Metallopeptide–DNA Interactions: Site-Selectivity Based on Amino Acid Composition and Chirality

Qi Liang, Paula Denney Eason, and Eric C. Long*

Contribution from the Department of Chemistry, Indiana University Purdue University–Indianapolis, Indianapolis, Indiana 46202-3274

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Abstract: DNA cleavage by synthetic tripeptides of the form NH_2-Xaa-Xaa-His-$CONH_2$ was investigated in the presence of Ni(II) and the oxygen activating agent oxone. Studies with Ni(II)·Gly-Gly-His, Ni(II)·Lys-Gly-His, and Ni(II)·Arg-Gly-His indicated that each metallopeptide is capable of inducing strand scission via a non-diffusible oxidant at mixed A/T-rich regions, albeit with an avoidance of homopolymeric A/T sites, in a reaction that can be inhibited by the minor groove binding drug distamycin. Additional metallopeptides containing substitutions of D-His for L-His and neutral metallopeptides containing Asn residues demonstrate the ability to alter site-selectivity through subtle structural changes; the metallopeptide Ni(II)·Gly-D-Asn-His displayed a selectivity for 5′-CCT sites while other substitutions varied with respect to their levels of site discrimination in comparison to Ni(II)·Gly-Gly-His. These observations indicate that the metallopeptides in question are sensitive to DNA structure and are capable of interacting selectively with the minor groove, suggesting the possibility of exploiting the chemical diversity and chirality of peptides in the further design of site-selective DNA binding and modifying agents.

Introduction

Recent investigations using low molecular weight metal complexes of unnatural ligand systems have increased our understanding of nucleic acid recognition and have provided a diverse array of "tools" for the chemical manipulation of nucleic acids.[1] Surprisingly, however, while considerable attention has focused on the design and synthesis of ligands to tune the selectivity and reactivity of such complexes, few studies have sought to harness the potential of *peptide* ligands containing the same chemical functional groups (e.g., guanidinium, amino, or amide moieties) employed by nucleic acid binding proteins or antitumor natural products.[2] Given the ability to easily alter the chirality and individual amino acid residues of a peptide, such ligands may serve as a basis for the design of selective binding agents while also bridging the study of artificial metal complexes and naturally-occurring systems.

Presently, we report an initial examination of DNA cleavage induced by a series of peptides of the general form NH_2-Xaa-Xaa-His-$CONH_2$ that contain positively-charged or amide-containing amino acids important in protein–nucleic acid interactions. The design of these peptides is based on the established metal chelating and substrate oxidation exhibited by Gly-Gly-His.[3] This tripeptide, which mimics the amino-terminal, square-planar Cu(II)-chelating domain of serum albumins, binds Cu(II) or Ni(II) in a 1:1 complex at physiological pH through the histidine imidazole nitrogen, two deprotonated amide nitrogens, and the terminal α-amine with a dissociation constant on the order of 10^{-16}–10^{-17} M.[3] Most importantly, however, for the purposes of this study, the Gly-Gly-His ligand system can be chemically activated in the presence of Cu(II) or Ni(II) to produce oxidizing equivalents capable of effecting DNA[4] or protein cleavage.[5] This relatively simple tripeptide thus provides an ideal framework through which to explore the effect(s) of alternative amino acids, with established roles in nucleic acid recognition, on the binding of metallopeptides to DNA. Indeed, previous studies of amino acid substitutions with side chains bulkier than glycine within the parent Gly-Gly-His tripeptide have shown little effect on metal complexation at or above physiological pH values.[6]

Included herein are studies of the reactivity, binding, and site-selectivity exhibited by the above metallopeptides in the presence of DNA. These experiments indicate that when bound to Ni(II) and appropriately activated, peptides of this series can *selectively* modify DNA. Furthermore, our initial studies also include structural modifications within this interesting new class of DNA binding metal complexes that indicate the site-selectivity observed can be altered through the chemical nature of the amino acid substitution employed, its position within the tripeptide sequence, and, importantly, its chirality. *These findings therefore suggest an opportunity to exploit the chemical diversity and chirality of low molecular weight peptides in the further design of novel, site-selective DNA binding and modifying agents.*

Experimental Section

Peptide Synthesis. All protected amino acids were purchased from Bachem California. Peptide syntheses were carried out by conventional solid-phase methodologies[7] followed by reverse-phase HPLC purifica-

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(1) (a) Sigman, D. S.; Mazumder, A.; Perrin, D. M. *Chem. Rev.* **1993**, *93*, 2295. (b) Pyle, A. M.; Barton, J. K. *Prog. Inorg. Chem.* **1990**, *38*, 413. (c) Long, E. C. *J. Inorg. Organomet. Polym.* **1993**, *3*, 3.

(2) (a) Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1376. (b) Zimmer, C.; Wahnert, U. *Prog. Biophys. Mol. Biol.* **1986**, *47*, 31. (c) Rhee, Y.; Wang, C.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1993**, *115*, 8742. For a recent example of a metal complex–peptide conjugate that binds and induces strand scission of DNA selectively see: Sardesai, N. Y.; Zimmerman, K.; Barton, J. K. *J. Am. Chem. Soc.* **1994**, *116*, 7502.

(3) (a) Camerman, N.; Camerman, A.; Sarkar, B. *Can. J. Chem.* **1976**, *54*, 1309. (b) Lau, S.-J.; Kruck, T. P. A.; Sarkar, B. *J. Biol. Chem.* **1974**, *249*, 5878.

(4) (a) Chiou, S.-H.; Chang, W.-C.; Jou, Y.-S.; Chung, H.-M. M.; Lo, T.-B. *J. Biochem.* **1985**, *98*, 1723. (b) Mack, D. P.; Iverson, B. L.; Dervan, P. B. *J. Am. Chem. Soc.* **1988**, *110*, 7572. (c) Mack, D. P.; Dervan, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 4604. (d) Mack, D. P.; Dervan, P. B. *Biochemistry* **1992**, *31*, 9399. (e) Shullenberger, D. F.; Eason, P. D.; Long, E. C. *J. Am. Chem. Soc.* **1993**, *115*, 11038.

(5) Cuenoud, B.; Tarasow, T. M.; Shepartz, A. *Tetrahedron Lett.* **1992**, *33*, 895.

(6) (a) Lau, S.-J.; Laussac, J.-P.; Sarkar, B. *Biochem. J.* **1989**, *257*, 745. (b) Iyer, K. S.; Lau, S.-J.; Laurie, S. H.; Sarkar, B. *Biochem. J.* **1978**, *169*, 61.

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tion and verification by FAB-MS. Each purified, synthetic carboxamide tripeptide, upon complexation to either Cu(II) or Ni(II), yielded visible absorbance spectra (Cu: λ_{max} = 525−527 nm, ϵ = ~108 M^{-1} cm^{-1}; Ni: λ_{max} 420−422 nm, ϵ = ~120 M^{-1} cm^{-1}, pH 7.5, 25 mM sodium cacodylate) and 1:1 titrations of peptide−metal ion characteristic of square-planar Gly-Gly-His metallopeptides.[3,4e] All molecular models of the metallopeptides employed throughout this study were based on the reported crystal structure of Cu(II)·Gly-Gly-His.[3a]

Cleavage of Supercoiled DNA. Peptides were pre-incubated with $Ni(OAc)_2$ in 10 mM sodium cacodylate buffer (pH 7.5) for 10 min, followed by the addition of ΦX174 RF plasmid DNA (50 μM base pair concentration) in a total volume of 20 μL. Reactions with Ni(II)·peptide were initiated with oxone and quenched after 1.0 min with EDTA (60 mM) loading buffer. Reactions were analyzed on 0.9% agarose gels which were electrophoresed at 60 V for 2 h followed by ethidium bromide staining and visualization on a UV transilluminator.

Sequence-Specific Cleavage of DNA Restriction Fragments. All enzymes utilized were from commercial sources. [α-^{32}P]dATP and [γ-^{32}P]ATP were obtained from NEN-Dupont. End labeled restriction fragments were prepared by digesting supercoiled pBR322 plasmid (BRL) with Eco RI restriction endonuclease followed by 3′-end labeling with terminal deoxynucleotidyl transferase and [α-^{32}P]dATP.[8] Alternatively, 5′-end labeling was achieved by sequential treatment of the restricted DNA with bacterial alkaline phosphatase followed by treatment with [γ-^{32}P]ATP and T4 polynucleotide kinase.[8] After labeling, the DNA was further digested with Rsa I to yield 167 and 514 base pair fragments that were purified by 6% preparative non-denaturing gel electrophoresis and isolated by electroelution.

Cleavage reactions were carried out in a 20 μL total volume containing calf thymus DNA (50 μM base pair concentration) and 3 × 10^4 cpm ^{32}P-end-labeled restriction fragment in 10 mM sodium cacodylate buffer, pH 7.5. Reactions were initiated through the admixture of DNA and equimolar amounts of preformed Ni(II)·peptide and oxone and quenched after 1 min with the addition of 3 μL of a 0.2 M EDTA solution. All reaction mixtures were ethanol precipitated with the addition of 10 μL of 3 M sodium acetate and 60 μL of EtOH. The precipitated DNA was washed with a small volume of 70% EtOH, dried, and resuspended in 3 μL of 80% formamide loading buffer (80% v/v formamide, 1 mM EDTA, 0.1% xylene cyanol, and 0.1% bromphenol blue). All reactions, along with Maxam-Gilbert G + A sequencing reactions,[8] were heat denatured at 90 °C for 5 min and quick-chilled on ice. The samples were loaded onto 8 or 12% (19:1) polyacrylamide/7.5 M urea sequencing gels and electrophoresed at 1500 V for approximately 4 h and transferred to a cassette and stored at −70 °C with Kodak X-omat film.

Quantitative Distamycin Competition Reactions. Approximate metallopeptide−DNA binding constants were determined at distinct sites on DNA restriction fragments using distamycin as a competitive binding agent.[9] The concentration of distamycin was determined optically using ϵ_{302} = 3.4 X 10^4 M^{-1} cm^{-1}. Different amounts of distamycin and constant amounts of Ni(II)·Lys-Gly-His were incubated with the 3′-end-labeled 514 base pair DNA restriction fragment and calf thymus DNA (50 μM base pair concentration). All other conditions were identical to those employed in the DNA cleavage reactions described previously.

Autoradiograms were scanned using a Bio Rad GS-670 imaging densitometer over the width of each lane. To correct for minor variability in the radioactivity loaded into each lane, the apparent distamycin protection fraction (θ_{app}) was calculated as described previously[10]

$$\theta_{app} = 1 - \frac{OD_{prot}/OD_{unprot}}{OD_{prot\ ref}/OD_{unprot\ ref}}$$

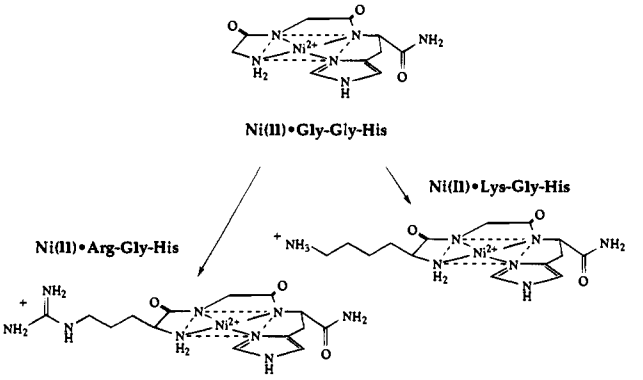
where OD_{prot} and OD_{unprot} refer to the optical densities of the protected and unprotected sites, which is directly proportional to the amount of cleavage at the distamycin protected and unprotected sites, respectively. $OD_{prot\ ref}$ and $OD_{unprot\ ref}$ refer to the amounts of cleavage at these same sites in a reference control lane to which no distamycin was added. For the fragment studied, the 5′-TATTATC-3′ sequence was chosen as the distamycin protection site, and the flanking 5′-CAT-3′ and 5′-ATGAC-3′ sequences at either side of the above protected site were chosen as the unprotected sites. Average θ_{app} values were determined from three independent gels. The binding constant for the Ni(II)·Lys-Gly-His complex to DNA was determined when 50% of the cleavable DNA sites were protected by distamycin (θ_{app} = 50%) as previously described.[9] The apparent binding constant was calculated from:

$$K_{distamycin}[\text{distamycin}] = K_{app}[\text{Ni(II)·peptide}]$$

Given the binding constant of distamycin to DNA (~10^8 M^{-1})[2b] and the concentrations of distamycin and Ni(II)·peptide complexes used in this experiment, K_{app} was determined directly using the relationship shown above.

Results and Discussion

Selective DNA Cleavage by the Ni(II) Complexes of Gly-Gly-His, Arg-Gly-His, and Lys-Gly-His. Initially, the DNA cleavage activities of Ni(II)·Gly-Gly-His and two derivatives of this "parent" tripeptide were examined. The derivatives chosen included substitutions of the amino-terminal glycine of Gly-Gly-His with either lysine (Lys) or arginine (Arg), positively-charged amino acids vital to protein−DNA interactions that also facilitate an electrostatic association with DNA. In each case, the peptides were synthesized as a carboxy-terminal amide to eliminate electrostatic repulsion with the DNA polyanion. Additionally, the modification involving an Arg residue permitted the inclusion of a guanidinium functional group within the metallopeptide structure as also found in the distamycin/netropsin class of natural product minor groove DNA binders;[2a-c] a Lys *and* Arg modification would therefore allow assessment of whether the overall charge of the complex or the chemical structure of the side chain influenced any selectivity observed.



The ability of each metallopeptide to cleave a DNA substrate was verified through the conversion of supercoiled DNA (form I) to nicked-circular (form II) and linear (form III) DNAs. Using an oxone activation protocol employed previously,[4e] the Ni(II) complexes of each tripeptide were found to readily convert supercoiled form I ΦX174 RF plasmid DNA to forms II and III in a reaction that is entirely dependent upon peptide; complete conversion of form I DNA to form II was achieved with 5 μM Ni(II)·Lys-Gly-His or Ni(II)·Arg-Gly-His while a similar extent of plasmid conversion required a 20-fold higher concentration of Ni(II)·Gly-Gly-His. As expected, the positively-charged complexes containing Arg or Lys residues produced substantially more plasmid conversion than the neutral complex containing only glycine residues, an effect no doubt promoted by their electrostatic association with the negatively-charged DNA backbone.

(7) Stewart, J. M.; Young, J. D. *Solid-Phase Peptide Synthesis*; Pierce Chemical Co.: Rockford, IL, 1984.

(8) Maniatis, T.; Fritsch, E. F.; Sambrook, J. *Molecular Cloning*; Cold Spring Harbor Laboratory Press: Plainview, NY, 1982.

(9) Uchida, K.; Pyle, A. M.; Morii, K.; Barton, J. K. *Nucl. Acids Res.* **1989**, *17*, 10259.

(10) Brenowitz, M.; Senear, D. F.; Shea, M. A.; Ackers, G. K. *Methods Enzymol.* **1986**, *130*, 133.

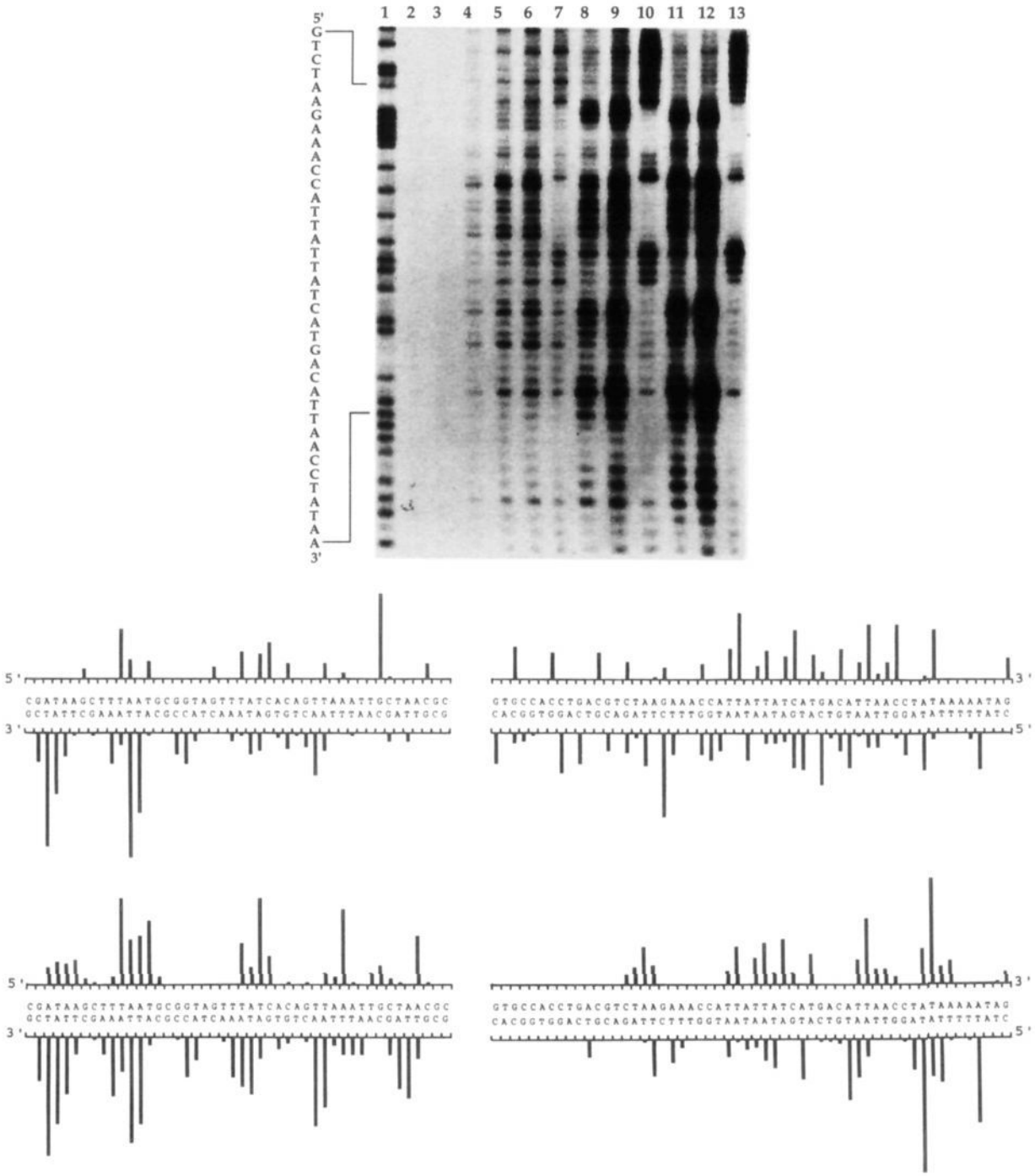


Figure 1. (top) Autoradiogram of Ni(II)•peptide induced cleavage of a 3′-^{32}P-end-labeled 514 base pair EcoRI → RsaI restriction fragment from pBR322. Lane 1, Maxam-Gilbert G + A reaction; Lane 2, intact DNA; Lane 3, reaction control [20 μM $Ni(OAc)_2$, 20 μM oxone; reaction controls conducted at higher concentrations of $Ni(OAc)_2$ + oxone yielded identical results]; Lane 4, 20 μM Ni(II)•Gly-Gly-His; Lane 5, 50 μM Ni(II)•Gly-Gly-His; Lane 6, 100 μM Ni(II)•Gly-Gly-His; Lane 7, 120 μM Ni(II)•Gly-Gly-His + 10 μM distamycin; Lane 8, 7.5 μM Ni(II)•Lys-Gly-His; Lane 9, 15 μM Ni(II)•Lys-Gly-His; Lane 10, 30 μM Ni(II)•Lys-Gly-His + 10 μM distamycin; Lane 11, 5 μM Ni(II)•Arg-Gly-His; Lane 12, 10 μM Ni(II)•Arg-Gly-His; Lane 13, 20 μM Ni(II)•Arg-Gly-His + 10 μM distamycin. The varied concentrations of Ni(II) + peptide listed were chosen based on cleavage of plasmid DNA to assist in normalizing for differences in cleavage efficiency of the individual metallopeptides. Quantitation of the cleavage of this fragment induced by Ni(II)•Arg-Gly-His is shown in the lower half of this figure. (bottom) Histogram of the major sites of cleavage induced by Ni(II)•Arg-Gly-His (lower histogram) and Ni(II)•Gly-Gly-His (upper histogram) on the 5′- and 3′-^{32}P-end-labeled EcoRI → RsaI fragments of pBR322. Portions of the 167 bp (left) and 514 bp (right) restriction fragments are shown including nucleotide numbers 25 to 70 and 4277 to 4332, respectively. Bar lengths are proportional to the intensity of cleavage observed; essentially identical results were obtained by Ni(II)•Arg-Gly-His and Ni(II)•Lys-Gly-His.

Having verified the ability of each metallopeptide to cleave DNA, end-labeled restriction fragments were employed to assess their relative site-selectivities. As shown in Figure 1, when 5′- or 3′-^{32}P-end-labeled restriction fragments were used as substrates for each metallopeptide, patterns of DNA modification are revealed which indicate that the positively-charged complexes display a selectivity for mixed A/T-rich sites[11] while Ni(II)•Gly-Gly-His appears to only "loosely" target similar DNA

regions. Figure 1 emphasizes, however, that while the cleavage efficiency of the charge-neutral complex Ni(II)·Gly-Gly-His was less than that of its positively-charged derivatives, the overall patterns of DNA modification exhibited by all three complexes were centered about similar points of intensity along the DNA strand suggesting that the addition of a positive charge served predominantly to focus a common underlying selectivity. Also apparent from the data is cleavage involving single residues of all four nucleobases; such a pattern suggests the operation of a non-diffusible oxidizing equivalent which, unlike previously studied Ni complexes useful in examining nucleic acid structures,[12] does not display a preference for G residues even upon *n*-butylamine or piperidine treatment.[13] Thus, it seems quite likely that the cleavage of DNA by these complexes occurs through a mechanism that employs deoxyribose modification.

In addition to their common selectivity for mixed A/T sites, a careful analysis of the cleavage data revealed that all three peptides also displayed a notable *lack* of scission activity at homopolymeric A/T regions that further decreases toward the 3′-end of these sites. Given the anomalous structures that can be formed by such regions of DNA (e.g., a narrowed minor groove, bent structures, etc.)[14] and the apparent targeting of each peptide for mixed A/T-rich regions,[15] it appears as if these metallopeptides are sensitive to structural differences found along the minor groove of a DNA helix. Minor groove binding is also supported by an analysis of the cleavage produced on *both* the 5′- and 3′-^{32}P-end-labeled strands (Figure 1) which revealed a pattern of DNA modification with an inherent 3′-asymmetry suggesting an association of each metallopeptide within the minor groove of the DNA structure.[16]

Quantitation of Peptide Binding through Distamycin Competition. To further support a mechanism involving minor groove binding and to characterize the binding affinity of the positively-charged metallopeptides, cleavage experiments were performed in the presence of the well-characterized minor groove associating drug distamycin.[2] As shown in Figure 1 (lanes 7, 10, and 13), reactions[17] performed in the presence of distamycin greatly inhibited metallopeptide-induced cleavage in regions of preferred drug interaction (e.g., the 5′-TATTAT site at the top half of the autoradiogram) implying a competition for similar binding sites along the DNA helix.

Using this competition for binding, DNA cleavage reactions (Figure 2) involving fixed amounts of Ni(II)·Lys-Gly-His in the presence of increasing amounts of distamycin were performed, as described previously,[9,10] to determine the DNA binding affinity of this metallopeptide. As shown (Figure 2), several A/T-rich regions produced a competition for binding between Ni(II)·Lys-Gly-His and distamycin; using one of these sites (bracketed) the binding affinity for DNA displayed by Ni(II)·Lys-Gly-His (K_b) was determined to be $\sim 5 \times 10^5$ M^{-1}.[18]

A Working Model of DNA Binding. The similarity in cleavage patterns induced by all three peptide structures suggests

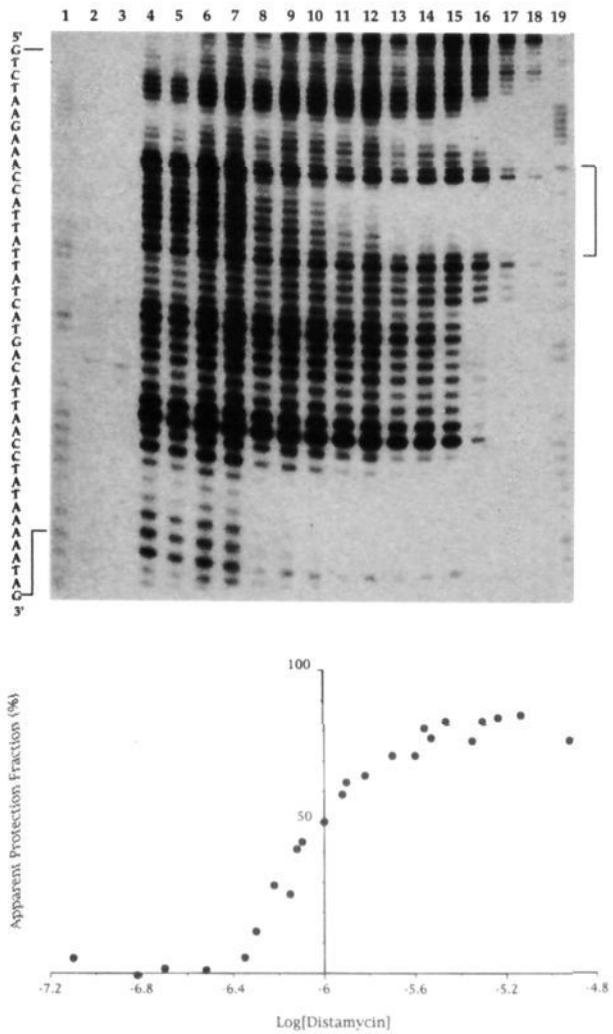


Figure 2. (top) Cleavage of DNA in the presence of Ni(II)·Lys-Gly-His and increasing amounts of distamycin. Lane 1, Maxam-Gilbert G + A reaction; Lane 2, DNA blank; Lane 3, reaction control, DNA + 200 μM Ni(OAc)$_2$ + 200 μM oxone; Lanes 4−18, DNA + 200 μM Ni(II)·Lys-Gly-His + oxone in the presence of increasing amounts of distamycin—0, 0.075, 0.20, 0.50, 0.75, 1.0, 1.25, 1.5, 2.0, 2.75, 3.5, 5.0, 7.5, 12.0, and 20.0 μM, respectively; Lane 19, Maxam-Gilbert G + A reaction. (bottom) Plot of log[distamycin] vs apparent protection fraction (θ_{app}) for cleavage of DNA in the presence of Ni(II)·Lys-Gly-His and increasing amounts of distamycin as listed above.

that association of the metallopeptides discussed so far and the A/T-rich regions of their DNA substrates may be mediated by the carboxy-terminal His residue and the terminal α-amino group, as also supported by a spectroscopic investigation of DNA binding by similar peptides.[19] Such an arrangement, with the edge of the square-planar peptide structure inserted into the minor groove of the DNA helix, would permit the side chain of the amino-terminal or interior amino acid to reach out of the minor groove permitting, at least in the case of the Arg- and Lys-containing peptides, an ionic interaction with the phosphate backbone further explaining their similarities in binding behavior; examination of molecular models revealed that the thickness of the square-planar peptide structure complements the width of the minor groove of DNA. In order to test this hypothesis, an additional tripeptide was synthesized, Ni(II)·Gly-Lys-His; the

(11) For examples of other metal complexes that target A/T-rich regions see: Gravert, D. J.; Griffin, J. H. *J. Org. Chem.* **1993**, *58*, 820. Raner, G.; Ward, B.; Dabrowiak, J. C. *J. Coord. Chem.* **1988**, *19*, 17.

(12) (a) Chen, X.; Rokita, S. E.; Burrows, C. J. *J. Am. Chem. Soc.* **1991**, *113*, 5884. (b) Burrows, C. J.; Rokita, S. E. *Acc. Chem. Res.* **1994**, *27*, 295.

(13) We have also carried out preliminary investigations of the chemistry of DNA strand scission mediated by the metallopeptides; upon cleavage of calf thymus DNA, free nucleic acid bases are released in amounts (A > T ≫ C/G) that parallel the observed site selectivities.

(14) (a) Burkhoff, A. M.; Tullius, T. D. *Cell* **1987**, *48*, 935. (b) Koo, H.-S.; Wu, H.-M.; Crothers, D. M. *Nature* **1986**, *320*, 501. (c) Alexeev, D. G.; Lipanov, A. A.; Skuratovskii, I. Y. *Nature* **1987**, *325*, 821. (d) Levene, S. D.; Wu, H.-M.; Crothers, D. M. *Biochemistry* **1986**, *25*, 3988.

(15) Rhoades, D.; Klug, A. *Nature* **1981**, *292*, 378.

(16) Dervan, P. B. *Methods Enzymol.* **1991**, *208*, 497.

(17) Cleavage reactions shown in Figure 1 that included distamycin (lanes 7, 10, and 13) were carried out at slightly elevated Ni(II)·peptide concentrations to improve the visibility of the resulting distamycin "footprints".

(18) In addition to the cleavage competition assay described, a fluorescence displacement assay employing ethidium bromide also allowed quantitation of metallopeptide−DNA binding; affinities similar to those calculated through cleavage competition were obtained.

(19) Chikira, M.; Sato, T.; Antholine, W. E.; Petering, D. H. *J. Biol. Chem.* **1991**, *266*, 2859.

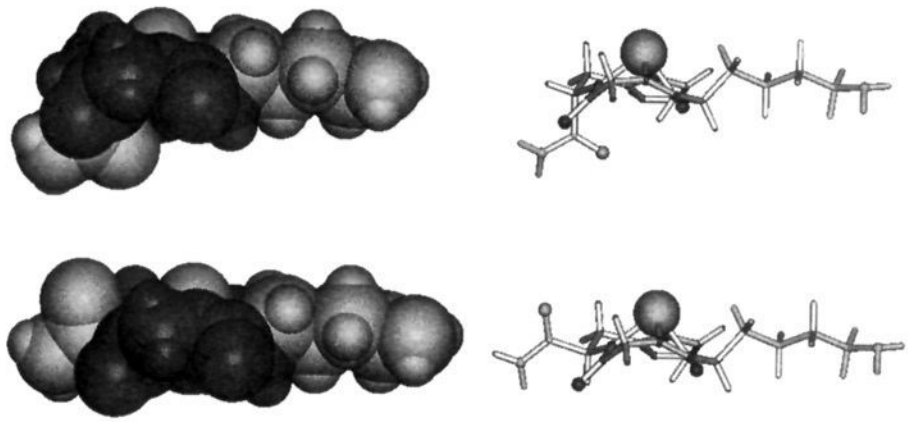


Figure 3. Comparison of the structures of Ni(II)·Lys-Gly-His (top) and Ni(II)·Lys-Gly-D-His (bottom). The metallopeptides are oriented edge-on with the Lys side chain to the right and the carboxy-terminal amide to the left of each structure.

pattern of DNA modification observed was again similar to that displayed by Ni(II)·Arg-Gly-His and Ni(II)·Lys-Gly-His.

Alteration of Site-Selectivity through Additional Amino Acid Substitutions and Changes in Chirality. Given the selectivity exhibited by the tripeptides explored above, the question arises as to whether alternative amino acid substitutions could lead to differing DNA cleavage selectivities. A demonstration of the ability to control sequence selectivity through a change in amino acid side chains could provide the wherewithal to design additional complexes with increasing levels of specificity or an entirely different spectrum of activity. Consequently, two different forms of modification were chosen for this initial determination: (1) inversion of selected stereocenters by point substitution of D-amino acid residues and (2) substitution of glycine within the parent Ni(II)·Gly-Gly-His tripeptide for a neutral, amide-containing amino acid (Asn) also exploited in protein–nucleic acid interactions.

Initially, a simple modification within the A/T-selective complex Ni(II)·Lys-Gly-His was made involving substitution of L-His for D-His. This modification, as shown in Figure 3, permits the overall complex to adopt a more planar structure due to a repositioning of the carboxy-terminal amide, perhaps allowing a tighter binding to A/T-rich regions of DNA or a better overall fit with the minor groove of the DNA helix. This modification was also chosen in light of previous observations[4c,d] which indicated that a substitution of D-His within Gly-Gly-His, when employed as an appendage to a much larger DNA binding oligopeptide, resulted in a shift in strand selectivity.

As shown in Figure 4, substitution of a D-His residue within Ni(II)·Lys-Gly-His resulted in a subtle *decrease* in the overall selectivity of this complex; cleavage occurs now at mixed sequence sites along with the A/T-rich regions of the original Ni(II)·Lys-Gly-His complex. Also apparent from the data shown in Figure 4 are differences in the extent of cleavage at the homopolymeric DNA regions; while Ni(II)·Lys-Gly-His induced cleavage is inhibited, as noted previously, Ni(II)·Lys-Gly-D-His cleavage in this region is slightly increased, albeit still reduced in comparison to the overall DNA substrate. It is also worthy of note that in the case of *both* metallopeptides, cleavage does not occur at a 5′-CCT site that, in the fragment studied, forms a junction between two A/T-rich regions.

Overall, these results suggest that the chirality and positioning of the carboxy-terminal amide appears to contribute to the selectivity of these complexes either through the formation of a directed, weak molecular interaction or, more likely, steric complementarity leading to a discrimination between available binding sites. Thus, with Ni(II)·Lys-Gly-D-His, the complex may fit more readily into the minor groove of any available

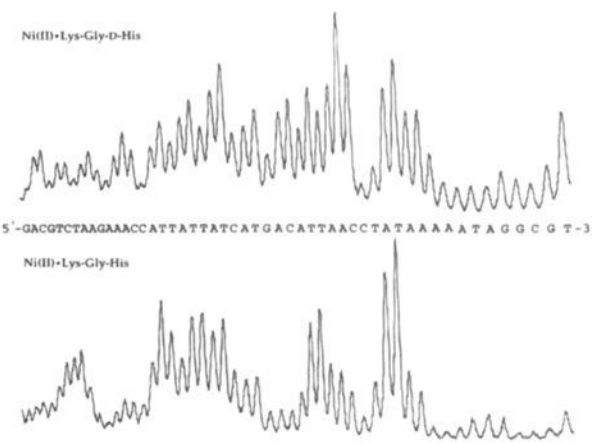


Figure 4. Comparison of densitometric analyses of autoradiograms generated through cleavage of the Eco RI → Rsa I 514 bp DNA restriction fragments of pBR322 by Ni(II)·Lys-Gly-His and Ni(II)·Lys-Gly-D-His.

DNA site indicating that, while an electrostatic interaction assists in determining selectivity, subtle structural factors within these *relatively simple metallopeptides can lead to binding site* discrimination.

Alterations in Site-Selectivity through Asparagine Substitutions. Having determined that a change in chirality of a single amino acid and an accompanying amide functionality can influence the interaction of the positively-charged metallopeptides with DNA, neutral complexes containing asparagine (Asn) substitutions were studied. These peptides were chosen to determine if amino acids containing amide side chains positioned at key locations within the Gly-Gly-His framework could drive an alternative selective interaction with the DNA helix without an electrostatic contribution to the binding.

Initially, several Asn-containing peptides were prepared and examined including Ni(II)·Asn-Gly-His, Ni(II)·Gly-Asn-His, Ni(II)·Gly-D-Asn-His, and Ni(II)·Gly-Asn-D-His. These choices present a range of molecular shapes and the possibility of forming hydrogen bonds at multiple points between metallopeptide amide functionalities and the DNA backbone. Like the positively-charged metallopeptides, each complex was tested to determine its relative DNA cleavage propensity using supercoiled plasmid as described earlier. Results obtained from these studies indicated that all the neutral metallopeptides listed above induced strand scission of DNA within a similar concentration range as Ni(II)·Gly-Gly-His (with Ni(II)·Gly-Gly-His being consistently more active in DNA strand scission);

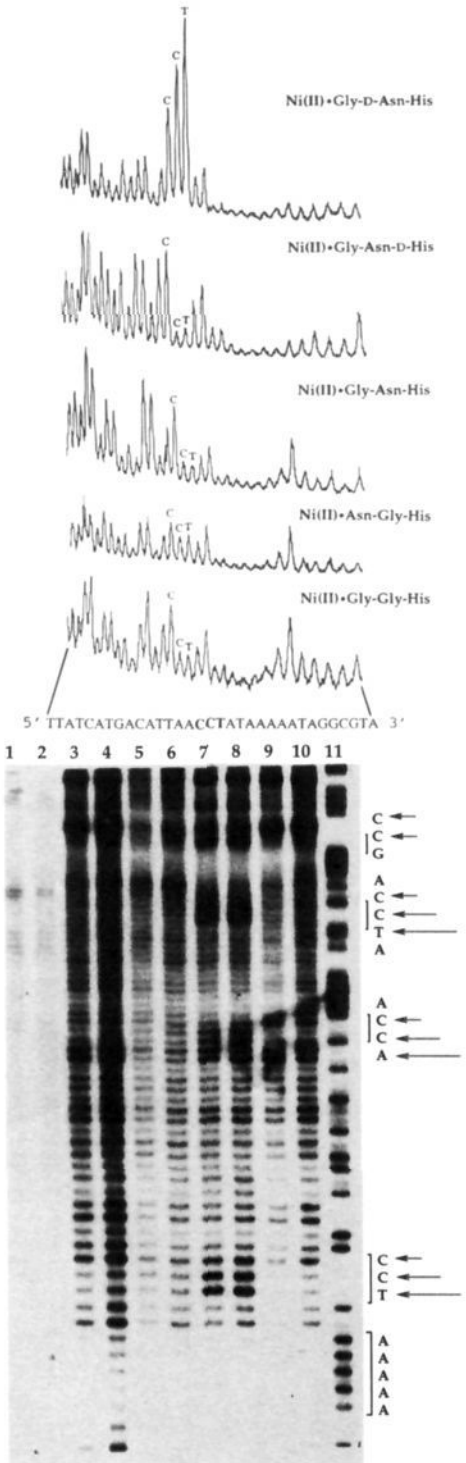
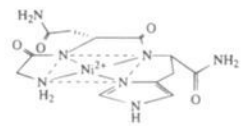


Figure 5. (top) Densitometric analysis of autoradiograms generated through cleavage of the Eco RI → Rsa I 514 bp DNA restriction fragments by Ni(II)•Gly-Gly-His (50 μM), Ni(II)•Asn-Gly-His (100 μM), Ni(II)•Gly-Asn-His (100 μM), Ni(II)•Gly-Asn-D-His (100 μM), and Ni-(II)•Gly-D-Asn-His (100 μM). (bottom) Autoradiogram of Ni(II)•peptide induced cleavage of a 3′-^{32}P-end labeled 514 base pair EcoRI → RsaI restriction fragment from pBR322. Lane 1, intact DNA; Lane 2, reaction control [100 μM $Ni(OAc)_2$, 100 μM oxone]; Lane 3, 50 μM Ni(II)•Gly-Gly-His; Lane 4, 100 μM Ni(II)•Gly-Gly-His; Lane 5, 50 μM Ni(II)•Asn-Gly-His; Lane 6, 100 μM Ni(II)•Asn-Gly-His; Lane 7, 50 μM Ni(II)•Gly-D-Asn-His; Lane 8, 100 μM Ni(II)•Gly-D-Asn-His; Lane 9, 50 μM Ni(II)•Gly-Asn-His; Lane 10, 100 μM Ni(II)•Gly-Asn-His; Lane 11, Maxam-Gilbert G + A reaction. Arrows at the right edge of the autoradiogram indicate cleavage sites unique to Ni(II)•Gly-D-Asn-His; note also the lack of cleavage by all complexes at the poly A sequence (bottom of autoradiogram).

total conversion of form I DNA to form II was obtained at ~100 μM metallopeptide concentrations.[20]

Subsequently, high resolution gel analyses of cleavage induced by the neutral complexes was conducted using the same DNA restriction fragments employed as substrates for the previous metallopeptides (Figure 5). As shown, consistent with results obtained with Ni(II)•Gly-Gly-His and all other metallopeptides studied in this series, the cleavage patterns revealed by the Asn-containing peptides maintained their lack of selectivity for homopolymeric sites. Additionally, two of the four peptides (Ni(II)•Asn-Gly-His, Ni(II)•Gly-Asn-His) appeared to target DNA in a fashion that was virtually identical to Ni(II)•Gly-Gly-His, suggesting that this particular positioning of the amide side chain of the Asn residue had little influence on their interaction with the DNA. This observation is consistent with our previously described model of binding where the side chain found at these metallopeptide positions is directed out of the minor groove which, in the case of the amide side chains, does not influence their interaction with the target DNA. Consistent also with results obtained with Ni(II)•Lys-Gly-D-His, cleavage induced by Ni(II)•Gly-Asn-D-His appeared somewhat less able to discriminate between available sites supporting further our model of DNA binding and the influence of the chirality of the His position.

In stark contrast, however, to the other peptides of this series, Ni(II)•Gly-D-Asn-His was found to display a selectivity for 5′-CCT-3′ sites (Figure 5). This selectivity was actually observed



Ni(II)•Gly-D-Asn-His

in four independent 5′-CCT-3′ sites within the 514 base pair restriction fragment of pBR322 (nucleotide numbers 4282 → 4284, 4319 → 4321, 4330 → 4332, and 4342 → 4344; in addition, this metallopeptide also displayed a less pronounced selectivity for a 5′-CCA site and a 5′-CCG site: nucleotides 4298 → 4300 and 4269 → 4271, respectively). What makes this metallopeptide different from the others? Careful examination of its structure reveals that both amide functionalities are directed toward the same "face" of the square-planar complex. Using a similar argument to rationalize the results obtained upon changes in chirality of the His residue, it appears that the positioning of the Asn side chain, perhaps in concert with the carboxy-terminal amide, leads to an increased discrimination of the available DNA sites. Whether or not the selectivity observed has more to do with the overall shape and steric interactions of the tripeptide with the DNA minor groove or a precise positioning of the amide side chain for interaction with a portion of the DNA helix cannot be determined from the present data.[21] However, these findings do support the notion that selective metal complexes can be derived from simple structural modifications of these tripeptides and, in the process, features of DNA molecular recognition worthy of further exploration may be elucidated.

Conclusions. The foregoing results demonstrate the feasibility of designing low molecular weight *peptide-based* reagents for the *selective* modification of DNA. While the peptides employed in the present investigation certainly only touch the

(20) The K_b of these metallopeptides to DNA was found to be ~5-fold lower than the positively-charged metallopeptides discussed earlier.

(21) For examples of other synthetic metal complexes that interact selectively with DNA based on these principles see: Sitlani, A.; Barton, J. K. *Biochemistry* **1994**, *33*, 12100. Krotz, A. H.; Kuo, L. Y.; Shields, T. P.; Barton, J. K. *J. Am. Chem. Soc.* **1993**, *115*, 3877.

surface of the possible combinations of amino acids and chiralities that can be employed in a tripeptide ligand, the results presented do serve to establish several guiding design principles for future generations of metallopeptides of this nature: (1) the chemical characteristics of the amino acid side chain can influence site selectivity; (2) the chirality of a single amino acid residue can also influence selectivity; (3) the chemistry involves a minor groove interaction with the DNA helix; and (4) an active species is generated that is capable of deoxyribose modification in a non-base-specific fashion.

Overall, metallopeptides such as those described above can be envisioned as having several potential future applications. While subsequent studies are likely to reveal that a judicious incorporation of D- vs L-amino acids into these metallopeptides lead to alternative DNA target sites *in vitro*, thus testing principles of nucleic acid recognition, such structures could also result in protease resistant molecules suitable for *in vivo* investigations. Additionally, given the non-diffusible nature of the oxidant formed, similar peptides of rational design may also provide catalysts capable of mediating the *atom selective* oxidation of DNA. Of further importance, since the relative ease of synthesis of these metallopeptides provides accessibility to most researchers, an examination of similar agents may assist in the rational design or redesign of antitumor agents with controlled selectivities and insight into the mechanism of action of naturally occurring peptide-based chemotherapeutics.[22] Ongoing studies in our laboratory seek to evaluate further the mechanism of action of these metallopeptides and to establish structure–function relationships to determine if any generalizable principles govern their interaction with DNA.

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(22) Long, E. C.; Eason, P. D.; Liang, Q. *Met. Ions Biol. Syst.* **1995**, *33*, in press.