Nuclease activity of transition metal complexes – a review

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The review deals with basic elements of DNA structure, DNA interactions, importance of non-specific DNA cleavage, transition metal complexes that cleave DNA through redox chemistry and observations of the author and his associates on gel electrophoresis experiments using plasmid DNA with a series of 2-substituted heteroaromatic semicarbazones and thiosemicarbazones and their copper(II) complexes.

Nucleic acids provide exciting and difficult challenges for chemists and biochemists. They are inherently important, yet highly complex to study. Many aspects of nucleic acid biochemistry are widely appreciated throughout the scientific community.

The recognition that DNA serves as a target for natural and artificial molecules in the inhibition of cellular disorders and in therapy of certain diseases is of paramount importance in inorganic biochemistry. There has been an explosion in the research effort directed towards the isolation and evaluation of naturally occurring DNA cleaving agents and towards the design and synthesis of model compounds that can (specifically) cleave DNA.

The utility of these compounds (nucleases) is enormous and ranges from the creation of synthetic restriction enzymes for use by molecular biologists to the development of chemotherapeutic agents that may be effective against a variety of neoplastic disease. Nucleases have become the molecular scalpels of the biochemists and the indispensable tools for analyzing DNA structure, sequencing DNA molecules and isolating and cloning genes.

Basic elements of DNA structure

DNA may be viewed\(^1\) as a double-helical assembly of the polynucleotides held together by hydrogen bonding and hydrophobic forces. Each polynucleotide includes a backbone of 2′-deoxyribose and phosphate moieties condensed together to form an alternating polymer (Fig. 1). The phosphodiester links involve the 5′ and 3′ oxygens of sugar and as a result, the polymer normally has terminal 5′- and 3′-phosphate ends. In addition, each sugar residue in the chain has a purine or a pyrimidine base covalently linked to its 1′-carbon. Under normal circumstances in aqueous solution the bases tend to stack one upon the other along the chain. In standard B-form DNA two complementary chains come together to form a duplex. The inside of the duplex contains a column of complementary base pairs. In the extended chain, the phosphate-phosphate distances is about 6 Å, but the thickness of a typical base is about 3.3 Å. Thus there has to be horizontal displacement of the phosphate groups in order for stacking to occur\(^2\). As a consequence, each sugar-phosphate backbone winds around the column of base pairs in a helical fashion. The twist angle between the long axes of adjacent base pairs is around 32°, and a stack of about 11 base pairs makes a complete turn of double helix. In a crude fashion, one can view B-form DNA as a twisted ladder with base pairs as the rungs and sugar-phosphate chains as the sides. The C(1′)-N bonds that link the bases to the deoxyribose units occur at an angle with respect to the long axis of the base pair. Hence the C(1′)-C(1′)-vector that connects the two sugars lies off the axis towards one of the edges of the base pair. Consequently, the spacing between the sugar-phosphate chains is not uniform up and down the helix, and there are two different types of grooves that wind along the surface of the DNA duplex. The minor groove has a depth of about 7.5 Å and a width of 5.7 Å, while the major groove has a nominal depth of 8.5 Å and width of 11.7 Å. The deoxyribose groups form the walls of the grooves and the opposite edges of the base pairs form the floors. Although the phosphate groups attract an atmosphere of cations that practically neutralize the charge, there is a residual electric field that is relatively strong within the minor groove.

There are two other important but less common forms of double-helical DNA. The A-form of DNA entails a different sugar conformation and represents a coiled form of a double helix. In this structure the major groove is narrower and deeper, and the bases are no longer perpendicular to the helix axis. Double helical RNA usu-
ally adopts this conformation. The third structure, Z-form DNA, is left-handed. At high ionic strength sequences that involve alternating runs of cytosine and guanine bases tend to adopt this structure.

Nucleases have been defined as molecules which cleave DNA either at specific sites along the strand or in an indiscriminant non-specific fashion. Therefore, the molecules which cleave DNA, are called nucleases.

**DNA interactions**

The reactions performed by both site-specific and non-specific molecules are quite remarkable and intriguingly complex. These reactions can be broken down in terms of two distinct chemical events: (i) the binding step, involving recognition of particular aspect of DNA base sequence or structure, and (ii) the cleaving step, involving a series of transformations which lead either directly or indirectly to hydrolysis of the phosphodiester linkage and scission of the DNA backbone.

A variety of nucleolytic reactions have been reported using both true enzymes and synthetic molecules, and all of which require metal ion for activity. Thus nucleases may be broadly classified into two categories: natural metallo-nucleases and artificial metallonucleases.

An enzyme which degrades nucleic acids and in doing so, has an absolute requirement for metal ion, is called natural metallonuclease. A synthetic molecule also degrades nucleic acids and depends on metal ion for activity, but does not react catalytically in the true sense. For natural nucleases, numerous examples exist in which metal ion have been shown to be essential to the reactivity of the enzyme. Only in a few cases, however, the role of metal ion has been elucidated. For synthetic nucleases, the primary role of the metal ion has been generally to serve as a center for the generation of metal-mediated redox reactions in close proximity to the DNA helix leading to scission of the DNA strand. The reactions mediated by these redox-based nucleases, therefore, are clearly quite different from the types of reactions performed by the DNA-cleaving enzymes.

**Scope of the review**

This review deals with nuclease activity of transition metal complexes of synthetic molecules that cleave DNA through redox chemistry.

**Importance of non-specific DNA cleavage**

Transition metal complexes of synthetic ligands usually accomplish non-specific DNA cleavage. The molecules which cleave DNA in a random non-specific fashion are often quite useful as tools to examine other molecules specifically bound to DNA, and therefore the lack of inherent specificity in these reagents themselves is essential.
To cleave DNA in a nondiscriminant manner requires both a binding interaction, which involves no site on sequence selectivity, and a cleaving reaction which shows no dependence on base composition or conformation. Non-specific nucleases appear to recognize changes in the local conformation of DNA, such as groove widths and orientations about the phosphate backbone; therefore, some sequence of structural selectivity is encountered. Metal ions facilitate the non-specific cleavage of DNA. With respect to sequence neutrality in the cleavage reaction, the ideal reaction might be that involving hydrolysis of the phosphodiester. One obvious role for metal ions in hydrolysis of DNA is their function as electrophilic catalysts, i.e. in polarizing bonds and reducing the negative charge on the phosphate backbone rendering the phosphodiester linkage more vulnerable to nucleophilic attack. However, model systems have elucidated the importance of metal ions as a source of metal-bound hydroxide ions, a potent nucleophile at neutral pH. The highest degree of non-specific cleavage of DNA with synthetic molecules is found through an innocent delivery of hydroxyl radicals to the DNA helix. Site-specific DNA cleavage has been reviewed by Basile and Barton.

**Transition metal complexes as chemical nucleases**

As in the case of the naturally occurring enzymes for the synthetic complexes, the primary role of the metal rests in strand cleavage reactivity. For the enzymes, however, the metal appears to aid in hydrolysis of the phosphate ester, either by providing Lewis acidity or through the delivery of a coordinated nucleophile. In the case of synthetic molecules, the cleavage is not primarily hydrolytic. Instead, primary oxidative damage to the sugar leads eventually to scission of the sugar-phosphate backbone. In these complexes, the metal provides neither acidity nor coordinated nucleophilicity but the redox source to effect the oxidative damage, either indirectly through the delivery of reactive hydroxyl radicals or singlet oxygen, or directly through hydrogen abstraction reactions or direct binding and activation of metal oxo species. Synthetic metallonucleases are discussed below.

**Methodidiumpropyl-EDTA-Fe**

It may be considered to be the first example of synthetic metallonuclease to be widely used as a probe for protein-nucleic interactions as well as for the interactions of DNA with smaller ligands, such as antiviral, antibiotic and antitumour drugs. These interactions can be revealed through the use of footprinting techniques. Previously the nuclease, DNase I, had been successfully used as an enzymatic probe of the sequence preferences of DNA-binding proteins in footprint experiments. In contrast to DNase I, however, MPE-Fe offers the distinct advantage of yielding a higher resolution footprint of the interaction of small molecules, as well as proteins with DNA. This increase in resolution is due to the smaller size of the synthetic footprinting tool compared with the enzymatic probe and also because MPE-Fe cleaves DNA with a lower sequence selectivity than does DNase I.

The synthetic nuclease MPE-Fe was elegantly designed by Hertzberg and Dervan. In the presence of ferrous ion and oxygen, this synthetic nuclease causes single-strand breaks in DNA in a reaction that is made more efficient upon the addition of reducing agents, such as dithiothreitol. The reactive species responsible for the cleavage of DNA in this metal-mediated reaction is presumably a diffusible hydroxyl radical emanating from the iron center. For this non-metallic metallonuclease, binding is coupled to reactivity owing to the presence of the metal ion. The metal ion is essential to the activity of MPE, as it is for the metalloenzyme system. The metal chelator, EDTA, found to be arranged in a slightly distorted octahedral geometry (Fig. 2) about the central ferrous ion is necessary to the reaction.

**Fig. 2. Structure of methodidium propyl-EDTA-Fe complex.**

The presence of an open coordination site or one that is occupied by a readily dissociable ligand, such as H$_2$O has been suggested as important to the reaction chemistry. The reaction mechanisms of MPE-Fe with DNA gave several key mechanistic features. From product analysis, it was suggested that strand scission proceeds via oxidative degradation of the deoxyribose ring. The MPE cleavage reaction depends on ferrous ion and oxygen and is enhanced in the presence of reducing agents, allowing the system to become catalytic by regeneration of the reduced form of the metal. This metal-mediated...
reaction is also inhibited by superoxide dismutase, which converts superoxide to hydrogen peroxide and oxygen. Both superoxide and \( \text{H}_2\text{O}_2 \) are thought to be necessary for the generation of hydroxyl radicals in a metal catalyzed Haber-Weiss reaction\(^{15}\).

\[
\text{O}_2^- + \text{M}^{n+} \rightarrow \text{O}_2 + \text{M}^{(n-1)+}
\]

\[
\text{M}^{(n-1)+} + \text{H}_2\text{O}_2 \rightarrow \text{M}^{n+} + \cdot\text{OH} + \text{OH}^-
\]

Therefore, the results are consistent with the iron mediated reduction of oxygen to hydroxyl radical.

**Bis(1,10-phenanthroline)cuprous ion complex, \([\text{Cu}^\text{(phen)}_2]^+\):**

It acts as an efficient oxidative nuclease. The reaction requires binding of the complex to its nucleic acid substrate and \( \text{H}_2\text{O}_2 \) as a coreactant\(^{16-21}\). In this reaction, \( \text{Cu}^\text{(phen)}_2^+ \) generated in situ is reduced to the cuprous form \( \text{Cu}^\text{(phen)}_2^- \) in the presence of thiol. As with MPE-Fe\(^{11}\), strand scission is seen to result from oxidative destruction of the deoxyribose moiety of DNA. The reaction involves generation of metal ion-associated radical species through a one-electron oxidation of the bound cuprous complex by \( \text{H}_2\text{O}_2 \).\(^{21}\) From analysis of the products of the scission reaction, it was concluded that the initial site of attack by this hydroxyl radical like species is the C-1' hydrogen of the deoxyribose ring, with minor site of attack at the C-4' hydrogen\(^{22}\).

Two possible binding modes have been proposed for the interaction of \( \text{Cu}^\text{(phen)}_2^+ \) with DNA: (i) interaction of one of planar phenanthroline ligands between adjacent base pairs and/or (ii) insertion of the coordination complex into a groove of duplex DNA\(^{23,24}\).

**Metalloporphyrins:**

Metal complexes of the water-soluble tetracationic porphyrin *meso*-tetrakis(N-methyl-4-pyridinium)porphin (\( \text{H}_2\text{TMPyP}\))\(^{25-30}\) are most widely studied DNA-binding metalloporphyrins, and many of these complexes have been shown to act as metallonucleases either through chemical\(^{29,30}\) or photochemical activation. These metallonucleases appear to cleave preferentially at AT sequences contained within a restriction fragment of pBR 322 DNA. These metalloporphyrins are, however, capable of cleaving at all four nucleotide positions suggesting that it is deoxyribose moiety which is the primary site of attack in the strand scission process. The preferential association in the minor groove with AT-rich sequences may be an indication that it is the groove-bound rather than intercalative interaction of porphyrins with DNA that leads to the cleavage chemis-

\[\text{Base} \rightarrow \text{SB1} \rightarrow \text{SB2} \rightarrow \text{Base} \]

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**Fig. 3. Different routes for oxidative attacks on C-H bonds of DNA deoxyriboses by chemical nuclease.**
try. A series of iron porphyrins tethered to an acidine ligand, in contrast, have been shown to cleave DNA in a sequence neutral manner\textsuperscript{31}, thus it is probably the binding interaction rather than the cleavage chemistry.

Porphyrin derivatives have been used in the diagnosis and treatment of malignant diseases. A hemin derivative such as HPD (hematoporphyrin derivative) tends to accumulate specifically in neoplastic tissues and produces irreversible damages via singlet oxygen when the dye is photoactivated by visible light\textsuperscript{32-34}. Efforts have been made to prepare new porphyrin derivative in cancer phototherapy\textsuperscript{35-37}. Manganese(III)-meso-(p-sulfonato-phenyl)porphine has been used as a tumour-specific contrast enhancing agent in medical resonance imaging (MRI)\textsuperscript{38,39}.

Cationic porphyrin molecules are able to bind to DNA\textsuperscript{40,41} and RNA\textsuperscript{42}. Most of the studies have been focussed as free meso-tetrakis(4-N-methylpyridinium)-porphine (TMPyP) or its metallated derivatives. Several exhaustive reviews in this field are available\textsuperscript{40,41}. For the metallated TMPyP derivatives three modes of binding have been recognized: (i) interaction when metalloporphyrins do not have axial ligands\textsuperscript{40,41}, (ii) outside DNA binding for complexes with axial ligands, and (iii) minor groove binding in the case of MnTMPyP. Recent advances in the oxidative DNA cleavage by iron and manganese porphyrin complexes including different pathways (Fig. 3) for oxidative attacks on C–H bonds of DNA deoxyribose by chemical nucleases have been reviewed by Meunier\textsuperscript{43}.

Peroxynitrite induced DNA strand scission mediated by a manganese porphyrin [5,10,15,20-tetrakis(N-methyl-4-pyridyl)porphatinomanganese, MnTMPyP] has been reported by Grooves and Sudhakar\textsuperscript{44}. Peroxynitrite (ONOO\textsuperscript{−}) is a potent oxidant\textsuperscript{45} generated by the reaction between nitric oxide (NO\textsuperscript{−}) and superoxide ion (O\textsuperscript{2−})\textsuperscript{46}. Peroxynitrite\textsuperscript{47} reacts rapidly and efficiently with MnTMPyP under physiological conditions to generate an oxomanganese intermediate; a 1:1 stoichiometry is observed for the reaction, even though the half-life of ONOO\textsuperscript{−} is \textless 3 s under these conditions\textsuperscript{48}. An oxidative DNA cleavage and phenol nitration reactions proceed via the oxo-Mn\textsuperscript{V} and oxo-Mn\textsuperscript{IV} intermediates, respectively\textsuperscript{49,50}. Thus these metallopor-porphyrins efficiently catalyze the decomposition of ONOO\textsuperscript{−} and mediate the oxidative cleavage of plasmid DNA. An oxo-Mn\textsuperscript{IV} intermediate was observed. Adventitious metal ions known to be present under conditions of cell stress\textsuperscript{51} would accelerate oxidation and nitration of cellular components in regions of high ONOO\textsuperscript{−} flux, thereby potentiating its toxicity. Alternatively, metal complexes designed to intercept reactive oxygen intermediate in cells would provide an avenue for pharmacological intervention.

\[
[\text{Fe}^{II}\text{-EDTA}]^{2−}:
\]

It is the simplest inorganic coordination complex capable of exhibiting nuclease activity. This complex provides a very simple yet powerful tool to probe the structure of DNA free in solution or bound to other molecules\textsuperscript{52-54}. Its mode of action as well as the function served by the metal are similar to that described for MPE-Fe\textsuperscript{II}, since in both cases, it is the [Fe\textsuperscript{II}-EDTA]\textsuperscript{2−} moiety that is responsible for the DNA strand scission reaction.

Tris(phenanthroline)metal complexes:

Barton \textit{et al.}\textsuperscript{55-59} developed a family of tris(1,10-phenanthroline)metal complexes which display stereoselective binding to duplex DNA and can be activated in the presence of light to produce strand scission reactions\textsuperscript{59-63}. These cationic complexes display octahedral coordination about a central metal ion, yielding complexes which are rigid in structure and exist in one of the two enantiomeric configurations: a right-handed spiral, the \(\Lambda\) enantiomer, or a left-hand spiral, the \(\Sigma\) enantiomer. Spectroscopic studies using ruthenium complexes are extremely valuable in elucidating the binding characteristics of the complexes to DNA\textsuperscript{56-58}. Ru(phen)\textsuperscript{3+} complexes are intensely coloured, highly luminescent and these spectroscopic features are perturbed upon bindnig to DNA. At least two binding modes of the complexes with DNA are apparent – interaction and groove (surface). In binding studies of Ru(phen)\textsuperscript{3+} to a right-handed B-DNA helix, the \(\Lambda\) enantiomer is favored for interaction, whereas for surface binding, it is the \(\Sigma\) enantiomer that is found to be preferred.

Tris(phenanthroline) metal complexes furthermore can serve to cleave the DNA strand. Ru(phen)\textsuperscript{3+}, Co(phen)\textsuperscript{3+} and Rh(phen)\textsuperscript{3+} have been shown to induce strand scission upon photoactivation\textsuperscript{64-68}.

\[
[Rh(phen)]_3\phi\text{ii}^{3+} \text{ and } \{Rh(DIP)\}_3\phi\text{ii}^{3+}:
\]

In contrast to [Rh(phen)]\textsuperscript{3+}, bis(phenanthroline)-phenanthroquinonediimine)rhodium, [Rh(phen)\textsuperscript{3+}] and tris(4,7-diphenyl-1,10-phenanthroline)rhodium, [Rh(DIP)]\textsuperscript{3+} show specific scission patterns. For example, the latter complex can recognize G-U mismatches in double-stranded regions of RNA\textsuperscript{69,70}, and also nicks DNA near cruciform structures\textsuperscript{71}. The former complex nicks RNA at a range of tertiary structures including G-U mismatches, bulges containing more than one unpaired nucleotide and stem loops.
The reactivities and applications of a biological, nucleolytic, redox active coordination complexes have been exhaustively reviewed by Sigman et al.\textsuperscript{72}.

**Macrocyclic Ni\textsuperscript{II} complexes:**

Nickel\textsuperscript{II} complexes of 13-14 membered tetradentate macrocyclic ligands are typically square-planar diamagnetic compounds. Common examples (Fig. 4) include Ni\textsuperscript{II} complex of the quadridentate Schiff base macrocyclic ligand resulting from condensation of 2,6-diacetylpyridine with the open-chain triamine H\textsubscript{2}N(CH\textsubscript{2})\textsubscript{4}NH(CH\textsubscript{2})\textsubscript{4}NH\textsubscript{2}, first described by Karn and Busch\textsuperscript{73}, and the well known Ni(cyclam), whose chemistry and derivatives are described in literally hundreds of publications. NiCR and Ni(cyclam) were found to be effective agents for oxidation of guanine residues in nucleic acids.

Nickel compounds have two characteristics in common with leading antitumour drugs: (i) direct metal binding to N7 of guanine is possible and (ii) nickel complexes are able to catalyze oxidative damage to nucleic acids.

Nickel is a remarkably versatile metal in biological chemistry\textsuperscript{74}. It is a necessary component of certain metallo-proteins and is at the same time an environmental carcinogen causing DNA damage and protein-DNA crosslinks. For the molecular biologist, nickel offers a tool for the study of nucleic acid structure in the form of tetraaza-macrocyclic complexes.

**Desferal complexes:**

Desferal, a well known siderophore\textsuperscript{75-78} and a highly effective drug in chelation therapy of iron overload diseases, forms a stable octahedral Fe\textsuperscript{III} complex. In contrast of \begin{math} ^{6} \text{FeJTA}, \end{math} it cannot undergo a redox cycling, thus preventing iron catalyzed hydroxyl radical formation\textsuperscript{79}, which is a useful property for its clinical applications. Indeed desferal is employed to arrest hydroxyl radical production in DNA scission reactions caused by Fe\textsuperscript{II} complexes\textsuperscript{80,81}, while the Fe\textsuperscript{III} complex of desferal is passive in DNA scission, the corresponding Cu\textsuperscript{II}, Co\textsuperscript{III} and Ni\textsuperscript{II} complexes of desferal actively cleave DNA\textsuperscript{82}, similar to other metallonucleases. The cleaving reaction with Cu\textsuperscript{II} complex requires a reducing agent such as 2-mercaptoethanol, dithiothreitol or ascorbate and the reaction is made more efficient by addition of H\textsubscript{2}O\textsubscript{2}. The cleavage reactions with Co\textsuperscript{III} and Ni\textsuperscript{II} proceed even in the absence of a reducing agent. The cleavage efficiency is dependent on metal ion concentration, and optimal efficiency for 100% cleavage being 235, 42.5 and 10 \textmu M for Cu\textsuperscript{II}, Co\textsuperscript{III}, and Ni\textsuperscript{II} complexes, respectively. Among the three, the Ni\textsuperscript{II} complex showed maximum efficiency at low concentrations and the excess complex did not further degrade the DNA to the linear form.

**Metal-oximates:**

Copper\textsuperscript{II}, nickel\textsuperscript{II} and iron\textsuperscript{II} complexes of 2-acetylpyridine oxime (APO) were synthesized and characterized and their nuclease activity was investigated\textsuperscript{83}. A gel electrophoresis experiment using pBR 322 (a circular plasmid DNA) was performed with these oximates in the absence and in the presence of H\textsubscript{2}O\textsubscript{2} as an oxidant. Only Fe\textsuperscript{II} complex, [Fe(APO)\textsubscript{2}]SO\textsubscript{4} affords a discernible DNA cleavage at 30 min incubation period, as evidenced by the disappearance of form I (supercoiled) of plasmid. This is due to the stepwise conversion of form I (supercoiled) plasmid to form III (linear) through the transient formation of form II (open circular). This is consistent with the increased production of radicals mediated by ferrous ions by the well known Fenton reaction\textsuperscript{84}. Further incubation (>40 min) results in complete degradation of DNA. This is probably due to the single cut in circular DNA giving rise to a linear form having open ends and eventual fragmentation of plasmid DNA.

**Copper-hydroxamates:**

The DNA nicking ability of copper\textsuperscript{II} complexes of the three hydroxamic acids was investigated by plasmid cleavage assay\textsuperscript{85}. All the three metal complexes were able to convert supercoiled DNA (form I) into open circular (form II) DNA. Neither the free ligand nor the H\textsubscript{2}O\textsubscript{2} was found to have any DNA cleavage activity under identical reaction conditions. Among the three copper\textsuperscript{II} complexes, that of p-TBHA showed maximum efficiency. Free hydroxamic acid in the presence of either an oxidizing agent or a reducing agent or both did not exhibit any DNA cleavage. The presence of hydroxyl radical scavengers such as mannitol and glycerol significantly inhibited the cleavage. All the three copper\textsuperscript{II} complexes were able to cleave DNA only in the presence of H\textsubscript{2}O\textsubscript{2} indicating that the Cu\textsuperscript{II}-DNA complex may be directly reduced by the peroxide to produce the corresponding DNA-Cu-hydroperoxo species\textsuperscript{86,87} thereby leading to DNA damage.
Reddy: Nuclease activity of transition metal complexes—a review

It has been assumed that OH radical is formed as follows

\[
\begin{align*}
\text{Cu}^{2+} + \text{H}_2\text{O}_2 & \rightleftharpoons \text{Cu}^+ + \text{HO}_2^- + \text{H}^+ \quad (1) \\
\text{Cu}^+ + \text{H}_2\text{O}_2 & \rightarrow \text{OH}^* + \text{HO}^- + \text{Cu}^{2+} \quad (2)
\end{align*}
\]

The general oxidative mechanisms proposed account for DNA cleavage by hydroxy radicals via abstraction of an H atom from sugar units.

**Copper(II) complexes of carboximide and thiocarboximide acids:**

Heteroaromatic thiosemicarbazones and semicarbazones have been rather neglected in the main stream of chelation chemistry\(^8\) of biological relevance. Perrin and Stunzi\(^9\) have reviewed the applications of thiosemicarbazones as antiviral agents. The report that the 2-substituted heteroaromatic thiosemicarbazones show appreciable antiviral activity\(^9\) has evoked much interest in coordination chemistry. There is also much interest in the development of artificial nuclease. The continued interest in the copper complexes as chemical nuclease prompted us to synthesize the copper complexes\(^9\) (Fig. 5) of carboximide \([(\text{furan-2-yl})\text{ethylidene}]\text{hydrazide}\) (1, CFMH); thiocarboximide \([(\text{furan-2-yl})\text{ethylidene}]\text{hydrazide}\) (2, TFMH); carboximide \([(\text{furan-2-yl})\text{ethylidene}]\text{hydrazide}\) (3, CFEH); thiocarboximide \([(\text{furan-2-yl})\text{ethylidene}]\text{hydrazide}\) (4, TFEH); carboximide \([(\text{thiophene-2-yl})\text{ethylidene}]\text{hydrazide}\) (5, CTMH); thiocarboximide \([(\text{thiophene-2-yl})\text{ethylidene}]\text{hydrazide}\) (6, TTMH); carboximide \([(\text{thiophene-2-yl})\text{ethylidene}]\text{hydrazide}\) (7, CTEH); thiocarboximide \([(\text{thiophene-2-yl})\text{ethylidene}]\text{hydrazide}\) (8, TTEH).

![Fig. 5. Structures of copper complexes: Cu(CFMH)₂Cl₂ (X = O, R = H, Y = O), Cu(TFMH)₂Cl₂ (X = O, R = H, Y = S), Cu(CFEH)₂Cl₂ (X = O, R = Me, Y = S), Cu(TFEH)₂Cl₂ (X = O, R = Me, Y = O), Cu(TTMH)₂Cl₂ (X = S, R = H, Y = S), Cu(CTEH)₂Cl₂ (X = O, R = Me, Y = O), Cu(TTEH)₂Cl₂ (X = S, R = Me, Y = O).](image)

A gel electrophoresis experiment using pBR 322, a circular plasmid DNA, was performed with ligands and complexes in the presence and in the absence of hydrogen peroxide as oxidant\(^9\). At micromolar concentrations, all the ligands exhibited no significant activity in the absence and in the presence of oxidant.

The nuclease activity is greatly enhanced by the incorporation of copper ion in the respective ligands. \([\text{Cu(CTMH)}]_2\text{Cl}_2\) in the presence of the oxidant degraded all the two forms of DNA completely, whereas \([\text{Cu(TFMH)}]_2\text{Cl}_2\) and \([\text{Cu(CTEH)}]_2\text{Cl}_2\) led to a discernible DNA cleavage as indicated by the disappearance of form I (supercoiled) of plasmid. This is due to the stepwise conversion of form I of plasmid DNA to form III (linear) through the transient formation of form II (nicked) (open circular). \([\text{Cu(CFEH)}]_2\text{Cl}_2\) and \([\text{Cu(TTMH)}]_2\text{Cl}_2\) in the absence of oxidant afforded least discernible DNA cleavage when compared to other complexes. With the \([\text{Cu(CFEH)}]_2\text{Cl}_2\) form III (linear) was observed, but the intensity of the supercoiled form decreases. Several other comparisons are available\(^9\). In overall view, all the complexes showed increased activity in the presence of oxidant may be due to the formation of hydroxy free radical\(^9\) which involves oxidation of the deoxyribose moiety followed by breakage of the sugar-phosphate backbone\(^9\).

The nuclease activity of the complexes in the absence of oxidant may be due to the binding of metal ions to DNA and these metal ions can be reduced and then oxidized by dioxygen, leading to the hydroxyl radical production close to the metal binding site which can damage DNA in site-specific reactions\(^2\) or due to the reactive oxygen species.

**Mixed ligand copper(II) complexes:**

Bis-pyridine adducts of the above mentioned parent copper(II) complexes have been synthesized and characterized. The nuclease activity of the adducts\(^6\) was carried out on a double stranded pBR 322 circular plasmid DNA by using the gel electrophoresis experiments in the presence and absence of oxidant \((\text{H}_2\text{O}_2)\). In the presence of oxidant all the adducts showed increased nuclease activity. The more pronounced nuclease activity of these adducts compared to parent complexes\(^9\) may be due to increased production of hydroxyl radicals. In the absence of oxidant, the adducts exhibit low DNA cleavage when compared to parent complexes\(^9\). The low activity of these bis-pyridine adducts may be due to six-coordination of copper(II), which is consequently unable to bind with DNA due to the absence of an available site on Cu\(^1\). In the parent complexes, the availability of coordinated sites at the metal centre...
may facilitate the binding of DNA to allow nucleolytic cleavage.

Bis-picoline adducts\textsuperscript{97} of the above mentioned parent copper complexes have been prepared and characterized by analytical, physicochemical and spectral techniques. All adducts showed increased nuclease activity in the presence of oxidant. The nuclease activity of picoline adducts are compared with parent copper(II) complexes.

Nucleolytic activity of natural molecules such as bleomycin and their metal complexes\textsuperscript{98-102} are not mentioned in this review because of intrinsic limitations of the subject. Metallo-intercalators\textsuperscript{103} and \textit{cis}-platin-DNA adducts\textsuperscript{104} are not dealt in this review as these have been exhaustively reviewed.

Conclusions

Transition metal complexes of synthetic ligands are of paramount importance for the designing of chemotherapeutic drugs. DNA helices are chiral. They would thus be expected to interact with chiral metal complexes in an enantioselective manner. We can expect further progress in enantioselective probes.

Copper complexes of synthetic ligands appear to be promising chemical/artificial nucleases. Studies on metal complexes of nitrogen-containing ligands, especially heteroaromatic nitrogen bases are of much interest.

The present review exemplifies that simple metal complexes with suitable organic ligands and appropriate overall geometries can be tailored to produce synthetic chemical nucleases having specific nucleolytic activity. However, more work is needed to quantify the available methods and to draw structure-function relationship and this continues to be a promising area of research in bioinorganic chemistry.

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