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Review

Design of artificial metallonucleases with oxidative mechanism

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Contents

Abbreviations: AFM, atomic force microscopy; Arg, arginine; Asc, ascorbic acid; BDEs, bond dissociation energies; BLM, bleomycin; bpy, bipyridine; BQQ, benzoquinoquinoxaline; DL, dinucleating ligand; dppt, 3-(1,10-phenanthrolin-2-yl)-5,6-diphenylas-triazine; dppz, dipyrido[3,2-*a*:2′,3′-*c*]phenazine; dpq, dipyrido[3,2-*d*:2′,3′-*f*]quinoxaline; ds, double strand; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; EPR, electron paramagnetic resonance; GGH, glycine-glycine-histidine; Gly, glycine; Gs, guanosine; GSH, glutathione; HAT, hydrogen abstraction transfer; Hapt, 5-amino-3-pyridin-2-yl-1,2,4-triazole; His, histidine; HPLC–MS, high performance liquid chromatography–mass spectrometry; HTH, helix-turn-helix; KGHK, lysine-glycine-histidine-lysine; lc, linear conformer; MALDI, matrix-assisted laser desorption ionization; MCD, magnetic circular dichroism; MPA, 3-mercaptopropionic acid; MS, mass spectrometry; NMR, nuclear magnetic resonance; NS oligo, non-specific oligonucleotide; oc, open-circular conformer; ODNs, oligodeoxyribonucleotides; ONT, oligonucleotide; OP, 1,10-phen-anthroline; ORNs, oligoribonucleotides; PAGE, polyacrylamide gel electrophoresis; PCET, proton-coupled electron transfer; phen, 1,10-phenanthroline; pta, 3-(1,10-phenanthrolin-2-yl)-as-triazino[5,6-*f*]acenaphthylene; RMOS, reactive metal-oxo species; ROS, reactive oxygen species; salen, *N*,*N*′-ethylenebis (salicylidene aminato); sc, supercoiled conformer; SECM, scanning electrochemical microscopy; SET, single electron transfer; TFO, triple-helix-forming oligonucleotide; TL, trinucleating ligand; Xaa, amino acid; XAS, X-ray absorption spectroscopy

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Abstract: The rational design of artificial metallonucleases capable of cleaving DNA in a controllable manner is highly desired due to their potential application in biology and medicine. This review will highlight the most recent progress in the area of artificial metallonucleases employing an oxidative mechanism, focusing on the development of methodologies for cleavage detection, understanding of the factors governing the cleavage efficacy and selectivity, and design strategies for the achievement of high cleavage efficacy. The challenging problems in mimicking the reactivity of natural nucleases will also be discussed.

Keywords: Artificial metallonucleases; Design strategy; DNA; Oxidative cleavage; Selectivity

1. Introduction

Artificial metallonucleases can be potentially used in gene regulation, mapping of protein and DNA interactions, probing of DNA specific structures, and in cancer therapy [1–4]. In 1998, a thematic issue of *Chemical Reviews* was devoted to the development of natural and synthetic reagents for DNA-cleavage, emphasizing the mechanism of oxidative and photo-induced cleavage [5–9]. These reviews paved the way for the understanding of the DNA-cleavage and for the further design of artificial nucleases. Six years later, another thematic issue appeared in *Chemical Reviews* in which some excellent reviews were included focusing mainly on the development of metallonucleases based on iron, zinc, manganese, vanadium and copper centers. The structure and function relationships were outlined [10–14]. More recently, some other reviews appeared, summarizing the progress of hydrolytic cleavage and photo-cleavage of DNA [15–18]. A number of photoactive rhodium and ruthenium intercalators have been used to probe the long-range oxidative damage of DNA. Radical cation migration was found to play the key role through a thermally activated hopping mechanism with base sequence-dependence [19–22]. The observation provided the possibility to recognize or even repair the aberrant sites in DNA [23–25]. Conjugates of metal-ONT and metal-peptides offer the possibility for the development of biocompatible metallonucleases with desired selectivity [26,27]. To our surprise, a comprehensive review on oxidative cleavage of DNA is lacking despite the extensive original work being published in the last few years.

Analytical, computational and molecular biology techniques have played an essential role in the design of artificial metallonucleases. They provide solutions for real biological problems and guidance for detailed molecular insight into the cleavage mechanism. For example, *ab initio* molecular dynamics simulations indicated that guanine residues and the phosphate backbone play crucial roles in the initial steps of oxidative damage of DNA via radical cation formation [28]. Cysteine biotin probe can selectively detect and quantify the oxidized abasic lesion, 2-deoxyribonolacton, in DNA exposed to various forms of oxidative stress [29]. MALDI-TOF mass spectroscopy is a useful technique for the identification of the DNA-cleavage sites and products [30]. It is highly sensitive and does not require radioactive labeling. The incorporation of macromolecules such as synthetic polymers in the artificial metalloenzymes could fine tune the hydrophobicity/hydrophilicity of the molecules and provide microenvironments that are needed for the enhanced catalytic activity of the enzymes [31].

In this article, we will focus mainly on metal complexes that mediate oxidative cleavage rather than the hydrolytic or photo-induced cleavage of DNA. The coverage extends over the past few years. The purpose of this review is to highlight factors influencing the oxidative cleavage behavior of the metallonucleases, strategies to develop efficient DNA-cleaving agents with high levels of site/structure selectivity, the most recent progress in developing an accurate method for cleavage detection, and the difficulties in mimicking the reactivity of natural metallonucleases.

Transition metal complexes of Fe, Cu, Ni, Pt, Ru, Rh, V, Cr, Co, Mn, Os and Pd have been reported to mediate DNA oxidation in the presence of oxidants or reductants or without any assistant agents [7,8,10–14]. These complexes attack the sugar or base moieties of DNA [7,8]. Several well-known and best-characterized nucleolytic agents are: $[Fe(EDTA)]^{2-}$ (EDTA = ethylenediaminetetraacetic acid) [32], $[Cu(OP)_2]^{+}$ (OP = 1,10-phen-anthroline) [33], Fe-BLM (BLM = bleomycin) [9,34], metalloporphyrins [35], Ni-peptides [36], and metal-salen [salen = *N*,*N*′-ethylenebis (salicylidene aminato)] [37]. They are typically mononuclear complexes. Recently, however, multi-nuclear complexes have attracted more and more interest as metallonucleases for the potential cooperative effects between the metal centers [38,39]. These oxidative cleavage agents are highly efficient in inducing strand scission of DNA.

In practical application, however, the high selectivity is more important when they are used as anticancer drugs or specific gene modifiers. Therefore, the key issue in the rational design of metallonucleases is the acquirement of selective scission of DNA.

2. Mechanism of oxidative cleavage of DNA

The mechanism of oxidative cleavage of DNA can be divided into different categories based on electron-transfer processes, the active species involved and target sites. In these mechanisms, reactive oxygen or metal-oxo species (ROS/RMOS), nucleobase modification or hydrogen abstraction of sugar moiety including single electron transfer (SET), hydrogen atom transfer (HAT) or proton-coupled electron-transfer (PCET) processes are involved [7,8,40].

2.1. Reactive oxygen or metal-oxo species (ROS/RMOS)

The DNA-cleavage ability of different metallonucleases is largely determined by the reactivity of reactive species. As shown in Table 1, most reactive intermediates are produced in an aerobic environment or in the presence of co-oxidants. The sequential reduction of molecular oxygen can generate a group of ROS such as superoxide anion (O_2^{-}), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$). The $^{\bullet}OH$ radical is an extremely strong and highly diffusible oxidant with redox potential of 2.8 V, which can mediate DNA damage by adding to double bonds of DNA bases or abstracting hydrogen atoms from sugar moiety [41]. Singlet oxygen ($^1O_2$) is another radical species derived from oxygen, which is often involved in the oxidative cleavage of DNA with energy transfer. Transition metal ions in a variety of ligand environments play a key role in the redox cycle to form primary and secondary ROS as well as the RMOS. The typical Fenton-type reaction produces hydroxyl radicals which are responsible for the non-selective DNA scission induced by $[Fe(EDTA)]^{2-}$ [32]. RMOS is more diversified than ROS, which includes end-on and side-on peroxo/superoxo metal species (M–OH/M–OOH) and even high-valent metal-oxo species (M=O) [14,42]. These radical intermediates may result in DNA-cleavage at certain domains or sites by binding DNA in a selective way. For example, Fe-BLMs can promote DNA-cleavage through the reaction of Fe^{III}–OOH or Fe^{IV}=O with C′4–H in the minor groove [9,34,43]. The detailed comparison in Table 1 also shows that mononuclear copper nucleases

Table 1
Oxidative cleavage of DNA induced by some metal complexes

Metal complexes	Co-factors	ROS/RMOS	DNA target/lesion	Reference
Cu^{II}-Hapt, Cu^{II}-$Hapt_2$	Ascorbate/H_2O_2	$^{\bullet}OH$ or $^1O_2$	Unknown	[44]
Cu^{II}-benzoguanamine-sulfonamide derivative	Ascorbate/H_2O_2	$^{\bullet}OH$ and peroxide copper	Unknown	[45]
Cu^{II}-benzothiazolesulfonamide derivative	Ascorbate/H_2O_2	$^{\bullet}OH$	C1′ oxidation (major), G oxidation (mild)	[46]
Cu^{II}-aminoglycosides	H_2O_2, ascorbate/$O_2^{\bullet}$	Unknown	C4′H abstraction	[47]
Cu^{II}-peptides	Ascorbate/O_2	$^{\bullet}OH$	C1′, C4′H abstraction	[48]
Cu^{II}-kanamycin A	H_2O_2	Unknown	Convert dG to 8-oxo-dG	[49]
$[\{Cu^{II}(L)\}_2(\mu\text{-}OH)_2]$, Cu^{II}-L, L = phen, dpq and dppz	MPA	$^{\bullet}OH$	Sugar-hydrogen abstraction	[50,51]
Cu^{II}-*N*,*N*′-ditert-butyl-1,10-phenanthroline-2,9-dimethanamine	H_2O_2	$^{\bullet}OH$	Unknown	[52]
$[Cu^{II}(L)(phen)]^+$, L: *N*-quinolin-8-yl-*p*-toluenesulfonamidate	Ascorbate/H_2O_2	$^{\bullet}OH$ and O_2^{-}	Unknown	[53]
$[Cu(L')L]^+$, L = phen, dpq; L′: 2-((methylthio)phenyl)salicylaldimine	MPA	$^{\bullet}OH$	Unknown	[54]
$[Cu^{II}(TpPh)(L)]^+$, L = phen, dpq, dppz, TpPh: tris(3-phenylpyrazolyl)borate	Ascorbic acid	$^{\bullet}OH$	Sugar-hydrogen abstraction	[55]
Cu^{II}_2-bipyridyl porphyrins	DTT and/or H_2O_2	$^{\bullet}OH$	Unknown	[56]
Cu^{II}-flavonoids	None	$^{\bullet}OH$, $^1O_2$/copper-peroxide	Unknown	[57]
Cu^{II}_2-(N*n*) (*n* = 4 and 5)	MPA/O_2	Side-on peroxodicopper^{II} Cu_2–O_2	Single-strand/double-strand junction (AGG)	[58]
Cu^{II}-TMPA, Cu_2^{II}-TMPA	MPA	Cu–O_2^{2-}–Cu	Sugar moiety oxidation	[59]
Cu^{II}_2-pyridylalkylamine	MPA	Cu_2–O_2	G	[60]
Cu^{II}_3-benzenesulfonylamide derivatives	Ascorbate/H_2O_2	$^{\bullet}OH$ and $^1O_2$	Unknown	[61]
Cu^{II}-Mn^{II}-oxime derivatives	MMPP	$^{\bullet}OH$	Unknown	[62]
Co-bithiazole	O_2	$Co^{III}OH$?	G	[63]
Fe^{III}-BLM analogues	H_2O_2	$^{\bullet}OH$	Unknown	[64]
Fe^{III}-BLMs	H_2O_2	Fe^{III}–OOH or Fe^{IV}=O	C4′H abstraction	[9,34]
Mn^{III}-salens	MMPP, $KHSO_5$, PAA	Mn^{V}=O	G (+ribose)	[65,66]
Mn^{III}-porphyrins	$KHSO_5$	Mn^{V}=O	G (+ribose)	[35,67]
Ni^{II}–Gly-Gly-His	S(IV)/O_2	$SO_3^{\bullet-}$, $SO_5^{\bullet-}$, $SO_4^{\bullet-}$	8-oxo-dG	[68]
Ni^{II}–peptides	$KHSO_5$, MMPP, or H_2O_2	Ni^{III}–$SO_4^{\bullet}$	G or C-4′H abstraction	[69–71]
Ni^{II}–macrocycles	MMPP, $KHSO_5$	Ni^{III}–$SO_4^{\bullet}$	G (>C > T)	[72–75]
Ni^{II}-salen	MMPP, $KHSO_5$	Alkalation	G	[37,76]

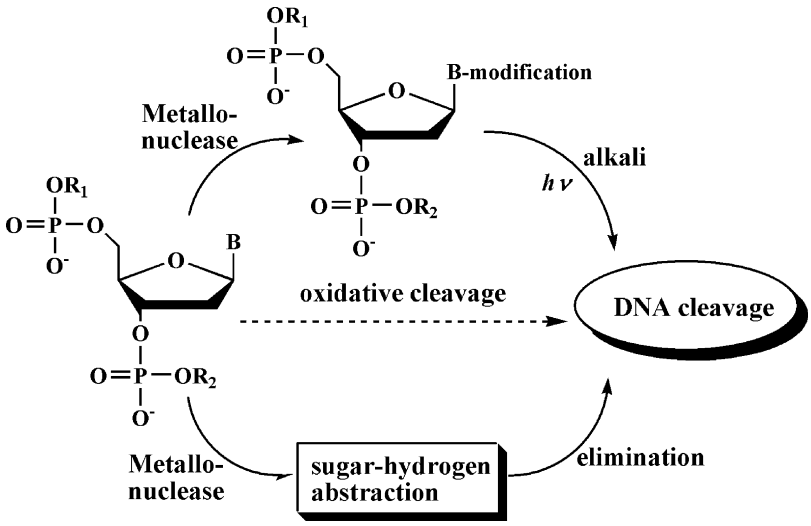

Fig. 1. DNA strand scission induced by nucleobase modification or sugar-hydrogen abstraction (modified based on Refs. [7,8]).

often induce DNA-cleavage through a sugar-hydrogen abstraction pathway by $^{\bullet}OH$ or O_2^- [44–55], while for dinuclear copper nucleases, the dicopper-end-on-peroxo (Cu_2–OOH) or dicopper-side-on-peroxo intermediate (Cu_2–O_2) may play a key role in the DNA selective nucleobase modification [14,57–60]. Mn^V=O is the major RMOS for DNA-cleavage promoted by manganese complexes [65–67]. Nickel-containing nucleases especially nickel-peptides exhibit specific DNA-cleavage with G recognition in the presence of $KHSO_5$, MMPP or S(IV)/O_2, and the RMOS involved is Ni^{III}–$SO_4^{\bullet}$ [69–75]. RMOS-nucleobase binding may be important for specific base recognition [60].

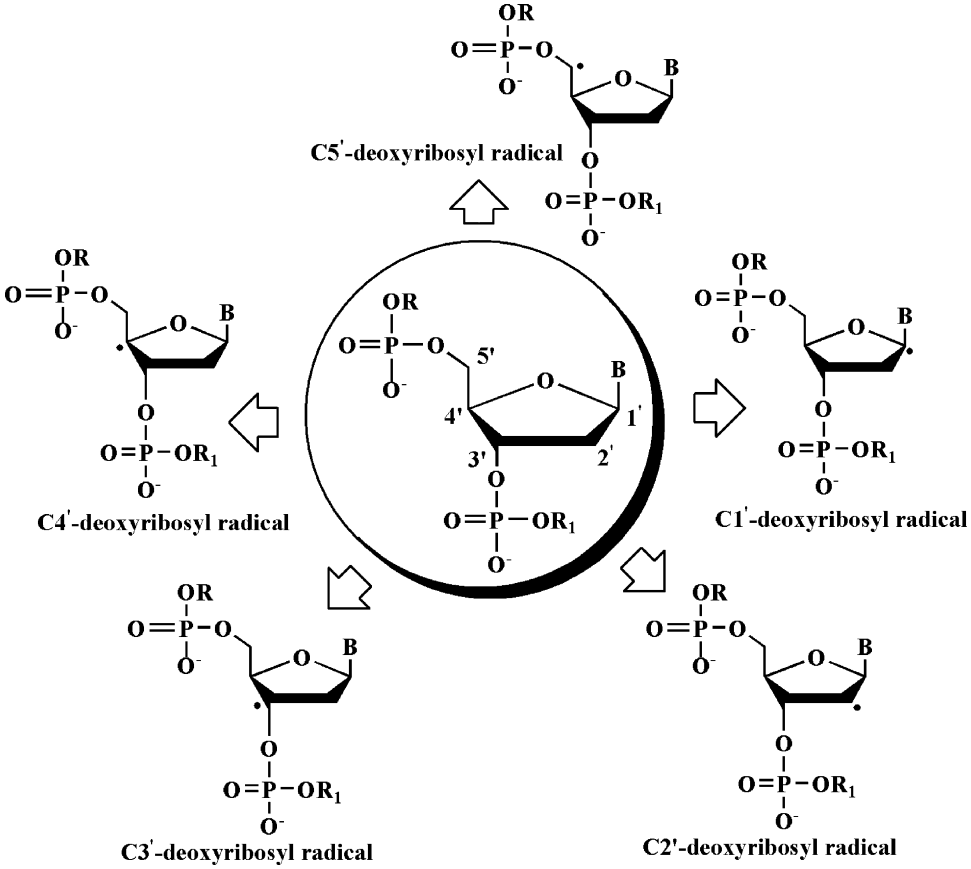

Fig. 2. Five deoxyribosyl radicals produced by hydrogen abstraction.

2.2. Nucleobase modification or sugar-hydrogen abstraction

Reactions of ROS and/or RMOS with nucleic acid result in sugar-hydrogen abstraction and/or nucleobase modification (Fig. 1) [7,8]. Strand scission at sugar moiety is governed by the reactivity of its seven hydrogen atoms. Weaker C–H bond strength and more accessibility to oxidizing agents favor the hydrogen abstraction. The overall preference for hydrogen abstraction is in the order of C5′ > C4′ > C2′ ≈ C3′ > C1′ [7]. Fig. 2 shows the five potential radicals derived from sugar-hydrogen abstraction. Hydrogen atoms at C1′, C4′ and C5′ are accessible from minor grooves of B-DNA while those at C2′ and C3′ are accessible from major grooves. Most metallonucleases are designed as minor groove binders so that C2′ and C3′ H abstraction pathway are rarely observed as a consequence of their limited accessibility and/or the low reactivity [7,77]. Hydrogen abstractions at C4′ and C5′ are easier than those at C1′, since the latter is normally hidden in minor grooves and almost inaccessible to metallonucleases [78].

Hydrogen abstraction from one of the five ribose carbon atoms is followed by a series of elimination reactions, which give a variety of characteristic products shown in Fig. 3 [7]. By analysis of these scission products, DNA-cleaving mechanisms can be speculated although the exact site of hydrogen abstraction may be ambiguous. For example, the cleavage site of the well-known minor groove binder, $Cu(OP)_2$, is still debatable. There is no conclusive evidence to show whether the direct cleavage occurs at the C1′ or C4′ and C5′ sites [79–83]. Although the major cleavage site of Fe-BLMs is known to occur at C4′ site, the mechanism of C–H bond cleavage reaction and the active oxidizing species are still open issues to be investigated [34,84].

Most nucleobase lesions do not lead to direct strand scission but rather result in the formation of alkali-labile or light-labile base modifications (Fig. 1). Since the different property of four nucleobases, strand scissions at nucleobases are more site-

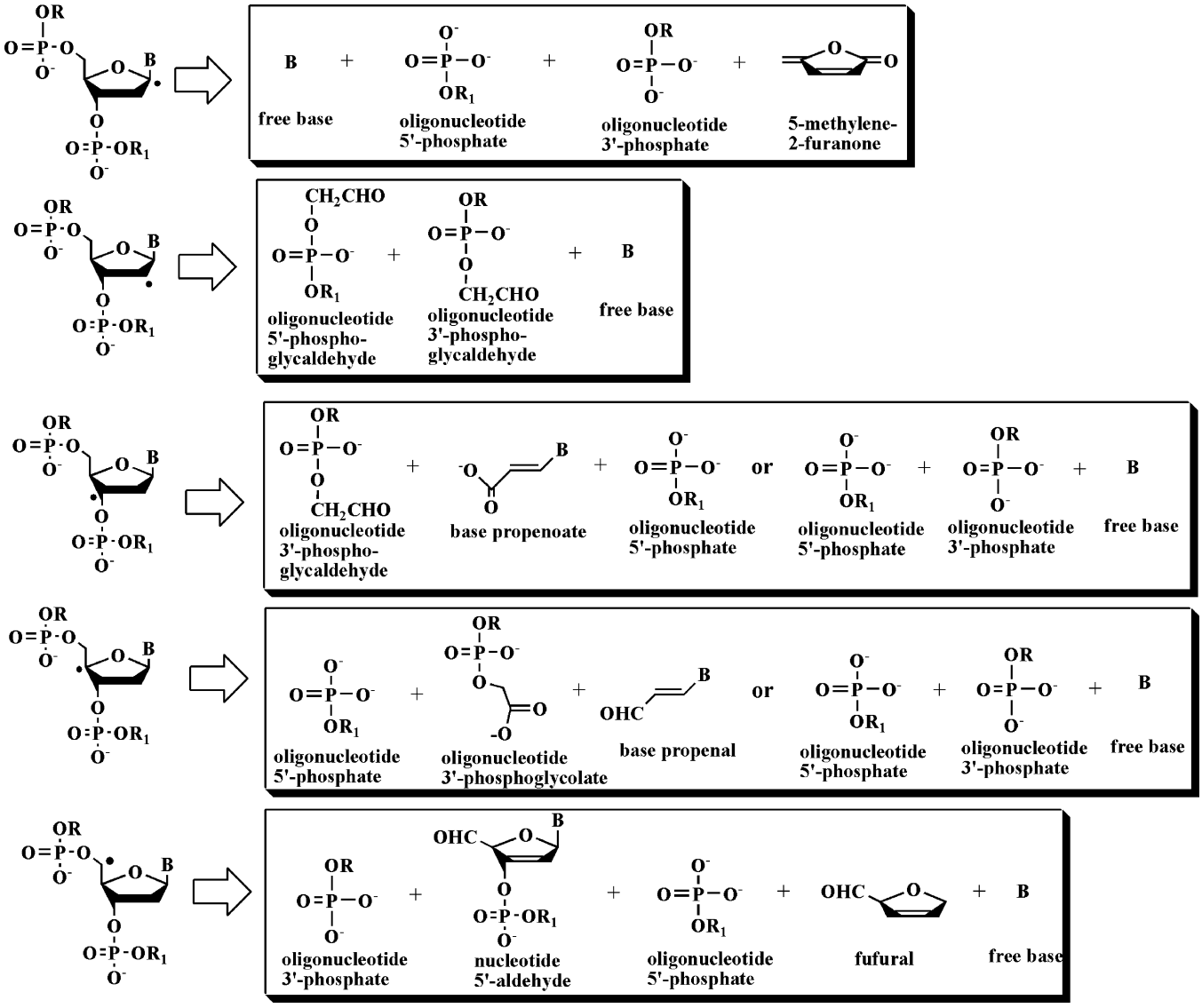

Fig. 3. Scission products from hydrogen abstraction (modified based on Ref. [7]).

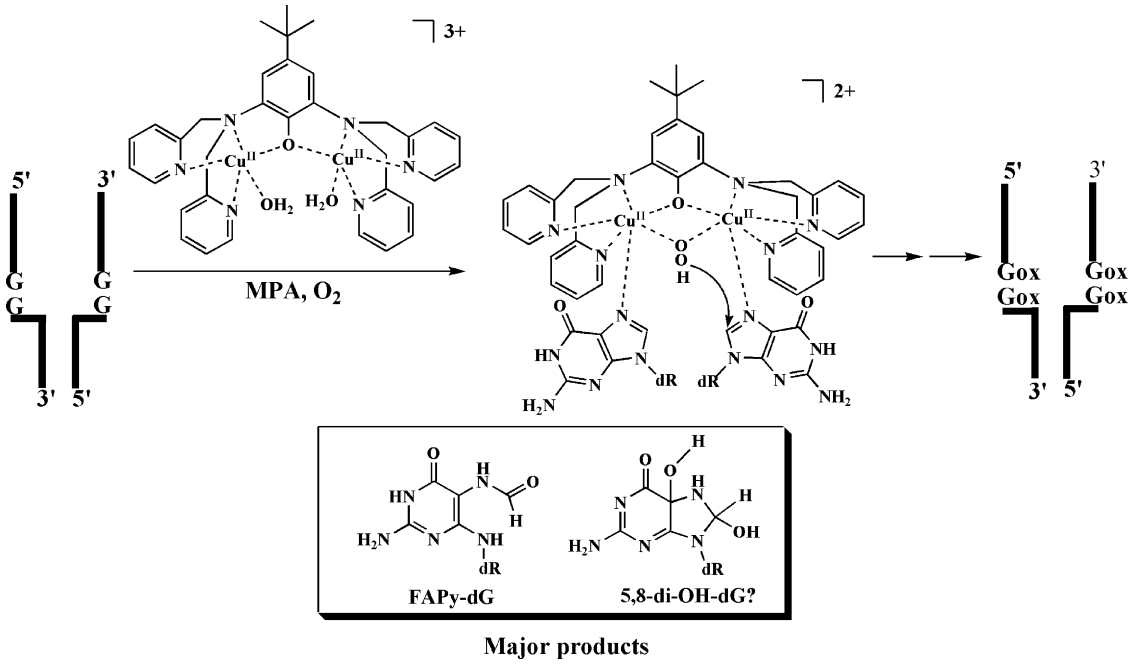

Fig. 4. Mechanism of targeted guanine oxidation by a dinuclear Cu(II) complex at single/double stranded DNA junction and major oxidative products (modified based on Ref. [87]).

specific than at sugar moieties. Guanine is the most labile base to be attacked and 8-oxo-dG is the most common product of guanine oxidation [8]. However, recent kinetic data showed that the difference in one-electron oxidation potentials of the four DNA bases is too small to account for the selective oxidation of GMP with metallo-oxidants such as $Fe(bpy)_3^{3+}$ or $Ru(bpy)_3^{3+}$ [85]. The most essential origin for the selective oxidation of GMP among DNA bases may come from the facile deprotonation from $GMP^{\bullet+}$ followed by the electron-transfer oxidation of the deprotonated radical [85]. In the RMOS mechanism, the nucleobase is bound by metal-oxo intermediates before oxidative reaction occurs. The N7 position of purine is the main target

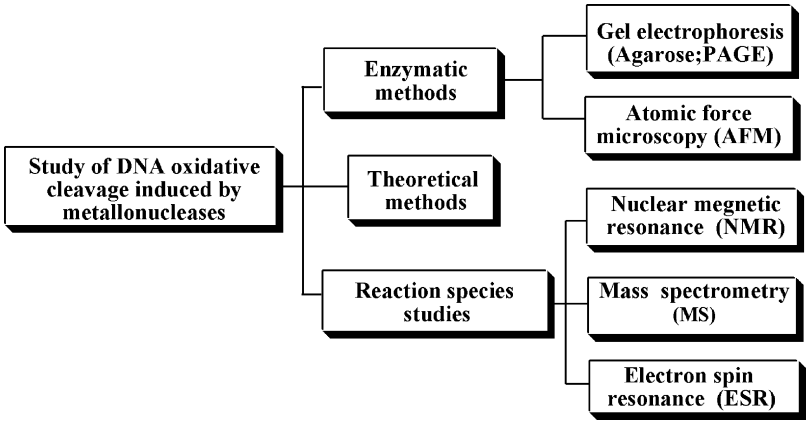

Fig. 5. Methodologies often used for the study of DNA oxidative cleavage.

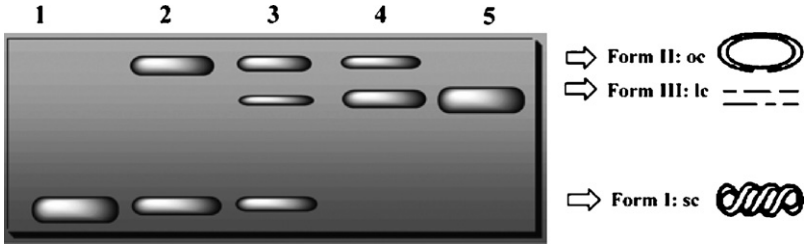

Fig. 6. Typical agarose gel electrophoresis of plasmid dsDNA.

site for base modification [86]. For example, the X-ray structural data revealed that a targeted guanine oxidation by a dinuclear Cu(II) complex at single/double stranded DNA junction was achieved by the coordination of O6 and N7 atoms of guanine with the two copper centers (Cu···Cu, 3.18 Å) [87]. The proposed mechanism and the major oxidative products are shown in Fig. 4.

3. Methodologies

Efficient evaluations of cleavage reaction of metallonucleases rely largely on the methodologies that could provide accurate determination of the reaction rates and unambiguous information on active intermediates and DNA fragments. Thus tremendous efforts have been made to develop and implement feasible and reliable methods and techniques applicable to complicated biological system. Some of the major methodologies frequently used in the field of metallonucleases are listed in Fig. 5. For example, the fast development of MALDI-TOF mass spectrometry greatly improved the identification of DNA fragments [88]. In practical application it is common to combine two or more techniques to get more valuable information. Typical example is the combination of HPLC–MS with ^{32}P-post-labeling/PAGE methods [89].

3.1. Investigation of enzymatic action

Gel electrophoresis is the most frequently used technique for the investigation of the enzymatic action of metallonucleases, including agarose gel electrophoresis and polyacrylamide gel electrophoresis (PAGE). Recently, atomic force microscopy (AFM) as more intuitionistic tool becomes an alternative technique for direct observation of enzymatic action.

3.1.1. Gel electrophoresis

Fig. 6 shows the schematic illustration of typical agarose gel electrophoresis of plasmid dsDNA. Different conformers and adducts of DNA can be visually distinguished from their migration speed in gel. Simple qualitative analysis of the DNA-cleavage ability of specific metallonuclease can be obtained based on the disappearance of *sc* as well as the appearance or disappearance of *oc* and *lc* DNA. As shown in Fig. 6, the cleavage efficiency increases from lane 2 to lane 5 (lane 1, control). Quantitative analysis can be realized by calculating the percentage of conversion of *sc* DNA to *oc* or *lc* forms. The rate constant of the cleavage reactions can be obtained from the time-dependent variation of DNA and nuclease concentration.

The ^{32}P-post-labeling analysis coupled with non-denaturing 30% polyacrylamide gel electrophoresis (^{32}P-post-labeling/PAGE) can be used to detect the cleavage site of DNA, oligonucleotides or DNA fragments. The major advantages of this technique include: (a) simultaneous analysis of multiple samples; (b) high speed and (c) less exposure to ^{32}P [90]. Fig. 7 is a typical example of 5′-^{32}P-labeling PAGE analysis, in which A Rxn. and G Rxn. represent the specific cleavage reaction at adenine and guanine, respectively. It clearly shows that nucleotide A13 is the major cleavage site,

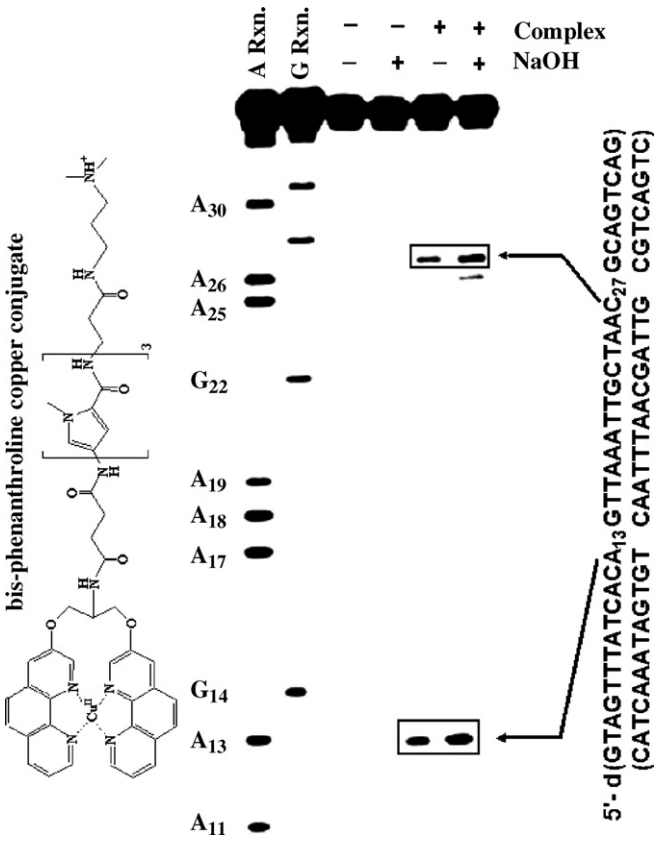

Fig. 7. PAGE analysis on sequence selectivity of the DNA-cleavage induced by a bis-phen–Cu(II) conjugate (modified based on Ref. [91]).

and nucleotide C27 is the second most frequently damaged nucleotide by the bis-phen–Cu(II) conjugate [91].

3.1.2. Atomic force microscopy (AFM)

Direct observation of three conformers of plasmid DNA can be achieved by AFM, although it does not provide information on the cleavage site or cleavage modes due to the limited resolution. Using tapping-mode AFM, plasmid DNA-cleavage by metallonucleases can be visualized [92]. As shown in Fig. 8, the conversion of supercoiled DNA to linear form in the presence of metallonuclease can be imagined clearly.

3.2. Identification of reaction species

Mass spectrometry (MS), electron paramagnetic resonance (EPR), nuclear magnetic resonance (NMR), magnetic circular dichromism (MCD), X-ray absorption (XAS) and spectroscopy are powerful techniques for DNA-cleavage studies. Circular dichromism, UV spectrometry, fluorescence spectrometry, and viscosity measurement are routine methods for studying the interactions of metallonuclease and DNA, and will not be discussed here.

3.2.1. Mass spectrometry (MS)

The high sensitivity of mass spectrometry makes it essential for the rapid detection of DNA binding using only small amounts (picomoles) of metal complexes and DNA [88]. Based

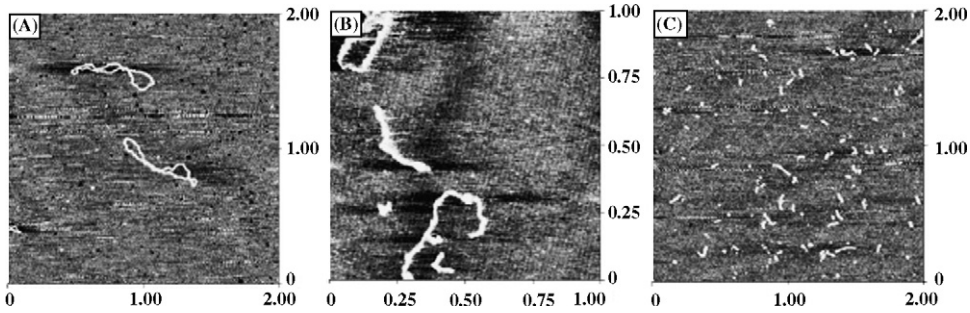

Fig. 8. The cleavage of plasmid DNA detected by AFM (the dimensions are in μm, (A) sc pBR 322 DNA; (B) linearization of DNA at low concentration of nuclease; (C) complete conversion to linear DNA at high concentration of nuclease; modified based on Ref. [92]).

on the molecular weight information of the cleavage products, the mechanism of the DNA-cleavage can be speculated. ESI-MS is powerful for characterization of non-covalent complexes of nucleic acids [93,94]. Combination of ESI with tandem mass spectrometry or MS/MS significantly enhances the potential of the technique, and ESI-MS/MS has been used to determine the sequence location of modified bases [95].

MALDI-TOF MS provides a rapid, sensitive and no-sample-damage method with wider application than ESI-MS. As shown in Fig. 9, 10 products can be detected by MALDI-TOF MS [30]. Peaks 1, 2, 7 and 9 represent the oligonucleotide a^{1+}, b^{1+}, b^{2+} and b^{3+}, respectively. Peak 3 is the 2′-deoxyribonolactone (a-C_{11}) from hydrogen abstraction at C-1′ of base C_{11} (the unpaired cytosine in oligonucleotide a), which form complexes 5 and 10 by β-elimination. Peaks 4 and 8 are assigned to cleavage products at C-3′ of base C_{11} while peak 6 indicates the cleavage at C-1′ of base C_{11}. All these information support the fact that the cleavage occurs at the unpaired cytosine in oligonucleotide no matter which hydrogen is abstracted. So MALDI-TOF MS is one of the most powerful techniques at present for the analysis of reaction mechanism and cleavage products.

3.2.2. Electron paramagnetic resonance (EPR)

EPR is often used to detect the ROS or active intermediates in the reaction of metallonucleases with DNA. For example, the involvement of oxygen atom of water in the oxidation of guanine which leading to formation of •GOH and to 8-oxo-$G^{\bullet+}$ can be demonstrated by EPR (Fig. 10) [96].

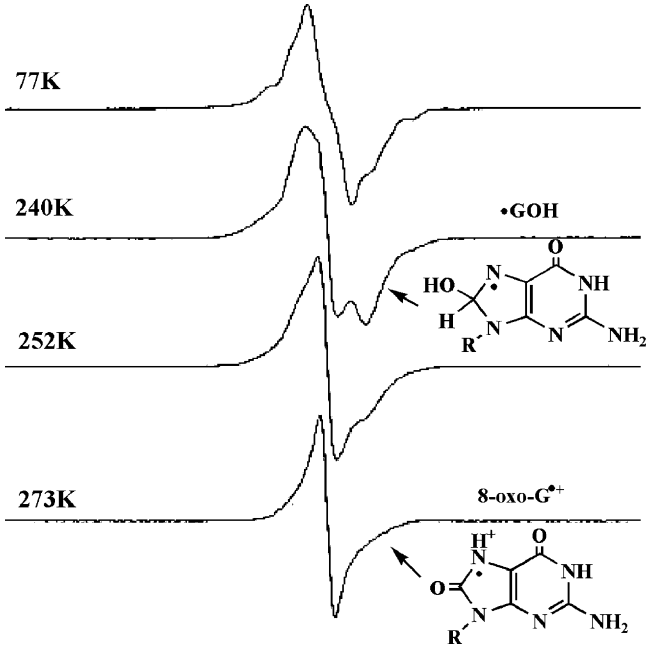

Fig. 10. The application of EPR in the detection of the ROS in G oxidation (modified based on Ref. [96]).

DNA fiber EPR can be used to illustrate the DNA-binding properties of metallonucleases. For example, DNA binding sites (major or minor groove) of the Cu(II) complexes of tetra-peptides can be derived from the *g*// value changes. The final

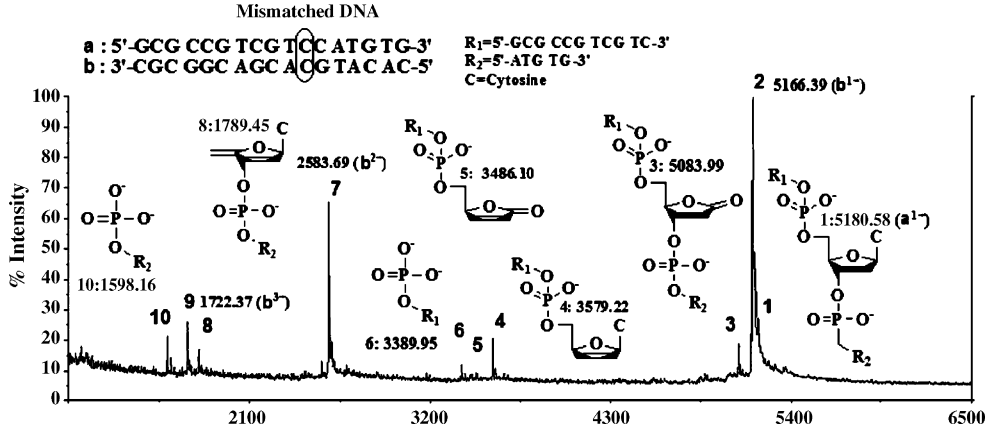

Fig. 9. Application of MALDI-TOF in the characterization of oxidative products of DNA oligonucleotide (modified based on Ref. [30]).

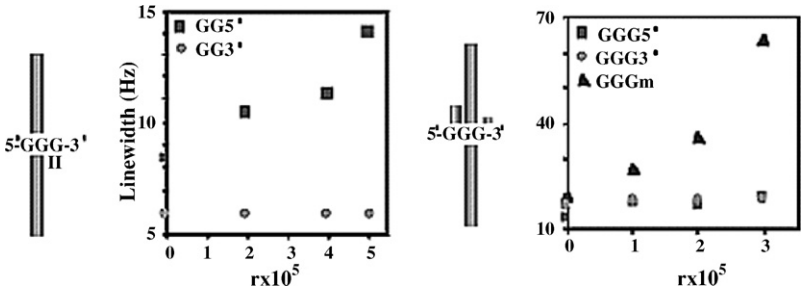

Fig. 11. ^{15}N NMR line broadening of G runs (GG doublet and GGG triplet) induced by Mn(II) ions, $r = [Mn^{2+}]/[phosphate]$ (modified based on Ref. [98]).

structure of complexes in the DNA fiber can also be obtained [97].

3.2.3. Nuclear magnetic resonance (NMR)

Isotopic labeling allows accurate detection of the DNA binding site and identification of cleavage products and intermediates by NMR spectroscopy. Information on the DNA binding property of metallonucleases has important implication for their selective DNA-cleavage. For example, the line broadening of ^{15}N NMR signals of G runs such as GG doublet or GGG triplet in DNA changes differently with increase of the concentration of Mn(II) (Fig. 11) [98]. This can be used for detection of the site selective binding of Mn(II) to DNA. Using ^{13}C- and ^{15}N-guanidinohydantoin, guanine oxidation by singlet oxygen can be followed by NMR spectroscopy. Guanidinohydantoin was identified as the main product of guanine in both nucleoside and in dsDNA [99].

3.2.4. Other techniques

Scanning electrochemical microscopy (SECM) in a G/C nanogap configuration is shown as a useful technique for observing short-lived electrogenerated intermediates of electrochemical oxidation of guanine (Gs). The advantage of the SECM technique is that the measurements can be made under steady-state conditions, short time measurements are not needed [100].

Magnetic circular dichroism (MCD) and X-ray absorption spectroscopy (XAS) spectroscopies have been used to study the interaction of Fe(II)-BLMs with DNA. Variable-temperature and variable-field (VTVH) MCD can be used to detect catalytically relevant Fe(II) species in non-heme iron systems. XAS provides an important complement to the MCD methodology in that the pre-edge can also provide information on coordination numbers while the extended X-ray absorption fine structure (EXAFS) provides metal–ligand bond lengths. The binding of Fe(II)-BLMs to either ct DNA or oligonucleotides perturbs the Fe(II) site, resulting in a change in intensity ratio of the d → d transitions, a decrease in the excited-state orbital splitting, and a reduction in pre-edge intensity. These data suggested a strong axial ligand interaction [101].

Resonance Raman spectra can also be used for the study of the interaction of metallonucleases with DNA. For example, the vibrational modes, ν(Co–OOH) and ν(CoO–OH) of Co-bleomycin, are unperturbed by the complexation of ct DNA, suggesting that the attack on the DNA substrate C4′–H bond by Co-bleomycin does not involve Co–O or O–OH bond cleavage [102].

3.3. Theoretical calculations

DNA-cleavage induced by natural or artificial nucleases can be studied by theoretical methods. For example, the initial attack of DNA by Fe-BLM can be examined by combining the kinetic measurements with density functional theory calculations. The theoretical data support the hypothesis of direct H-atom abstraction from the C-4′ of the DNA deoxyribose sugar by hydroperoxo complex, ABLM, which could generate a reactive Fe^{IV}=O species capable of a second DNA strand cleavage [84] (Fig. 12).

4. Factors influencing oxidative cleavage efficiency

The DNA-cleavage ability of metallonucleases can be affected by metal centers, ligands, co-factors as well as pH, ion strength, temperature and the reaction time. The metal centers largely control the redox potential, the formation of reactive species and DNA binding ability. Ligands act as the unit of functional regulation. Some ligands alone are responsible to a large degree for the overall cleavage ability of the metallonucleases such as metal-BLMs. Measurements of the bond dissociation

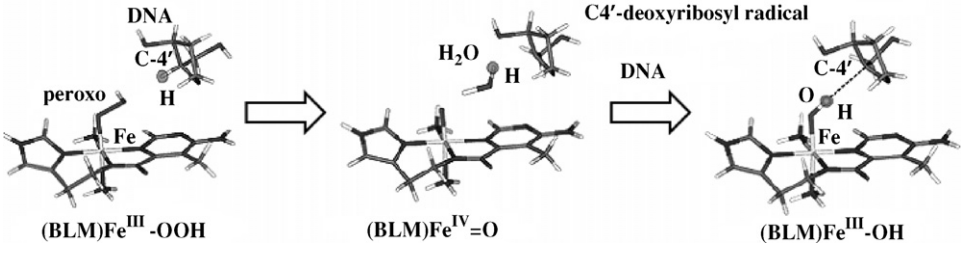

Fig. 12. Direct H-atom abstraction of deoxyribose by ABLM (modified based on Ref. [84]).

energies (BDEs) of many metal complexes in biological systems indicated that metal–ligand BDEs are influenced mainly by the properties of the ligand, generally scaling with the number of electrons donated, the magnitude and alignment of the ligand dipole (or local dipole at the bonding site), and the polarizability of the ligand [86]. Experimental conditions such as the cooperators, pH, ionic strength, temperature and reaction time are also factors that cannot be neglected.

4.1. Metal ions

4.1.1. Metal centers

The intrinsic property of metal ions plays an important role in the cleavage ability of metallonucleases. Burrows and Muller have reviewed, in detail, the nucleobase oxidation mediated by transition metals such as Cr, Mn, Re, Fe, Ru, Os, Co, Rh, Ni, Pd and Cu, and summarized three major mechanisms including electronic transfer, ROS and metal-oxo mediated oxidations [8]. Most novel DNA oxidative cleaving agents reported in the last 6 years are Cu-, Fe-, Mn- and Ni-based metallonucleases [42–76]. Ru-, Rh- and V-based artificial nucleases are often involved in the photo-cleavage of DNA while Zn-based nucleases cleave DNA in a hydrolytic pathway [11,103–106].

4.1.2. Redox potential of metal ions

The oxidative DNA-cleavage induced by metallonucleases often proceed via redox cycles between different oxidation states of the metal ions. Therefore, the redox potential is a useful index for the evaluation of the cleavage ability. For example, the DNA-cleavage ability of a series of Cu-based nucleases derived from *N*,*N*′-dialkyl-1,10-phen-2,9-dimethanamine is highly dependent on the Cu(II)/Cu(I) redox potentials. The most effective complex is the one with the highest redox potential [52]. A series of mononuclear Cu(II) complexes of planar heterocyclic bases like phen, dipyrido[3,2-*d*:2′,3′-*f*]quinoxaline (dpq) and dipyrido[3,2-*a*:2′,3′-*c*] phenazine (dppz) also showed a clear correlation between their redox potential and DNA-cleavage ability [51,55].

4.2. Ligands

Ligands play a key role in the design of metallonucleases. We summarize here some of the most important factors that should be considered in ligand design.

4.2.1. Structural requirements

Some structural features are important for metallonuclease systems and cannot be replaced. Typical examples include Cu-prodigiosin and Fe-BLMs. Prodigiosin (Fig. 13) has been shown to induce oxidative cleavage of dsDNA in the presence of Cu(II) without the need of an added reductant and exhibits regiospecific oxidation of the C-pyrrole [107]. The cleavage activity originates from the complexation of Cu(II) by the electron-rich tripyrrole moiety. Any replacement of A-, B- and C-pyrrole ring by a weaker Cu-ligating group will diminish the Cu^{II}-mediated cleavage activity. As shown in Fig. 13, the active analogue of **1** keeps all three pyrrole rings with only modification of the side chain, while the inactive analogues (**2**–**6**) either lack one of the rings or bear extra donating groups which disturb the chelating environment of Cu center [108–110].

The N- and C-terminus unit of BLM act as the metal binding domain and the DNA binding domain, respectively. The latter increases the affinity of BLM to DNA, thereby enhancing the efficacy of the metal binding unit to cleave DNA (Fig. 14). The Fe-BLM analogue with a N_4Py unit linked via a spacer to an acridine unit, can instantaneously cleave DNA in the presence of O_2, indicating its efficient mimic to both metal binding and DNA binding ability of Fe-BLM [111]. The carbohydrate moiety of a Cu(II)-BLM analogue is essential for the oxidative cleavage of DNA since its precursor complex (without kanamycin A unit) is cleavage inactive [112].

4.2.2. Charge

Since DNA is negatively charged, the higher positive charges of metallonucleases could enhance their affinity to DNA and increase their DNA-cleavage ability. Fig. 15 shows an example of two metal complexes with different overall cleavage. The

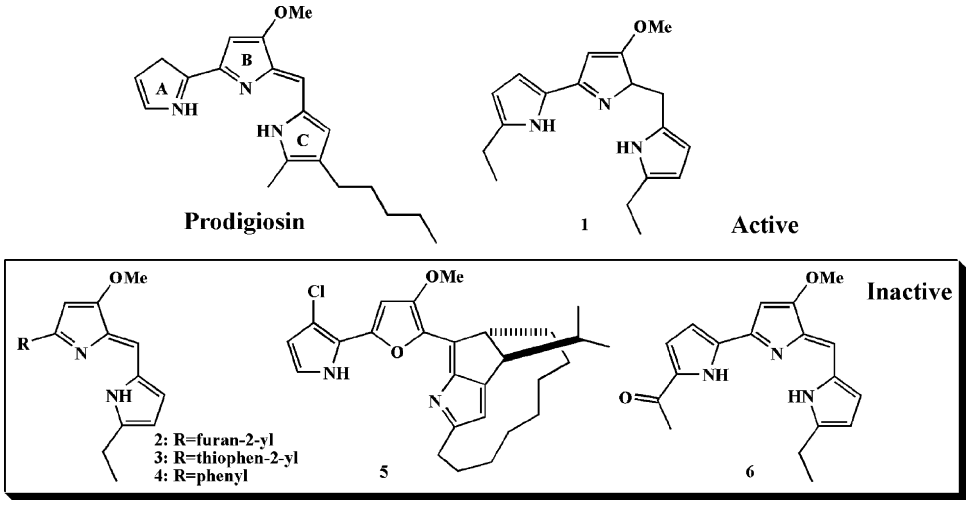

Fig. 13. Prodigiosin and its active or inactive analogues (modified based on Refs. [108–110]).

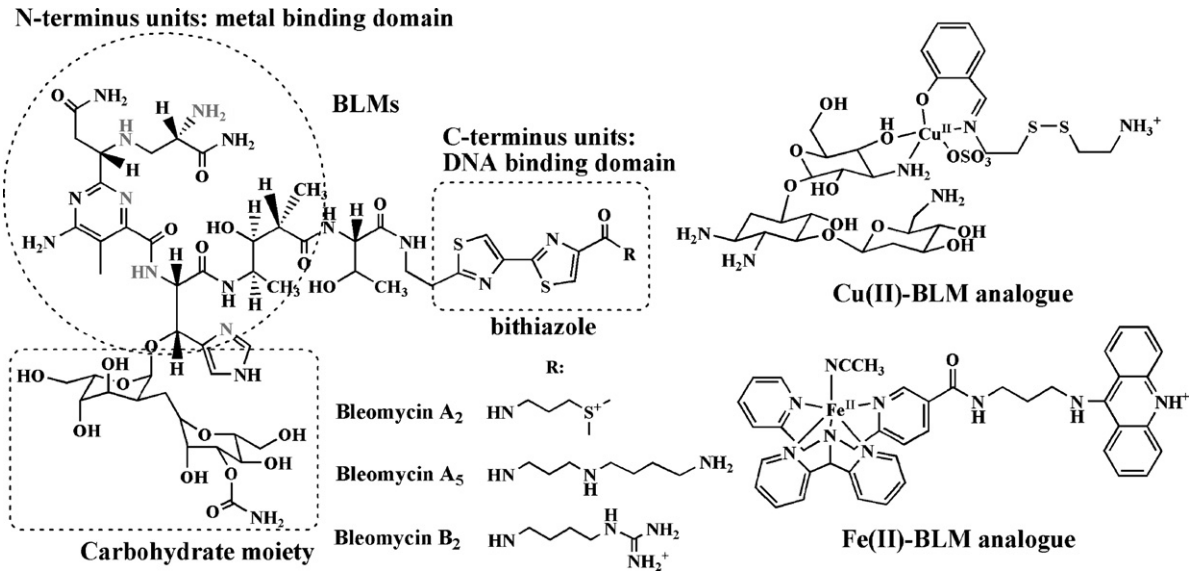

Fig. 14. Structural feature of BLMs and effective metal-BLM analogues (modified based on Refs. [111–113]).

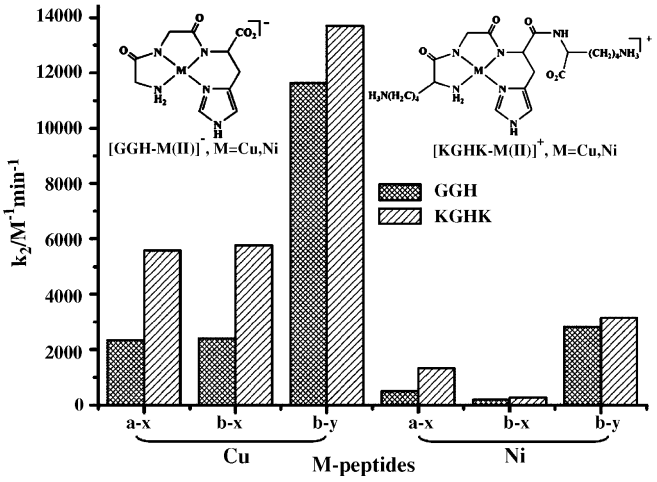

Fig. 15. DNA-cleavage ability of metal-peptides (a, 10 mM Tris buffer, pH 7.4; b, 20 mM Na–cacodylate buffer, pH 7.5; x, oxidative cleavage in the presence of ascorbic acid; y, oxidative cleavage in the presence of $KHSO_5$ (modified based on Ref. [48])).

higher positive charge on $[KGHK\text{-}M]^+$ results in a more effective cleavage efficiency over $[GGH\text{-}M]^-$ [48]. In the case of copper nucleases, monoanionic ligands create Cu(I) complexes that are more reducing and yield less oxidizing Cu/O_2 products, which makes the oxidative cleavage of DNA occur more readily [14].

4.2.3. Planarity

Ligand planarity is another favorable factor for improving DNA-cleavage ability of metallonucleases. The planar structure such as phen can strengthen the DNA binding potential of metallonuclease by intercalation (Fig. 16).

Two Cu(II) complexes of phen derivatives (dppt and pta) are shown in Fig. 17. The former ligand contains a non-planar group while the latter contains a planar one. This difference leads to different DNA binding and cleavage capability of the two Cu(II) complexes. The favorable effect of the planar group is evident [114].

Another planar ligand enhanced DNA-cleavage effect is shown in Fig. 18. The cleavage ability of Cu(II) complexes follows the order of dpq > phen > bpy, suggesting the larger planar structure enhances the DNA-cleavage efficiency [115,116]. The dppz complexes behave quite differently from other complexes, which may be attributed to its distinct DNA binding site compared with that of the other three kinds of complexes [51,55,117,118].

Ligand planarity also has major impact on the mechanism of DNA-cleavage. As Fig. 19(A) shows, complexes with larger planar structure ($[CuL(dpq)]^+$) could be sensitized by both photo-irradiation and co-factors [54]. With the increase of steric encumbrance of L (dmq > phen), oxidative cleavage efficiency of complexes evidently decreases ($[CuL(dmq)]^+ < [CuL(phen)]^+$) while their photo-induced DNA-cleavage efficiency only

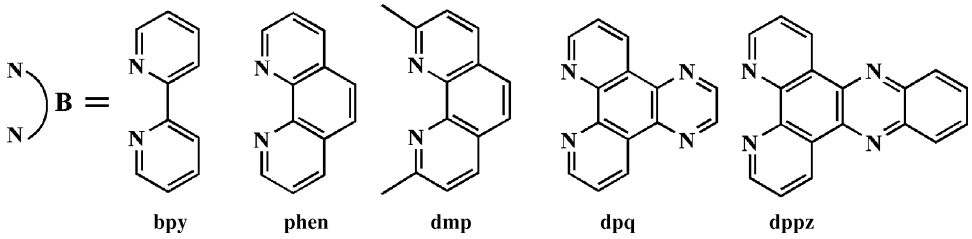

Fig. 16. Several planar ligands as DNA binding groups.

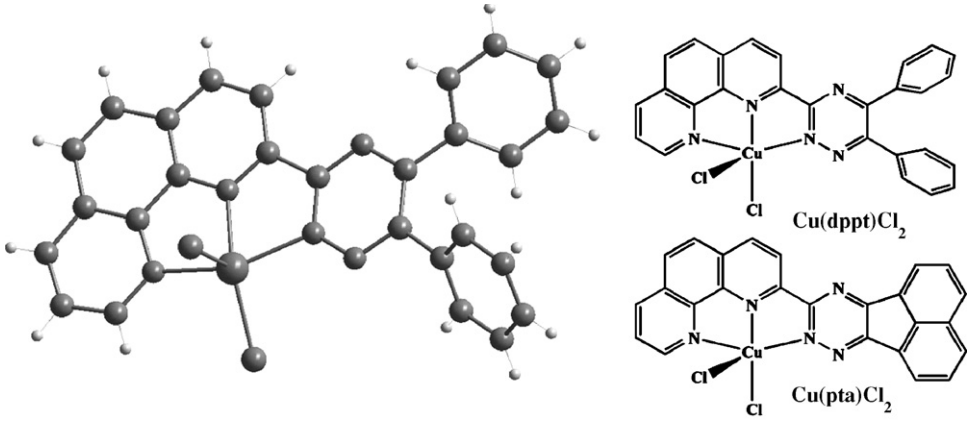

Fig. 17. Structures of $Cu(dppt)Cl_2$ and $Cu(pta)Cl_2$ (modified based on Ref. [114]).

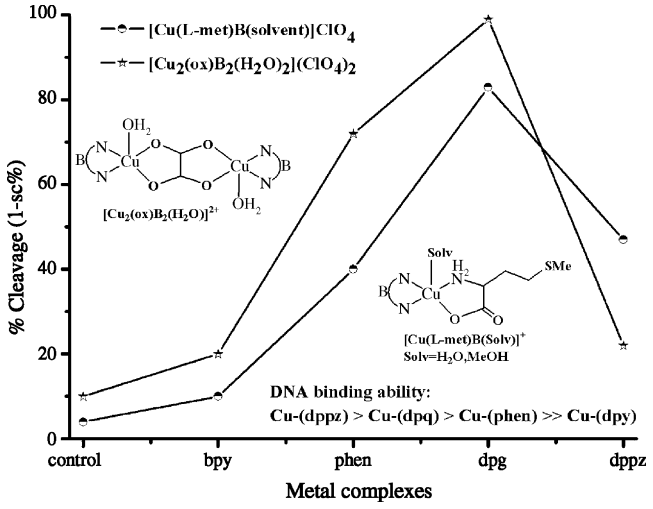

Fig. 18. Influence of ligand planarity (Refs. [115,116]).

changes slightly. Therefore, the oxidative cleavage is more DNA binding-dependent than photo-cleavage. Extensive π-conjugation has the same effect on DNA-cleavage as the ligand planarity. Fig. 19(B) shows that the oxidative cleavage ability of four $[CuL(phen)]^+$ complexes follows the order phen > L^2 > L^1 > L^3, indicating that the presence of extensive π-conjugation can efficiently enhance the DNA-cleavage under dark condition but reduce the photo-cleavage activity by possibly reducing the triplet state lifetime [119].

4.2.4. Geometric configuration

Geometric configuration, including *cis*/*trans* and D/L configuration of metallonucleases, can significantly influence their efficiency and selectivity on DNA-cleavage.

For Ni(II) complexes of dioxocyclams containing quinoxaline moieties (Fig. 20), the cleavage ability is primarily controlled by the appended side arms [120]. *cis*-Quinoxaline amide complex exhibits much higher effective cleavage ability than its *trans*-isomer. It is not known why such a conformational change induced such a drastic change of activity.

The DNA-cleavage abilities of Ni-peptides, Ni(II)-Xaa_1-Xaa_2-His_3 (Fig. 21), were summarized as a function of Xaa position and configuration [36,121]. The cleavage efficiency is higher for D-Xaa than for L-Xaa. When Xaa or His residue adopts L-configuration, an A/T site selective cleavage can be achieved. Steric hindrance of the C-terminal amide in L-His is larger than that in D-His, which makes D-His metallopeptides fit more easily into the minor groove of DNA.

4.2.5. Steric factors

The steric effect of the metallonucleases can influence their DNA binding hence resulting in different cleavage efficiency

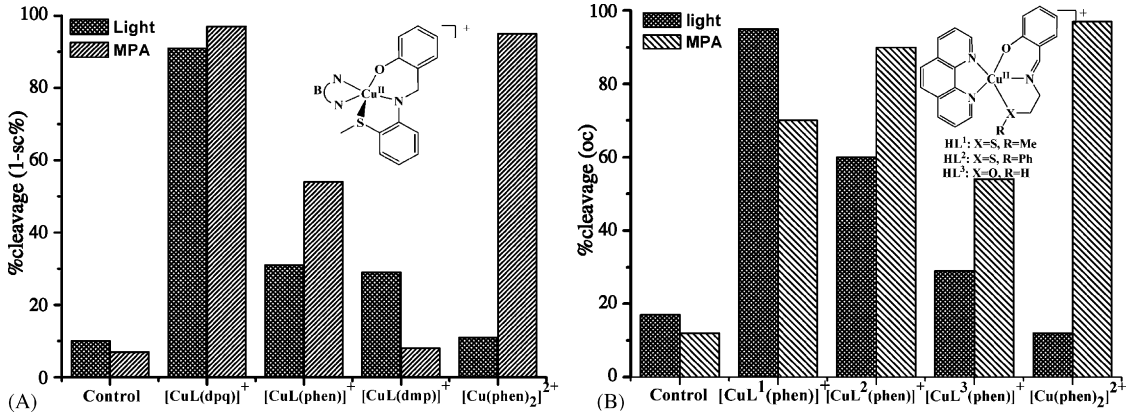

Fig. 19. The comparison of (A) photo-irradiated and (B) oxidative cleavage ability of the complexes $[CuLB]^+$ (based on Refs. [54,119]).

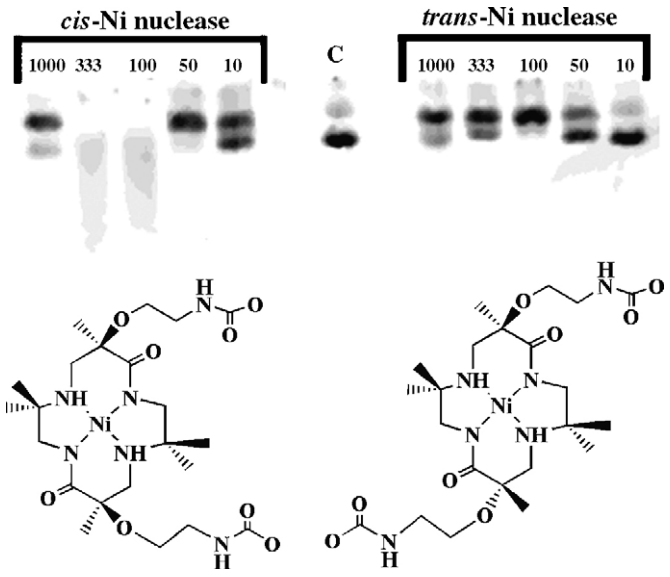

Fig. 20. DNA-cleavage induced by *cis*- and *trans*-Ni-nucleases (modified based on Ref. [120]).

and selectivity. In mono-ligand complexes, steric hindrance disfavors the cleavage efficiency as shown in the case of [Cu(dppt)Cl_2] and [Cu(pta)Cl_2] (Fig. 17). A similar effect is also observed with bis-ligand complexes (Fig. 22). A well-known example is the case of $[Cu(phen)]^{2+}$ and $[Cu(phen)_2]^{2+}$, the activity of $[Cu(phen)_2]^{2+}$ decreased remarkably compared with that of $[Cu(phen)]^{2+}$ due to the block effect of second phen ligand [122,123]. Interestingly, a reverse effect was found for the analogues of Cu-phen, the bis-phen copper complex has more efficient activity than the mono-phen copper complex in a site-specific mode (Fig. 22) [91,124]. This may be due to the fact that the DNA damage mediated by mono- and bis-phen copper complexes proceeds through different reactive species [91]. The same effect is found for $[Cu(Hapt)]^{2+}$ and $[Cu(Hapt)_2]^{2+}$. Studies with inhibiting reagents suggest that the ROS involved in the DNA strand scission for $[Cu(Hapt)]^{2+}$ and $[Cu(phen)_2]^{2+}$ are hydroxyl radical ($^{\bullet}OH$) and singlet oxygen ($^1O_2$), respectively [44]. So the major contribution to positive cooperative effect comes from the ROS.

4.3. Co-factors and reaction conditions

4.3.1. Co-factors

The DNA-cleavage abilities of many metallonucleases are affected greatly by the nature and concentration of the co-factors. Common co-factors include oxidants such as $KHSO_5$ and H_2O_2 and reductants such as ascorbic acid (Asc), 3-mercaptopropionic acid (MPA), glutathione (GSH) and dithiothreitol (DTT). Excess of co-reductants do not favor nucleobase damage since they may reduce the active species (ROS/ROMS/oxidized nucleobases) to inactive species [125].

MPA usually plays dual roles in the strand scission not only as the reductant but also as the reactant with the metallonuclease to form an intermediate. Fig. 23(A) gives an example of the copper–phen complexes. At three cleavage sites A13, C23 and T24, all the rate constants obtained in the existence of Asc are about one order of magnitude larger than that obtained in the existence of MPA. This difference may be due to the following two reasons: the formation of an intermediate inactive to DNA oxidation in the case of MPA or the increase of the H_2O_2 production by autoxidation of Asc [91]. Another example comes from a dinuclear copper complex (Fig. 23(B)). When the extent and pattern of DNA scission were assessed by testing different reductants MPA, GSH, and DTT, the efficiency of the three reductants to promote scission of the target DNA follows the order MPA > GSH > DTT [126].

The influence of co-factor concentration is complicated since they can help producing ROS and are also involved in the redox cycles of the metal complexes. The increase of concentration often improves the cleavage efficiency of the metallonuclease (Fig. 24(A)) [68]. However, the ratio of metallonuclease and co-factor should be kept appropriate. For example, Cu(II)/tetraglycine complex catalyzes the autoxidation of sulfite (S(III)), resulting in oxidative DNA damage. The enhancement effect of S(IV) was observed up to 0.5 mM, while at higher S(IV) concentration, the enhancement effect decreased (Fig. 24(B)) [127]. This can be explained by the competitive redox reaction of excess S(IV) with the active intermediate Cu(III) complex rather than with the oxygen in solution, which may affect the oxidative progress of efficient cleavage.

If the redox potential of the complex is considered, the cleavage efficiency varies greatly when replacing reductant with

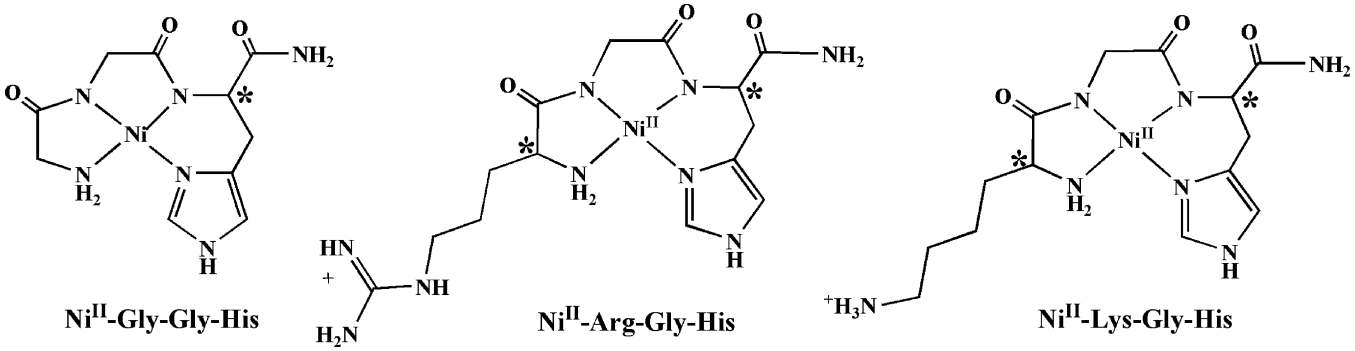

Fig. 21. Structure of Ni-Xaa_1-Xaa_2-His (Xaa = Gly, Arg or Lys, the stereo carbon atoms are labeled in asterisk, modified based on Ref. [36]).

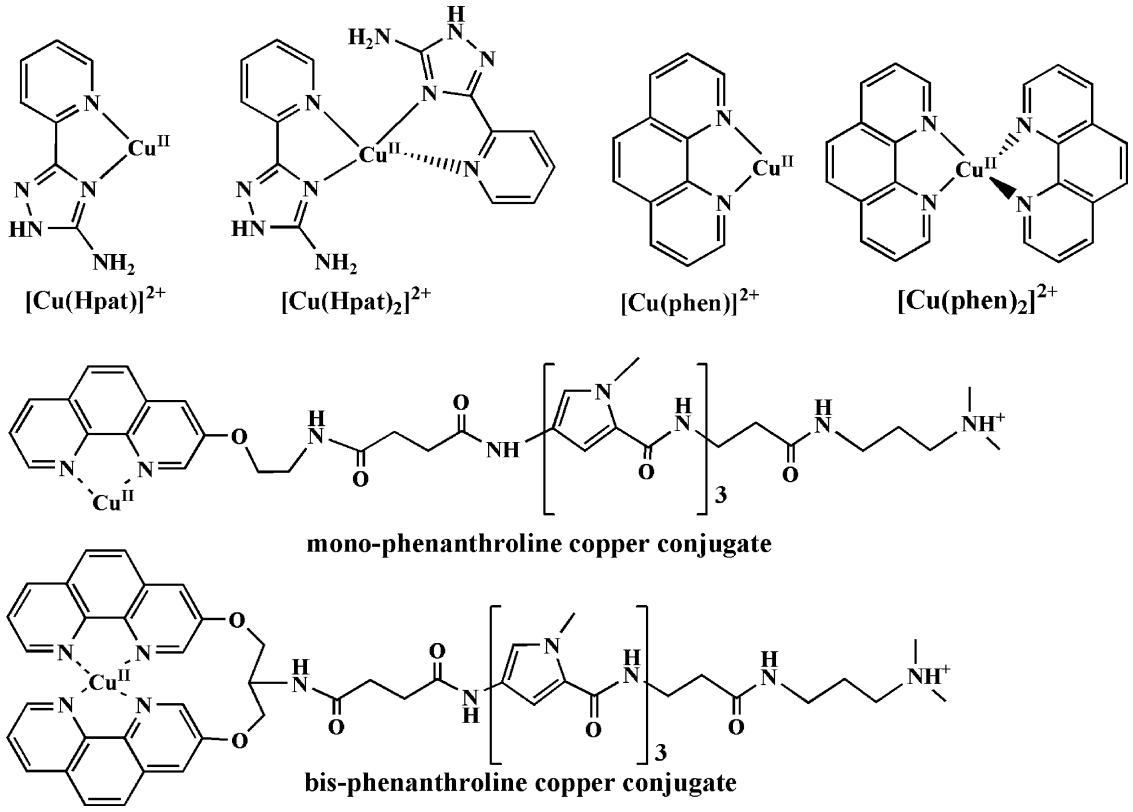

Fig. 22. Structure of CuL^{2+} and CuL_2^{2+}.

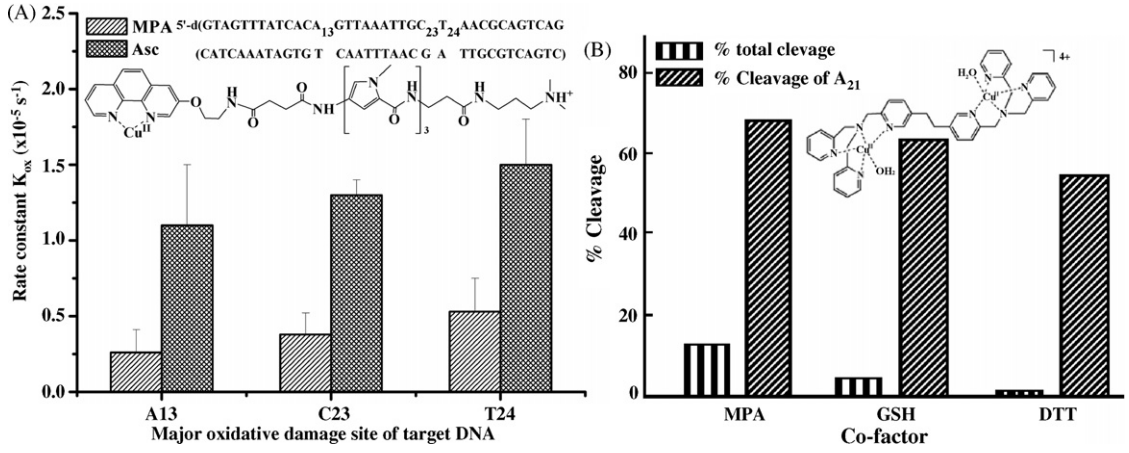

Fig. 23. (A and B) Influence of co-factors on DNA oxidation cleavage (modified based on Refs. [91,126]).

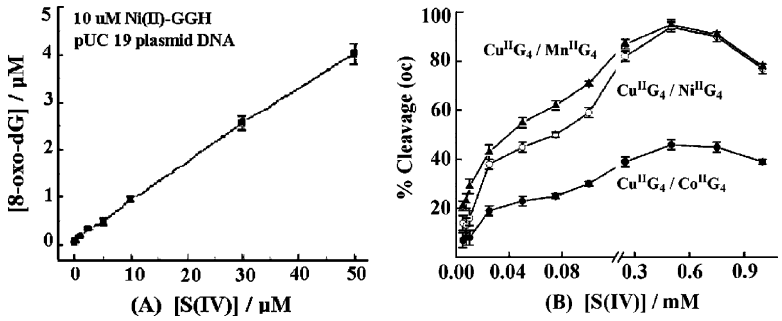

Fig. 24. (A and B) Influence of the co-factor concentration (modified based on Refs. [68,127]).

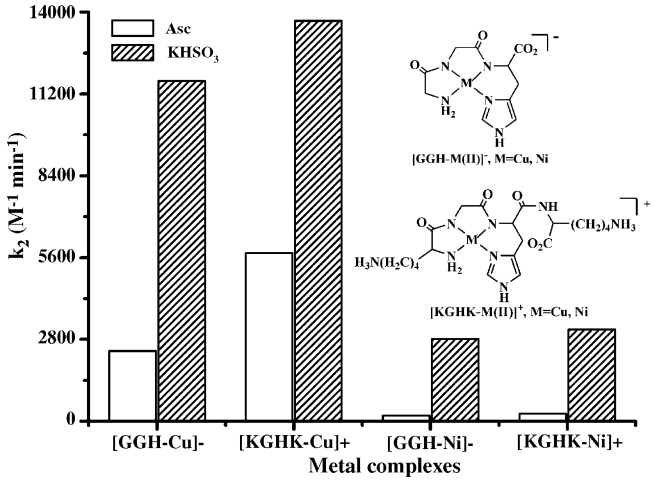

Fig. 25. Influence of oxidant and reductant to DNA oxidative cleavage (based on Ref. [48]).

oxidant. As shown in Fig. 25, metal-peptides (M = Cu, Ni; peptides = GGH, KGGH) have exhibited much faster second order rate constant (k_2) in the existence of $KHSO_5$ (oxidant) compared with that in the existence of Asc (reductant), and those Cu-peptides are more effective than the Ni-peptides, again the effect of redox potentials is evident [48].

4.3.2. pH values and ionic strength

Both ionic strength and pH of the reaction medium are relevant to the activity of some metallonucleases. For those metallonucleases containing amino- or carboxyl-residues, the pH effect may be primary, similar to the mechanism of the acid–base catalysis of natural enzyme. The selection of optimum pH is crucial for the efficient cleavage of DNA by metallonucleases, which may vary according to metal ions, co-factors and the concentration of substrates. Usually, neutral pH is adopted in the investigation of metallonucleases.

DNA damage promoted by Ni(II)-peptides can be dependent on the ionic strength of the reaction medium since the process is diffusion-controlled [69,70]. A high ionic strength hinders the approach of nickel complex to DNA, resulting in a decrease of cleavage efficacy [68].

5. Design strategies

5.1. Multinuclearity

Multinuclearity is one of the successful strategies to increase the efficiency and selectivity of the metallonucleases. The cooperative effect between metal centers can facilitate the formation of the active intermediate involving multinuclear species and play an important role in DNA recognition. An appropriate internuclear distance is the precondition of an internuclear positive cooperative effect [14]. A slight change in ligand structure may alter the selective reaction of multinuclear complexes with nucleic acids [128]. Many studies thus have focused on the cooperative effect of multinuclear complexes. Fig. 26 lists several ligands employed for the synthesis of multinuclear metallonucleases.

The linker and chelater of the ligands shown in Fig. 26 play a determining role in the DNA-cleavage activity of Cu^{II}_2DLn (n = 1–7). For example, Cu_2DL6 showed evident photo-cleavage ability due to the introduction of anthracene [130]. The dinuclear copper complex, Cu_2DL1, is bridged by a phenolate oxygen with Cu···Cu distance of 4.05 Å. It mainly affects DNA base (rather than ribose) oxidation on G residues in single-stranded regions of DNA with the major oxidation products, FAPy-dG [60]. For Cu^{II}_2DLn (n = 2–5), direct DNA strand scission can be induced in the existence of appropriate reductants [39,126]. The Cu^{II}_2DL2 and Cu^{II}_2DL3 are complexes with two copper ions located at the *meta*- and *para*-positions of phenyl group, respectively. The Cu^{II}_2DL3 facilitates the formation of lc DNA while the Cu^{II}_2DL2 can convert sc DNA to oc DNA and then to small fragments directly, reflecting more synergistic effect from *meta*-dicopper motif than *para*-dicopper motif [39]. For Cu_2DL4 and Cu_2DL5, the longer linker reduced the site-specificity and the cleavage ability. The former complex selectively cleaves oligonucleotide strands that extend from the 3′ side of frayed duplex structures at a site two residues displaced from the junction, independent of the nature of nucleobases. The cleavage reaction requires a guanine in the first unpaired position of the 3′ overhang directly adjacent to the central duplex and an adenine on the 5′ overhang in the same position [126]. However, Cu_2DL5 exhibits only a very weak preference for reaction at a helix/coil junction although it can produce a similar yield of sequence-neutral DNA-cleavage as Cu_2DL4 does [126]. The distinction probably derives from the formation and orientation of their μ-peroxo derivative. The Cu^{II}_2(DL7) (n = 4 and 5) other than Cu^{II}_2(DL7) (n = 3) can mediate specific cleavage at helix-coil junctions of DNA evidenced the importance of the ligand-induced copper-dioxygen species [58]. In the presence of oxidants, Cu_2DL7 (n = 3) produced dicopper-end-on-peroxo intermediate (Cu^{II}_2–OOH) while Cu_2DL7 (n = 4 and 5) formed the side-on bridging peroxodicopper intermediate (Cu^{II}_2(DL7)–O_2), which is capable of promoting direct DNA strand scission (Fig. 27).

The presence of three proximate copper ions can facilitate the cleavage efficiency through both the cooperative effect of two metal centers and the orientation of the third to the target DNA. Sometimes, the effect from the third nuclear is negative. Fig. 28 shows three trinucleating complexes Cu_3TLn (n = 1–3) and two mononuclear analogues, Cu-BPA and Cu-DPA [38,39,129]. Comparison between their cleavage abilities indicates that Cu_3TL1 has the lowest DNA-cleavage efficiency, but is more efficient than the mononuclear complex Cu-DPA at the same concentration of copper (Fig. 29(A)) [129]. Compared with the poor cleavage ability of mononuclear analogue Cu-BPA, which cannot convert sc DNA to lc DNA even at high concentration of 18 μM, there is 18% lc DNA appeared for 4 μM Cu_3TL2-treated sc DNA [39]. These data indicate that trinuclear Cu-nucleases have more effective cleavage abilities than their mononuclear analogues. X-ray data show the internuclear distances are 7.353, 6.883 and 8.041 Å for Cu_3TL2 and 5.834, 6.239 and 8.774 Å for Cu_3TL3, respectively [131].

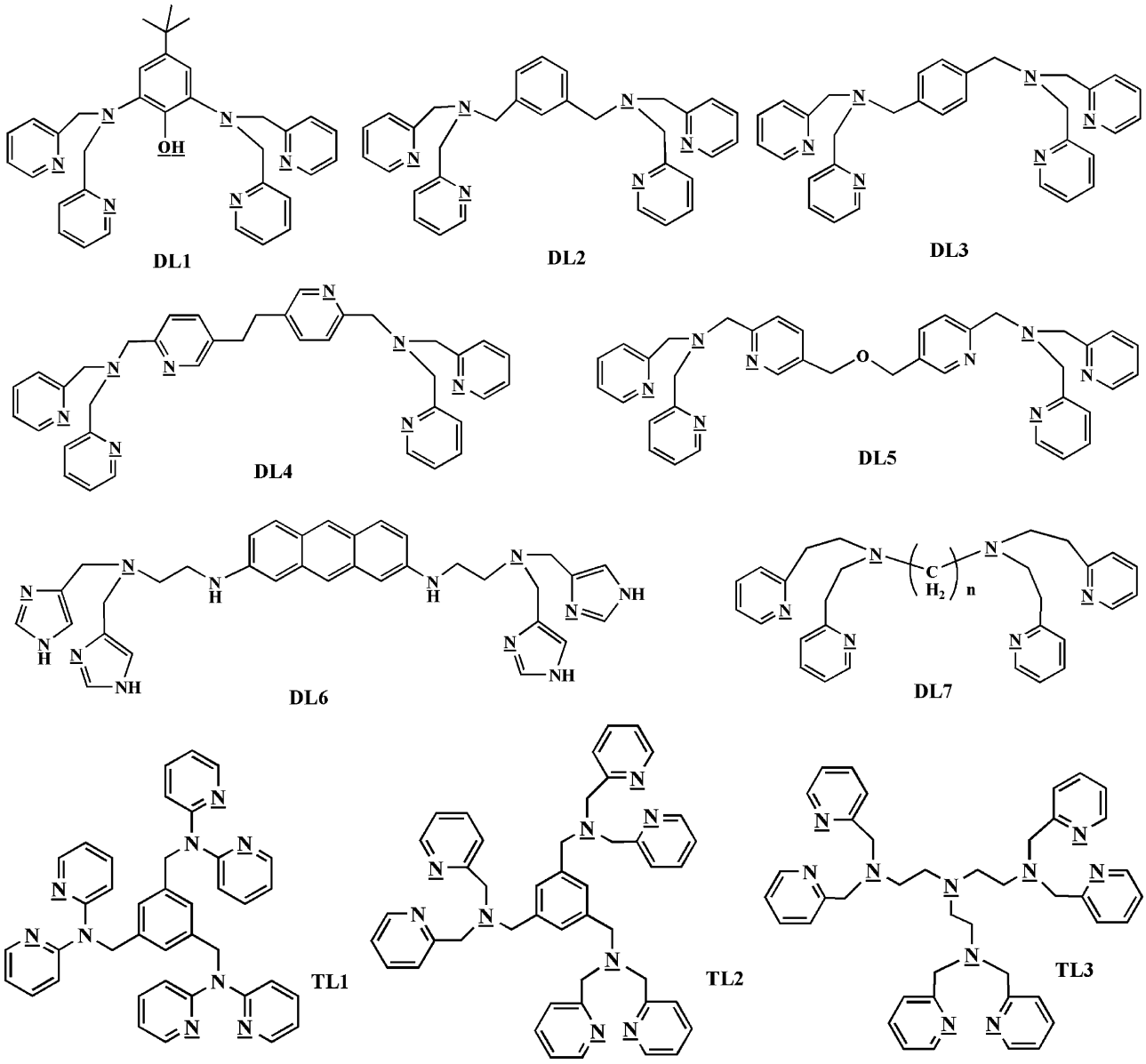

Fig. 26. Dinucleating ligands (DL) and trinucleating ligands (TL) (with the chelate atoms underlined, based on Refs. [38,39,58–60,126,129–132]).

CuTL3 possesses two shorter Cu···Cu distances than CuTL2 does. Their DNA oxidative cleaving ability follows the order of Cu_3TL3 > Cu_3TL2 at low concentrations (≤5 μM) (Fig. 29(B)), suggesting the flexible structure of TL3 is superior to the rigid structures of TL2 for the better inter-nuclear cooperation for the regulation of inter-nuclear distances. However, when the complex concentration increased to 10 μM, the activity of Cu_3TL2 is higher than that of Cu_3TL3 with more lc DNA formed, which can be explained by supposing that the rigid structure of Cu_3TL2 may favor the second strand scission on oc DNA. Through examination of a series of oligonucleotide targets, it was found that Cu_3TL3 promoted specific cleavage at the 3′ overhang of the

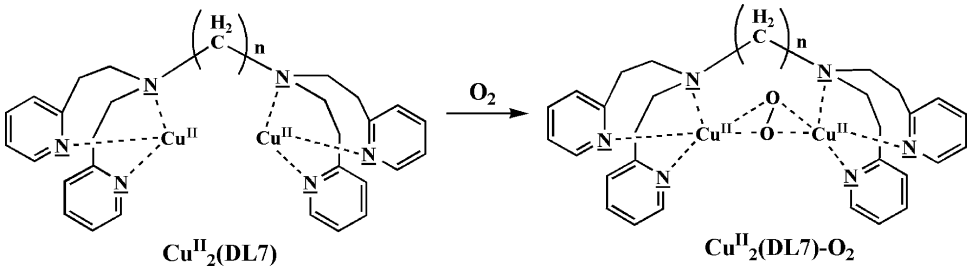

Fig. 27. The formation of the side-on-peroxo-dicopper(II) (Cu_2–O_2) complex (n = 4 and 5) modified, based on Ref. [58].

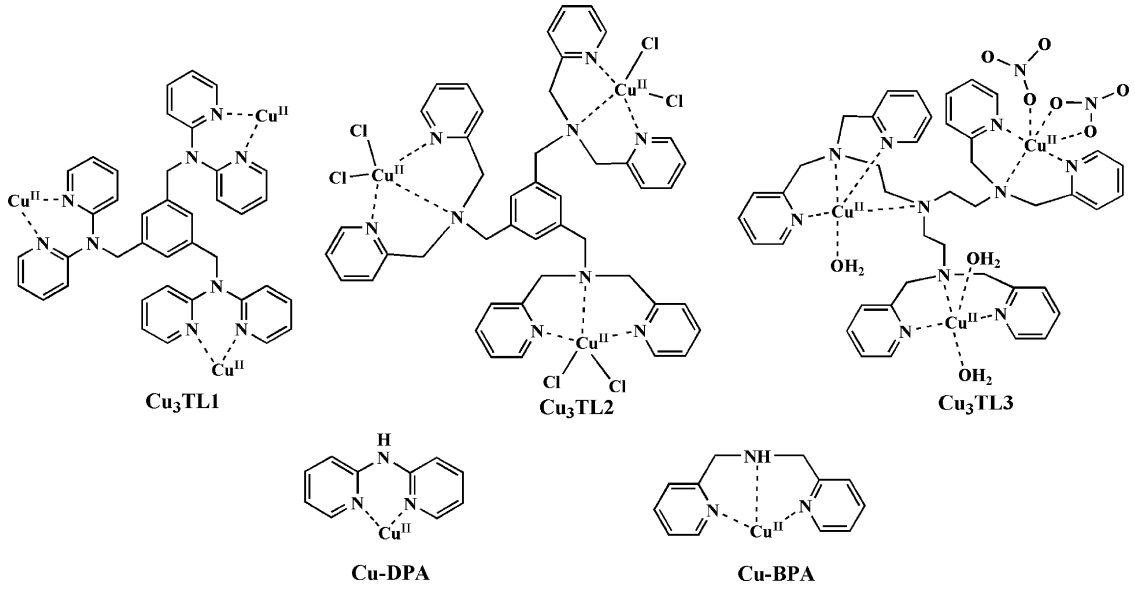

Fig. 28. Three trinucleating complexes Cu_3TLn (n = 1–3) and two mononuclear analogues, Cu-BPA and Cu-DPA (modified based on Refs. [38,39,129]).

junction of a hairpin or frayed duplex structure. Strand scission is not dependent on the identity of the base at cleavage site but requires a purine at the first unpaired position and a guanine at the second unpaired position of the 5′ overhang [38,132].

5.2. Introduction of DNA site/sequence-recognition group

To date, the most common method to increase the DNA recognition of metallonucleases is to combine the nuclease-active motifs with the DNA site/sequence-specific groups. Targeting of different DNA domains such as the minor groove, the major groove and the quadruplex structure of telomeric DNA or a certain sequence can be realized.

5.2.1. Small molecules targeting DNA

Small molecules such as BLMs, DAPI, netropsin, Hoeschst dye, distamycin, porphyrin and perylane derivatives are introduced in the design of metallonucleases. These molecules are well known for minor groove or quadruplex targeting.

For example, a ternary Fe(II)-BLM complex binds to the minor groove of double stranded DNA with a K_{app} of $4.1 \times 10^5\ M^{-1}$ and cleaves sc DNA in the presence of H_2O_2 (Fig. 30(b)) [64]. The iron(II) complex of benzoquinoquinoxaline (BQQ) derivatized EDTA (BQQ–EDTA·Fe) exhibited selective DNA-cleavage at a single site on a 2718 bp plasmid DNA in the presence of a site-specific, triple-helix-forming oligonucleotide (TFO) (Fig. 30(a)) [133,134]. As shown in Fig. 30(c), when BQQ–EDTA·Fe intercalated within a triple helix composed of T·A × T base triplets, the aromatic rings of BQQ intercalated within the triplex and the EDTA·Fe moiety located in the minor groove.

Telomerase activity has been found in 85–90% of all human tumor cells but not in normal cells [135]. Since the aberrant telomere length regulation was discovered in numerous neoplasias, G-quadruplexes, a four-stranded guanine quadruplex secondary structure that generated in the single strand of the telomere, is being considered as a new molecular target for cancer therapy. Metalloporphyrins and perylene derivatives have

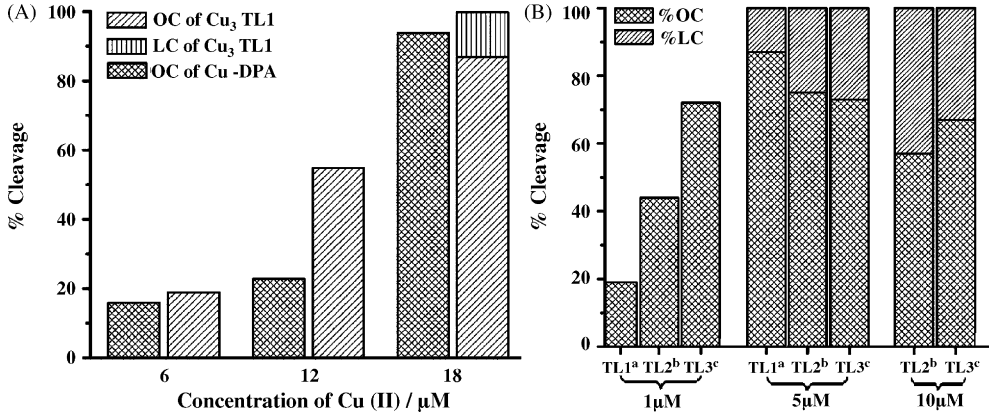

Fig. 29. The DNA-cleavage ability of trinuclear copper complexes CuTLn: (A) comparison of CuTL1 with Cu-DPA and (B) comparison of three CuTLn (n = 1–3) (a: reactions were carried out at pH 7.4, 37 °C, 25 min, 40 mM MOPS buffer, the concentration are 2 and 6 μM, respectively, Ref. [129]; b: pH 7.4, 37.8 °C, 30 min, 50 mM tris–HCl buffer, Ref. [39]; c: pH 8.0, 25 °C, 30 min, 0.1 M tris–HCl and 2 mM EDTA buffer, from Ref. [131]).

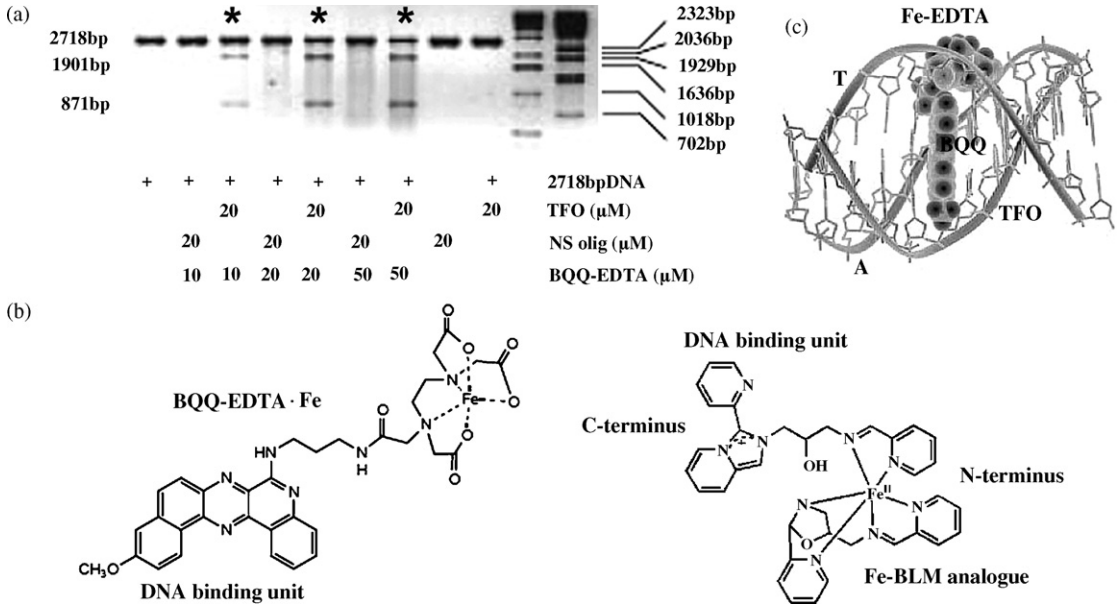

Fig. 30. (a) The selective cleavage of 2718 bp plasmid DNA induced by the BQQ–EDTA·Fe; (b) structures of the BQQ–EDTA·Fe and a Fe-BLM analogue; (c) the energy-minimized model of BQQ–EDTA·Fe intercalated within a triple helix composed of T·A × T base triplets. TFO and NS oligo represent a site-specific triple-helix-forming oligonucleotide and non-specific oligonucleotide, respectively (modified based on Refs. [64,133,134]).

been shown to target the quadruplex structure of telomeric DNA (Fig. 31).

Metalloporphyrins such as Mn-porphyrins interact with the intra-molecular quadruplex by a one-side external π-stacking mainly at the "bottom" tetrad [136]. Modification on the cationic tetramethylpyridiniumyl porphyrins by replacement of one methylpyridiniumyl group by one 4-aminoquinoline moiety can give a more site-specific Mn-nuclease. However, when Mn-porphyrin was modified by two 4-aminoquinoline moieties, their DNA binding ability decreased. These data suggest that the tetramethylpyridiniumyl porphyrin may be partly inserted into the quadruplex [137]. Perylene–Fe(II)·EDTA has exhibited similar binding and cleavage properties at the "bottom" tetrad like PIPER [138–140]. Besides, a more localized cleavage of the G-quadruplex region has been found with increase of pH, indicating a positive effect due to a more effective localization of Fe(II)

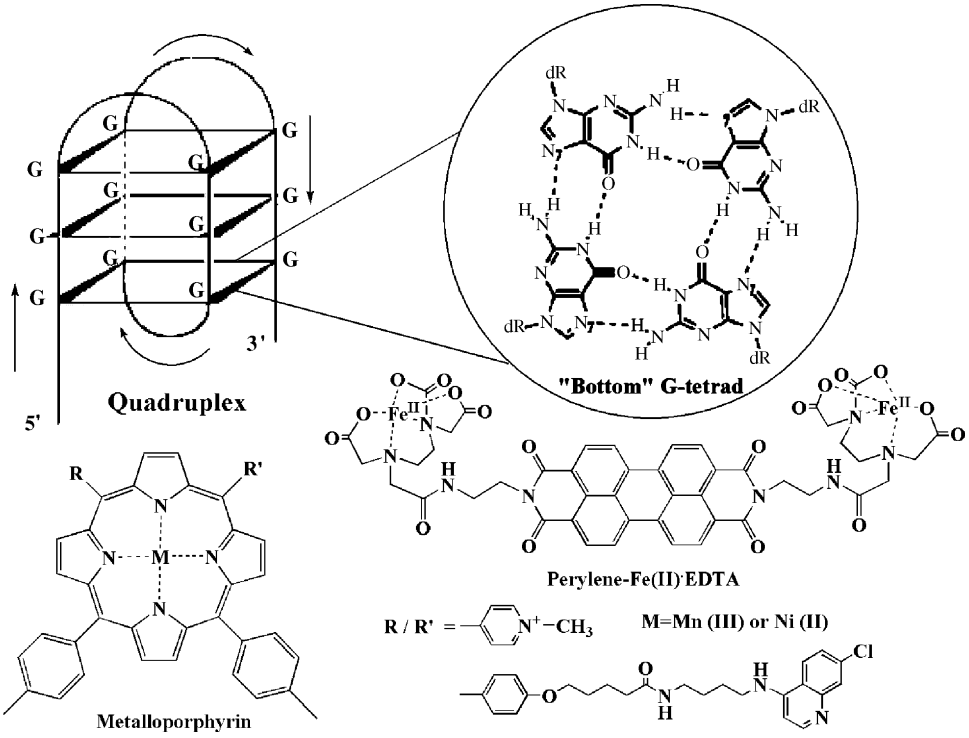

Fig. 31. Metallonucleases targeting the quadruplex structure of telomeric DNA (modified based on Refs. [135–141]).

by perylene–EDTA [141]. These cleaving agents may function as telomere-interactive anticancer agents as well as probes for G-quadruplex structures in chromatin.

5.2.2. Oligonucleotides complementary to the template strand of DNA

The conjugation of specific oligonucleotides (RNA or DNA) with chemical nucleases usually exhibits selective strand scission to the template strand of DNA complementary to the oligonucleotides. The attachment of 1,10-phen–Cu(I) chelate $[Cu(OP)_2]^+$ to different site of oligoribonucleotides (ORNs) can form a series of OP-linked ORNs (Fig. 32), which demonstrates a template-specific DNA scission and could potentially become gene-specific transcription inhibitors [142]. Novel metal-DNA hybrid, Ni^{II}salen-DNA has been assembled for the site-specific cleavage of complementary DNA sequence. Characterization of the DNA-cleavage products by gel electrophoresis and MALDI-TOF MS suggest that Ni^{II}salen-DNA targeted at the dG sites of the complementary strand, even if the nearest dG residue was not directly opposite to the metal-complex in the primary sequence (Fig. 32) [37].

5.2.3. DNA-binding peptides or proteins

Attachment of chemical nucleases to a peptide or a protein has also been attempted to achieve sequence-specific DNA-cleavage [26,143].

Ni-peptides are among the most extensively investigated systems. Ni(II) binding to some chromatin proteins in somatic and sperm cells may result in oxidative and structural damage to the DNA, and these effects may alter the fidelity of DNA replication and gene expression and thus facilitate carcinogenesis, including paternally mediated cancer in the progeny [144]. A Ni(II)-ATP-His system can result in the oxidative cleavage of plasmid DNA but no sequence-specificity is found [145]. Ni-Xaa_1-Xaa_2-His (Xaa are Gly, Lys, Arg, et al.) represents a class of metallonucleases with short peptide chains. The target of these metallonuclease is primary at the minor groove of DNA or the AT-rich domain of DNA, possibly due to the hydrogen bonds between peptide and DNA [121]. The activities of these metallonucleases are dependent on the position and configuration of Xaa residues [36].

A helix-turn-helix (HTH) metallopeptide has been proposed for the purpose of sequences-specific DNA strand scission (Fig. 33). The peptides P3W (33 aa, based on engrailed) and P5b (32 aa, based on antennapedia) comprise both HTH as DNA binding motif and the consensus EF-hand Ca-binding loop as the metal binding motif [146]. Both metallopeptides, Ce(IV)P3W and Eu(III)P3W, cleave the 5′-position of a 121-mer DNA fragment and the 3′-position of DNA oligonucleotides and release 3′-OPO_3 termini and 5′-OPO_3 termini, respectively, suggesting a regioselective mechanism [147]. However, no obvious site/sequence-specific selectivity has been found for these HTH metallopeptides.

5.3. Constraining molecular shapes to fit target DNA

By constraining the molecular shape, a specific domain of DNA can be targeted. For example, Hannon etc. designed a series of Fe(II)-, Cu(I)- and Ru(II)-helicates with imine-based ligands. The shape and size of the helicates fit nicely in the major groove of DNA but are too big for the minor groove [149,150]. A Cu cylinder was found to exhibit unusual tendency to perform a

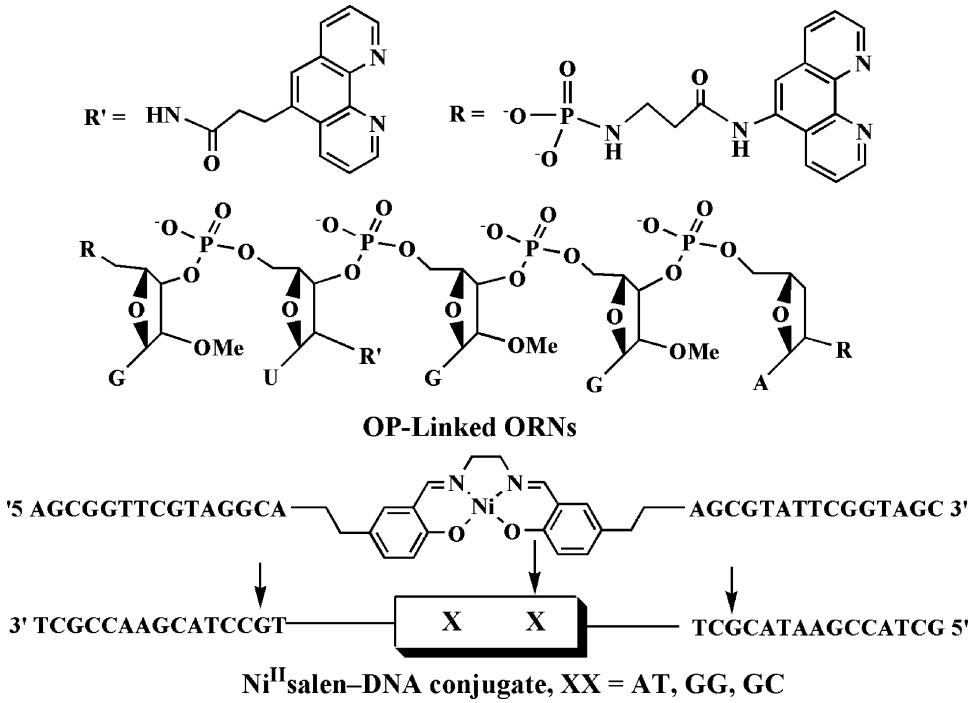

Fig. 32. OP-linked ORNs and Ni^{II}salen-DNA conjugate (the cleavage sites are indicated by the arrowheads, cleavage occurs at X when XX = GG, modified based on Refs. [37,142]).

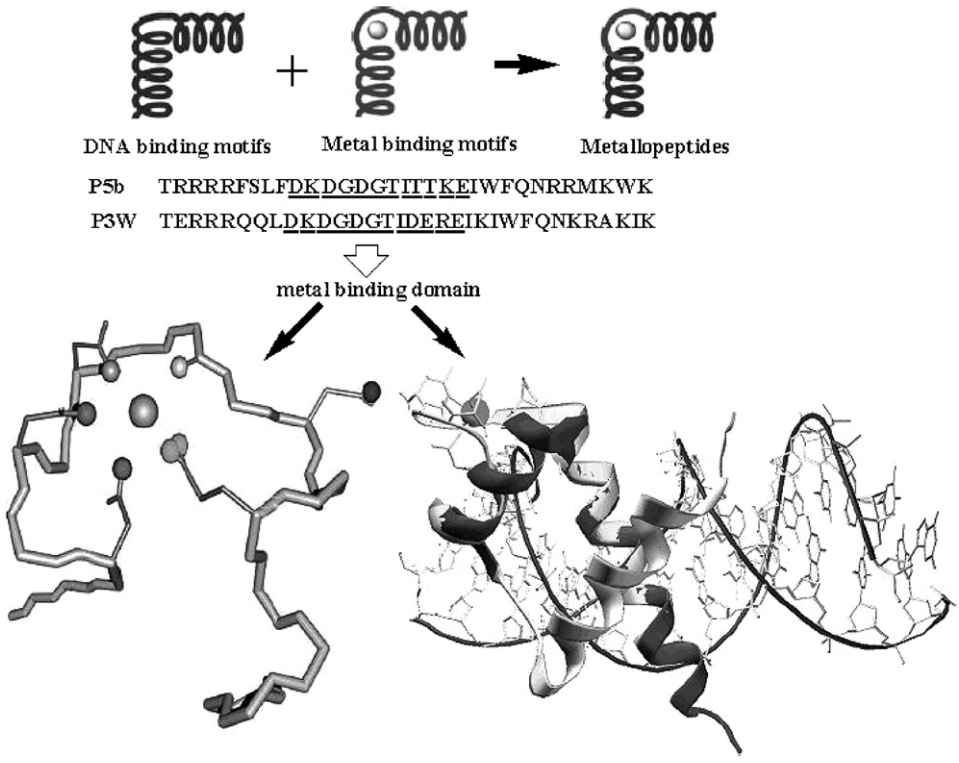

Fig. 33. The design of helix-turn-helix (HTH) metallopeptides (modified based on Refs. [146–148]).

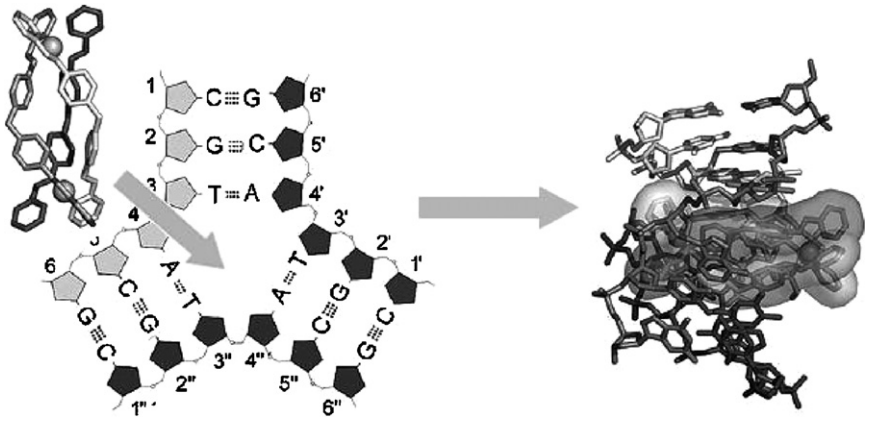

Fig. 34. Specific binding of $[Fe_2L_3]^{4+}$ to three-way DNA junction (modified based on Ref. [151]).

double-strand DNA-cleavage activity in the presence of peroxide, possibly a reflection of its dinuclear nature [92]. A triple helical complex $[Fe_2L_3]^{4+}$ can even selectively bind the three-way DNA junction (Fig. 34) [151]. MD study of the $[Fe_2L_3]^{4+}$ showed this cylinder is approximately 19 Å in length and 11 Å in diameter [152]. These open up a new route for targeted cleavage of DNA by metallonucleases.

6. Concluding remarks

In summary, current design strategies of metallonucleases are focused on two major aspects: (1) integration of both the structural and functional elements. This includes the combination of two or more motifs with different functions in one ligand or two or more ligands with different functions to one metal center; (2) optimization of factors that govern the cleavage ability of metallonucleases, including the selection of the metal ions, ligands, co-factors and reaction conditions.

The purposeful design of metallonucleases with desired structure and function is challenging but promising. A range of DNA recognition groups has been adopted. From simple molecules to large biomolecules such as ORNs, ODNs, peptides or even protein have been employed in the development of nucleases with desired activity and selectivity. Groups sensitive to detectors are introduced in the design to facilitate the prompt detection and accurate characterization of the enzymatic action. Cytotoxic groups are enclosed to study the potential relationship between DNA-cleavage and cytotoxicity. The substrates other than dsDNA such as telomere DNA, PNA and the DNA with pathological changes can also be targeted.

There is a huge scope for the exploration of the mechanism of action of metallonucleases. Even the mechanisms of many well-known systems are sometimes controversial, therefore, a general rationale for effective design of artificial metallonucleases is currently lacking. Future work will be devoted to the accomplishment of artificial metallonucleases with the activity and selectivity comparable to natural enzymes.

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References

[1] M.E. Núñez, J.K. Barton, Curr. Opin. Chem. Biol. 4 (2000) 199.
[2] S.E. Wolkenberg, D.L. Boger, Chem. Rev. 102 (2002) 2477.
[3] T.D. Tullius, J.A. Greenbaum, Curr. Opin. Chem. Biol. 9 (2005) 127.
[4] J.D. West, L.J. Marnett, Chem. Res. Toxicol. 19 (2006) 173.
[5] B. Armitage, Chem. Rev. 98 (1998) 1171.
[6] D.R. McMillin, K.M. McNett, Chem. Rev. 98 (1998) 1201.
[7] W.K. Pogozelski, T.D. Tullius, Chem. Rev. 98 (1998) 1089.
[8] C.J. Burrows, J.G. Muller, Chem. Rev. 98 (1998) 1109.
[9] R.M. Burger, Chem. Rev. 98 (1998) 1153.
[10] M. Costas, M.P. Mehn, M.P. Jensen, L. Que Jr., Chem. Rev. 104 (2004) 939.
[11] G. Parkin, Chem. Rev. 104 (2004) 699.
[12] A.J. Wu, J.E. Penner-Hahn, V.L. Pecoraro, Chem. Rev. 104 (2004) 903.
[13] D.C. Crans, J.J. Smee, E. Gaidamauskas, L. Yang, Chem. Rev. 104 (2004) 849.
[14] L.M. Mirica, X. Ottenwaelder, T.D.P. Stack, Chem. Rev. 104 (2004) 1013.
[15] F. Mancin, P. Scrimin, P. Tecillab, U. Tonellato, Chem. Commun. (2005) 2540.
[16] J.A. Cowan, Chem. Nucl. 5 (2001) 634.
[17] C. Liu, M. Wang, T. Zhang, H. Sun, Coord. Chem. Rev. 248 (2004) 147.
[18] L.J. Boerner, J.M. Zaleski, Curr. Opin. Chem. Biol. 9 (2005) 135.
[19] J.P. Dandliker, R.E. Holmlin, J.K. Barton, Science 275 (1997) 1465.
[20] A. Joy, G.B. Schuster, Chem. Commun. (2005) 2778.
[21] P.K. Bhattacharya, J.K. Barton, J. Am. Chem. Soc. 123 (2001) 8649.
[22] M.E. Núñez, D.B. Hall, J.K. Barton, Chem. Biol. 6 (1999) 85.
[23] S.R. Rajski, B.A. Jackson, J.K. Barton, Mutat. Res. 447 (2000) 49.
[24] J.L. Kisko, J.K. Barton, Inorg. Chem. 39 (2000) 4942.
[25] B.A. Jackson, J.K. Barton, Biochemistry 39 (2000) 6176.
[26] C.B. Chen, L. Milne, R. Landgraf, D.M. Perrin, D.S. Sigman, Chembiochem 2 (2001) 735.
[27] K.D. Copeland, M.P. Fitzsimons, R.P. Houser, J.K. Barton, Biochemistry 41 (2002) 343.
[28] F.L. Gervasio, A. Laio, M. Iannuzzi, M. Parrinello, Chem. Eur. J. 10 (2004) 4846.
[29] K. Sato, M.M. Greenberg, J. Am. Chem. Soc. 127 (2005) 2806.
[30] J. Brunner, J.K. Barton, J. Am. Chem. Soc. 128 (2006) 6772.
[31] J. Suh, Acc. Chem. Res. 36 (2003) 562.
[32] W.J. Pogozelski, T.J. McNeese, T.D. Tullius, J. Am. Chem. Soc. 117 (1995) 6428.
[33] D.S. Sigman, Acc. Chem. Res. 19 (1986) 180.
[34] C.A. Claussen, E.C. Long, Chem. Rev. 99 (1999) 2797.
[35] B. Mestre, A. Jakobs, G. Pratviel, B. Meunier, Biochemistry 35 (1996) 9140.
[36] Y.-Y. Fang, C.A. Claussen, K.B. Lipkowitz, E.C. Long, J. Am. Chem. Soc. 128 (2006) 3198.
[37] J.L. Czlapinski, T.L. Sheppard, Chem. Commun. (2004) 2468.
[38] K.J. Humphreys, K.D. Karlin, S.E. Rokita, J. Am. Chem. Soc. 124 (2002) 8055.
[39] Y. Zhao, J. Zhu, W. He, Z. Yang, Y. Zhu, Y. Li, J. Zhang, Z. Guo, Chem. Eur. J. 12 (2006) 6621.
[40] J.W. Whittaker, Chem. Rev. 103 (2003) 2347.
[41] L.J. Marnett, J.N. Riggins, J.D. West, J. Clin. Invest. 111 (2003) 583.
[42] G. Roelfes, V. Vrajmasu, K. Chen, R.Y.N. Ho, J.-U. Rohde, C. Zondervan, R.M.l. Crois, E.P. Schudde, M. Lutz, A.L. Spek, R. Hage, B.L. Feringa, E. Munck, L. Que Jr., Inorg. Chem. 42 (2003) 2639.
[43] M.L. Neidig, E.I. Solomon, Chem. Commun. (2005) 5843.
[44] S. Ferrer, R. Ballesteros, A. Sambartolomé, M. González, G. Alzuet, J. Borrás, M. Liu, J. Inorg. Biochem. 98 (2004) 1436.
[45] B. Macías, M.V. Villa, F. Sanz, J. Borrás, M. González-Álvarez, G. Alzuet, J. Inorg. Biochem. 99 (2005) 1441.
[46] M. González-Álvarez, G. Alzuet, J. Borrás, M. Pitié, B. Meunier, J Biol. Inorg. Chem. 8 (2003) 644.
[47] A. Patwardhan, J.A. Cowan, Chem. Commun. (2001) 1490.
[48] Y. Jin, J.A. Cowan, J. Am. Chem. Soc. 127 (2005) 8408.
[49] V.G. Vaidyanathan, B.U. Nair, J. Inorg. Biochem. 94 (2003) 355.
[50] A.M. Thomas, M. Nethaji, A.R. Chakravarty, J. Inorg. Biochem. 98 (2004) 1087.
[51] A.M. Thomasa, M. Nethajia, S. Mahadevanb, A.R. Chakravarty, J. Inorg. Biochem. 94 (2003) 171.
[52] T. Hirohama, Y. Kuranuki, E. Ebina, T. Sugizaki, H. Arii, M. Chikira, P.T. Selvi, M. Palaniandavar, J. Inorg. Biochem. 99 (2005) 1205.
[53] B. Macías, I. García, M.V. Villa, J. Borrás, M.G. Álvarez, A. Castiñeiras, J. Inorg. Biochem. 96 (2003) 367.
[54] P.A.N. Reddy, B.K. Santra, M. Nethaji, A.R. Chakravarty, J. Inorg. Biochem. 98 (2004) 377.
[55] S. Dhar, P.A.N. Reddy, M. Nethaji, S. Mahadevan, M.K. Saha, A.R. Chakravarty, Inorg. Chem. 41 (2002) 3469.
[56] M. Lainé, F. Richard, E. Tarnaud, C. Bied-Charreton, C. Verchére-Béaur, J. Biol. Inorg. Chem. 9 (2004) 550.
[57] M. González-Álvarez, G. Alzuet, J. García-Giménez, B. Macías, J. Borrás, Z. Anorg. Allg. Chem. 631 (2005) 2181.
[58] S. Thyagarajan, N.N. Murthy, A.A.N. Sarjeant, K.D. Karlin, S.E. Rokita, J. Am. Chem. Soc. 128 (2006) 7003.
[59] K.J. Humphreys, A.E. Johnson, K.D. Karlin, S.E. Rokita, J. Biol. Inorg. Chem. 7 (2002) 835.
[60] L. Li, K.D. Karlin, S.E. Rokita, J. Am. Chem. Soc. 127 (2005) 520.
[61] M. González-Álvarez, G. Alzuet, J. Borrás, B. Meunier, A. Castiñeiras, Inorg. Chem. 42 (2003) 2992.
[62] N. Saglam, A. Colak, K. Serbest, S. Dülger, S. Güner, S. Karaböcek, A. Osman Beldüz, BioMetals 15 (2002) 357.
[63] S.A. Kane, H. Sasaki, S.M. Hecht, J. Am. Chem. Soc. 117 (1995) 9107.
[64] A. Mukherjee, S. Dhar, M. Nethaji, A.R. Chakravarty, Dalton Trans. (2005) 349.
[65] D.J. Gravert, J.H. Griffin, Inorg. Chem. 35 (1996) 4837.
[66] C.J. Burrows, J.G. Muller, G.T. Poulter, S.E. Rokita, Acta Chem. Scand. 50 (1996) 337.
[67] B. Mestre, G. Pratviel, B. Meunier, Bioconjug. Chem. 6 (1995) 466.
[68] M.V. Alipázaga, R.G.M. Moreno, E. Linares, M.H.G. Medeiros, N. Coichev, Dalton Trans. (2005) 3738.
[69] J.G. Muller, R.P. Hickerson, R.J. Perez, C.J. Burrows, J. Am. Chem. Soc. 119 (1997) 1501.
[70] Q. Liang, P.D. Eason, E.C. Long, J. Am. Chem. Soc. 117 (1995) 9625.
[71] Q. Liang, D.C. Ananias, E.C. Long, J. Am. Chem. Soc. 120 (1998) 248.
[72] C.J. Burrows, S.E. Rokita, Acc. Chem. Res. 27 (1994) 295.
[73] X. Chen, C.J. Burrows, S.E. Rokita, J. Am. Chem. Soc. 114 (1992) 322.
[74] J.G. Muller, X. Chen, A.C. Dadiz, S.E. Rokita, C.J. Burrows, J. Am. Chem. Soc. 114 (1992) 6407.
[75] J.G. Muller, X. Chen, A.C. Dadiz, S.E. Rokita, C.J. Burrows, Pure Appl. Chem. 65 (1993) 545.
[76] J.G. Muller, L.A. Kayser, S.J. Paikoff, V. Duarte, N. Tang, R.J. Perez, S.E. Rokita, C.J. Burrows, Coord. Chem. Rev. 185–186 (1999) 761.
[77] A. Sitlani, E.C. Long, A.M. Pyle, J.K. Barton, J. Am. Chem. Soc 114 (1992) 2303.
[78] K. Miaskiewicz, R. Osman, J. Am. Chem. Soc. 116 (1994) 232.
[79] M. Pitié, C.J. Burrows, B. Meunier, Nucleic Acids Res. 28 (2000) 4856.
[80] T. Chen, M.M. Greenberg, J. Am. Chem. Soc. 120 (1998) 3815.

[81] M.M. Meijier, O. Zelenko, D.S. Sigman, J. Am. Chem. Soc. 119 (1997) 1135.
[82] T.E. Goyne, D.S. Sigman, J. Am. Chem. Soc. 109 (1987) 2846.
[83] T. Oyoshi, H. Sugiyama, J. Am. Chem. Soc. 122 (2000) 6313.
[84] A. Decker, M.S. Chow, J.N. Kemsley, N. Lehnert, E.I. Solomon, J. Am. Chem. Soc. 128 (2006) 4719.
[85] S. Fukuzumi, H. Miyao, K. Ohkubo, T. Suenobu, J. Phys. Chem. A 109 (2005) 3285.
[86] M.T. Rodgers, P.B. Armentrout, Acc. Chem. Res. 37 (2004) 989.
[87] L. Li, N.N. Murthy, J. Telser, L.N. Zakharov, G.P.A. Yap, A.L. Rheingold, K.D. Karlin, S.E. Rokita, Inorg. Chem. 45 (2006) 7144.
[88] J.H. Banoub, R.P. Newton, E. Esmans, D.F. Ewing, G. Mackenzie, Chem. Rev. 105 (2005) 1869.
[89] C. Vialas, C. Claparols, G. Pratviel, B. Meunier, J. Am. Chem. Soc. 122 (2000) 2157.
[90] I. Terashima, N. Suzuki, S. Shibutani, Chem. Res. Toxicol. 15 (2002) 305.
[91] B.C. Bales, T. Kodama, Y.N. Weledji, M. Pitié, B. Meunier, M.M. Greenberg, Nucleic Acids Res. 33 (2005) 5371.
[92] L.J. Childs, J. Malina, B.E. Rolfsnes, M. Pascu, M.J. Prieto, M.J. Broome, P.M. Rodger, E. Sletten, V. Moreno, A. Rodger, M.J. Hannon, Chem. Eur. J. 12 (2006) 4919.
[93] S.A. Hofstadler, R.H. Griffey, Chem. Rev. 101 (2001) 377.
[94] T. Urathamakul, J.L. Beck, M.M. Sheil, J.R. Aldrich-Wrightb, S.F. Ralph, Dalton Trans. (2004) 2683.
[95] P. Iannitti-Tito, A. Weimann, G. Wickham, M.M. Sheil, Analyst 125 (2000) 627.
[96] L.I. Shukla, A. Adhikary, R. Pazdro, D. Becker, M.D. Sevilla, Nucleic Acids Res. 32 (2004) 6565.
[97] R. Nagane, T. Koshigoe, M. Chikira, J. Inorg. Biochem. 93 (2003) 204.
[98] A. Okamoto, K. Kanatani, T. Taiji, I. Saito, J. Am. Chem. Soc. 125 (2003) 1172.
[99] Y. Ye, J.G. Muller, W. Luo, C.L. Mayne, A.J. Shallop, R.A. Jones, C.J. Burrows, J. Am. Chem. Soc. 125 (2003) 13926.
[100] S. Bi, B. Liu, F.-R.F. Fan, A.J. Bard, J. Am. Chem. Soc. 127 (2005) 3690.
[101] J.N. Kemsley, K.L. Zaleski, M.S. Chow, A. Decker, E.Y. Shishova, E.C. Wasinger, B. Hedman, K.O. Hodgson, E.I. Solomon, J. Am. Chem. Soc. 125 (2003) 10810.
[102] C. Rajani, J.R. Kincaid, D.H. Petering, J. Am. Chem. Soc. 126 (2004) 3829.
[103] E. Boseggia, M. Gatos, L. Lucatello, F. Mancin, S. Moro, M. Palumbo, C. Sissi, P. Tecilla, U. Tonellato, G. Zagotto, J. Am. Chem. Soc. 126 (2004) 4543.
[104] C.-T. Chen, J.-S. Lin, J.-H. Kuo, S.-S. Weng, T.-S. Cuo, Y.-W. Lin, C.-C. Cheng, Y.-C. Huang, J.-K. Yu, P.-T. Chou, Org. Lett. 6 (2004) 4471.
[105] M. Sam, J.H. Hwang, G. Chanfreau, M.M. Abu-Omar, Inorg. Chem. 43 (2004) 8447.
[106] V.G. Vaidyanathan, B.U. Nair, Dalton Trans. (2005) 2842.
[107] G. Park, J.T. Tomlinson, M.S. Melvin, M.W. Wright, C.S. Day, R.A. Manderville, Org. Lett. 5 (2003) 113.
[108] M.S. Melvin, K.E. Wooton, C.C. Rich, G.R. Saluta, G.L. Kucera, N. Lindquist, R.A. Manderville, J. Inorg. Biochem. 87 (2001) 129.
[109] M.S. Melvin, J.T. Tomlinson, G.R. Saluta, G.L. Kucera, N. Lindquist, R.A. Manderville, J. Am. Chem. Soc. 122 (2000) 6333.
[110] M.S. Melvin, M.W. Calcutt, R.E. Noftle, R.A. Manderville, Chem. Res. Toxicol. 15 (2002) 742.
[111] G. Roelfes, M.E. Branum, L. Wang, L. Que, B.L. Feringa, J. Am. Chem. Soc. 122 (2000) 11517.
[112] S. Dhar, M. Nethaji, A.R. Chakravarty, Dalton Trans. (2004) 4180.
[113] A.T. Abraham, X. Zhou, S.M. Hecht, J. Am. Chem. Soc. 123 (2001) 5167.
[114] X.-L. Wang, H. Chao, H. Li, X.-L. Hong, L.-N. Ji, X.-Y. Li, J. Inorg. Biochem. 98 (2004) 423.
[115] A.M. Thomas, G. Neelakanta, S.M. devan, M. Nethaji, A.R. Chakravarty, Eur. J. Inorg. Chem. (2002) 2720.
[116] A.K. Patra, S. Dhar, M. Nethaji, A.R. Chakravarty, Dalton Trans. (2005) 896.
[117] S. Dhar, A.R. Chakravarty, Inorg. Chem. 44 (2005) 2582.
[118] A.K. Patra, M. Nethaji, A.R. Chakravarty, Dalton Trans. (2005) 2798.
[119] S. Dhar, D. Senapati, P.K. Das, P. Chattopadhyay, M. Nethaji, A.R. Chakravarty, J. Am. Chem. Soc. 125 (2003) 12118.
[120] L.S. Hegedus, M.M. Greenberg, J.J. Wendling, J.P. Bullock, J. Org. Chem. 68 (2003) 4179.
[121] Y.Y. Fang, B.D. Ray, C.A. Claussen, K.B. Lipkowitz, E.C. Long, J. Am. Chem. Soc. 126 (2004) 5403.
[122] J.M. Veal, K. Merchant, R.L. Rill, Nucleic Acids Res. 19 (1991) 3383.
[123] D.S. Sigman, R. Landgraf, D.M. Perrin, L. Pearson, in: A. Sigel, H. Sigel (Eds.), Metal Ions in Biological Systems, vol. 33, Marcel Dekker, New York, 1996, p. 485 (Chapter 16).
[124] B.C. Bales, M. Pitié, B. Meunier, M.M. Greenberg, J. Am. Chem. Soc. 124 (2002) 9062.
[125] J. Cadet, in: A. Hemminki, A. Dipple, D.E.G. Shuker, F.F. Kadlubar, D. Segerbäck, H. Bartsch (Eds.), DNA Adducts: Identification and Biological Significance, IARC Scientific, Lyon, 1994.
[126] K.J. Humphreys, K.D. Karlin, S.E. Rokita, J. Am. Chem. Soc. 124 (2002) 6009.
[127] R.G.M. Moreno, M.V. Alipázaga, M.H.G. Medeiros, N. Coichev, Dalton Trans. (2005) 1101.
[128] L.Q. Hatcher, K.D. Karlin, J. Biol. Inorg. Chem. 9 (2004) 669.
[129] C. Tu, Y. Shao, N. Gan, Q. Xu, Z. Guo, Inorg. Chem. 43 (2004) 4761.
[130] B. Wilson, L. Gude, M.-J. Fernández, A. Lorente, K.B. Grant, Inorg. Chem. 44 (2005) 6159.
[131] S.T. Frey, H.H.J. Sun, N.N. Murthy, K.D. Karlin, Inorg. Chim. Acta 242 (1996) 329.
[132] K.J. Humphreys, K.D. Karlin, S.E. Rokita, J. Am. Chem. Soc. 123 (2001) 5588.
[133] H.C. Escudé, C.H. Nguyen, S. Kukreti, Y. Janin, J.-S. Sun, E. Bisagni, T. Garestier, C. Hélène, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 3591.
[134] R. Zain, C. Marchand, J. Sun, C.H. Nguyen, E. Bisagni, T. Garestier, C. Hélène, Chem. Biol. 6 (1999) 771.
[135] N.W. Kim, M.A. Piatyszek, K.R. Prowse, C.B. Harley, M.D. West, P.L.C. Ho, G.M. Coviello, W.E. Wright, S.L. Weinrich, J.W. Shay, Science 266 (1994) 2011.
[136] C. Wialas, G. Pratviel, B. Meunier, Biochemistry 39 (2000) 9514.
[137] A. Maraval, S. Franco, C. Vialas, G. Pratviel, M.A. Blasco, B. Meunier, Org. Biomol. Chem. 1 (2003) 921.
[138] W. Tuntiwechapikul, M. Salazar, Biochemistry 40 (2001) 13652.
[139] S.M. Kerwin, Curr. Pharm. Des. 6 (2000) 441.
[140] L.H. Hurley, R.T. Wheelhouse, D. Sun, S.M. Kerwin, M. Salazar, O.Y. Fedoroff, F.X. Han, H. Han, E. Izbicka, D.D. Von Hoff, Pharmacol. Ther. 85 (2000) 141.
[141] W. Tuntiwechapi kul, J.T. Lee, M. Salazar, J. Am. Chem. Soc. 123 (2001) 5606.
[142] L. Milne, Y. Xu, D.M. Perrin, D.S. Sigman, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 3136.
[143] E.G. Prestwich, M.D. Roy, J. Rego, S.O. Kelley, Chem. Biol. 12 (2005) 695.
[144] K.S. Kasprzak, W. Balb, A.A. Karaczyn, J. Environ. Monit. 5 (2003) 183.
[145] P. Kaczmarek, W. Szczepanik, M. Jeżowska-Bojczuk, Dalton Trans. (2005) 3653.
[146] S.W. Wong-Deyrup, Y. Kim, S.J. Franklin, J. Biol. Inorg. Chem. 11 (2006) 17.
[147] R.T. Kovacic, J.T. Welch, S.J. Franklin, J. Am. Chem. Soc. 125 (2003) 6656.
[148] J.T. Welch, W.R. Kearney, S.J. Franklin, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 3725.
[149] C. Uerpmann, J. Malina, M. Pascu, G.J. Clarkson, V. Moreno, A. Rodger, A. Grandas, M.J. Hannon, Chem. Eur. J. 11 (2005) 1750.
[150] M.J. Hannon, V. Moreno, M.J. Prieto, E. Moldrheim, E. Sletten, I. Meistermann, C.J. Isaac, K.J. Sanders, A. Rodger, Angew. Chem. Int. Ed. 40 (2001) 880.
[151] A. Oleksi, A.G. Blanco, R. Boer, I. Usón, J. Aymamí, A. Rodger, M.J. Hannon, M. Coll, Angew. Chem. Int. Ed. 45 (2006) 1227.
[152] S. Khalid, M.J. Hannon, A. Rodger, P.M. Rodger, Chem. Eur. J. 12 (2006) 3493.