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Ni(II)·Xaa-Xaa-His Metallopeptide−DNA/RNA Interactions†

ERIC C. LONG*

Department of Chemistry, Purdue School of Science, Indiana University/Purdue University−Indianapolis, Indianapolis, Indiana 46202-3274

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Introduction

The past few decades have witnessed the development of a diverse array of metal complexes that interact with DNA and RNA. Interest in the design, synthesis, and study of these agents has been spurred by their ability to act as DNA or RNA conformational probes, footprinting reagents, and models to understand the selective recognition of these biopolymers.[1,2] In addition, the antitumor activity of several metal-based, DNA binding drugs has also provided an impetus for the study of metal complex−nucleic acid interactions.[3] In general, metal complexes are distinctly suited to the study of nucleic acids, since a metal center can impart a well-defined shape to an otherwise unstructured ligand and, importantly, often endows a complex with redox activity, enabling it to oxidatively modify or cleave a nucleic acid target.[1]

While many useful ligand systems have been designed, several years ago this laboratory noticed an opportunity to develop DNA binding metal complexes with peptide-based ligands.[4] Peptide ligands would enable a metal complex to present to a nucleic acid target the same chemical functional groups, such as guanidinium, amino, and amide moieties, employed by proteins[5] or peptide-derived natural products[6] for the selective recognition of DNA and RNA. In addition, the metal center imparts structure and reactivity to the peptide, which alone would lack these attributes. These features, coupled to the ease of peptide synthesis and the ability to alter readily the chirality of select α-carbon stereocenters, suggested that metallopeptides could function as useful models to further our knowledge of protein− and small molecule−nucleic acid recognition principles.

In light of the above, this laboratory has exploited the metal binding properties of peptides of the general form NH_2-Xaa-Xaa-His-$CONH_2$ (where Xaa is an α-amino acid) to generate nucleic acid binding and cleaving metallopeptides. Our goal is to understand how amino acids and peptides, within the three-dimensional constraints imposed by a metal center, can be used to selectively and efficiently target nucleic acid biopolymers. In the long term, information derived from these studies, such as particular spatial arrangements of chemical functional groups, could be used to augment the activity of established DNA-interactive agents or assist in the development of biosynthetic structures targeted to nucleic acids. The following Account seeks to review the status of our metallopeptide−nucleic acid studies and begins with a brief historical perspective on their development.

DNA Cleavage by Cu(II)/Ni(II)·Gly-Gly-His

The tripeptide Gly-Gly-His represents the consensus sequence of the amino-terminal Cu(II) or Ni(II) chelating domain of the serum albumins.[7] With Cu(II) or Ni(II) at physiological pH, peptide complexation occurs through chelation of the histidine imidazole nitrogen, two intervening deprotonated amide nitrogens, and the terminal α-amine [as shown for Cu(II)·Gly-Gly-His, **1**]. The tripep-

Eric C. Long was born November 20, 1962, in Reading, Pennsylvania. He received his B.S. degree (1984) in biochemistry from Albright College and his Ph.D. (1988) from the University of Virginia under the direction of S. M. Hecht. After postdoctoral work as a Jane Coffin Childs Fellow with J. K. Barton at Columbia University and the California Institute of Technology, he joined the faculty of the Department of Chemistry within the Purdue School of Science at Indiana University/Purdue University−Indianapolis (1991). He is currently an Associate Professor with research interests in drug− and peptide−nucleic acid interactions.

† This manuscript is dedicated to the birth of Dylan Warren Long (8/10/98), who made his presence well-heard throughout its writing.

* Address correspondence to Department of Chemistry, Indiana University/Purdue University−Indianapolis, 402 N. Blackford St., Indianapolis, IN 46202-3274. E-mail: long@chem.iupui.edu. Telephone: (317) 274−6888. Fax: (317) 274−4701.

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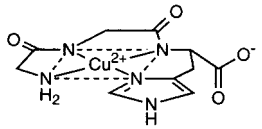

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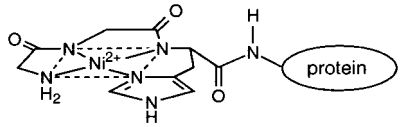
tide binds quite avidly to both Cu(II) and Ni(II) with a dissociation constant on the order of 10^{-16}−10^{-17} M. Importantly, previous studies of amino acid substitutions with side chains bulkier than Gly within the Gly-Gly-His ligand have shown little effect on metal complexation at or above physiological pH values.[7]

Initially, the DNA cleavage ability of Cu(II)·Gly-Gly-His was reported to be dependent upon the reductant L-ascorbic acid and, quite likely, endogenously generated hydrogen peroxide.[8] Subsequently, the attachment of Gly-Gly-His to the amino terminus of DNA binding protein motifs (**2**) has permitted their development as affinity cleavage reagents.[9−11] The Gly-Gly-His-modified DNA



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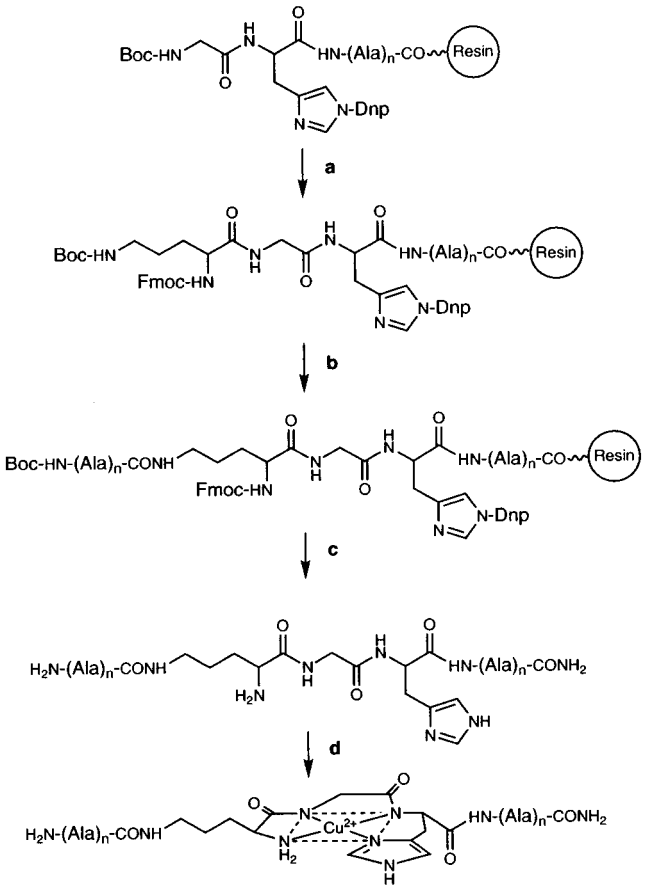
binding domain of Hin recombinase [Gly-Gly-His(Hin 139−190)] was observed, in the presence of Cu(II)[9] + sodium ascorbate/hydrogen peroxide or Ni(II)[10] + MMPP (magnesium monoperoxyphthalic acid), iodosobenzene, or hydrogen peroxide, to promote DNA strand scission at the binding site of Hin recombinase. Key observations[9,10] with Cu(II)/Ni(II)·Gly-Gly-His(Hin 139−190) suggested that the metallopeptide domain resides in the minor groove of the targeted DNA sequence and generates a nondiffusible oxidant that results in deoxyribose-centered damage. Evidence[10] for deoxyribose damage included the formation of DNA fragments bearing 3′- and 5′-phosphorylated termini, a kinetic isotope effect upon C4′-deuteration of a target nucleotide, and the observation that DNA cleavage was enhanced upon postreaction workup with *n*-butylamine in a fashion reminiscent of the breakdown of the C4′-hydroxylated nucleotide lesion produced by Fe·bleomycin.[3a] Interestingly, these data also indicated that, unique from other metal-based DNA cleavage reagents (e.g., Fe·EDTA)[1], strand scission by Ni(II)·Gly-Gly-His(Hin 139−190) was highly focused to the site of binding, suggesting the formation of a metallopeptide-bound oxidizing equivalent, perhaps a high-valent nickel-bound oxygen.[10]

Upon report of the above, examples of Cu(II) or Ni(II) complexes of Xaa-Xaa-His-modified proteins derived biosynthetically have appeared in the literature as DNA affinity cleavage reagents;[11] these include Gly-Gly-His-modified Sp1 and Gly-Lys-His-modified Fos. In addition, Ni(II)·Xaa-Xaa-His-modified biomolecules have also been employed to examine PNA−DNA interactions,[12] protein−protein interactions through oxidative cross-linking,[13] directed protein cleavage,[14] and low-molecular-weight agent−DNA interactions.[15]

Scheme 1. Stepwise Solid-Phase Peptide Synthesis of Orn-Based Metal-Binding Oligopeptides[a]



[a] (a) Coupling of Boc-Orn(Fmoc) to peptides terminating in Gly-His; (b) additional cycles of Boc-benzyl peptide synthesis; (c) side-chain deprotection and cleavage from resin; (d) $Cu(OAc)_2$ [or $Ni(OAc)_2$] in 10 mM sodium cacodylate, pH 7.5

Clearly, the above examples indicate that Cu(II)/Ni(II)·Xaa-Xaa-His-modified systems can assist in our understanding of macromolecule− and low-molecular-weight agent−macromolecule interactions. Due, however, to the central role played by the terminal amine in metal complexation by Xaa-Xaa-His tripeptides, the incorporation of this moiety is limited to the amino terminus of natural polypeptide structures.[7] To circumvent this requirement, this laboratory developed a straightforward method to place the unique cleavage abilities of Ni(II)/Cu(II)·Gly-Gly-His at any location within a synthetic polypeptide (i.e., the amino or carboxy termini and interior sequences).[16] As summarized in Scheme 1, instead of completing the required tripeptide sequence with an amino-terminal Gly residue, an N^{δ}-Boc-N^{α}-Fmoc-protected Orn residue was used that, when deprotected at the side chain, allows continued peptide synthesis for attachment to the carboxy terminus or internal sequences of a polypeptide. Upon final deprotection, this sequence permits the α-amine of the (δ)-Orn residue to participate in metal ion binding in a fashion analogous to the amino-terminal nitrogen of Gly-Gly-His; the metal binding and DNA cleavage properties of this modified domain were found to be virtually identical to those exhibited by amino-

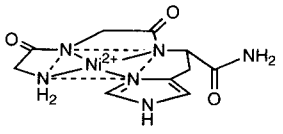
terminal Gly-Gly-His.[7] Interestingly, this modified sequence has also aided the development of Cu(II) chemosensors.[17]

During the study of model peptides containing the (δ)-Orn-modified sequence, our laboratory observed their ability to selectively cleave DNA, even in the absence of a defined nucleic acid binding domain. These early observations suggested that the core Cu(II)/Ni(II)·Xaa-Xaa-His motif alone may be capable of selectively recognizing DNA. This thought also warranted development in light of concurrent observations which indicated that the "metallopeptide" portion of the antitumor agent bleomycin contributes significantly to its 5′-GT and 5′-GC site selectivity.[18] While metallo·Gly-Gly-His and metallobleomycin are dissimilar in many structural aspects, their overall features and ability to generate nondiffusible oxidants provided impetus for the idea that appropriate amino acid compositions of Ni(II)·Xaa-Xaa-His may be capable of selective DNA damage.

Ni(II)·Xaa-Xaa-His metallopeptides thus appeared ideally suited to explore peptide−nucleic acid interactions, an endeavor which is difficult otherwise due to the lack of a defined structure in most low-molecular-weight peptides. The metal center imparts structure to the peptide and enables it to generate a discrete, nondiffusible oxidant. In addition, the tripeptide ligand binds avidly to Cu(II) or Ni(II) and does so without the formation of often complicating isomers at the metal center. The peptide ligand is also easy to synthesize and amenable to combinatorial methodologies and exposes the side chains of the L- or D-amino acids incorporated, allowing an examination of their effects on DNA site selectivity.

Ni(II)·Xaa-Xaa-His DNA Cleavage Selectivity

The initial investigation of selective DNA cleavage by Ni(II)·Xaa-Xaa-His metallotripeptides utilized Ni(II)·Gly-Gly-His (**3**) in a side-by-side comparison to metallopeptides that included positively charged Arg and Lys residues [Ni(II)·Arg-Gly-His (**4**) and Ni(II)·Lys-Gly-His (**5**) respectively].[19] In these and all subsequent studies, Ni(II) was



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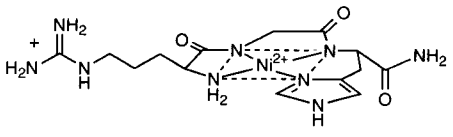

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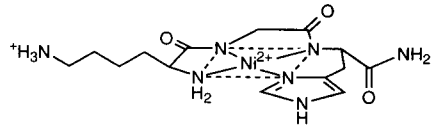

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used exclusively as the metal center due to its demonstrated ability to form a highly focused, nondiffusible oxidizing equivalent. In addition, Ni(II) + activating agent was unreactive in control experiments in the absence of a peptide ligand, whereas free Cu(II) + ascorbate avidly cleaves nucleic acids.[8]

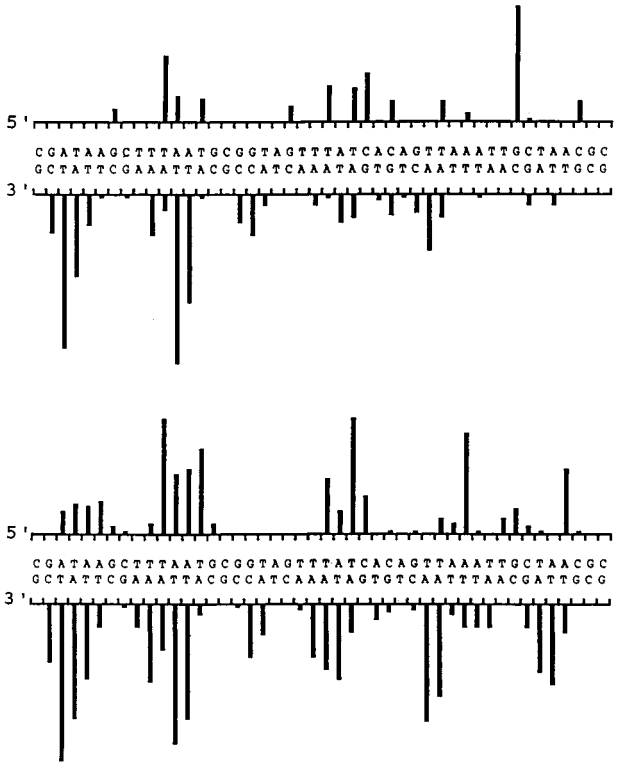


FIGURE 1. Representative histograms illustrating the sequence selectivity of DNA restriction fragment cleavage by Ni(II)·Gly-Gly-His (top) and Ni(II)·Arg-Gly-His (bottom). Essentially identical results are obtained for Ni(II)·Arg-Gly-His and Ni(II)·Lys-Gly-His.

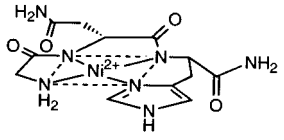
While previous examination of DNA cleavage by the carboxylate analogue of Ni(II)·Gly-Gly-His (which carries an overall charge of −1) did not reveal significant amounts of DNA modification,[20] our laboratory chose to examine $KHSO_5$-activated Ni(II) complexes of carboxamide derivatives of the peptide−ligand (NH_2-Xaa-Xaa-His-$CONH_2$) to create an overall charge-neutral complex, as Ni(II)·Gly-Gly-His (**3**), and positively charged complexes when Arg and Lys are included. Further, these ligands, in contrast to their carboxylate analogues, do not readily decarboxylate[21] nor contain negatively charged moieties with an inherent electrostatic repulsion to a polyanionic DNA substrate.

The DNA cleavage selectivities of Ni(II)·Gly-Gly-His, Ni(II)·Lys-Gly-His, and Ni(II)·Arg-Gly-His in the presence of equimolar amounts of $KHSO_5$ were examined through the use of 5′-^{32}P end-labeled DNA restriction fragments and polyacrylamide gel electrophoresis (PAGE). As summarized in Figure 1, direct DNA cleavage by Ni(II)·Gly-Gly-His was relatively random, with a slight preference for A/T-containing sites. However, upon inclusion of Lys or Arg, DNA cleavage increased and became focused to A/T-rich regions.

In addition to revealing an underlying selectivity for A/T-rich regions, these studies also allowed observation

of several important aspects of the recognition and cleavage of DNA by the metallopeptides: (1) direct strand scission was observed to occur at single residues of all four nucleotides present in the substrate, suggesting, as noted,[10] that metallopeptide activation results in a nondiffusible oxidant of the deoxyribose moiety; (2) while the intensity of cleavage at most modified sites was increased slightly upon postreaction workup with *n*-butylamine or piperidine, the site selectivity observed was not altered, thus differentiating the activity of the metallopeptides, under the conditions employed, from other Ni complexes, which display a preference for G residues alone;[20] (3) while cleavage occurred readily at A/T-rich sites, cleavage at homopolymeric A/T sequences [i.e., poly(dA)·poly(dT) sites] diminished toward their 3′-ends, suggesting that the narrowing minor groove of these regions[22] limited metallopeptide binding; and (4) cleavage of both complementary strands of a DNA substrate revealed a 3′-asymmetric cleavage pattern indicative of minor groove association,[1,10] as also supported through distamycin competition experiments.[19]

This laboratory also explored the effect of amino acid chirality within the peptide−ligand on DNA cleavage.[19] The substitution of two D-amino acids was examined: D-His for L-His in the carboxy-terminal peptide position, and the positioning of D- vs L-Asn within the remaining Xaa positions. These alterations placed an amide functionality, commonly exploited in the molecular recognition of nucleic acids, at various locations around the metallopeptide. Upon L- to D-His substitution, the site-selectivity of Ni(II)·Lys-Gly-D-His became more random; molecular modeling suggested that D-His repositions the carboxy-terminal amide, allowing the complex to fit more easily within the minor groove of DNA at all sites. In contrast, most substitutions of L- and D-Asn within the Xaa positions of the peptide−ligand did not affect the Ni(II)·Gly-Gly-His-like selectivity of the metallopeptides. However, one metallopeptide, Ni(II)·Gly-D-Asn-His (**6**) exhibited a preference for 5′-CCT sites that were ignored by all other complexes; it is likely that a combination of steric interactions and perhaps hydrogen bonding via the amide functional group(s) and the DNA backbone leads to the site discrimination observed.



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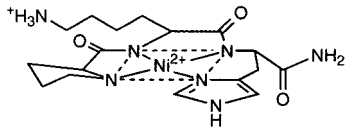
Overall, the above results verified that amino acid composition and chirality can influence Ni(II)·Xaa-Xaa-His DNA cleavage activity. To further examine alternative amino acid compositions of Ni(II)·Xaa-Xaa-His and their effect(s) on the cleavage efficiency and selectivity for B-form DNA, a positional scanning-based combinatorial procedure was employed to generate and examine nearly all combinations of naturally occurring L-α-amino acids within the Xaa positions of the tripeptide ligand.[23] This procedure employed 18 naturally occurring amino acids (excluding Cys and Trp) to generate two libraries in which the first and second positions of the peptide ligand were varied systematically. Results from these studies indicated that direct B-form DNA strand scission was enhanced between 5- and 8-fold relative to Ni(II)·Gly-Gly-His when Arg, Lys, Met, or Pro (Pro > Met > Arg > Lys) was located in the amino-terminal peptide position and between 3- and 5-fold when Arg, Lys, Met, Ser, or Thr (Lys > Arg > Met/Ser/Thr) was located in the second peptide position. These results predicted that Ni(II)·Pro-Lys-His (**7**), containing the most active amino acids in their respective positions, would substantially increase the direct strand scission of B-form DNA in comparison to Ni(II)·Gly-Gly-His; independent synthesis of this tripeptide and subsequent DNA cleavage studies verified that Ni(II)·Pro-Lys-His cleaved DNA an order of magnitude better than Ni(II)·Gly-Gly-His.[23] Surprisingly, however, further analysis



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indicated that the overall (1) metal binding ability, (2) DNA binding affinity, and (3) site selectivity of the active metallopeptides for A/T-rich regions did not differ appreciably. These observations again suggested that their selectivity for A/T-rich regions is predominantly influenced by the "core" metallopeptide structure as opposed to the side chains present at the Xaa positions of the tripeptide unless D-amino acids are employed.

To probe the possible basis for the increased activity of the combinatorially selected metallopeptides and their A/T-rich selectivity in general, molecular modeling was performed using Ni(II)·Pro-Lys-His.[23] Given that A/T selectivity is observed regardless of the L-amino acids located in the two amino-terminal positions of the peptide−ligand, it is likely that the His imidazole is a major determinant of selectivity. This hypothesis is supported by the following: (1) Ni(II)·Xaa-Xaa-His complexes containing a His N3 methyl substituent do not cleave DNA[24] and (2) the N3 pyrrole nitrogen of His is a very good hydrogen bond donor functionality.[25] In addition, (3) spectroscopic investigations of similar metallopeptides have indicated that His residues were a key determinant in DNA binding.[26]

Given the above, models were constructed in which the N3 hydrogen of the His imidazole acts as a hydrogen bond donor to the most prominent hydrogen bond acceptors found at the floor of the minor groove of A/T-rich regions, the exocyclic O2 of thymine or the N3 of adenine (Figure 2).[27] Examination of such models indicated that the side-chain carbons of the Pro residue are inserted deeply into the minor groove and in van der Waals contact with the sugars that form the walls of this groove; the narrow structure of A/T-rich regions[28] appears to complement well the overall structure of Ni(II)·Xaa-Xaa-His metal-

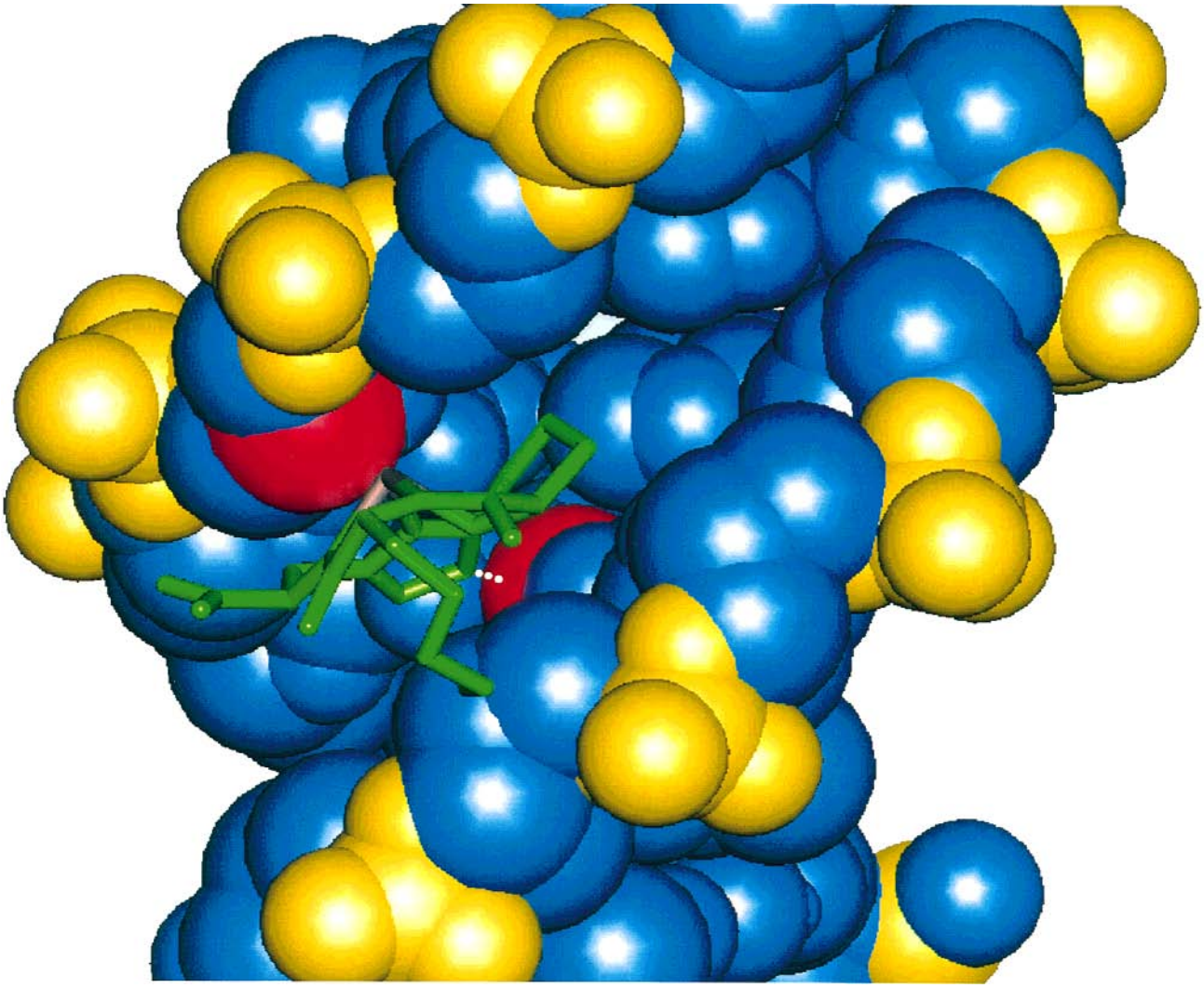


FIGURE 2. Molecular model of Ni(II)·Pro-Lys-His bound to the minor groove of an A/T-rich region of B-form DNA. DNA, phosphates, and metallopeptide are blue, yellow, and green, respectively. The red van der Waals surface on the left side of the groove is a C4′ H, and the red surface on the floor of the groove is the N3 of adenine connected via an H bond (dotted line) to the imidazole of the metallopeptide.

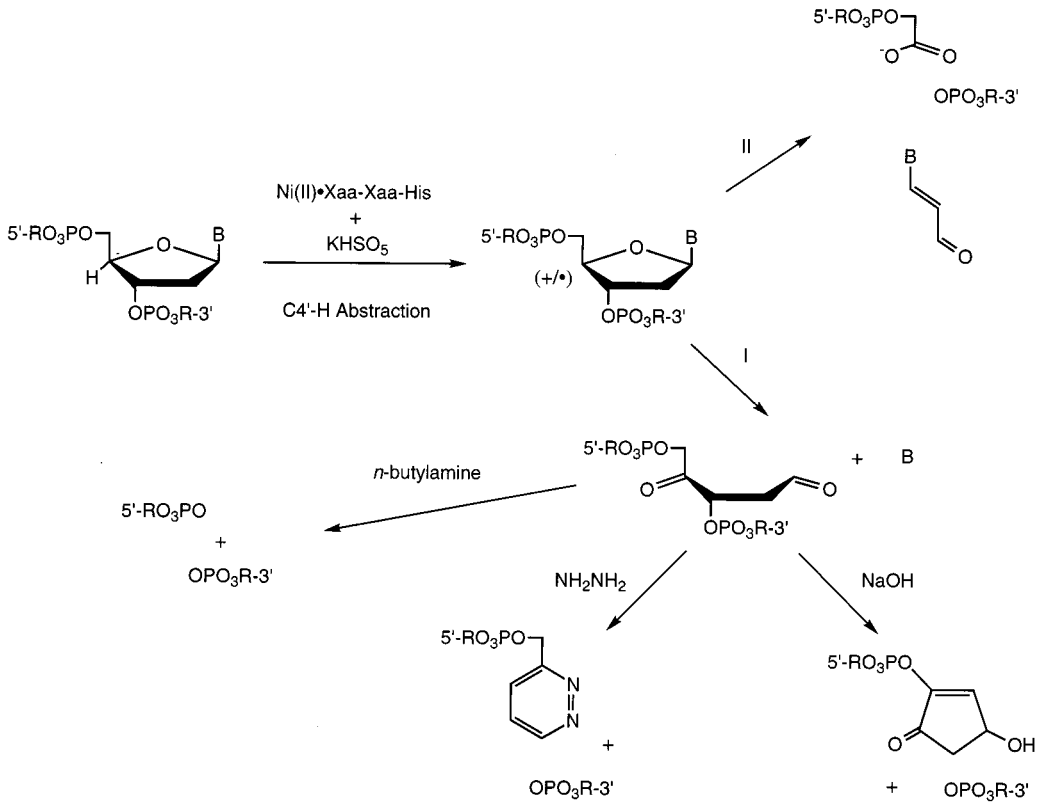
lopeptides, features which also promote the selectivity of other agents for these DNA sites.[27−29] Positioned as such, a Lys residue located within the second peptide position would be exposed at the surface of the DNA and capable of forming a salt bridge between its ϵ-amino functionality and a phosphate group proximal to the site of binding. Importantly, with the metallopeptide docked as described, the metal center is poised to interact with the C4′ H of an adjacent nucleoside. With this model as a guide, ongoing studies are seeking to examine the details of this peptide−DNA interaction via high-resolution methods.

The above studies address fundamental issues surrounding how amino acids can be used to provide contacts to the minor groove. It is worthy of note that most of the amino acids found to enhance cleavage by the metallopeptides are also those found in several important classes of minor groove binding protein motifs, such as the repeating Ser-Pro-Lys/Arg-Lys/Arg motifs of some histones.[30] These findings indicate that the activity of Ni(II)·Xaa-Xaa-His metallopeptides may be influenced by and serve to model the same amino acid−minor groove interactions that Nature has chosen to employ in protein−DNA minor groove recognition events.

Mechanism of DNA Cleavage

Molecular modeling predicts that the metal center of Ni(II)·Xaa-Xaa-His is poised to abstract the deoxyribose C4′ H, consistent with cleavage results obtained with Ni(II)·Gly-Gly-His-modified proteins[10] and the metallotripeptides alone.[19] While these studies have strongly suggested a mechanism of DNA damage involving deoxyribose modification, the orientation of the metallopeptide domain within the minor groove may differ when connected to a protein in comparison to the metallotripeptide alone. In addition, one report has also indicated that the metallotripeptides were capable of modifying G residues without deoxyribose modification when reaction conditions included excess $KHSO_5$ or sulfite in the presence of 100 mM NaCl.[31] Thus, knowledge of the exact details of cleavage were determined to facilitate a further under-

Scheme 2. Summary of Cleavage Products Observed and Pathways of Deoxyribose-Based DNA Degradation by Ni(II)·Xaa-Xaa-His Metallopeptides Activated with $KHSO_5$, MMPP, or H_2O_2



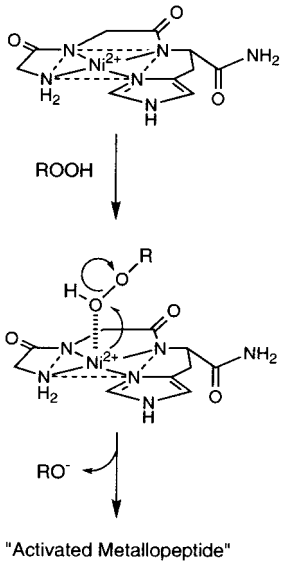
standing of the nature of the reactive metallopeptide generated and its location of DNA binding.

Initially, our laboratory identified[32] the DNA cleavage products formed by the metallopeptides under the reaction conditions employed to examine their site selectivity.[19] Using 5′- and 3′-^{32}P end-labeled DNA restriction fragments, which permit the analysis of the newly formed 3′- and 5′-termini at a site of metallopeptide-induced cleavage, respectively, it was found that the 5′- and 3′-termini formed were consistent with the chemistry of C4′ H abstraction (Scheme 2). The products found included 5′-phosphorylated termini and 3′-termini that were phosphorylated, or possessed moieties upon postreaction workup with NaOH or NH_2NH_2 that comigrated with the breakdown products of the keto-aldehyde abasic lesion formed by C4′ hydroxylation (Scheme 2, path I). In addition, 3′-phosphoroglycolate termini were identified, consistent with the intermediacy of a C4′-hydroperoxy lesion (Scheme 2, path II). Along with these termini, free nucleobases and nucleobase propenals, representing the remaining deoxyribose fragmentation products released into solution, were also found. Thus, paralleling the chemistry of Fe•bleomycin,[3a] DNA cleavage by Ni(II)·Xaa-Xaa-His appears, under the conditions employed, to result from an initial act of deoxyribose modification via C4′ H abstraction, leading to the formation of either C4′ hydroxy or C4′ hydroperoxy nucleotides; like other metal complexes examined under similar conditions,[33] the ratio between these lesions was found to be dependent upon the binding affinity of the cleaving agent and the microheterogeneity of the sequence targeted.

The generality of the above mechanism was also examined using two additional activating reagents, MMPP and H_2O_2. Results from these studies[32] indicated that the site selectivity and product formation of Ni(II)·Lys-Gly-His remained the same, suggesting the formation of a common metallopeptide-based oxidant upon activation with all three reagents. Furthermore, the presence of radical scavengers exhibited no effect on direct DNA strand scission by the metallopeptides utilizing these three activating reagents; the conditions employed[32] ruled out the intermediacy of diffusible hydroxyl radicals and diffusible or metal-bound sulfate radicals (when $KHSO_5$ is employed in activation) in the direct, deoxyribose-based mechanism of DNA cleavage.

What is the nature of the activated Ni(II)·Xaa-Xaa-His responsible for deoxyribose C4′ H abstraction? Given that a "common" nondiffusible active species appears to be formed from three diverse activating reagents that can act as oxygen atom donors, our laboratory has proposed that the active species responsible for deoxyribose damage is a peptide-bound Ni(III)−HO• or Ni(IV)═O. This species is likely generated through the heterolytic splitting of the oxygen−oxygen bond present in $KHSO_5$, MMPP, or H_2O_2 (Scheme 3). This proposal is supported also by the observation[32] that the relative abilities of these reagents to activate the metallopeptides ($KHSO_5$ > MMPP ≫ H_2O_2) closely parallels the acidity of their leaving group upon oxygen−oxygen bond heterolysis (H_2SO_4 > $COOHC_6H_4$-$COOH$ ≫ H_2O), a factor known to influence the ability of a peroxide functional group to act as an oxygen atom donor to a metal center.[34] In addition, upon Ni(II)·Xaa-

Scheme 3. Proposed Mechanism of Ni(II)·Xaa-Xaa-His Activation through Ni-Promoted Heterolytic Cleavage of a Peroxide/Peracid Oxygen−Oxygen Bond



Xaa-His activation, a UV−vis absorption shift from 425 nm to a more intense absorption at 375 nm is observed, indicative of an octahedral Ni(III) or Ni(IV) species.[35] Along with the above, Ni(II)·Gly-Gly-His has been shown to catalyze alkene epoxidations;[10] in similar reactions with other Ni complexes, a ligand-bound Ni(III)−HO• or Ni(IV)═O has been implicated as an active intermediate.[36] While definitive proof of the above intermediate awaits further characterization, such a species positioned in the minor groove of DNA (Figure 2) would likely lead to C4′ H abstraction.

In light of the above, why was selective guanine oxidation observed in one instance? To address this issue, this laboratory performed a direct comparison[32] of the reaction conditions reported to result in deoxyribose modification vs those found to lead to guanine oxidation. As illustrated in Figure 3, cleavage of 3′-^{32}P end-labeled restriction fragments under conditions employed[19] in a determination of the site selectivity of Ni(II)·Lys-Gly-His [equimolar $KHSO_5$ + Ni(II)·Lys-Gly-His, 15 μM, 10 mM sodium cacodylate, pH 7.5, 1 min] led to a pattern of A/T-rich region modification directly or upon postreaction workup with piperidine/90 °C. In comparison, cleavage of the same DNA restriction fragment under conditions reported[31] to result in guanine oxidation [excess $KHSO_5$ (100 μM) + 15 μM Ni(II)·Lys-Gly-His, 100 mM NaCl, 10 mM phosphate, pH 7.0, 30 min] led to less direct strand scission; however, postreaction workup with piperidine/90 °C revealed predominant oxidation at guanine residues. Further, while MMPP and H_2O_2 were capable of supporting A/T-selective cleavage as with $KHSO_5$, these reagents were unable to support guanine oxidation under the conditions described above.[32]

These studies indicate that $KHSO_5$, MMPP, and H_2O_2 can activate Ni(II)·Xaa-Xaa-His metallopeptides to produce a common activated metallopeptide that leads to deoxyribose-centered strand scission under relatively low ionic strength conditions. Meanwhile, guanine oxidation occurs solely upon metallopeptide activation with excess $KHSO_5$ and relatively high ionic strength conditions. Thus, it appears that Ni(II)·Xaa-Xaa-His metallopeptides activated with $KHSO_5$ are capable of forming two "active" DNA-modifying species, one in common with those derived from MMPP and H_2O_2 and another, perhaps a "caged" sulfate radical.[31] In the case of low ionic strength conditions and equimolar $KHSO_5$, the metallopeptides generate a Ni-based oxidizing equivalent (in common with MMPP and H_2O_2 activation) that is able to recognize and bind to the minor groove of DNA, resulting in C4′ H abstraction. In contrast, with excess $KHSO_5$ and high ionic strength conditions, the formation of a metallopeptide-bound sulfate radical may predominate which sterically is prevented from binding to the narrow minor groove and screened from a tight association with DNA; these conditions thus limit deoxyribose-based chemistry and facilitate guanine oxidation, perhaps via the intermediate modification[31] of the N7 position of this nucleobase exposed in the major groove.

RNA Recognition and Cleavage

This laboratory has recently demonstrated that Ni(II)·Xaa-Xaa-His metallopeptides are capable also of selective RNA cleavage.[37] As with DNA, metallopeptides have the wherewithal to contribute to our knowledge of RNA molecular recognition by providing unique structures to complement the rich diversity of three-dimensional shapes presented by a structured RNA.[2] As illustrated in Figures 4 and 5, cleavage of 3′-^{32}P end-labeled $tRNA^{Phe}$ [38] and the TAR RNA[39] of HIV-1 by Ni(II)·Gly-Gly-His, Ni(II)·Lys-Gly-His, and Ni(II)·Arg-Gly-His resulted in the modification of the loop structures of these RNAs upon postreaction aniline−acetate workup. With $tRNA^{Phe}$, metallopeptide-induced cleavage was observed to occur within the D, anticodon, and the TΨC loops; with TAR RNA, cleavage primarily occurred within the apical loop. As with DNA, cleavage was observed to occur at a variety of nucleobases, indicating a selectivity that is likely due to a distinct act of binding rather than reactivity with a particular nucleobase. However, cleavage of several highly exposed guanine residues within $tRNA^{Phe}$ (G18 and G19) also suggests some level of RNA modification that parallels other Ni complexes.[40] In contrast, though, to when DNA is employed as a substrate, the Ni(II)·Xaa-Xaa-His metallopeptides (1) produced identical RNA cleavage patterns regardless of the terminal Xaa amino acid and (2) required aniline−acetate for strand scission, suggesting a mechanism involving initial nucleobase damage.[41] Overall, the metallopeptide selectivities observed indicate a preference for RNA loop structures; unlike several other RNA cleaving agents examined to date,[2] the metallopeptides are unique in that they do not cleave the single-stranded bulge of TAR RNA nor single-stranded regions in $tRNA^{Phe}$. Interestingly, within the RNA loops targeted, the pattern of metallopeptide-induced modification occurred mainly within their 3′-halves. These observations suggest a distinct metallopeptide binding

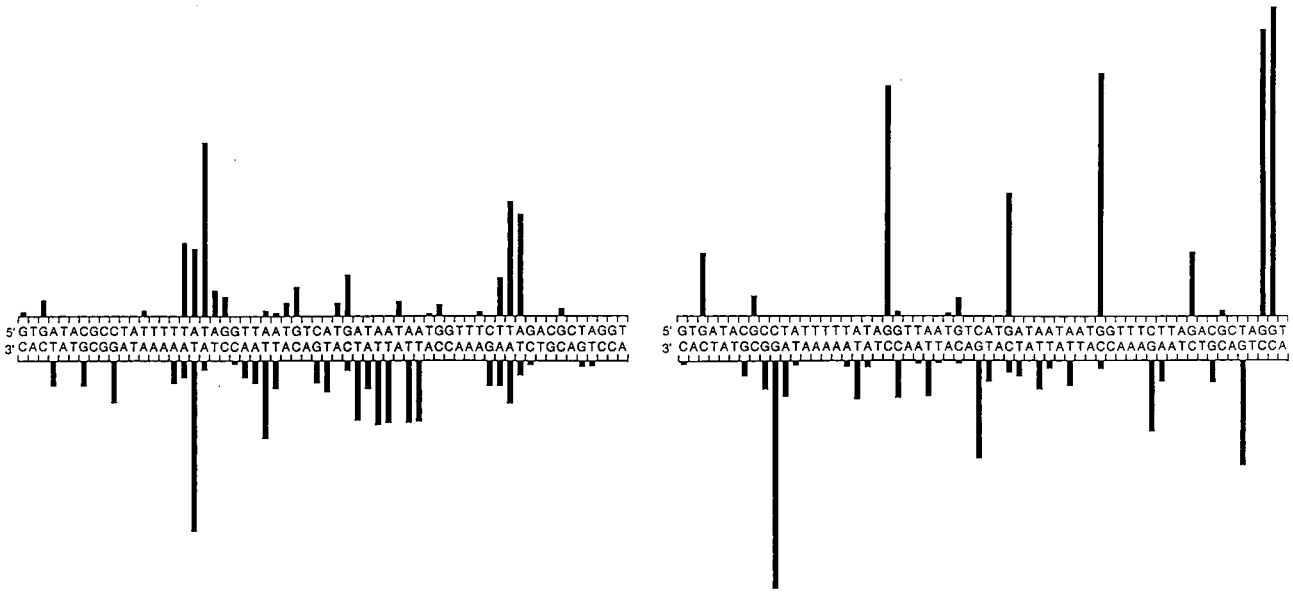


FIGURE 3. Histograms generated from the densitometric analysis of 3′-^{32}P end-labeled DNA restriction fragment cleavage by Ni(II)·Lys-Gly-His under "low" ionic strength conditions (left) and "high" ionic strength conditions (right). Both reactions included a postreaction workup with piperidine/90 °C.

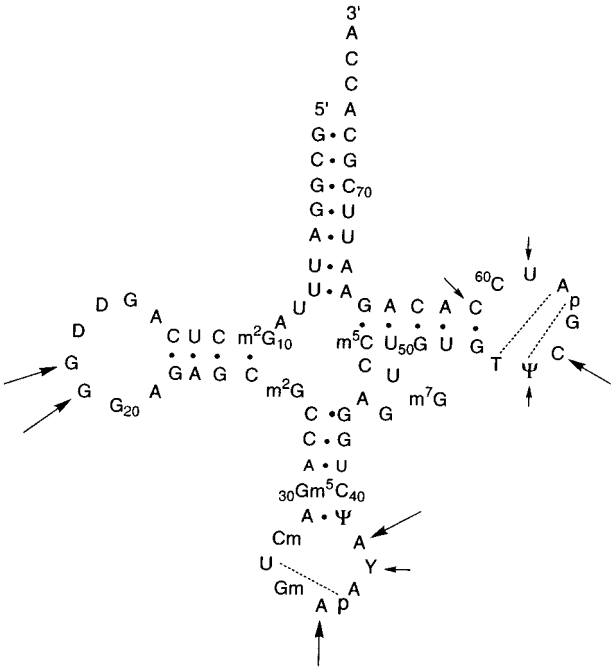


FIGURE 4. Secondary structure of tRNA^{Phe}, indicating the sites of Ni(II)·Xaa-Gly-His cleavage. Dashed lines indicate the locations of documented intraloop hydrogen bonding.

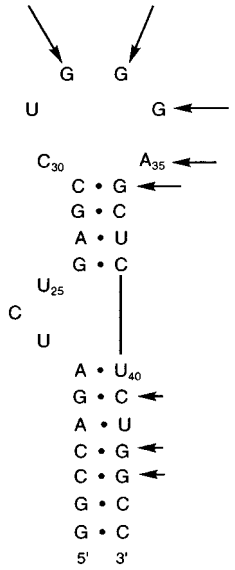


FIGURE 5. Secondary structure of the TAR RNA of HIV-1, indicating the sites of Ni(II)·Xaa-Gly-His cleavage.

orientation, likely leading to the modification of exposed nucleobases within these loops. Thus, differing from several reagents known to cleave single-stranded RNA,[2] the metallopeptides appear to interact within a particular loop like many RNA binding proteins[42] as opposed to reacting with single-stranded character alone.

The above results suggest that RNA loops may present a binding pocket complementary to the size and shape of Ni(II)·Xaa-Xaa-His metallopeptides. With, perhaps, the His portion of the metallopeptide inserted into the loop as with the DNA minor groove, the metallopeptide becomes distinctly oriented, leading to modification of the nucleobases in close proximity. Although only speculation, metallopeptide binding may sense unique loop structural features. In the case of the anticodon and TΨC loops of tRNA^{Phe}, structural investigations have indicated their participation in the formation of "uridine turns" (U-turns),[38] where the U residues of these loops are hydrogen-bonded to a phosphate on the opposite side of the loop. Curiously, the nucleotides immediately adjacent to the phosphates involved in U-turn formation are not cleaved by the metallopeptides (Figure 4). In contrast, with TAR RNA, the accessibility[39] of its apical loop and its lack of intraloop hydrogen bonding may allow cleavage throughout the 3′-half of this loop upon metallopeptide binding. Overall, these findings may assist in furthering our understanding of RNA recognition phenomena.

Summary

Ni(II)·Xaa-Xaa-His metallopeptides have proven to be unique agents targeted to either the minor groove of B-form DNA or loop regions of structured RNAs. With the DNA minor groove, metallopeptide binding is influenced by the character of the amino acids included in the ligand and positions the reactive metal center generated to abstract the C4′ H. In constrast, with RNA, loop association leads to the modification of nucleobases that are exposed within the lining of these potential binding "pockets". Ni(II)·Xaa-Xaa-His metallopeptides thus provide fundamental information pertaining to amino acid and peptide contacts to these nucleic acid regions. The information provided to date, along with higher resolution studies and a greater understanding of the effects of functional group orientation, will further our knowledge of protein−nucleic acid complexation and perhaps assist in developing wholly synthetic structures targeted to these sites.

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