Interaction of DNA with small molecules: Role of copper histidyl peptide complexes in DNA binding and hydrolytic cleavage

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Received 19 March 2010; revised and accepted 17 July 2010

Deoxyribonucleic acid is the site of storage and retrieval of genetic information through interaction with proteins and other small molecules. Hydrolysis of the phosphodiester bond of DNA is of critical importance at several stages in a cell cycle. Thus, the development of metal complexes that cleave nucleic acids hydrolytically at physiological conditions is of great interest in the field of artificial metallonucleases. Among transition metals, Cu(II) complexes have been extensively studied in promoting hydrolysis of DNA. Histidine is a biologically important ligand for Cu(II) binding in many biological systems. Since imidazole is an efficient catalyst for ester hydrolysis at neutral pH and histidine residues are often involved in neutral hydrolytic metalloenzyme active centres and have the potential to bind to DNA, an attempt is made in this review to highlight the importance of DNA binding and cleavage and the role of a few copper histidyl peptide complexes in DNA binding and hydrolytic cleavage.

Keywords: Bioinorganic chemistry, DNA cleavage, Copper, Hydrolysis, Metalloenzymes

IPC Code: Int. Cl. D CO7F1/08

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The deoxyribonucleic acid (DNA) plays the key informational role in the cell. The DNA in the nucleus bearing the genetic blue print is frequently copied and transcribed by DNA and RNA polymerases. Yet DNA is wrapped up in chromatin structure and must be manipulated by topoisomerases and helices to allow its access and usage. Like proteins DNA has primary, secondary and tertiary structures. Since primary and secondary structures are well known a brief mention of tertiary structure is made here. The tertiary structure of DNA is important in view of its interaction with several anticancer agents.

DNA is an extremely long molecule, so long in fact that it would not fit into the nucleus of the cell if it existed as a linear molecule. It has to be coiled into a more compact three dimensional shape which can fit into the nucleus – a process known as supercoiling. This process requires the action of a family of enzymes called topoisomerases. Supercoiling allows the efficient storage of DNA, but the DNA has to be uncoiled again if replication and transcription are to take place. The same topoisomerase enzymes are responsible for catalyzing this process, so inhibition of these enzymes can efficiently block transcription and replication. Therefore, cleavage of supercoiled (SC) DNA into nicked circular (NC) and linear forms

1.
are necessary biological process. Mimicking such a process in the laboratory is not only important but also challenging to the scientists. Another important property of DNA is its ability to interact reversibly with a broad range of chemical species that include water, metal ions and their complexes, small organic molecules and proteins. All the intricate DNA conformations which exist are stabilized and are only possible because of these reversible interactions. Because of their relative simplicity, the interactions of small molecules with nucleic acids have provided accurate information about nucleic acid binding specificity. Incidentally, this (specificity) differentiates between the two types of DNA cleavage i.e. oxidative and hydrolytic. Hence it is important to know the types of reversible interactions. Molecules and ions interact with DNA in three primary ways which are significantly different (Scheme 1).

(a) binding along the exterior of the helix through interactions which are generally non-specific and are primarily electrostatic in origin,

(b) groove binding interactions which involve direct interactions of the bound molecule with edges of base pairs in either of the major (G-C) or minor (A-T) grooves of nucleic acids, and,

(c) intercalation of planar or approximately planar aromatic ring system between base pairs.

The cleavage of nucleic acids may be considered as an enzymatic reaction which comprises various biological processes as well as the biotechnological manipulation of genetic material. One of the most important approaches to drug development and current chemotherapy against some cancers and viral and parasitic diseases involve drugs which interact reversibly with DNA. Therefore, design of new metal complexes which can bind with specificity to DNA and bring about its (DNA) cleavage are of importance in the development of new antitumor agents. In particular the development of reagents which cleave nucleic acids hydrolytically under mild conditions is attracting great interest in the field of artificial metallonucleases. Possessing higher reactivity in the cleavage of DNA than other transition metal complexes, Cu(II) complexes have attracted the most interest. Many Cu(II) complexes have been synthesized as artificial nucleases. A thorough literature survey reveals that much of the attention has been focused on complexes of copper and histidine/histidyl peptides due to their coordinating abilities and their biological importance. In view of the above an attempt has been made to review the importance of DNA binding and cleavage and compare the DNA binding and hydrolytic cleavage activities of some ternary copper complexes of histidyl peptides and phen/Hist/En (where phen=phenanthroline, Hist=histamine, En=ethylenediamine).

While several inorganic and organic systems that mediate the hydrolysis of RNA or simple phosphate monoesters have been developed, the hydrolysis of the DNA backbone has been much more difficult to achieve. This is due to the absence of 2'-OH group, which when deprotonated, can assist in the cleavage of phosphodiester bonds between ribonucleotides. The half-life of the phosphodiester linkage in DNA at pH 7.0 and 30 °C is estimated to be 200 million years. The exceptional stability of phosphodiester backbone of DNA has been suggested as one reason why nucleic acids have evolved as genetic material. However DNA cleavage is an essential phenomenon in an organism.

**Importance of DNA cleavage**

Hydrolysis of phosphodiester bond of DNA is of critical importance at several stages in a cell cycle (including DNA repair excision, integration and signal transduction). Hence, DNA strand scission reactions are of considerable interest both in understanding the ubiquitous phosphate ester hydrolysis reactions carried out in nature and also in designing new artificial restriction enzymes. Double strand breaks in duplex DNA are thought

![Scheme 1](image)
to be more significant sources of cell lethality than single strand breaks, as they appear to be less readily repaired by DNA repair mechanisms. Metal ion mediated hydrolysis of phosphate esters by metallo-nuclease enzymes is a common catalytic pathway in nucleic acid biochemistry, and such enzymes are ubiquitous and essential for living organisms. Hence, there is a considerable interest in the design, synthesis and characterization of molecules that target RNA or DNA motifs, either to inhibit the binding of cognate proteins or enzymes, or to mediate strand scission. DNA cleavage agents find many potential applications in molecular biology, biotechnology and medicine, DNA footprinting for locating base mismatches and loop regions, conformational variations in DNA and as chemotherapeutic agents.

The interaction of transition metal complexes with nucleic acids is a major area of research due to the utility of these complexes in the design and development of synthetic restriction enzymes, spectroscopic probes, site specific cleavers and molecular photoswitches. The reason for using transition metal complexes as artificial nuclease is because of their diverse structural features, and the possibility to tune their redox potential through the choice of proper ligands. Inorganic constructs such as cis platin and bimetallic rhodium acetate, etc., exert antitumor activity by inner sphere coordination.

There are some distinct advantages of chemical nucleases over conventional enzymatic nucleases. The former are smaller in size rendering quick stretching in the hindered sites of a macromolecule and have a quick reach to sterically hindered regions of DNA. Hence, considerable attention has been focused towards the development of DNA cleavage agents. Ce complexes have been reported to hydrolyze single and double strand DNA, several dinuclear complexes have been reported to be very efficient in hydrolytic cleavage of plasmid DNA, while Cu(II) complexes of linear or macrocyclic polyamines, aminoglycosides and histidine show good nuclease activity. It has also been demonstrated that many compounds of transition metals are capable of promoting efficient hydrolytic cleavage of DNA.

The DNA cleavage can occur by two major pathways, i.e., hydrolytic and oxidative:

(a) Hydrolytic DNA cleavage involves cleavage of phosphodiester bond to generate fragments which can be subsequently religated. Hydrolytic cleavage active species mimic restriction enzymes.

(b) Oxidative DNA cleavage involves either oxidation of the deoxyribose moiety by abstraction of sugar hydrogen or oxidation of nucleobases. The purine base guanine is most susceptible for oxidation among the four nucleobases. Oxidative cleavage of DNA occurs in the presence of additives or photoinduced DNA cleavage agents. Photocleavers require the presence of a photosensitizer that can be activated on irradiation with UV or visible light. The redox active ‘chemical nucleases’ are effective cleavers of DNA in the presence of a reducing agent or H2O2 as an additive.

Oxidative cleavage agents require the addition of an external agent (e.g. light or H2O2) to initiate cleavage and are thus limited to in vitro applications. Since these processes are radical based and deliver products lacking 3' or 5' phosphate groups that are not amenable to further enzymatic manipulation, the use of these reagents has been limited in the field of molecular biology and their full therapeutic potential has not been realized. Hydrolytic cleavage agents do not suffer from these drawbacks. They do not require co-reactants and, therefore, may be more useful in drug design. Also, they produce fragments that may be religated enzymatically. The metal complexes that catalyze DNA hydrolytic cleavage may be useful not only in gene manipulation but also in mimicking and elucidating the important roles of metal ions in metalloenzyme catalysis.

**Metal ions in biology**

Hydrolytic degradation of nucleic acids by nuclease enzymes is a critical biological reaction and metal ions play a central role in mediating such cleavage pathways. Many hydrolytic enzymes contain metal ions in their active sites, which have an important roles in enzymes and all ribosome mediated scission reactions. Small metal complexes that promote the hydrolytic cleavage of DNA, therefore, are useful not only in molecular biology and drug design but also in elucidating the precise role of metal ions in enzyme catalysis.

Hydrolysis of DNA by small molecules is primarily hindered by the repulsive interaction of negatively charged phosphate group towards an incoming nucleophile. However, various transition
metal and lanthanide cations have been shown to alleviate this repulsive interaction by direct inner sphere binding of the phosphate ester. The redox properties in metal centers are essential to generate reactive oxygen species for DNA cleavage. By changing the metal ions, the geometry of the complex (square planar, tetrahedral, octahedral, etc) and consequently its photophysical properties, may be modified and may affect its interaction with nucleic acids.

The key role of metal ions in promoting hydrolysis of phosphate esters appears to be an intramolecular delivery of nucleophile by reducing the $pK_a$ of coordinated water molecules, resulting in good nucleophiles at neutral pH. Therefore, the design of metal complexes as potential synthetic hydrolytic agents requires:

(a) two cis oriented labile coordination sites in order to bind both the phosphodiester substrate and a water molecule in an orientation appropriate for intramolecular attack, and

(b) a strong lewis acid metal in order to facilitate deprotonation of the coordinated water molecule in order to generate the hydroxide nucleophile.

Role of copper in DNA cleavage

Copper, a natural constituent of the cell nuclei, has been suggested to play a key role in structural organization and function of chromosomes. It is one of the most abundant transition metals found in living systems, and is an integral component of many enzymes, e.g., superoxide dismutase, tyrosinase, ceruloplasmin, etc. A driving force in the activity of these enzymes is the folding of a peptide which consists of amino acid side chains around the Cu(II) cation. It is involved in mixed ligand complex formation in a number of biological processes.

The biological role of Cu(II) and its synergetic activity with drugs have been the focus of a large number of research studies. The antifungal and antibacterial properties of a range of Cu(II) complexes have been evaluated against several pathogenic fungi and bacteria. Coordination compounds of copper, in both its oxidation states (+1 and +2), have been extensively used in metal mediated DNA cleavage through the generation of hydrogen abstracting activated oxygen species. Cu(II) complexes under certain ligand environments have also been shown to bring about photocleavage of DNA as well as RNA and DNA scission via phosphodiester transesterification. In recent years there has been substantial interest in the design and study of lewis acidic, redox potential and spectroscopically active Cu(II)/Cu(I) complexes of synthetic and naturally occurring ligands as nuclease mimics.

Cu(II) complexes have been extensively studied in the past decade in promoting hydrolysis of DNA or model substrates because of its similar coordination chemistry, superior lewis acidity to that of Zn(II) which has been observed as cofactor in many nucleases, biologically accessible redox potential and relatively high affinity for nucleobases.

Sigman and co-workers have shown that the cationic complex $[Cu(phen)_2]^{+}$ in the presence of molecular oxygen and a reducing agent, acts as an efficient nuclease by oxidative cleavage mechanism with high preference for the double stranded DNA. There are also reports of copper complexes cleaving DNA hydrolytically and several other copper based synthetic nucleases have also been reported.

A wide range of ligand environments for Cu(II) has been found to be effective in DNA and phosphodiester hydrolysis. Some examples include simple macrocycles, amino acid residues and complex polysaccharides, etc. Many Cu(II) complexes including mononuclear, dinuclear and trinuclear and macromolecular catalytic systems have been synthesized as artificial nucleases.

Role of 1, 10 phenanthroline and histidine in DNA cleavage

1,10 Phenanthroline (phen) is the parent molecule of an important class of chelating agents. The consequence of the planar nature of phen is its ability to participate as either an intercalating or a groove binding species with DNA and RNA. There has been substantial interest in understanding the DNA binding properties of transition metal polypyridyl complexes in the hope of developing novel probes of nucleic acid structures, DNA cleavage agents and antitumor drugs. Transition metal complexes of bipy, phen or their modified variants are widely employed in studies of DNA in view of their applications in several research areas including bioinorganic and biomedical chemistries.

Copper complexes containing phen bases have received considerable current interest in the nucleic acids chemistry due to their various applications.
following the discovery of the “chemical nuclease” activity of bis-phen complex of Cu(I) in presence of H₂O₂ and a reducing agent by Sigman and coworkers. Subsequent studies have shown that molar ratio of 1:2 for Cu and phen is necessary to observe efficient DNA cleavage activity. Yang and coworkers have reported that the mono-(phen)Cu complex in the presence of H₂O₂, exhibits higher nuclease activity than bis-(phen)Cu species. However mono-phen Cu(II) complexes cleave SC DNA less effectively in presence of a reducing agent.

Copper complexes containing polypyridine ligands and their derivatives exhibit numerous biological activities such as antitumor, antimicrobial, antimicrobial, and antitumor activities, etc. Cu(II) complexes of phen have been used to inhibit DNA and RNA polymerase activities, to induce strand scission of DNA in the presence of H₂O₂, exhibits higher nuclease activity than bis-(phen)Cu species. However mono-phen Cu(II) complexes cleave SC DNA less effectively in presence of a reducing agent.

A driving force in the activity of galactose oxidase and superoxide dismutase is the folding of peptide which consists of amino acid side chains around the Cu(II) cation. His moieties are known to be of major importance in the process because this type of N-coordinating ligand forms stable complexes with the copper cation. To unravel the formation processes of these types of enzymes and to find the exact coordination geometry of the Cu/His moieties, extensive research has been carried out in this area much of which has been summarized by Sarkar et al. The coordination ability of His containing peptides towards Cu(II) ion has been discussed by Kozlowski et al.

His residues are important binding sites for Ni(II). In human serum albumin, the binding site for nickel is the N-terminal Asp-Ala-His. Serum albumin is believed to be the primary nickel transport protein in human blood. Additionally, Ni(II) complexes with bound histidine nitrogens are present in, at least, one nickel containing enzyme. Enzyme superoxide dismutase contains histidyl nitrogen coordinated to the axial position of nickel.

Due to the presence of an imidazole ring, histidine exhibits metal binding and DNA binding activities different from those of other amino acids. It has been reported that Cu(II)-L-His complex aggregates in a groove or along a phosphodiester chain of the double helical DNA and shows hydrolytic cleavage activity of plasmid DNA and a dinucleotide at physiological pH and temperature. His also shows specific interaction with DNA as a protein residue. Peptides containing histidine residues at the third position from the N-terminus are well characterized specific sequences for metal binding in several proteins. Ni(II) complexes of Xaa-Xaa-His have shown sequence selective DNA cleaving activities and have been employed as a specific metal binding and DNA cleaving motif in several DNA binding molecules.

Ni(II) peptide complexes containing His as the third amino acid residue has been used in site specific DNA cleavage, RNA cleavage, protein–protein cross linking, and protein affinity labelling. DNA cleavage activity of histidine, histidine derivative, Cu(II)-Histidine complex, diiron-histidine complex, seryl-histidine, seryl-histidine derivative, etc., has been reported.

**Cu(II)-L-His systems**

Cu(II)–L-His systems which are more stable in comparison with the other metal(II)-L-His complexes (metal=Co, Ni, Zn, Cd) are of particular interest due to the biochemical and pharmacological properties, as well as the different possible coordination modes. Since the discovery in 1966 of the Cu(II)–L-His species in human blood, extensive research has been carried out to determine its role in copper transport. Copper transport in the tissues is facilitated by L-His while albumin inhibits the copper uptake into the cells. The exchange of Cu(II) between L-His and albumin modulates the availability of copper to the cell. Evidence has been presented for the existence of a ternary coordination complex between albumin, Cu(II) and L-His under physiological conditions. The physiological importance of Cu(II)–L-His and its therapeutic applications are well known. Studies...
on the transport properties of Cu(II)–L-His has led to its application in the treatment of Menkes disease, a fatal genetic neurodegenerative disease\textsuperscript{125}. Recently, the use of Cu(II)–L-His in the treatment of infantile hypertrophic cardiomegaly has also been reported\textsuperscript{126}. Experiments with hepatic tissues demonstrated that L-His enhances the uptake of copper in hepatic cells\textsuperscript{127}. Similar results have been obtained in placental cells\textsuperscript{128}. \textit{In vitro} studies have demonstrated that L-His can facilitate copper uptake in mammalian brain\textsuperscript{129}.

Considering the fact that L-His has significant importance in biology, we have reported studies on copper complexes of histidyl peptides, viz., histidyl serine (HisSer), histidyl phenylalanine (HisPhe) and histidyl leucine (HisLeu) (Fig. 1) and reported their DNA binding and cleavage activity.

**DNA binding and cleavage activity of ternary Cu(II) complexes of histidyl peptides**

The DNA binding and cleavage activity of [Cu(II)(HisSer)(phen)]\textsuperscript{+}(1), [Cu(II)(HisLeu)(phen)]\textsuperscript{+}(2), [Cu(II)(Hist)(HisPhe)]\textsuperscript{+}(3), [Cu(II)(Hist)(HisSer)]\textsuperscript{+}(4), [Cu(II)(Hist)(HisLeu)]\textsuperscript{+}(5), [Cu(II)(En)(HisLeu)]\textsuperscript{+}(6) have been reported from our laboratory. Since DNA binding by nuclease is a prerequisite for its DNA cleavage activity, an attempt is made here to summarize some of the important and commonly used DNA binding techniques along with the trends observed for the complexes (1-6).

**DNA binding**

The mode and extent of binding of complexes to DNA can be determined by various techniques i.e., absorption spectroscopy, thermal denaturation and fluorescence spectroscopy\textsuperscript{133-142}. Natural biomolecules often contain chromophoric functional groups that have characteristic UV spectra. For example, DNA has significant absorption in the UV range because of the presence of aromatic bases like adenine, guanine, cytosine and thymine. Thus, by monitoring the absorbance at a fixed wavelength with changes in the concentration of reactants, the biomolecular interactions have been assessed. Hyperchromism and hypochromism are both the spectral features of DNA concerning its double helix structure. The UV absorption spectra of the complexes in the absence and presence of calf-thymus DNA (CT-DNA) were monitored. Figure 2 shows absorption spectra of (1) in the absence and presence of increasing amounts of CT-DNA (peak at 394 nm was monitored). In the presence of CT-DNA, a decrease in absorption intensities (hypochromism) was observed for (1), (2), (5) and (6) while hyperchromism was observed.

![Geometry of Cu(II) complexes of histidyl peptides](image-url)
for (3) and (4). Hypochromism was suggested to arise due to the interaction between the electronic state of the intercalating chromophore and that of the DNA bases. These spectral changes are consistent with the intercalation of (1), (2), (5) and (6) into the DNA base stack. Hyperchromism for (3) and (4) indicates base binding. The intrinsic binding constants ($K_b$) of the complexes (Table 1) with CT-DNA were determined using following function equation:

$$[\text{DNA}] / (\varepsilon_a - \varepsilon_t) = [\text{DNA}] / (\varepsilon_b - \varepsilon_t) + 1 / K_b (\varepsilon_b - \varepsilon_t) \quad ...(1)$$

The ‘apparent’ extinction coefficient ($\varepsilon_a$) was obtained by calculating $A_{\text{absd}} / [\text{Cu}]$. The terms $\varepsilon_t$ and $\varepsilon_b$ correspond to the extinction coefficients of free (unbound) and the fully bound complexes, respectively. A plot of $[\text{DNA}] / (\varepsilon_a - \varepsilon_t)$ versus $[\text{DNA}]$ will give a slope $1/(\varepsilon_b - \varepsilon_t)$ and an intercept $1/K_b (\varepsilon_b - \varepsilon_t)$. $K_b$ is the ratio of the slope and the intercept. The $K_b$ values of complexes (1-6) are presented in Table 1.

The melting of CT-DNA can be used to distinguish between those molecules which bind via intercalation and those binding externally. The interaction of small molecules with double helical DNA may increase or decrease $T_m$, the temperature at which double helix is broken up into single stranded DNA. An increase in $T_m$ value is indicative of an intercalative or phosphate binding, while a decrease is attributed to base binding. The thermal denaturation profiles of DNA in the absence and presence of complexes were monitored (Fig. 3, for 1). An increase of $5 - 7 \degree C$ was observed in the $T_m$ profile of (1), (2), (5) and (6) whereas a decrease of $4 - 6 \degree C$ was observed for (3) and (4) as compared to that of free DNA. These results provide an evidence for intercalative and/or phosphate binding for (1), (2), (5) and (6) and base binding for (3) and (4) with DNA.

Fluorescence spectroscopy is an emission phenomenon, which depends upon radiation excitation rather than thermal excitation, of an atomic vapor. Fluorescence quenching experiments were

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**Table 1** - Binding and cleavage of copper complexes of histidyl peptides

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Intrinsic binding constant $K_b$ (M$^{-1}$)</th>
<th>[Complex]</th>
<th>NC (Linear) DNA (%)</th>
<th>Rate constant (h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Cu(II) (HisSer)(Phen)]* (1)</td>
<td>4.9 x 10$^3$</td>
<td>125, 187, 378, 437, 500</td>
<td>45, 54, 69, 95, 97</td>
<td>1.40</td>
</tr>
<tr>
<td>[Cu(II) (HisLeu)(Phen)]* (2)</td>
<td>4.2 x 10$^3$</td>
<td>125, 187, 250, 312, 378, 437, 500</td>
<td>50, 53, 55, 60, 65, 85, 90</td>
<td>1.32</td>
</tr>
<tr>
<td>[Cu(II) (Hist)(HisPhe)]* (3)</td>
<td>2.9 x 10$^3$</td>
<td>13.3, 21.4, 26.7, 31.2, 62.2, 93.7</td>
<td>38, 56, 69, 97, 98, 100</td>
<td>1.29</td>
</tr>
<tr>
<td>[Cu(II) (Hist)(HisSer)]* (4)</td>
<td>2.2 x 10$^3$</td>
<td>13.3, 21.4, 26.7, 31.2, 62.2, 93.7</td>
<td>35, 44, 64, 95, 97, 100</td>
<td>1.24</td>
</tr>
<tr>
<td>[Cu(II) (Hist)(HisLeu)]* (5)</td>
<td>7.8 x 10$^3$</td>
<td>125, 250, 375</td>
<td>43(15), 34(27), 27(43)</td>
<td>0.97</td>
</tr>
<tr>
<td>[Cu(II) (En)(HisLeu)]* (6)</td>
<td>5.9 x 10$^2$</td>
<td>125, 250, 375, 500</td>
<td>58, 62, 64, 70</td>
<td>0.81</td>
</tr>
</tbody>
</table>
performed with ethidium bromide (EB) bound DNA with increasing concentrations of complexes to determine the extent of binding between the second molecule and DNA. EB, a typical indicator of intercalation strongly fluoresces in the presence of DNA due to complete intercalation between the adjacent DNA base pairs, a process that can be reversed by addition of a competing molecule (fluorescence quenching)\(^{144}\). Two mechanisms have been proposed in this context; the replacement of molecular fluorophores, and/or electron transfer. The quenching extent of fluorescence EB bound to DNA can be used to determine the DNA-binding strength of a given molecule. The fluorescence quenching curves of EB bound to DNA in the absence and the presence of complexes were monitored (Fig. 4 for I). The addition of complex to DNA pretreated with EB caused appreciable reduction in emission intensity, indicating that the complex binds to DNA at the sites occupied by EB. The quenching plots (Fig. 4 inset for I) indicate that the quenching of EB bound to DNA by the complexes is in good agreement with the linear Stern-Volmer equation, which indicates that the complexes bind to DNA. In the plot of \(I_0/I\) versus [complex]/[DNA], \(K_{sv}\) is given by the ratio of slope to intercept.

Fluorescence Scatchard plots for the binding of EB to CT-DNA in the presence of complexes were obtained and \(r_{EB}\) values were determined (Fig. 5 for I). The term \(r_{EB}\) = [EB]/DNA is the concentration ratio of bound EB to total DNA and \([EB]_f\) is the concentration ratio of free EB (i.e. \([EB] = [EB]_b + [EB]_f\)). The binding mode of complexes with the DNA can be inferred from the trends of Scatchard plots.

A decrease of slope and intercept upon the addition of complexes indicates an intercalative and covalent binding of the complexes with DNA, while a decrease of slope with no change in intercept as compared to complex free plot confirms an intercalative binding of complexes with DNA.

**DNA cleavage**

Even though atomic force microscopy\(^{145-147}\) is being used nowadays for monitoring DNA cleavage, gel electrophoresis technique\(^{148-156}\) is still widely used as it provides information regarding the percentage of cleavage as a function of concentration of nuclease. Hydrolytic cleavage which started in a modest way of converting SC DNA to the NC form, is now being used for identifying the % of cleavage as a function of concentration of nuclease. Recent advances in this area have also provided information regarding the site specific cleavage and religation of the cleaved products. Kinetic experiments also showed rate enhancements of DNA cleavage in comparison to non-catalyzed DNA cleavage. Despite the fact that rate enhancements produced by natural enzymes are much higher, the enhancement of rate constant for DNA cleavage by metal complexes in the range...
0.09–0.25 h\(^{-1}\) was considered impressive\(^{157,158}\). Several complexes have been reported which show higher rate enhancement than the 0.09-0.25 h\(^{-1}\) range.

The cleavage reaction on SC pUC19 DNA by Cu(II) complexes has been monitored by agarose gel electrophoresis. In general, when DNA is subjected to electrophoresis, a relatively fast migration will be observed for the intact SC DNA. If scission occurs on one strand (nicking), the SC DNA will relax to generate a slower moving NC form. If both strands are cleaved, a linear form that migrates between SC form and NC form will be generated\(^{159,160}\). When DNA was incubated with increasing concentration of the complexes, SC DNA was degraded to the NC form. The cleavage efficiency was found to increase with increase in the concentration of Cu(II) complexes. The agarose gel electrophoresis patterns for the cleavage of pUC19 DNA by (1) is shown in Fig. 6.

After confirming the cleavage, attention was focused towards the kinetic aspects of the reactions. The observed distribution of SC and NC DNA forms in an agarose gel provides a measure of the extent of hydrolysis of phosphodiester bond in each plasmid DNA. The time course plot for the decrease of form I (SC DNA) and formation of form II (NC DNA) during the reaction under physiological conditions by (1) is shown in Fig. 7. The decrease of form I and the increase of form II DNA fits well to a single exponential curve. From these curve fits, the hydrolysis rate constants at 37 °C and at a fixed concentration of the Cu(II) complexes (1-6) have been determined (Table 1).

The experimental results suggested that the above complexes effectively bind and cleave DNA under mild conditions. The order of DNA binding ability and the rate constants were found to be \(1 > 2 > 3 > 4 > 5 > 6\). The order of DNA binding ability is also reflected in their cleavage activity. Histidyl peptide in complexes (1-6) plays an important role in DNA binding and cleavage due to the presence of planar imidazole group which actively participates in DNA binding through intercalative mode and also provides an additional donor site in metal chelation through N-3 atom of imidazole ring.

When the binding \((K_b)\) and DNA cleavage constants of phen (1 and 2), Hist (3, 4 and 5), En (6) systems are compared, it can be seen that the binding ability \((K_b)\) and DNA cleavage rate constants follow the order: phen>Hist>En. Phen systems (1 and 2) shows higher efficiency due to the presence of planar aromatic system with 3 rings which can show effective stacking interaction with the DNA bases. However in Hist systems (3, 4 and 5) stacking interactions are weak due to the presence of only one ring in histamine (imidazole ring). The En system (6) shows lower efficiency since it is an aliphatic system and cannot participate in stacking interactions with DNA bases.

Within the phen systems (1 and 2), (1) show a slightly higher \(K_b\) and cleavage ability due to the OH side chain of HisSer dipeptides, which can involve in cleavage thereby enhancing the rate. To explain this, the following tentative mechanism for the hydrolytic pathway has been proposed: The \(\alpha\) amino group of His forms H-bonds with the phosphate and the hydroxyl group of serine moiety forms one H-bond with oxygen atom of another phosphate. There are two other H-bonds between the amide or the imidazole group of

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**Fig. 6** – Agarose gel electrophoresis patterns for the cleavage of pUC19 DNA by [Cu(II)(Phen)(HisSer)]\(^{+}\) (1). [Lane 1, DNA control; Lane 2, (125 \(\mu\)M); Lane 3, (187 \(\mu\)M); Lane 4, (378 \(\mu\)M); Lane 5, (437 \(\mu\)M); Lane 6, (500 \(\mu\)M)]. [Figure taken from Ref. 130].

**Fig. 7** – Disappearance of supercoiled form I plasmid DNA (■) and formation of nicked circular form II DNA (▲) in the presence of [Cu(II)(Phen)(HisSer)]\(^{+}\). [Cond.: [Cu] = 378 \(\mu\)M; in aq. buffer (pH 8.0) at 37 °C]. [Figure taken from Ref. 130].
His moiety and phosphate group of DNA. These interactions may play an essential role in the binding and facilitate nucleophilic attack by bringing the DNA closer to the complex. This is schematically depicted in Scheme 2. Similar mechanism for DNA cleavage has been reported for SerHis peptide.

Within the Hist systems (3, 4 and 5), (5) shows weak binding which is also reflected in its cleavage ability whereas (3) and (4) have comparable $K_b$ which is again reflected in their cleavage activity. However, the slightly higher binding and cleavage ability of (3) may be attributed to the presence of an aromatic phenyl ring, which is known to involve in stacking interaction with DNA bases. In (4), OH side chain of HisSer dipeptide is involved in phosphodiester bond hydrolysis thereby showing higher DNA cleavage rate constant than (5). In (5), the leucine part of HisLeu peptide has a simple carbon chain which cannot involve in stacking interaction with DNA, thereby showing less efficiency than (3) and (4).

**Conclusions**

In recent years copper containing complexes have received considerable interest in nucleic acid chemistry, following the discovery of the chemical nuclease activity of $[\text{Cu(phen)}_2]$ in the presence of $\text{H}_2\text{O}_2$ and a reducing agent. In particular, the investigations on Cu(II) peptide complexes are of scientific and technological importance, since such systems may be regarded as models for both protein-DNA and antitumor agent-DNA interactions. Therefore, it is important to rationalize how such complexes with peptides affect DNA binding and cleavage.

Future prospects in this area lie in the development of new chemical nucleases which are capable of achieving 100 % DNA cleavage with minimum concentration of nuclease. Further they should be able to enhance the rate of hydrolysis as close to that of natural enzymes but in the absence of external agents and under physiological conditions. This is important since these conditions are close to *in vivo* systems. Moreover, higher concentration of complexes and addition of external agents will have adverse effects on the target molecule. This will pave way for new drug designing which covers a wide spectrum of other disciplines and technologies such as chemistry, biochemistry, genetics, etc. leading to new products and technologies in the future.

**Acknowledgement**

PRR thanks the Council of Scientific and Industrial Research (CSIR), New Delhi, for financial assistance. He also thanks UGC, New Delhi, for the award of Emeritus Fellowship.

**References**


