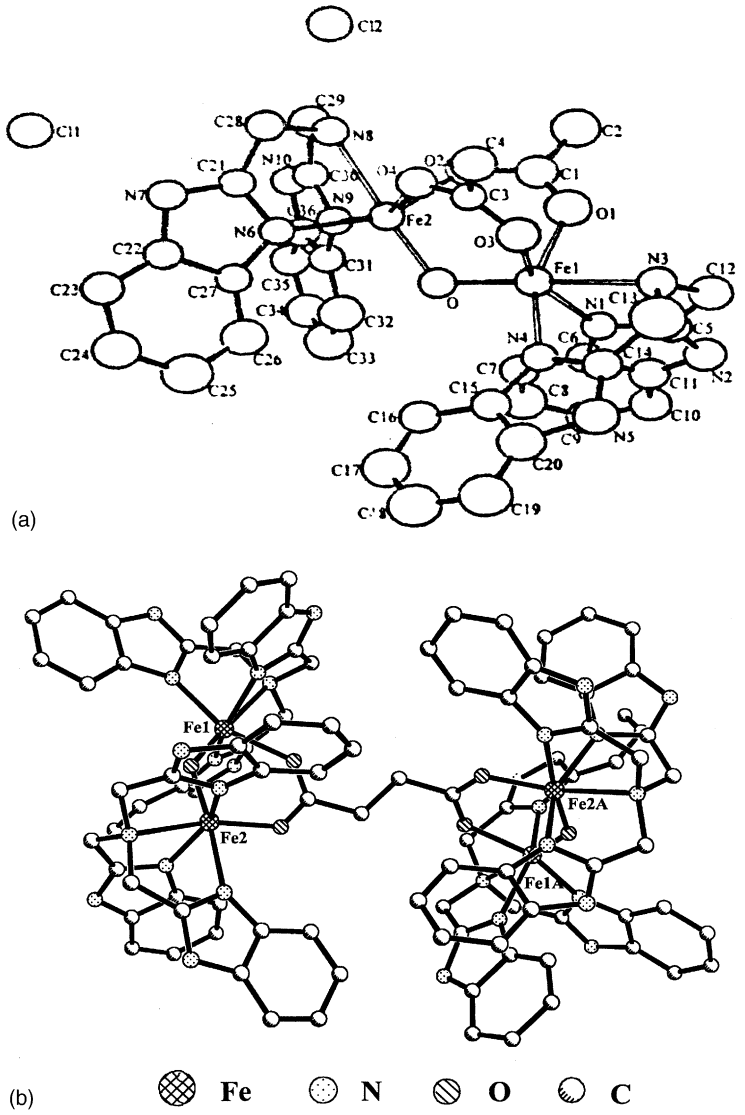


Fig. 5. Molecular structures of dinuclear and tetranuclear iron complexes $[Fe_2(IDB)_2(\mu_2\text{-}O)(\mu_2\text{-}OAc)_2]^{2+}$ [40] and $[Fe_4(NTB)_4(\mu\text{-}O)_2(\mu_4\text{-}Suc)]^{6+}$ [43].

(2) sites that serve only a structural role. The most common Zn sites in first category are 4- and 5-coordinate Zn complexes that are consisted of His_2(His/Asp/Glu)Water and are partially exposed to solvent [44]. The majority of di- and multi-nuclear Zn complexes that can promote nucleic acid hydrolysis have also geometries of 4-coordinate tetrahedron and 5-coordinate trigonal bipyramid, respectively.

The early reported dinuclear zinc complexes are Zn-aminomethylimidazole complexes held in proximity by flexible spacers. The activities are only two times higher ($\alpha = 2$) than their mononuclear analogue in the hydrolysis of the triester tris(*p*)nitrophenyl phosphate (TPNP), due to the lack of cooperative catalysis between two Zn^{2+} ions [45]. The catalytic phosphate ester cleavage by positively cooperating metal ions was reported by Breslow group by using a phenyl or alkyl spacer to orient two tridentate macrocyclic ligands known to form extremely stable Zn complexes [46]. These dinuclear zinc complexes exhibited rate accelerations of up to 10^4-fold ($\alpha \sim 10^4$), when the examined substrate were monoester *p*-nitrophenyl phosphate (PNP) or diester BNPP. The dinuclear zinc complexes based an azamacrocycle reported by Bianchi group also showed no or little positively cooperative character in the presence of BNPP as a substrate [7,22,47–49].

Lippard group has chosen a stable dinuclear complex $[Zn_2(BPAN)(\mu\text{-}OH)(\mu\text{-}O_2PPh_2)](ClO_4)_2$ (BPAN: 2,7-bis[2-(2-pyridylethyl)-aminomethyl]-1,8-naphthyridine) as a model to investigate the reactivity of di-zinc(μ-OH) centers in metallohydrolase [50]. They studied the hydrolysis of phosphate diesters in the presence of BNPP as the substrate of this dinuclear zinc complex to model process catalyzed in vivo by zinc-containing P1 nuclease. In the first step of hydrolysis promoted by this di-zinc complex, the substrate replaces the bridging diphenylphosphate. The bridging hydroxide serves as a general base to deprotonate water, which acts as a nucleophile in the ensuing

hydrolysis. Kinetic and mechanistic studies indicated that the bridging hydroxide in this complex is not very reactive, despite its low p*Ka*. Its reactivity is only 1.8 times more than that of a mononuclear analogue, $Zn(BPTA)(OTf)_2$ (BPTA: *N*,*N*-bis(2-pyridylmethyl)- *tert*-butylamine, OTf: $CF_3O_2SO^-$), in hydrolyzing phosphate diester. This low or negative cooperativity has been observed in other dinuclear zinc complexes and presumably arises from the two factors. First, the bridging hydroxide and coordinated substrate in $[Zn_2(BPAN)(\mu\text{-}OH)(substrate)]^{2+}$ are not aligned properly to favor nucleophilic attack. Second, the nucleophilicity of the bridging hydroxide is diminished because it is simultaneously bound to the two Zn^{2+} ions [50].

In order to study catalytic cooperativity between two metal centers, Reinhoudt group designed many dinuclear zinc complexes on a calix [4] arene-based molecular scaffold and found that their phosphate diesterase activity is much higher than their mononuclear zinc analogues. Yamada et al also reported phosphate diester bond cleavage mediated by a cyclic β–sheet peptide-based dinuclear zinc complex [51]. However, their substrates are mainly RNA and RNA models [9].

The dinuclear zinc complex $Zn_2(L2O)$ (see Table 1 and Fig. 6a) and its mononuclear analogue Zn(L1OH) (see Fig. 6b) were studied as catalysts of the cleavage of the phosphate diester 2-hydroxypropyl-4- nitrophenyl phosphate (HPNP) [52]. The alcohol linker in the $Zn_2(L2O)$ is ionized below pH 6.0, while the alcohol group in Zn(L1OH) remains protonated even at high pH. The pH-rate profiles show that the second-order rate constants for these zinc complex-catalyzed cleavage of HPNP are $0.71\,M^{-1}\,s^{-1}$ for $Zn_2(L2O)$ and $0.061\,M^{-1}\,s^{-1}$ for Zn(L1OH) at high pH, respectively. The larger catalytic activity of $Zn_2(L2O)$ ($\alpha = 11.6$) compared to Zn(L1OH) is due to the cooperative role of the metal ions in facilitating the formation of the ionized zinc-bound water at close to neutral pH and providing additional stabilization of the rate-limiting transition state for phosphodiester cleavage.

The well-characterized dinuclear zinc complexes are the dinuclear hydrolytic systems of DNA based on azamacrocycles. The dinuclear Zn^{2+} complex (Zn_2HP, see Table 1) of 3_{10}-helical synthetic heptapeptides modified with two triazacyclononane rings shows a nuclease activity [53]. Its two metal centers are faced each other and are placed ∼ 6 Å apart, a distance close to that (∼ 7 Å) between two consecutive phosphate groups in a B-DNA. This complex more efficiently catalyzes plasmid DNA hydrolytic cleavage with clear evidence of cooperativity between the two Zn^{2+} centers relative to the RNA substrate model 2-hydroxypropyl-*p*-nitrophenyl phosphate, possible reflecting stronger DNA binding. Its reactivity is >20 times more than that of its mononuclear analogue. The rate constant measured for the reaction of 3.6 μM of this dinuclear zinc complex with 12 μM (bp) pBR322 DNA at 37 °C and pH 7.0 is $\sim 10^{-5}\,s^{-1}$, a value that amount to a 10 million-fold rate acceleration over that for the uncatalyzed DNA hydrolysis. This value is comparable to that reported by Barton with a mononuclear Zn^{2+}-binding peptide tethered to a rhodium intercalator [54]. However, with a large excess of the complex, formation of the nicked product is somewhat limited, even after 24 h. Akkaya also observed remarkable cooperative action of two Zn^{2+} centers similar to those in Zn_2HP in plasmid DNA hydrolysis, while the spacer between these two Zn^{2+} centers is phenyl group [55].

We reported recently that the dinuclear zinc complex $Zn_2(DTPB)Cl_4$, first synthesized by Reedijk and co-workers [56], is also an efficient promoter for supercoiled DNA hydrolytic cleavage [57]. The estimation of disappearing

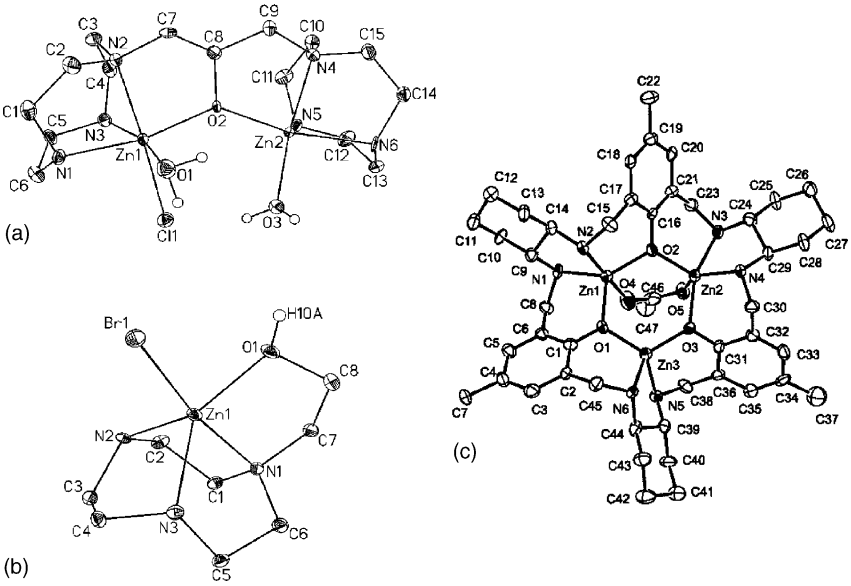


Fig. 6. Molecular structures of the dinuclear zinc complex $Zn_2(L2O)$ (a) and its mononuclear analogue Zn(L1OH) (b) [52] and trinuclear zinc complex $[Zn_3L1(\mu\text{-}OAc)]^+$ (c) [59].

rate of supercoiled DNA indicates that the approximate pseudo first-order rate constant is $2.4 \times 10^{-5}\,s^{-1}$, a value close to that by Zn_2HP. The linear calf thymus DNA hydrolysis promoted by this dinuclear zinc complex was observed. The reaction conditions for $Zn_2(DTPB)Cl_4$ to exhibit its optimal DNA hydrolytic activity are in the neutral or slightly acidic buffers with lower ion strength. The DNA fragments mediated by the hydrolytic cleaver can be enzymatically ligated and end-labeled. The restriction analysis reveals that pBR322 DNA is preferentially cut by $Zn_2(DTPB)Cl_4$ at the sites near bp 570, 1500, 2700 and 4160. What is interesting about these sites is that they consist mainly of multiplet nucleotides A and C. However, Kimura group demonstrated that the di- and tri-nuclear Zn^{2+} complexes of multi-azamacrocycle ligands selectively bind to T in single- and double-stranded DNA [10,58].

The cation structure of the trizinc complex $[Zn_3L1(\mu\text{-}OAc)](ClO_4)(PF_6)\cdot 5CHCl_3\cdot H_2O$ (L1: an optically active hexaaza triphenolic macrocycle, see Table 1 and Fig. 6c) closely resembles the trinuclear Zn^{2+} active site of P1 nuclease [59]. Although this complex is the first trizinc complex that can cleave linear calf thymus DNA at 37 and 50 °C, any kinetic data were not reported. In addition, the trinuclear Zn^{2+} calix[4]arene complexes can promoted RNA dinucleotide cleavage [9].

It is notable that an allosteric supramolecular catalyst ($TrenP_3$) obtained by connecting three copies of the heptapeptide P (*P*: H-Iva-Api-ATANP-Iva-Api-Iva-$NHCH_3$, Iva: (*S*)-isovaline, Api: 4-amino-4-carboxy piperidine, ATANP: (*S*)-2-amino-3-[1-(1,4,7-triazacyclononane)] propanoic acid) to a functionalized tris(2-aminoethyl)amine (Tren) platform was able to bind four Zn^{2+} ions, one in the Tren subsite and three in the azacyclononane subunits [60]. This molecule shows a peculiar behavior, i.e. the tetrazinc complex catalyzes the cleavage of 2-hydroxypropyl-*p*-nitrophenyl phosphate (HPNP), whereas the free ligand is a catalyst of the cleavage of an oligomeric RNA sequence with selectivity for pyrimidine bases. In the case of HPNP, Zn^{2+} acts as a positive allosteric effector by enhancing the catalytic efficiency of the system. In the case of polyanionic RNA substrate, Zn^{2+} switches off the activity, thus behaving as a negative allosteric regulator. The opposite behavior of the catalyst induced by Zn^{2+} seems to be associated with the change of conformation of the Tren platform, and consequently of the relative spatial disposition of the three linked peptides, that occurs after binding of the metal ions.

On the basis of the above-mentioned results and other currently available data, the complexes Zn_2HP and $Zn_2(DTPB)Cl_4$ only exhibit a relatively high reactivity, likely because of the presence of Zn^{2+}-coordinated water molecules as a nucleophile. It appears that dinuclear zinc complexes and even the enzymatic dinuclear zinc active sites are not very advantageous for nucleophilic reactivity over their mononuclear analogues [50,61].

3.3. Dinuclear copper complexes

Chin group is one of the first to report the high catalytic activity of dinuclear Cu^{2+} complexes in phosphate diester cleavage [6,7]. They demonstrated that many dicopper complexes based di-triazamacrocycle, di-pyridyl or tetra-benzimidazloe display a high cooperativity and an enormous rate acceleration in the RNA model compound or the RNA dinucleotides. Many di- and tri-nuclear copper complexes on a calix[4]arene-based molecular scaffold also display similar reactivity [9]. The recently reported dinuclear $Cu_2(tdciH_{-3})$ (tdci: 1,3,5-trideoxy-1,3,5-tris(dimenthylamino)-*cis*-inositol) species, formed around pH 8.5, provides an outstanding rate acceleration for the selective hydrolysis of the activated BNPP [62]. In addition, mononuclear complex $Cu(tach\text{-}Me_3)^{2+}$ (tach-Me_3: *N*,*N*′,*N*″-trimethyl-*cis*-1,3,5-triaminocyclohexane) is converted into a dicopper complex in the presence of BNPP and is selective for hydrolytic cleavage of BNPP [42]. Some dinuclear Cu^{II} complexes provide five orders of magnitude rate acceleration for cleaving ApA, but give eight orders of magnitude rate acceleration for hydrolyzing 2′,3′-cAMP [63]. This additional rate acceleration may be due to the intramolecular metal-hydroxide nucleophile in addition to the double Lewis acid activation because the metal-hydroxide could be providing general base catalysis [6].

Plasmid DNA cleavage promoted by the dinuclear copper complex $Cu^{II}_2(N_4S_4)$ (N_4S_4: 1,2,4,5-tetrakis(1′-amino-3′-thiobutyl)benzene) is significantly greater than that by its mononuclear analogues, which is essentially inactive [64]. An apparent rate constant of $3.3 \times 10^{-5}\,s^{-1}$ was reported at pH 7.8 and 37 °C, when concentrations of this complex and of plasmid DNA were 75 μM and 0.12 mg/ml, respectively. A synergy between the two metal ions was suggested, although the enhanced electrostatic effect of two cupric ions provides a reasonable explanation. Distinct binding of substrate and formation of an active Cu^{2+}–OH species were suggested.

The dinuclear divalent copper complex $[Cu_2(H_2bbppnol)(\mu\text{-}OAc)(H_2O)_2]Cl_2\cdot 2H_2O$ ($H_2bbppnol$: *N*,*N*′,*N*,*N*′-bis[(2-hydroxylbenzyl)(2-pyridylmethyl)]-2-ol-1,3-propanediamine) effectively promoted the cleavage of double-stranded plasmid and genomic DNA under physiological conditions [65]. Rossi et al. strongly suggested that this dicopper complex mediates a hydrolytic attack on the phosphate diester backbone through cooperativity between the two cupric ions, a mechanism proposed for the reaction of this complex with the model substrate BDNPP. The rate constant of $6.7 \times 10^{-5}\,s^{-1}$ at pH 6.0 and 50 °C represents about a 6.7×10^6-fold rate increase compared with the uncatalyzed hydrolysis rate of DNA.

Cowan group have shown that copper complexes of aminoglycoside antibiotics, such as neomycin B and kanamycin A, generate linear DNA using catalytic amounts of complex (~5 nM) and a large excess of plasmid DNA (~51 μM) in the presence of an external reducing

agent, such as ascorbic acid [66]. No linear DNA was observed in the absence of either external reducing agents or dioxygen. Moreover, these copper complexes might be mononuclear. The dinuclear Cu^{2+} compound $[Cu_2(D^1)(H_2O)_2](ClO_4)_4$ (see Table 1, D^1: dinucleating ligand with two tris(2-pyridylmethyl)-amine units covalently linked in their 5-pyridyl positions by a $–CH_2CH_2–$ bridge) reported by Karlin group selectively promotes DNA cleavage on oligonucleotide strands that extend from the 3′ side of frayed duplex structures at a site two residues displaced from the junction [67]. The DNA cleavage mechanism does not involve a diffusible radical species, whereas both a reductant and dioxygen are necessary. These two cases might be ascribed to DNA oxidative cleavage.

3.4. Dinuclear rare-earth complexes

There has been much interest in the development of lanthanide complexes as nucleic acid cleavage agents. This reflects the absence of redox chemistry and the high Lewis acidity associated with trivalent and tetravalent lanthanide ions. Also, these ions show fairly rapid ligand-exchange kinetics, favorable from the viewpoint of binding reactant and releasing product, but a problem that must be solved for the ligand itself [2].

It has been known for many decades that lanthanides as monomers, dimmers or gels are highly reactive for hydrolyzing phosphate ester bonds, including the robust bonds of DNA [4]. Dinuclear or higher order lanthanides for hydrolyzing phosphates are more difficult to solve than those of dinuclear transition metal complexes. The reasons are partly that ligand-free dinuclear or higher order lanthanides tend to be the most reactive but such species are difficult to solubilize and to let alone crystallize and characterize. Bracken et al has reported hydrolysis of BNPP as a DNA model by the lanthanide-containing micellar systems [68]. Chin group has proposed a unified mechanism for how these highly reactive lanthanide species may be working by combination of data from dinuclear transition metal complexes with the studies on lanthanide-promoted hydrolysis of phosphate diesters [6,7]. There is enormous cooperativity between hydrogen peroxide and Ln^{3+} ions for hydrolyzing activated phosphate diesters [69,70]. The simple active form of the Ln^{3+} complexes for hydrolyzing nucleic acids is a dinuclear species with two bridging peroxides as a nucleophilic catalyst, supported by a crystal structure of a dinuclear Ce^{4+} complex with bridging peroxide (Fig. 7a.) [71]. On the other hand, Chin group has also demonstrated by X-ray crystallography that phosphate diesters can bridge two Ln^{3+} centers as they bridge two alkaline earth or transition metal centers (Fig. 7b) [6]. Therefore, the mechanism of the hydrolysis reactions may involve nucleophilic attack of the bridging peroxide on the bridging nucleic acids.

The dinuclear hydroxo complexes $M_2(BTP)_2(OH)_n{}^{6-n}$ (M: La^{3+}, Pr^{3+}, Nd^{3+}, Eu^{3+}, Gd^{3+} and Dy^{3+}; $n = 2, 4, 5, 6$; BTP: 1,3-bis-tris(hydroxymethyl)aminomethane propane) are catalysts for hydrolysis of BNPP, mono-nitrophenyl phosphate (NPP) and 4-nitrophenyl acetate (NPA) [72]. For a given lanthanide ion, the second-order rate constants for the BNPP hydrolysis increase with increasing in the number n of coordinated hydroxide ions. In a series of complexes with a given n, the rate constants decrease in the order La > Pr > Nd > Eu > Gd > Dy, whereas the observed first-order rate constants at a given concentration and ∼pH 9 follow a different order La > Pr > Nd > Eu < Gd < Dy. NPP hydrolysis follows Michaelis–Menten-type "saturation" kinetics. This difference in kinetic behavior was attributed to stronger binding of NPP dianion than BNPP monoanion to the lanthanide species. Activities of $M_2(BTP)_2(OH)_n{}^{6-n}$ in the NPA hydrolysis, which is 10 times more reactive than BNPP in alkaline medium, are similar to those in BNPP hydrolysis, indicating unique capability of lanthanide cations to stabilize the transition state of phosphate diester hydrolysis. The results can be correlated with available data for other transition metal ions in terms of the Brønsted correlation and transition state-catalyst complexation strength [71]. Phosphate diester bonds can also be cleaved by dinuclear peroxide complexes $Y_2(O_2)_2{}^{2+}$ and $Y_2(O_2)_2(OH)_2$ via a hydrolytic pathway [73]. In addition, La^{3+} (Eu^{3+})-BTP complexes can also mediate phosphonoformate diester hydrolysis with C-OMe regiospecificity and substantial rate enhancement [74].

Schneider group reported that dinuclear Pr^{3+} and Eu^{3+} complexes (Pr_2/Eu_2(macro)) based on a range of supramolecular ligands consisted of 30-membered macrocyclic ring with six nitrogen and four oxygen ligating atoms accelerate the cleavage of supercoiled DNA by almost two orders of magnitude over that with the metal ions alone with apparent first-order rate constants of approximately $1 \times 10^{-4}\,s^{-1}$ in the case of Pr_2(macro) [75]. The relative

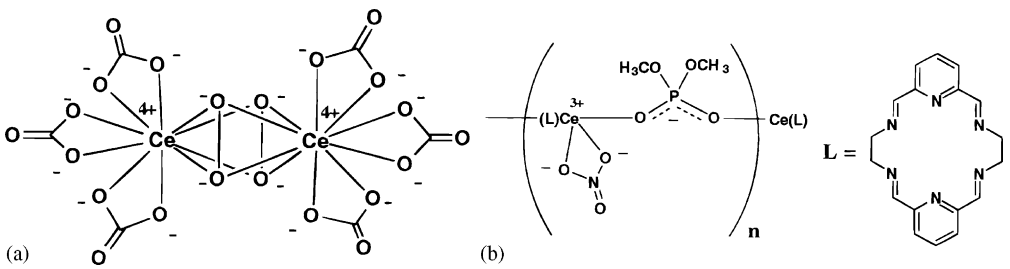


Fig. 7. Schematic illustration of a dinuclear Ce^{4+} complex with bridging peroxide (a) and phosphate diester- bridged two Ce^{4+} centers (b).

activity order of the complexes is similar to the cleavage of plasmid DNA and BNPP. Zhu et al have used dinuclear Ho^{3+} and Er^{3+} macrocyclic complexes at 37 °C and neutral pH to promote conversion of supercoiled DNA into nicked and linear forms [76]. Saturation kinetics performed under pseudo-Michaelis–Menten conditions yielded k_{obs} (k_{cat} ?) of $1.03 \times 10^{-3}\,s^{-1}$. The mechanisms of cleavage were not determined and are presumed to be hydrolytic.

Cerium, the only lanthanide that is easily oxidized to the tetravalent state, is the only lanthanide that hydrolyzes DNA at a reasonable rate [4,6,77]. A dinuclear tetravalent cerium complex Ce_2(HXTA) (HXTA: 5-methyl-2-hydroxyl-1,3-xylene-α,α-diamine-*N*,*N*,*N*′,*N*′-tetraacetic acid) has been shown to effect double-stranded cleavage by a hydrolytic pathway to yield linear plasmid DNA [78]. Although the yields of linear products were low (∼20%), the catalysts were functional with high regioselectivity at 37 °C and pH 8, and gave 5′-OPO_3- and 3′-OH-labeled end products in high yield (>90%), like the products of natural DNA hydrolyzing enzymes; a clear improvement over earlier complexes Ce_2(HPTA) and La_2(HPTA) (HPTA: 2-hydroxylpropenylene-1,3-diamine-*N*,*N*,*N*′,*N*′-tetraacetic acid) [79]. These two dinuclear complexes worked effectively at higher temperatures, i.e. 55 °C, but are inefficient at 37 °C. This group observed that the three dicerium complexes far to carry out double-strand hydrolysis of plasmid DNA exhibit the following order of increasing effectivity: Ce_2(HPTA) < Ce_2(HXTP) < Ce_2(HXTA) (HXTP: 5-methyl- 2-hydroxyl-1,3-xylene-α,α-diamine-*N*,*N*,*N*′,*N*′-tetrapropanoic acid). The kinetics of reactions leading to nicked and linear products for Ce_2(HXTA) were found to be similar, both being associated with apparent first-order rate constants of approximately $1 \times 10^{-4}\,s^{-1}$ for complex concentration of 10^{-5} to 10^{-4} M. The factors that affect the hydrolysis of the first and second DNA strands are similar. The rate determining step is likely cleavage of the phosphate diester bonds.

On the other hand, detail mechanistic studies on the DNA hydrolysis promoted by the dinuclear Ce^{4+} complexes have not been possible due to solubility problems with Ce^{4+} above pH 4. However, on the basis of studies related to other lanthanides and the detailed mechanistic evidence available from the dinuclear cobalt complexes, it is tempting to suggest that it is dinuclear Ce^{4+} with bridging peroxides, hydroxides or oxides that is the reactive species required for hydrolyzing DNA in acidic media. At near neutral pH, where the hydrolysis is biologically relevant, further aggregation is noticed, forming complicated gels. The dinuclear metallic hydroxo species in the gels seem to be the active species in hydrolysis [80].

3.5. Other dinuclear metal complexes

Many dinuclear nucleases are activated by Mg^{2+}, but Mg^{2+} is only a weak Lewis acid. To date very few functional dinuclear Mg^{2+} complexes have been developed. Carboxylate- bridged dimagnesium complex is the first dinuclear Mg^{2+} complex with a bridging phosphate [81]. The Yang group reported that the Mg complex of diethylenetriamine (dien) appeared in a Cl^--bridged dimer form of $[Mg_2(dien)_2Cl_3]^+$ and is a remarkably simple yet effective promoter for the hydrolytic cleavage of DNA [82]. In addition, Ye et al. synthesized an aqua-bridged dinuclear complex $M_2(\mu\text{-}H_2O)(\mu\text{-}OAc)_2(Im)_4(OAc)_2$ (M: Mg^{2+}, Mn^{2+}, Ni^{2+}; Im: imidazole) as a structural model for the active sites of dinuclear hydrolases [83].

Substitutionally inert Co^{3+} complexes consisting of a phosphate bound to one or two Co^{3+} centers can be considered as models for enzyme-substrate Michaelis–Menten complexes and can be unambiguously analyzed. In order to demonstrate phosphate monoesterase activity, Chin et al has prepared and characterized a dinuclear Co^{3+} complex and has proposed a mechanism in which the phosphate is doubly Lewis acid activated and subsequently cleaved by attack of a Co^{3+} bound hydroxide ion [6,7]. This group has also investigated the dependence of the rate acceleration for phosphate diester cleavage on the basicity of the leaving group by studying the reactivities of two hydroxide-bridged triazamacrocyclic dinuclear Co^{3+} complexes [6,7,84]. While double Lewis acid activation alone provides comparable rate acceleration for cleaving phosphate diesters with good or poor leaving groups, the bridging oxide nucleophile provides increasingly larger rate accelerations for phosphate diesters with better leaving groups. There has an enormous cooperative effect between nucleophile and leaving group activation.

Vance and Czarnik have reported that the dinuclear cyclen–Co^{3+} complexes based on both a very rigid anthracene spacer and a very flexible alkyl spacer promoted hydrolysis of phosphate diester BNPP [85]. The rigid complexes are more reactive than the flexible complexes, whereas no cooperativity between the metal ions is observed for the BNPP hydrolysis. Schneider group has increased the DNA affinity of dinuclear cyclen-Co^{3+} by modification with positively charged peralkylammonium groups that can interact with the anionic phosphate ester linkages in DNA. The presence of these groups leads to lower activities in the hydrolysis of the activated phosphate esters of BNPP, but to higher activities in transition of supercoiled to nicked form of plasmid DNA. The highest activity was observed for a complex in which two Co^{3+} centers can act simultaneously on the DNA grooves [9].

The dinuclear complexes M_2–L2 resulted from a macrocyclic ligand (M: Co^{2+}, Ni^{2+}, Cu^{2+}, and Ca^{2+}; L2: 3,13-dihydroxyl-1,5,8,11,15,18-hexaazacyclicamine) catalyze conversion of supercoiled pBR322 DNA into the nicked and linear forms under neutral pH conditions and at 37 °C, with 1.6–10 times rate enhancements over the corresponding mononuclear complexes [86]. Little cleavage was observed under comparable conditions by the metal ions or receptor alone. The efficiency of DNA cleavage mediated by the M_2-L2 series decreases in the order $Co^{2+} \sim Ni^{2+} > Cu^{2+} \sim Ca^{2+}$. Measurement of the rate of disapperance of supercoiled DNA indicated that the approximate

pseudo-first-order rate constant was $8.0 \times 10^{-4}\,s^{-1}$ for the system Co_2–L2. Although this is one of the largest known rate enhancement factors of $\sim 10^{10}$ against DNA, the concentration of this catalyst (100 μM) is enormous excess over the substrate (6.1 μM base pair).

3.6. Miscellaneous di- and multi-nuclear metal complexes

The miscellaneous dinuclear complexes shown in Fig. 8a and b have a higher affinity for DNA binding than the corresponding mononuclear complexes, whereas they did not show notable rate enhancements in the catalytic cleavage of BNPP [87]. The heterodinucleating ligand shown in Fig. 8c can bind an alkaline earth ion and a Zn^{2+} ion at addressed sites [88]. At pH 8.5, the Ba^{2+}–Zn^{2+} couple gave a rate enhancement of 1120-fold over background hydrolysis of BNPP. From saturation kinetic study it appeared that this dinuclear complex has a higher affinity for the substrate and that the mononuclear Zn^{2+} complex exhibits a higher turnover rate. The low cooperative catalysis is attributed to a nonideal geometrical relationship between the metal centers. In the hydrolysis of BNPP, the novel macrocyclic complex $Cu^{II}Cd^{II}$-BDBPH (BDBPH: 3,6,9,17,20,23-hexaaza-29,39-dihydroxy-13,27- dimethyl-tricyclo[23,3,1,1] triaconta-1(28),11, 13,15(30),25,25-hexene) can provide both Lewis acid and base sites to an active phosphate diester in which nucleophilic OH^- attacks the substrate to fulfill the hydrolysis cycle [89]. The synergic two functional groups in this catalyst molecule can exert remarkable catalytic activity towards hydrolysis of BNPP.

The heterotrinuclear Zn_2Cu complex on a calix[4]arene-based molecular scaffold (Fig. 8d) is more active than the Zn_3 analogue [90], indicating that the combination of metal ions with different properties can lead to an unexpected catalytic activity. This is also found in natural metallo-phosphoesterases, for example, the active site of alkaline phosphatase [15]. The homotrinuclear complex cation $[Zn_3L(\mu\text{-OAc})]^{2+}$ (see Table 1) can cleave linear calf thymus DNA at 37 and 50 °C [59].

Dulger group has shown double-stranded DNA cleavage mediated by heterotetranuclear $Cu^{2+}Mn^{2+}$ complexes based on a tetrathioether–tetrathiol ligand [91]. In the absence of external oxidants, the tetranuclear complex cleaved supercoiled DNA to yield linear DNA within 5 min at 10–50 μM concentrations. Although appreciable cleavage was observed, an addition of external oxidant increased the efficiency of double-stranded DNA cleavage (with 5–10 μM complex) within 3 min. While no kinetic details were described, nor the mechanism of cleavage established, the efficiency of double-stranded DNA cleavage mediated by the heterotetranuclear complexes was demonstrated.

4. Dependence of DNA hydrolysis on the structures of di- and multi-nuclear metal complexes

It is possible to indirectly compare with each other the kinetic behavior that has been reported for many di- and multi-nuclear metal complexes by using the combination of parameters α and β, and the catalyst concentrations used and summarized in Table 1, when the supercoiled DNA or phosphate ester has been used as a substrate for the reaction catalyzed by a di- and multi-nuclear complex. From Table 1, we can find that the complexes with the highest hydrolysis rate enhancement β values are the tetranuclear complex $[Fe_4(NTB)_4(\mu\text{-O})_2(\mu_4\text{-Suc})]^{6+}$, and dinuclear complexes $Fe_2(DTPB)(\mu\text{-O})(\mu\text{-Ac})Cl(BF_4)_2$ and $Ce_2(HXTA)$. When compared to DNA hydrolyzing enzymes, however, these three complexes are about 1–4 orders of magnitude less than type-II restriction endonucleases, which typically exhibit rate enhancements in 10^9–10^{10} for the formation of linear DNA [92]. Moreover, much higher concentrations are required to be comparable to that by the natural enzymes. The complexes far to carry out double-stranded

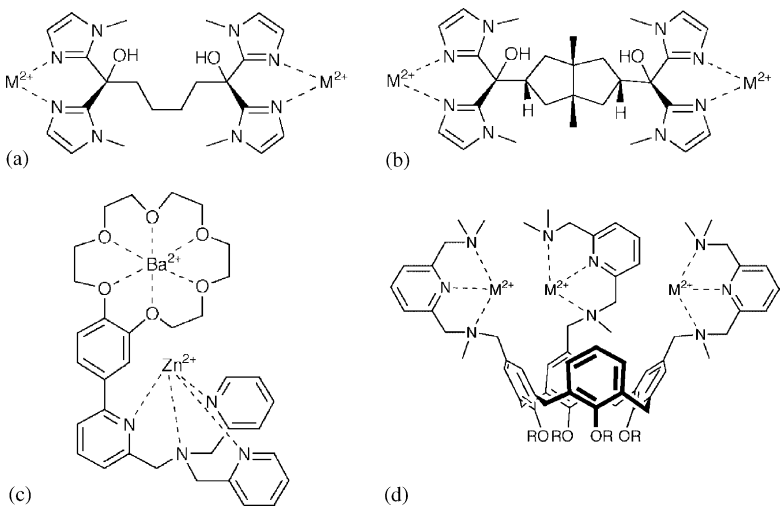


Fig. 8. Schematic illustration of some miscellaneous di-nuclear metal complexes.

Table 2
pBR322 DNA cleavage by various dinuclear metal complexes (% estimated)

	DNA forms		
	Supercoiled	Nicked	Linear
$[Fe_2(DTPB)(\mu\text{-}O)(\mu\text{-}Ac)Cl](BF_4)_2$	8	51	41
$Zn_2(DTPB)Cl_4$	30	42	28
$Zn_2(EDTB)Cl_4$	41	45	14
$Fe_2(EGTB)Cl_6$	38	48	14
$[Fe_2(IDB)_2(\mu\text{-}O)(\mu\text{-}Ac)_2]Cl_2$	23	70	7
$Cu_2(DTPB)Cl_4$	37	44	21
$Cu_2(EGTB)(NO_3)_4$	49	51	
$Zn[Zn_2(TTHA)]$	49	51	
$Cu_2(EGTB)(Im^-)Cl_3$	57	43	
$Fe_2(EGTB)(Im^-)Cl_5$	58	42	
Control	62	38	

hydrolysis of supercoiled DNA exhibit the following order of increasing β values: $Zn_2HP \sim Cu_2(N_4S_4) \sim [Cu_2(D^1)(H_2O)_2](ClO_4)_4 < Zn_2(EDTB)Cl_4 \sim Pr_2(Macro) < Fe_2(DTPB)(\mu\text{-}O)(\mu\text{-}Ac)Cl(BF_4)_2 \sim Ce_2(HXTA) < [Fe_4(NTB)_4(\mu\text{-}O)_2(\mu_4\text{-}Suc)]^{6+}$.

Table 2 exhibits the plasmid DNA hydrolytic efficiency mediated by a variety of dinuclear metal complexes containing different metal ions under the identical conditions [38]. It is clear from Table 2 that $Fe_2(DTPB)(\mu\text{-}O)(\mu\text{-}Ac)Cl(BF_4)_2$ results in the nearly complete disappearance of supercoiled DNA and the appearance of linear DNA and exhibits the most significant DNA cleavage chemistry. The dinuclear metal complexes $M_2(DTPB)Cl_4$ (M: Cu and Zn) can effectively cleave the DNA under the identical conditions. In addition, the complexes $Zn_2(EDTB)Cl_4$, $Fe_2(EGTB)Cl_6$ and $Fe_2(IDB)_2(\mu\text{-}O)(\mu\text{-}Ac)_2Cl_2$ can also degrade the DNA to a certain extent. However, the dinuclear complexes $Cu_2(EGTB)(NO_3)_4$, $Zn[Zn_2(TTHA)]$, $Fe_2(EGTB)(Im^-)Cl_5$, and $Cu_2(EGTB)(Im^-)Cl_3$ are not effective promoters for the DNA hydrolytic cleavage. As a result, the competence that the complexes degrade DNA under the hydrolytic conditions decrease in the order of appearance of linearized DNA: $Fe_2(DTPB)(\mu\text{-}O)(\mu\text{-}Ac)Cl(BF_4)_2 > Zn_2(DTPB)Cl_4 > Cu_2(DTPB)Cl_4 > Zn_2(EDTB)Cl_4$, $Fe_2(EGTB)Cl_6 > Fe_2(IDB)_2(\mu\text{-}O)(\mu\text{-}Ac)_2Cl_2 > Zn[Zn_2(TTHA)]$, $Cu_2(EGTB)(Im^-)Cl_3$, $Fe_2(EGTB)(Im^-)Cl_5$, $Cu_2(EGTB)(NO_3)_4$ (TTHA: triethylene tetraaminehexaacetic acid, EDTB: *N*,*N*′-bis(2′-benzimidazol-2-ylmethyl)-ethylenediamine).

It is not difficult to understand the DNA hydrolytic reactivity shown in Tables 1 and 2 according to the composition of ligand spheres and structures of the di- and multi-nuclear metal complexes. First, the concerted effect between or among metal ions and between ligands and metal ions can contribute to the DNA hydrolytic reactions. Then, the di- and multi-nuclear metal complexes with high β values contain an μ-oxo or -hydroxide bridge, open coordination site(s) or/and one or more labile ligands. This is consistent with the previously proposed hydrolytic mechanism of phosphate diester [92]. On the other hand, the DNA hydrolytic chemistry by the di- and multi-nuclear metal complexes containing aromatic nitrogen heterocycles and polyamine backbone are strengthened with pH decrease [38].

There is a considerable distinction among the DNA hydrolytic chemistry exhibited by the di- and multi-nuclear metal complexes that contain different metal ions. Although the complexes $Zn_2(DTPB)Cl_4$ and $Cu_2(DTPB)Cl_4$ can also hydrolyze DNA, in comparison with the complexes $[Fe_4(NTB)_4(\mu\text{-}O)_2(\mu_4\text{-}Suc)]^{6+}$, $Fe_2(DTPB)(\mu\text{-}O)(\mu\text{-}Ac)Cl(BF_4)_2$ and $Ce_2(HXTA)$, they can activate less effectively the phosphate diester bonds than the ferric ions in the di-iron complex. On the other hand, the distance between two zinc ions in $Zn_2(DTPB)Cl_4$ is ∼6.9 Å [56], while the corresponding distances in $Fe_2(DTPB)(\mu\text{-}O)(\mu\text{-}Ac)Cl(BF_4)_2$ and $[Fe_4(NTB)_4(\mu\text{-}O)_2(\mu_4\text{-}Suc)]^{6+}$ are ∼3.1 Å [38]. It has been reported that if the cooperativity between two metal ions is utilized to hydrolyze phosphate diester bonds, their separation must be ∼3.9 Å [93]. Therefore, the DNA hydrolytic activity by the dinuclear zinc complex is weaker than that by the di- and multi-nuclear iron and cerium complexes. In addition, the affinity of zinc ion to DNA phosphate oxygens is larger than that of cupric ion, since copper atoms tend to coordinate to DNA bases [94]. Thus, $Zn_2(DTPB)Cl_4$ is a more efficient DNA hydrolytic agent than $Cu_2(DTPB)Cl_4$.

The ability of lanthanide and ferric complexes to readily catalyze the DNA hydrolysis is notable, particularly in comparison to biologically relevant transition metal or alkaline earth Lewis acids, such as Zn^{2+}, Ca^{2+} and Mg^{2+} [4]. This efficiency results from the conjunction of higher oxidation state, charge density, coordination number, and rapid ligand exchange rates. These characteristics make the lanthanide and ferric ions well-suited to be catalytic centers in the development of artificial enzymes. Based on the criteria of substitutional lability that is dependent on ligand field stabilization energy and Lewis acidity, Cu^{2+}, Zn^{2+}, Ca^{2+}, Fe^{3+} and $Ln^{3+/4+}$ ions would be the expected catalytic centers of natural hydrolases. Because 4f orbitals in $Ln^{3+/4+}$ ions are both diffuse and of an inner shell, these $Ln^{3+/4+}$ ions experience very little ligand field splitting effect. Thus the coordination number and geometry is primarily dependent on ligand-ligand repulsion and steric factors $Ln^{3+/4+}$ complexes.

Cerium is unique among the lanthanides in its ability to access a tetravalent oxidation state under aqueous conditions. The Ce^{4+} complexes have been found to be particularly effective in promoting nuclease activity, enhancing DNA hydrolysis by a factor of $\beta > 10^7$ [77]. This rate is 20 ∼ 1000 times faster than those in the presence of the trivalent Ln^{3+} complexes. This striking activity has been ascribed to the ability of Ce^{4+} to make coordination bonds with DNA and promote formation of the pentacoordinate phosphorus intermediate [4]. This coordination interaction, coupled with greater positive charge density and a tendency to form hydroxo-bridged dinuclear species in solution, causes Ce^{4+} to be significantly better in binding and activating

DNA than other Ln^{3+}, which only withdraw electron density electrostatically. Also, the 4f orbitals of Ce^{4+} are lower in energy than Ln^{III} ions and thus can interact efficiently with the orbitals of phosphates. This mixing of orbitals plus electron transfer from phosphate ester to Ce^{4+} decreases the activation free energy for DNA hydrolysis.

The composition of ligand spheres in the di- and multi-nuclear metal complexes contributes to the remarkable distinction in the DNA cleavage chemistry. The complexes Fe_2(DTPB)(μ-O)(μ-Ac)Cl$(BF_4)_2$ and $[Fe_4(NTB)_4(\mu\text{-}O)_2(\mu_4\text{-}Suc)]^{6+}$ contain an μ-oxo bridge, whereas Ce_2(HXTA) has many open coordinate sites. Although the symmetrical and stable complex Fe_2(IDB)$_2$(μ-O)$(\mu\text{-}Ac)_2Cl_2$ also contains an μ-oxo bridge, it contains no labile anionic ligand, and is six-coordinated and crowded in space conformation. Hence, its two ferric ions can not coordinated directly to the DNA phosphate oxygens. This may be a critical factor that results in its poor DNA hydrolytic activity. Because there is no μ-oxo bridge in the Fe_2(EGTB)Cl_6 structure, its unstable chloride ions also can not improve its DNA hydrolytic chemistry to the utmost extent. Consequently, the complexes $[Fe_4(NTB)_4(\mu\text{-}O)_2(\mu_4\text{-}Suc)]^{6+}$, Fe_2(DTPB)(μ-O) (μ-Ac)Cl$(BF_4)_2$ and Ce_2(HXTA) hydrolyze DNA duplex in the highest efficiency.

When studying catalytic reactions involving ligand exchange at a metal ion, it is important to consider the ligand exchange rates and Lewis acidity typical of that metal ion. In general, trivalent and tetravalent metal ions are stronger Lewis acids than divalent metal ions. However, ligand exchange reactions are generally slower at tri- and tetra-valent metal ions than at divalent metal ions [95]. Despite this apparent disadvantage of trivalent and tetravalent metal ions, several enzymes are now known to make use of the strong Lewis acidity of a trivalent metal ion while exhibiting fairly high turnover numbers. The strong Lewis acidity of Fe^{3+} and Ce^{4+} ions can efficiently activate the DNA hydrolysis. Moreover, the different ligand environments can also allow metal ions exhibit the extremely different Lewis acid activation. When the metal ions are bound more anionic ligands, for example, M_2(DTPB)Cl_4 (M: Zn or Cu) and Fe_2(EGTB)Cl_6, their Lewis acidity will be decreased. This may also contribute to the low DNA hydrolytic activity. For M_2(DTPB)Cl_4 (M: Zn or Cu), although Lewis acidity of the former is stronger than that of the later, their DNA hydrolytic efficiency is opposite. Therefore, it appears that Lewis acidity is only one of the dominant factors of DNA hydrolysis.

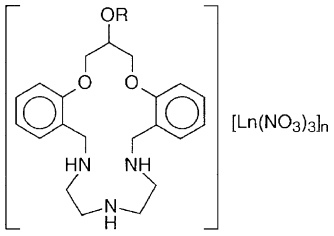
5. Toward the development of artificial restriction enzymes

It seems to be very difficult to develop type-II restriction nuclease-like artificial enzymes only based on the small-molecule di- and multi-nuclear metal complexes. Thus, along with the well-characterized small-molecule nucleases being developed, there has been interest in design of systems consisted of di- and multi-nuclear metals and biopolymer or heterogeneous phase. Micellar or bilayer surfaces have been shown to promote hydrolytic DNA cleavage in the presence of Ca^{2+}, Mg^{2+}, Cu^{2+} and Ln^{3+} ions [96,97].

Conjugates of metal complexes to biomacromolecules have myriad uses, because they might possess recognition motifs for selective binding (such as polycationic ligands, triplex-forming oligonucleotides, peptide nucleic acids, or structured oligopeptides). Utilizing an antisense approach involves the recognition of a single-stranded DNA or RNA target by a complementary oligonucleotide sequence (usually DNA) to which a nuclease or a metal complex have been appended. This antisense approach is extremely selective in recognition and cleavage of a target sequence. Komiyama demonstrated that the lanthanide-mediated antisense strategy could be employed to hydrolyze DNA site-specifically [98]. The DNA-Ce^{4+} complex promoted highly selective cleavage of two bases to the 3′-side of the complementary strand-binding site, as expected from the molecular design. Despite this early success, the antisense approach for DNA nucleases remain relatively unexplored.

Protein- and peptide-based artificial nucleases, an alternative approach to the antisense strategy, are an agent to specifically recognize double-stranded DNA in manner of transcription factors and restriction enzymes. This strategy employs the same flexible, yet sequence-selective, protein–DNA recognition. By incorporating both metal sites and protein-based DNA-binding elements into a nuclease design, the artificial enzymes utilize the protein–DNA interactions to deliver the hydrolytic metals to the backbone of DNA for cleavage. For example, a fusion protein generated by splicing a known endonuclease domain onto DNA-binding zinc-finger domain cleaves double-stranded DNA with high specificity [99]. Another strategy involved appending a Zn^{2+}-binding peptide onto an ancillary ligand of a rhodium metallointercalator [54]. The metallointercalators $[Rh(phi)_2bpy']^{3+}$ (phi: 9,10-phenanthrenequinone diimine; bpy′: 4-(butyric acid)-4′-methyl-2,2′-bipyridine) or $[Ru(DIP)_2Macro]^{2+}$ (DIP: diphenyl-1,10-phenanthroline; Macro: 4,7-$((NH_2CH_2CH_2)_2NCH_2CH_2NHSO_2C_6H_4)$-1,10-phenanthroline) tethered to the metal (Zn^{2+}, Fe^{2+}, Cu^{2+})-binding peptides by a rather flexible spacer provides the DNA affinity, delivering the Zn^{2+}-peptide to the major groove of DNA for hydrolytic cleavage [100–102]. Franklin group developed the chimeric metallopeptide nucleases by incorporating a Ca^{2+}-binding site into a helix-turn-helix DNA-binding domain to utilize native-like protein–DNA interactions to promote lanthanide-mediated DNA hydrolysis [103,104]. In addition, the Eu^{3+} or Ce^{4+}-peptide complexes are hydrolytically active toward supercoiled plasmid DNA, causing single-stranded breaks at lower concentrations than free Ln^{3+} [105,106].

The search for low molecular weight catalysts that can work with the efficiency of enzymes has been an area



Ln = Eu, Gd, Tb, Dy, Ho, Er
R = H, Me, C_7H_7, β-$C_{11}H_9$

Fig. 9. Aza crown ether lanthanide complexes [107].

of interest to chemists. Recent progress in the generation of catalysts for a number of processes linked to combinatorial methodology has yielded exciting results and remarkable properties. Janda et al reported the use of combinatorial catalysis to hydrolyze phosphate ester [107]. This group screened the ability of various azacrown ether lanthanide complexes (Fig. 9) to hydrolyze BNPP and double-stranded DNA using a parallel format on a 96-well plate reader. The results indicated that the Gd^{III} complex with β-naphthyl substitution showed the best rate enhancements (7.5×10^6), a value that is two orders of magnitude lower than the complexes $[Fe_4(NTB)_4(\mu\text{-}O)_2(\mu_4\text{-}Suc)]^{6+}$, $Fe_2(DTPB)(\mu\text{-}O)(\mu\text{-}Ac)Cl(BF_4)_2$ and $Ce_2(HXTA)$, in double-stranded DNA cleavage.

6. Prospects

Di- and multi-nuclear metal systems are particularly effective in promoting DNA hydrolysis, as the metals can function in tandem to activate both the P–O bonds and the water nucleophile. It is becoming increasingly clear that di- and multi-nuclear Fe^{3+} and Ce^{4+} complexes are the most effective hydrolytic systems available for DNA hydrolysis, although the coordination interactions between these systems and DNA remain to be understood. Nevertheless, the ability of the catalysts to bind to the DNA substrate is a critical and previously overlooked aspect in DNA hydrolysis. Most likely, many available complexes fails as effective catalysts as a result of their inability to form an enzyme-like Michaelian complex with substrate. Therefore, tuning the coordination interactions between the catalysts and DNA, and the activation of the P–O bond and the water nucleophile, and the stability of the intermediate and leaving groups mediated by these systems is the next challenge in the rational nuclease design. The key is to design ligand systems that could form stable di- and multi-nuclear Fe^{3+} and Ce^{4+} complexes with the ability to mediate hydrolysis of phosphate ester. With the advent of these di- and multi-nuclear Fe^{3+} and Ce^{4+} complexes, it seems reasonable to predict higher rate enhancement in DNA hydrolysis.

The exquisite sequence specificity of natural restriction enzymes has yet to be realized *in vivo* (or even *ex vivo*), nor has their catalytic efficiency been approached. The majority of protein and peptide-based artificial nucleases and DNA hydrolytic systems by antisense and combinatorial strategy have only incorporated mononuclear metal sites. Perhaps, greater improvements in the rate and specificity of metal complex-mediated DNA hydrolysis are the development of di- and multi-nuclear metal complexes with site selectivity based on ligand design and antisense methodology. With the lessons learned from the natural DNA hydrolases, therefore, the future is promising for the development of small-molecule and biopolymer-based di- and multi-nuclear Fe^{3+} and Ce^{4+} complexes to target DNA sequences at will, and to offer insights into the mechanism of natural nucleases.

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