Ni(II)·Xaa-Xaa-His Induced DNA Cleavage: Deoxyribose Modification by a Common "Activated" Intermediate Derived from $KHSO_5$, MMPP, or H_2O_2

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Abstract: Ni(II) chelated peptides of the form NH_2-Xaa-Xaa-His-$CONH_2$ (Ni(II)·Xaa-Xaa-His) mediate *deoxyribose* damage through C4′−H abstraction of a targeted nucleotide when activated with $KHSO_5$ (oxone), MMPP (magnesium monoperoxyphthalate), or H_2O_2. The products released and identified in comparison to the authentic C4′−H oxidant Fe(II)·bleomycin included fragmented DNA terminating in 5′-phosphates, 3′-phosphates, and 3′-phosphoglycolates; upon treatment of Ni(II)·Xaa-Xaa-His cleavage reactions with NaOH or NH_2NH_2, fragmented DNA 3′-termini were released consistent with the intermediate formation of keto-aldehyde abasic (alkaline-labile) sites. In addition, nucleobases and nucleobase propenals were detected in proportions consistent with abasic site and 3′-phosphoglycolate termini formation, respectively. These results indicate that Ni(II)·Xaa-Xaa-His metallopeptides, like Fe(II)·bleomycin, degrade DNA through two pathways resulting from an initial C4′−H modification. Importantly, the partitioning between these two pathways appears to be dependent on the structure of the Ni(II)·Xaa-Xaa-His metallopeptide employed in the cleavage reaction and the nucleotide sequence targeted. Further studies also indicate that metallopeptide activation with $KHSO_5$, MMPP, or H_2O_2 yields identical reaction products and sequence-selective DNA cleavage suggesting the formation of a common "activated" metallopeptide responsible for C4′−H deoxyribose damage, quite possibly a metal-bound hydroxyl radical. These studies also demonstrate that metallopeptide activation with $KHSO_5$ is *condition-dependent* resulting in (1) C4′−H damage in common with MMPP or H_2O_2 under relatively "low" ionic strength conditions (10 mM Na-cacodylate, pH 7.5, equimolar $KHSO_5$/metallopeptide) *or* (2) guanine nucleobase oxidation under higher ionic strength conditions (100 mM NaCl, 10 mM phosphate, pH 7.0, excess $KHSO_5$).

Introduction

Metallopeptides of the general form Ni(II)·Xaa-Xaa-His (where Xaa-Xaa-His is NH_2-Xaa-Xaa-His-$CONH_2$ and Xaa is any amino acid) can be activated to selectively degrade DNA via a minor groove binding interaction.[1] The site-selectivity exhibited by Ni(II)·Xaa-Xaa-His appears to derive from the identity and chirality of the amino acids included in the tripeptide, e.g., Ni(II)·Lys/Arg-Gly-His was found to modify A/T-rich regions while Ni(II)·Gly-D-Asn-His was found to modify 5′-CCT sites.[1] Given their use of amino acid side chains, Ni(II)·Xaa-Xaa-His metallopeptides stand unique among metal-based nucleic acid cleavage reagents[2] in their ability to incorporate and position within a metal complex framework the same chemical functionalities (e.g., guanidinium, amide, or amine moieties) used by proteins and antitumor natural products for the molecular recognition of DNA and RNA.[3] These features, coupled to their relative ease of synthesis and the ability to control the chirality of select stereocenters, make Ni(II)·Xaa-Xaa-His metallopeptides attractive models to further our knowledge of small molecule−nucleic acid recognition principles.[4] In addition to the tripeptides alone, similar metallopeptides employed as appendages to much larger DNA binding motifs have also contributed to our understanding of macromolecule−DNA interactions through affinity cleavage; in these experiments, purine and pyrimidine nucleotides adjacent to sites of affinity binding are cleaved by the oxidizing potential of the chemically activated metallopeptide moiety.[5]

The design of Ni(II)·Xaa-Xaa-His metallopeptides is based on the characterized metal chelation exhibited by Gly-Gly-His.[6] This tripeptide, which mimics the amino-terminal, square planar Cu(II)-chelating domain of serum albumins, binds Cu(II) or Ni-

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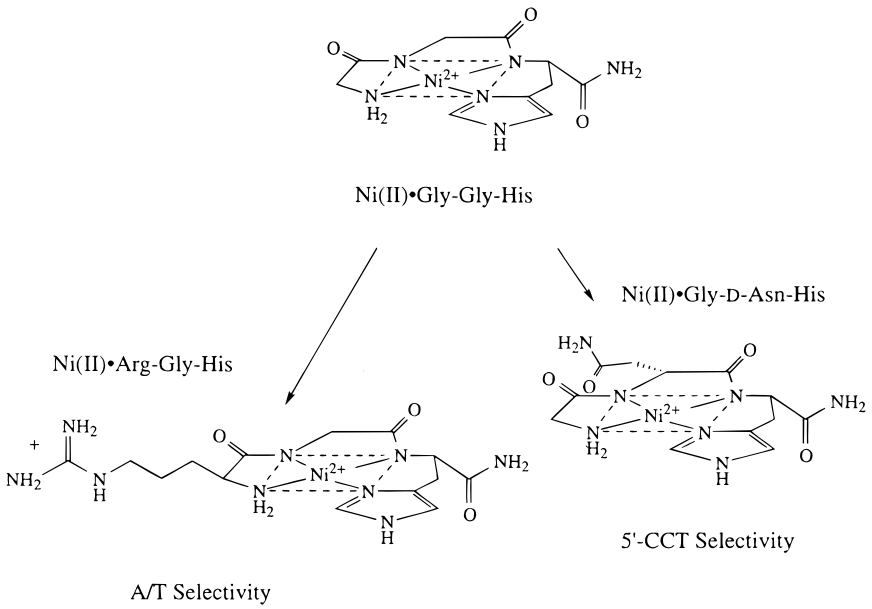

(II) in a 1:1 complex at physiological pH through the histidine imidazole nitrogen, two deprotonated amide nitrogens, and the terminal α-amine with a $10^{-16}-10^{-17}$ M dissociation constant. Importantly, the Xaa-Xaa-His ligand system can be chemically activated in the presence of Cu(II) or Ni(II) to produce oxidizing equivalents capable of effecting DNA cleavage[1,4,5,7] and protein[8] modification. In contrast to many synthetic, metal-based DNA oxidizing reagents,[2,4] activated Ni(II)·Xaa-Xaa-His peptides appear to operate via the formation of a nondiffusible, complex-centered oxidant; the active metallopeptide so-formed is bound in the DNA minor groove resulting in deoxyribose sugar ring modification of targeted purine *and* pyrimidine nucleotides.[1,4,5]

In addition to reports of deoxyribose modification,[1,4,5] a recent study[9] has shown that Ni(II)·Lys-Gly-His, when activated with excess oxone ($KHSO_5$) or through sulfite autoxidation, results in the oxidation of guanine nucleobases *without* deoxyribose modification. This nucleobase-centered oxidation was found to yield piperidine + heat-labile DNA lesions and implicated a "caged" (i.e., metallopeptide-bound) sulfate radical as the active DNA oxidant. In light of the possibility of both deoxyribose-*and* nucleobase-centered mechanisms of DNA degradation, we have sought to more clearly define the chemistry of Ni(II)·Xaa-Xaa-His-mediated *deoxyribose* modification and the active species responsible for this damage. Included herein are studies of the mechanism of DNA cleavage by Ni(II)·Xaa-Xaa-His peptides (e.g., Ni(II)·Lys-Gly-His and Ni(II)·Gly-Gly-His) which demonstrate (1) deoxyribose damage involving C4′−H abstraction that is comparable to that exhibited by the glycopeptide antibiotic Fe(II)·bleomycin[10] and (2) identical patterns of DNA damage regardless of the peracid/peroxide-based activating agent employed [$KHSO_5$ (oxone), magnesium monoperoxyphthalate (MMPP), or H_2O_2]. These results argue for the formation of a common "activated" metallopeptide which is responsible for *deoxyribose* damage under conditions used to examine the site-selectivity of these metallopeptides.[1] In addition, conditions are delineated under which deoxyribose modification by Ni(II)·Xaa-Xaa-His can be suppressed leading to the nearly exclusive oxidation of guanine nucleobases (piperidine/heat-labile sites).

Experimental Section

Peptide Synthesis. All protected amino acids were purchased from Bachem California. Peptide syntheses were carried out by conventional solid-phase methodologies[11] followed by reverse phase HPLC purification and verification by FAB-MS. Each purified, synthetic carboxamide tripeptide, upon complexation to either Cu(II) or Ni(II), yielded visible absorbance spectra (Cu: $\lambda_{max} = 525-527$ nm, $\epsilon = \sim 108$ M^{-1} cm^{-1}; Ni: λ_{max} 420−422 nm, $\epsilon = \sim 120$ M^{-1} cm^{-1}, pH 7.5, 25 mM Na-cacodylate) and 1:1 titrations of peptide:metal ion characteristic of square planar Gly-Gly-His metallopeptides.[6] All molecular models of the metallopeptides employed throughout this study were based on the reported crystal structure of Cu(II)·Gly-Gly-His.[6a]

Cleavage of DNA Restriction Fragments for Termini Analyses. All enzymes utilized were from commercial sources. [α-^{32}P] dATP and [γ-^{32}P] ATP were obtained from NEN-Dupont. Restriction fragments bearing 3′-^{32}P end-labeled termini 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 with a final digestion with *Aat* II.[12] Alternatively, 5′-^{32}P end-labeling was achieved by digesting pBR322 with *Aat* II followed by a sequential treatment of the restricted DNA with bacterial alkaline phosphatase, [γ-^{32}P] ATP and T4 polynucleotide kinase,[12] and a final digestion with *Eco* RI. The resulting 5′-^{32}P and 3′-^{32}P end-labeled 71 base pair fragment (which includes pBR322 nucleotides 4291 → 4361) was purified by 6% preparative nondenaturing 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 of ^{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)·Lys-Gly-His and oxone and quenched after 1 min with the addition of 3

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μL of a 0.2 M EDTA solution. Alternatively, Fe(II)·bleomycin-mediated cleavage reactions for comparative purposes were initiated through the admixture of equal amounts of bleomycin (Sigma) and freshly dissolved $Fe(NH_4)_2(SO_4)_2$, incubated at 0 °C for 30 min with labeled restriction fragment, and quenched with EDTA.

After treatment as described above, 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 either analyzed directly or chemically and enzymatically treated to determine the nature of the termini at the sites of cleavage (see below). Reactions with and without post-cleavage modifications were 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) and, along with Maxam−Gilbert G + A sequencing reactions,[12] heat denatured at 90 °C for 5 min and quick-chilled on ice. All samples were loaded onto 20% (19:1) polyacrylamide/7.5 M urea sequencing gels and electrophoresed at 1500 V for approximately 12 h, transferred to a cassette, and stored at −70 °C with Kodak X-omat film.

Chemical Treatment of Ni(II)·Peptide-Modified DNA for Termini Analyses. Dried reaction mixtures (produced as described above) were treated with either 10 μL of 0.1 N NaOH (60 °C for 5 min), 40 μL of 0.1 N *n*-butylamine (90 °C, 30 min), or 10 μL of 0.1 N NH_2NH_2 (25 °C for 1 h). Upon completion of each treatment, all reactions were EtOH precipitated in 0.3 M sodium acetate (reaction mixtures that were subsequently phosphatase-treated were neutralized with 0.1 N acetic acid before precipitation). Reaction mixtures treated with $NaBH_4$ were dissolved in 10 μL of 0.5 M $NaBH_4$, incubated at 0 °C for 1 h, and then quenched with 10 μL of 0.5 M acetic acid followed by EtOH precipitation. Reactions were analyzed by gel electrophoresis as described in the previous section.

Enzymatic Treatment of Ni(II)·Peptide-Modified DNA for Termini Analyses. The presence of 5′- or 3′-phosphate termini were detected through their selective enzymatic removal. The presence of a 3′-phosphorylated termini was tested using T_4 polynucleotide kinase. Cleavage reactions (produced as described above) were dissolved in 25 μL of H_2O, heat-denatured at 90 °C for 5 min, and quick-chilled on ice. Subsequently, 25 μL of buffer (20 mM Tris-HCl, pH 7.5, 20 mM $MgCl_2$, and 10 mM β-mercaptoethanol) was added to each denatured DNA sample followed by 2 μL of T_4 kinase (20 units). Reactions were incubated at 37 °C for 1 h followed by EtOH precipitation and gel analysis as described above. The presence of a 5′-phosphorylated termini was tested using bacterial alkaline phosphatase (BAP). Cleavage reactions were dissolved in 40 μL of 10 mM Tris-HCl, pH 8.0, 120 mM NaCl, and 20 units of BAP. Upon incubation at 65 °C for 1 h, reactions were EtOH precipitated and analyzed by gel electrophoresis.

Comparison of DNA Restriction Fragment Cleavage by Ni(II)·Xaa-Xaa-His Activated with $KHSO_5$, MMPP, or H_2O_2. Restriction fragment preparation and metallopeptide cleavage reactions were carried out essentially as described above. The 5′-^{32}P end-labeled restriction fragment employed in this analysis was prepared by digesting supercoiled p*BR*322 plasmid with *Eco* RI restriction endonuclease followed by a sequential treatment of the restricted DNA with bacterial alkaline phosphatase, [γ-^{32}P] ATP, and T_4 polynucleotide kinase followed by a second restriction with *Aat* II. Cleavage of the end-labeled DNA substrate by Ni(II)·Xaa-Xaa-His metallopeptides was carried out as described previously except that the reaction times and the concentration of activating agents varied as follows: 50 μM DNA was cleaved by 40 μM Ni(II)·Xaa-Xaa-His peptide in the presence of either 40 μM $KHSO_5$ for 1 min, 80 μM MMPP for 9 min, or 40 mM H_2O_2 for 20 min. Hydrazine-treated reactions were prepared as described above. Densitometric analysis of these data was performed on a Bio-Rad GS-670 Imaging Densitometer interfaced to an Apple computer with operative software for data recording and analysis.

Analysis of Monomeric Product Release by HPLC and Thiobarbituric Acid. Analysis and quantitation of free nucleobase release in Ni(II)·Xaa-Xaa-His + DNA reaction mixtures was achieved by preparing 10 μL reactions containing 500 μM calf thymus DNA (base pair concentration), 400 μM Ni(II)·Xaa-Xaa-His, and 400 μM oxone in 10 mM sodium cacodylate (pH 7.5). Each reaction mixture was incubated for 1 min followed by reversed phase HPLC analysis using a Rainin Microsorb C-18 column. Products were eluted isocratically with 0.1 M ammonium acetate, pH 6.8, using a flow rate of 1 mL/min. Product elution was monitored at 260 nm and quantitated through comparison to authentic standards of each nucleobase.

The analysis of thiobarbituric acid-reactive product release[13] was performed on reaction mixtures (300 μL total volume) containing 500 μM calf thymus DNA (base pair concentration) and 400 μM Ni(II)·Xaa-Xaa-His in 10 mM sodium cacodylate (pH 7.5). Reactions were initiated upon the addition of oxone (400 μM final concentration), allowed to react for 1 min, and quenched by heating at 90 °C for 5 min. Each reaction was then treated with 150 μL of a 1% 2-thiobarbituric acid (TBA) solution in 50 mM NaOH and 150 μL of 25% HCl (v/v) for 15 min at 100 °C. After completion, the absorbance of each reaction at 532 nm was measured. Product release was quantitated using an extinction coefficient for TBA-malondialdehyde of 1.6×10^5 M^{-1} cm^{-1}.

Determination of the Effect of Radical Scavengers on DNA Cleavage by Ni(II)·Lys-Gly-His Activated with $KHSO_5$, MMPP, or H_2O_2. In a total reaction volume of 20 μL, 15 μM preformed Ni(II)·Lys-Gly-His was incubated with 25 μM base pair of ϕX174 RF plasmid DNA in the presence or absence of either 50 or 100 mM ethanol, *tert*-butyl alcohol, DMSO, or mannitol. Reactions were initiated upon the addition of 15 μM $KHSO_5$ for 1 min, 30 μM MMPP for 9 min, or 15 mM H_2O_2 for 20 min. Reaction mixtures were quenched by the addition of 2 μL of 0.2 M EDTA and 4 μL of a gel loading buffer containing 0.25% xylene cyanol, 0.25% bromophenol blue, and 30% glycerol. Parallel analyses of the cleavage of plasmid DNA by Fe(II)·EDTA were performed similarly except that the supercoiled DNA substrate was cleaved upon the addition of 40 mM H_2O_2 and 40 mM sodium ascorbate to premixed Fe(II)·EDTA (4 μM $FeSO_4$ with 8 μM EDTA) in the presence or absence of the radical scavengers listed above. Fe(II)·EDTA reactions were quenched after 2 min by the addition of 2 μL of 0.4 M thiourea and 4 μL of the loading buffer described above.

All reactions listed above were analyzed on 0.9% agarose gels (containing 0.5 μg/mL ethidium bromide) which were electrophoresed at 70 V for 2 h followed by visualization on a UV transilluminator. Quantification of DNA cleavage was performed by dividing the total quantitated amounts of form II + form III DNA produced in each reaction by the total amount of DNA present (final value obtained multiplied by 100); a correction factor of 1.47 was multiplied to the values obtained for form I DNA to adjust for differential staining.[14]

Condition-Dependent Cleavage of DNA Restriction Fragments by Ni(II)·Lys-Gly-His + $KHSO_5$. Restriction fragments bearing 3′-^{32}P end-labeled termini were prepared by digesting supercoiled p*BR*322 plasmid with *Eco* RI restriction endonuclease followed by 3′ end-labeling with terminal deoxynucleotidyl transferase and [α-^{32}P] dATP with a final digestion with *Rsa* I. Alternatively, 5′-^{32}P end-labeling of the complementary strand was achieved by digesting p*BR*322 with *Eco* RI followed by a sequential treatment of the restricted DNA with bacterial alkaline phosphatase, [γ-^{32}P] ATP, and T4 polynucleotide kinase, with a final digestion with *Rsa* I. Each end-labeling produced a 167 base pair and a 514 base pair fragment which were separated by 6% preparative nondenaturing gel electrophoresis and isolated by electroelution.

Cleavage reactions were carried out in a 20 μL total volume containing calf thymus DNA (20 μM base pair concentration) and 3×10^4 cpm of ^{32}P end-labeled restriction fragment. The "low" ionic strength reactions[1] were performed in 10 mM sodium cacodylate buffer, pH 7.5, and were initiated through the admixture of equimolar amounts (15 μM) of preformed Ni(II)·Lys-Gly-His and $KHSO_5$. The "higher" ionic strength reactions contained 100 mM NaCl and 10 mM phosphate buffer, pH 7.0, and were initiated by the admixture of preformed Ni(II)·Lys-Gly-His and excess oxone (100 μM).[9] All reactions were quenched by the addition of 3 μL of a 0.2 M EDTA solution. The reaction time for the "low" ionic strength reactions was 1 min,[1] while the "higher" ionic strength reactions were quenched after 30 min.[9] After

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quenching, all reactions were ethanol-precipitated as described above. Reaction mixtures subjected to piperidine treatment were dissolved in 60 μL of freshly diluted 0.2 N piperidine, heated at 90 °C for 30 min, and then lyophilized. All reaction mixtures were then resuspended in 3 μL of an 80% formamide loading buffer (described above), heat denatured at 90 °C for 5 min, and quick-chilled on ice. The samples were then loaded onto 12% (19:1) polyacrylamide/7.5 M urea sequencing gels and electrophoresed at 600 V for approximately 12 h.

For comparison to the above, 3′-^{32}P end-labeled 514 base pair fragments (20 μM) from pB*R*322 DNA were cleaved also by the activation of preformed Ni(II)·Lys-Gly-His (40 μM) with MMPP (80 μM) for 9 min or H_2O_2 (40 mM) for 20 min. "Low" ionic strength reactions[1] were carried out in 10 mM cacodylate buffer, pH 7.5, while the "higher" ionic strength reactions[9] contained 100 mM NaCl and were carried out in 10 mM phosphate buffer, pH 7.0. Piperidine treatment of reaction aliquots was carried out as described above.

Results and Discussion

Analysis of DNA 5′-Termini at the Site of Deoxyribose Modification. The chemical nature of the new 5′-termini produced at sites of DNA strand scission by Ni(II)·Lys-Gly-His was examined by high-resolution gel electrophoresis using 3′-^{32}P end-labeled restriction fragments (under conditions employed in a previous determination of the site-selectivity of Ni(II)·Lys-Gly-His[1]). Results of these analyses (Supporting Information) indicated that Ni(II)·Lys-Gly-His produced DNA fragments with gel mobilities identical with those generated by Fe(II)·bleomycin[10] and Maxam−Gilbert sequencing reactions,[12] reagents known to produce authentic 5′-phosphorylated termini. To confirm that 5′-phosphorylated termini were produced, additional Ni(II)·Lys-Gly-His and Fe(II)·bleomycin reactions were treated with bacterial alkaline phosphatase (BAP) prior to gel analysis. BAP treatment resulted in products with decreased migration rates in comparison to the untreated reaction mixtures; these results indicate the successful enzymatic dephosphorylation of each of the 5′-phosphorylated termini confirming this species as a major product of strand scission by Ni(II)·Lys-Gly-His. In addition to the above, reaction aliquots were also treated with $NaBH_4$ to evaluate the presence of any reducible nucleoside fragment akin to the products of calicheamicin/esperamicin C5′−H induced DNA damage;[15] the fragmented DNA products generated by Ni(II)·Lys-Gly-His and, as expected, Fe(II)·bleomycin were unaffected by $NaBH_4$ suggesting the absence of any reducible nucleoside fragment that remains attached to the new 5′-termini produced upon strand scission.

Analysis of DNA 3′-Termini at the Site of Deoxyribose Modification. The new DNA 3′-termini produced at Ni(II)·Lys-Gly-His cleavage sites were examined by high-resolution gel electrophoresis using 5′-^{32}P end-labeled restriction fragments. In contrast to the release of one form of 5′-termini, as presented in the previous section, Ni(II)·Xaa-Xaa-His peptides were observed to produce several distinctly different 3′-termini depending upon the post-cleavage chemical treatment employed in the reaction workup; these termini, consistent with the chemistry of C4′−H abstraction,[10] occur to different extents based on the nucleotide sequence targeted by the metallopeptide and the identity of the amino acids employed in the tripeptide ligand.

To fully explore the nature of the 3′-termini observed, reactions with Ni(II)·Xaa-Xaa-His metallopeptides were carried out in parallel with Fe(II)·bleomycin under several conditions. As shown in Figure 1, high-resolution gel analyses of DNA cleavage initiated by Ni(II)·Lys-Gly-His, Fe(II)·bleomycin, and an additional Ni(II)·Xaa-Xaa-His metallopeptide, Ni(II)·Gly-Gly-His, were compared directly and upon treatment with NaOH or NH_2NH_2. From this analysis it is clear that each agent produces an identical set of 3′-termini cleavage products, albeit with differing site-selectivities, under the conditions employed.

In the absence of any post-cleavage chemical workup, each reaction produces 3′-phosphorylated termini (Figure 1, lanes 3, 6, and 9) as evidenced by their comigration with the products of Maxam−Gilbert sequencing reactions and their enzymatic dephosphorylation with T_4 polynucleotide kinase (Supporting Information). In addition, upon treatment with NaOH or NH_2NH_2 (which is known[16] to react with the keto-aldehyde abasic sites produced by Fe(II)·bleomycin to create strand breaks terminating in 3′-phosphopyridazine moieties) conspicuous bands appear with gel mobilities *less than* the 3′-phosphorylated species (Figure 1, lanes 4/5, 7/8, and 10/11). The autoradiogram presented in Figure 1 confirms that the NaOH-induced products formed by Ni(II)·Xaa-Xaa-His metallopeptides have identical gel mobilities in comparison to the authentic NaOH-induced alkaline-labile products formed by Fe(II)·bleomycin.[10,16] In the case of NH_2NH_2 treatment, the resulting 3′-phosphopyridazine termini appear as more pronounced bands in comparison to the NaOH-induced products and possess identical gel mobilities in all three of the cleavage agents employed. In addition, with both the NH_2NH_2- and NaOH-induced products, each appears to differ in intensity as a function of the sequence targeted. These data strongly support the notion that Ni(II)·Xaa-Xaa-His metallopeptides mediate C4′−H abstraction chemistry leading to the formation of alkaline-labile lesions in a fashion which appears identical with the documented activity of Fe(II)·bleomycin.[10,16]

In addition to alkaline-labile product formation by Ni(II)·Xaa-Xaa-His metallopeptides, inspection of the autoradiogram shown in Figure 1 also illustrates clearly (bottom portion of the autoradiogram) the presence of termini with mobilities slightly *increased* in comparison to the 3′-phosphorylated termini. While these bands appear to a lesser extent with Ni(II)·Lys-Gly-His (Figure 1, lanes 3−5), they appear consistently when Ni(II)·Gly-Gly-His was employed in the reaction mixtures (Figure 1, lanes 9−11); this product, formed by either metallopeptide, has a gel mobility identical with the authentic 3′-phosphoglycolate bands produced by Fe(II)·bleomycin (Figure 1, lanes 6−8). As an additional confirmation of this band assignment, the Ni(II)·Xaa-Xaa-His metallopeptides were examined in comparison to the products formed by Fe(II)·EDTA + ascorbate (which also produces an authentic 3′-phosphate and 3′-phosphoglycolate product per nucleotide cleavage site[17]); the bands produced by the metallopeptides were found to comigrate with the authentic products generated by this cleaving agent in side-by-side high-resolution electrophoretic analyses (data not shown). Overall, the presence of 3′-phosphoglycolate termini is consistent with a mechanism of DNA cleavage initiated by abstraction of the C4′−H of a target nucleotide.[10]

As mentioned, Ni(II)·Gly-Gly-His is observed to produce a larger amount of the putative 3′-phosphoglycolate termini in comparison to Ni(II)·Lys-Gly-His. Given the nature of the formation of this product, which requires dissolved O_2 to recombine with the initially modified C4′-position of a targeted nucleotide to produce a C4′-hydroperoxide,[10] the differences between these two product ratios for each metallopeptide can

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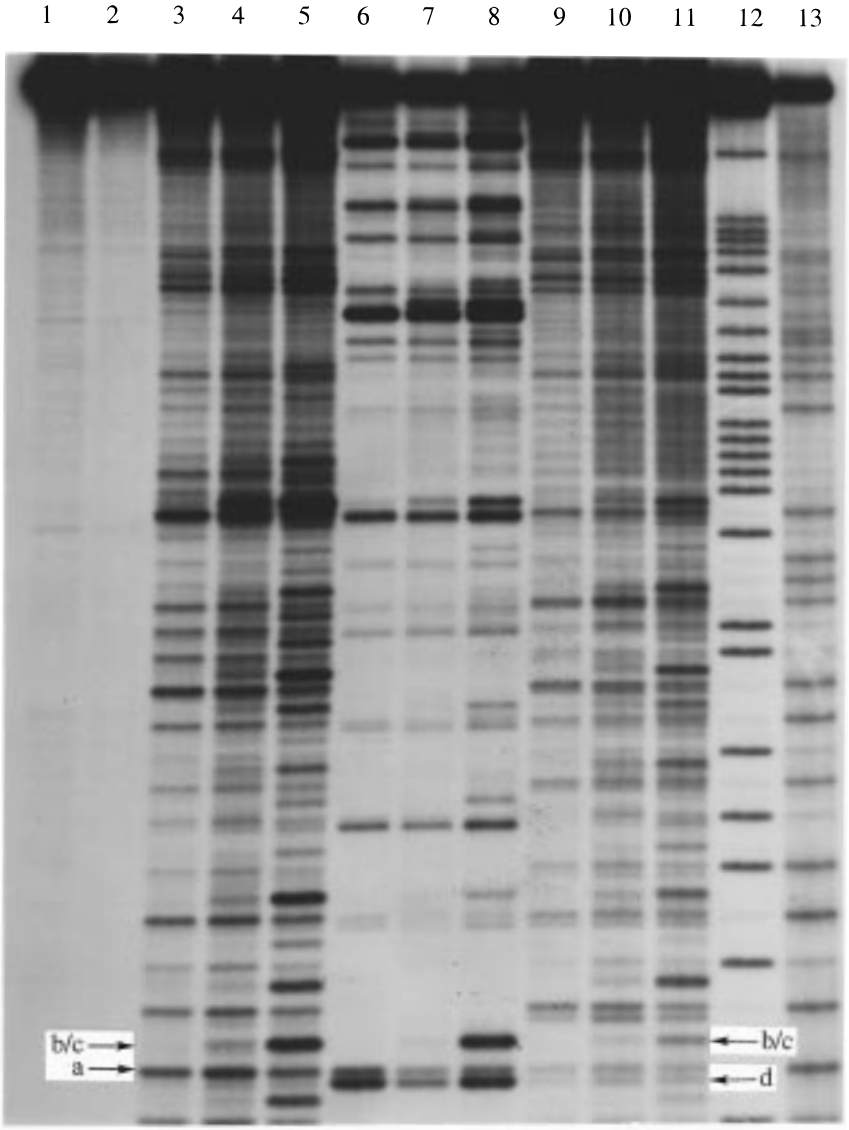

Figure 1. Autoradiogram of a high-resolution denaturing polyacrylamide gel analyzing the new 3′-termini produced at the sites of restriction fragment cleavage by Ni(II)·Lys-Gly-His or Ni(II)·Gly-Gly-His + oxone in comparison to Fe(II)·bleomycin. Lane 1, intact DNA; Lane 2, reaction control [150 μM $Ni(OAc)_2$, 150 μM oxone]; Lane 3, 40 μM Ni(II)·Lys-Gly-His + 50 μM oxone; Lanes 4 and 5, Ni(II)·Lys-Gly-His reactions followed by NaOH and NH_2NH_2 treatment, respectively; Lane 6, 5 μM Fe(II)·bleomycin; Lanes 7 and 8, Fe(II)·bleomycin reactions followed by NaOH and NH_2NH_2 treatment, respectively; Lane 9, 150 μM Ni(II)·Gly-Gly-His + 150 μM oxone; Lanes 10 and 11, Ni(II)·Gly-Gly-His reaction followed by NaOH and NH_2NH_2 treatment, respectively; Lanes 12 and 13, Maxam−Gilbert G + A and C + T reactions, respectively. Arrows indicate examples of (a) 3′-phosphorylated termini (lanes 3−11 and 13); (b/c) closely migrating NaOH-induced 3′-termini and 3′-phosphopyridazine termini (lanes 4/5, 7/8, and 10/11, respectively); and (d) 3′-phosphoglycolate termini (lanes 3−11).

be rationalized; in the case of positively charged Ni(II)·Lys-Gly-His, which binds more tightly to DNA in comparison to Ni(II)·Gly-Gly-His,[1] the complex formed may limit diffusion of O_2 to the modified C4′ "active site". In comparison, with the charge-neutral complex Ni(II)·Gly-Gly-His, diffusion of dissolved O_2 to the initially formed C4′-lesion may occur unimpeded resulting in a higher ratio of C4′-OOH to C4′-OH intermediate and ultimately in an increased production of the 3′-phosphoglycolate, direct strand scission product.[18] These data imply that, like Fe(II)·bleomycin, Ni(II)·Xaa-Xaa-His metallopeptides can also mediate DNA strand scission via two distinct pathways, one leading to keto-aldehyde abasic site formation which can react with NH_2NH_2 or NaOH and the other leading to production of 3′-phosphoglycolate termini.

While it is clear that the product ratios formed by Ni(II)·Lys-Gly-His vs Ni(II)·Gly-Gly-His are different, it is also worthy to note that the ratio of 3′-phosphate (which occurs as a breakdown product of the alkaline-labile lesion[10,16]) to 3′-phosphoglycolate appears, for Ni(II)·Gly-Gly-His, to differ as a function of the nucleotide sequence cleaved. This observation suggests that, as is also the case with Fe(II)·bleomycin[19] and synthetic diimine complexes of Rh(III),[20] the microheterogeneity of the DNA duplex influences the pathway to final product formation by producing DNA−metallopeptide complexes that

(18) In support of this hypothesis, agarose gel analyses of supercoiled plasmid cleavage reactions carried out under controlled atmospheric conditions (helium purge in an inert atmosphere bag) indicate that DNA cleavage by Ni(II)·Lys-Gly-His + oxone is insensitive to dioxygen, while Ni(II)·Gly-Gly-His + oxone cleavage is diminished slightly under conditions that significantly limit DNA cleavage by Fe(II)·bleomycin (in side-by-side reactions).

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Table 1. Quantitation of Nucleobase Release and Thiobarbituric Acid-Reactive Material in Ni(II)·Xaa-Xaa-His + Oxone Supported Calf Thymus DNA Cleavage Reactions

metallopeptide	nucleobase release (μM) T	G[a]	C	A	total	thiobarbituric acid reactive material (μM)
Ni(II)·Lys-Gly-His	7.8	1.2	3.8	1.9	14.7	1.6
Ni(II)·Gly-Gly-His	2.4	1.1	8.0	3.0	14.5	5.0

[a] Guanine was found to be partially degraded by Ni(II)·Xaa-Xaa-His + oxone; the value shown should be considered a conservative estimate of the amount actually released in the reaction.

alter the accessibility of the C4′-lesion to dissolved dioxygen. The above observations also suggest a reason for the lack of observed 3′-phosphoglycolate in previous studies[5c] of Ni(II)·Gly-Gly-His-modified Hin recombinase; the modified protein no doubt facilitates a much tighter binding of the Ni(II)·Gly-Gly-His moiety to DNA in comparison to the metallotripeptide alone and, given its highly selective binding, Ni(II)·Gly-Gly-His-modified Hin recombinase was unable to sample alternative sites which may have promoted the formation of 3′-phosphoglycolate terminating products.

Analysis of Monomeric Product Release. The DNA termini identified in Ni(II)·Xaa-Xaa-His-promoted DNA cleavage reactions, consistent with an initial C4′−H DNA modification, predict the release of several monomeric products resulting from the remaining portions of the fragmented target nucleotide.[10] These products include the release of (1) free nucleobases generated upon formation of the keto-aldehyde abasic (alkaline-labile) site and (2) nucleobase propenals (thiobarbituric acid reactive products)[21] which are generated upon release of the new 3′-phosphoglycolate (and 5′-phosphate) termini.

As shown in Table 1, both free nucleobase and nucleobase propenal products are released in cleavage reactions generated by treatment of calf thymus DNA with Ni(II)·Xaa-Xaa-His + oxone (or Fe(II)·bleomycin by direct comparison); in the case of free nucleobase release, direct HPLC quantitation could be carried out accurately, while nucleobase propenal release (observed also by HPLC) was most efficiently quantitated through treatment with thiobarbituric acid.[21] In parallel with the results of the 5′- and 3′-termini analyses, monomeric product release by the individual metallopeptides was found to reflect the formation of alkaline-labile and direct cleavage (3′-phosphoglycolate termini) sites; with Ni(II)·Lys-Gly-His, free nucleobases were found to be released to an extent greater than the generation of thiobarbituric acid reactive materials (base propenals), while Ni(II)·Gly-Gly-His released a greater proportion of thiobarbituric acid sensitive materials reflecting the increased amount of 3′-phosphoglycolate termini observed on high-resolution gels. The individual nucleobases released by each metallopeptide also reflect the site-selectivities reported previously;[1] with the A/T -selective complex Ni(II)·Lys-Gly-His, thymine was detected to an extent greater than cytosine and guanine, while the nucleobases released by Ni(II)·Gly-Gly-His reflect the greater pyrimidine selectivity of this complex.[1]

The results presented above indicate that under the conditions employed, oxone-activated Ni(II)·Xaa-Xaa-His metallopeptides, like Fe(II)·bleomycin, bind to the DNA minor groove resulting in C4′−H abstraction. Upon C4′−H modification, DNA cleavage by Ni(II)·Xaa-Xaa-His metallopeptides appears to occur via two pathways (Scheme 1). The first (I), results in the formation of keto-aldehyde abasic sites with concomitant loss of free nucleobase, and the other (II) results in direct strand scission with the formation of 3′-phosphoglycolate termini, 5′-phosphate termini, and nucleobase propenals; these two pathways occur most likely through the intermediacy of C4′-hydroxylated- and C4′-hydroperoxide-modified nucleotides, respectively.[10] In the case of abasic site formation, new 3′-phosphorylated termini are released consistent with the established chemistry of the alkaline-labile product of Fe(II)·bleomycin,[10,16] or the remaining, intact keto-aldehyde sites can be trapped with NaOH or NH_2NH_2 to produce modified termini with gel mobilities identical with authentic products generated in parallel with Fe(II)·bleomycin.

Activation of Ni(II)·Xaa-Xaa-His Metallopeptides with $KHSO_5$, MMPP, and H_2O_2: A Comparison of DNA Cleavage Selectivity, Product Formation, and the Effect of Radical Scavengers. The generality of the C4′-centered mechanism of deoxyribose modification determined for Ni(II)·Xaa-Xaa-His + $KHSO_5$ was probed through the use of two additional activating reagents, MMPP and H_2O_2. Previously, these reagents were employed in the activation of Ni(II)·Xaa-Xaa-His metallopeptides toward DNA[5] or protein[8] modification, albeit with differing levels of efficiency. In addition to understanding the requirements for C4′ deoxyribose modification, a direct comparison of DNA cleavage supported by these reagents was carried out to assist in determining the prerequisites for the formation of the "active" metallopeptide responsible for deoxyribose modification.

As shown in Figure 2 (and Supporting Information), treatment of a 5′-^{32}P end-labeled restriction fragment with Ni(II)·Lys-Gly-His activated with $KHSO_5$, MMPP, or H_2O_2 resulted in identical patterns of DNA cleavage selectivity. While the site-selectivity produced by Ni(II)·Xaa-Xaa-His was the same with all three activating reagents, the amounts of supporting oxidant, and the time of the reaction required to achieve comparable levels of DNA modification varied in the following order: $KHSO_5$ > MMPP ≫ H_2O_2 (see Figure 2 for concentrations and times). In addition to identical patterns of nucleotide cleavage, identical ratios of fragmented DNA 3′-termini at a given targeted nucleotide were also observed (i.e., 3′-phosphate, 3′-phosphoglycolate, and 3′-phosphopyridazine termini); these products are consistent with the initial modification of the C4′ position of a targeted nucleotide when Ni(II)·Xaa-Xaa-His is activated with $KHSO_5$, MMPP, *or* H_2O_2 under the reaction conditions employed. Importantly, given the established sensitivity of metallopeptide structure to DNA site-selectivity,[1] these data suggest the formation of a *common* metallopeptide-centered oxidant responsible for deoxyribose modification upon activation with $KHSO_5$, MMPP, or H_2O_2. Otherwise, differences in site-selectivities and product ratios would be expected as a function of the steric limitations of the active metallopeptides formed from these structurally diverse reagents.

To further probe the nature of the active metallopeptide responsible for C4′ deoxyribose damage, the effects of commonly employed radical scavengers on Ni(II)·Lys-Gly-His induced conversion of supercoiled form I DNA to relaxed circular form II DNA were compared in the presence of $KHSO_5$, MMPP, or H_2O_2. Under the conditions employed,[1] which assayed *direct* polymer strand scission *without* subsequent chemical workup, $KHSO_5$, MMPP, and H_2O_2 supported cleavage of plasmid DNA consistent with their ability to activate Ni(II)·Lys-Gly-His toward restriction fragment scission ($KHSO_5$ > MMPP ≫ H_2O_2). However, in the presence of the radical scavengers ethanol, *tert*-butyl alcohol, DMSO, or mannitol, little to no effect on DNA cleavage was apparent under conditions

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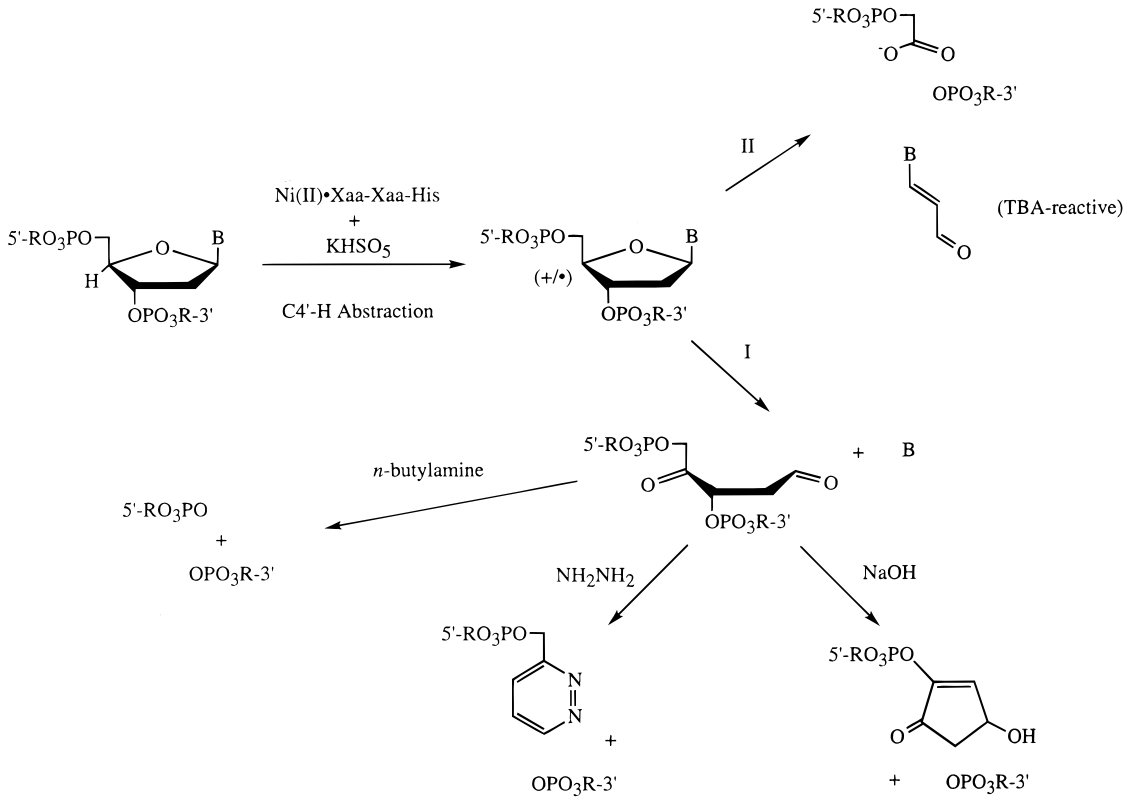
Scheme 1. Summary of Cleavage Products Observed and the Proposed Pathways of DNA Degradation by Ni(II)·Xaa-Xaa-His + $KHSO_5$[a]



[a] Pathway I involves nucleobase release and abasic (keto-aldehyde or alkaline-labile) site formation (*via* a C4′-OH nucleotide) that can result in DNA strand scission through treatment with alkaline reagents (*n*-butylamine, hydrazine, or NaOH) to produce modified 3′-termini and 5′-phosphorylated termini. Alternatively, pathway II results in direct DNA strand scission (*via* a C4′-OOH nucleotide) to produce 3′-phosphoglycolate termini, 5′-phosphorylated termini, and nucleobase propenals (TBA-reactive material).

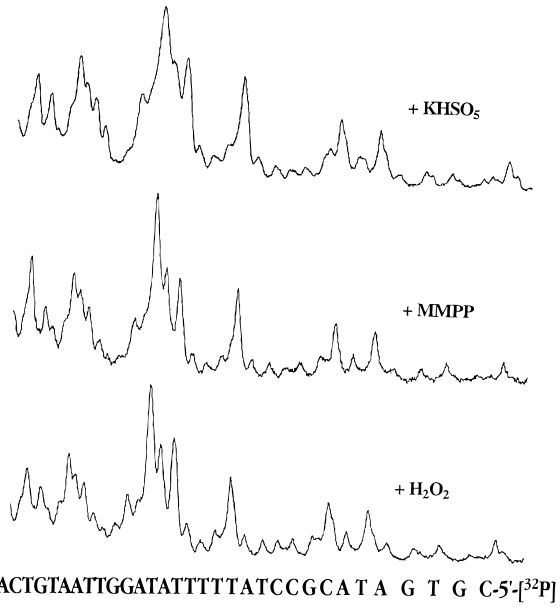


Figure 2. Densitometric analysis of an autoradiogram (Supporting Information) of a high-resolution denaturing polyacrylamide gel emphasizing the similarities in the DNA site-selectivity and termini products formed by Ni(II)·Lys-Gly-His cleavage of a 5′-^{32}P end-labeled restriction fragment from pB*R*322. Ni(II)·Lys-Gly-His (40 μM) was activated with 40 μM $KHSO_5$ (oxone)/1 min, 80 μM magnesium monoperoxyphthalate (MMPP)/9 min, or 40 mM H_2O_2/20 min.

that produced substantial inhibition of Fe(II)·EDTA induced DNA cleavage by hydroxyl radicals[17] in parallel control reactions (Supporting Information).

Overall, these data indicate that the "active" metallopeptides formed through the interaction of Ni(II)·Lys-Gly-His with $KHSO_5$, MMPP, or H_2O_2 result in identical cleavage site-selectivities and mechanistic products and also behave similarly in the presence of four well-established radical scavengers. These results clearly demonstrate that the active metallopeptides formed do not degrade DNA via freely diffusible hydroxyl radicals as also concluded in a Ni(II)·Gly-Gly-His induced protein cross-linking experiment.[8b] In addition, these data also indicate that the active intermediate involved in the direct strand scission of supercoiled plasmid DNA is not a freely diffusible or metal-bound sulfate radical ($SO_4^{\bullet-}$) when $KHSO_5$ is employed in metallopeptide activation; it has been established that 0.1 M ethanol is able to quench about 90% and 50% of the DNA cleavage induced by a free sulfate radical and a Ni(II)-complex bound sulfate radical, respectively.[22]

It is necessary to note, however, that while these results seemingly rule out the involvement of the above-named radical species in the *deoxyribose-based* strand scission of plasmid DNA, they do not preclude their formation and involvement in other pathways of DNA modification (e.g., nucleobase oxidation[9]). These data do, however, strongly suggest that, upon activation with $KHSO_5$, MMPP, or H_2O_2, a common "activated" metallopeptide is formed which interacts with the DNA helix leading to identical site-selective strand scission through initial C4′−H abstraction.

Condition-Dependent Modification of DNA by $KHSO_5$-Activated Ni(II)·Xaa-Xaa-His: C4′-Deoxyribose Modification *vs* Guanine Oxidation. The results outlined above

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implicate a common "activated" Ni(II)·Xaa-Xaa-His metallopeptide in the modification of the deoxyribose moiety of a DNA substrate when reacted with $KHSO_5$, MMPP, or H_2O_2. Given the recent report[9] of nearly exclusive guanine nucleobase oxidation by Ni(II)·Lys-Gly-His + $KHSO_5$, we sought to determine the differences in these observations. To accomplish this goal, a direct comparison of restriction fragment cleavage by Ni(II)·Lys-Gly-His + $KHSO_5$ was made using conditions that have been reported to result in (1) deoxyribose modification[1,4a,5] and (2) guanine nucleobase oxidation.[9]

As shown in Figure 3, cleavage of 5′-^{32}P end-labeled restriction fragments by Ni(II)·Lys-Gly-His + $KHSO_5$ under conditions employed previously[1] for an analysis of their site-selectivity [equimolar $KHSO_5$ + Ni(II)·Xaa-Xaa-His, 15 μM, 10 mM sodium cacodylate, pH 7.5, 1 min reaction] yielded the expected pattern of DNA cleavage at A/T rich regions.[1] Also consistent with previous observations, this pattern of A/T modification was virtually unaffected upon treatment with piperidine/90 °C. In contrast to the above reactions, however, restriction fragment cleavage reactions carried out under conditions reported[9] to yield guanine nucleobase oxidation [excess $KHSO_5$ (100 μM) + 15 μM Ni(II)·Xaa-Xaa-His, 100 mM NaCl, 10 mM phosphate, pH 7.0, 30 min reaction] produced slightly less intense *direct* DNA cleavage in the *absence* of piperidine treatment (compare Figure 3, lanes 6 and 8); further treatment of aliquots of these cleavage reactions with piperidine/90 °C predominantly yielded guanine-selective DNA damage as reported (Figure 3, lane 9).[9]

These data suggest that the conditions under which Ni(II)·Xaa-Xaa-His metallopeptides are activated with $KHSO_5$ strongly influence the observed chemistry and selectivity of DNA damage. Given the results presented earlier (Figure 2), which indicate a similar sequence-selective, deoxyribose-based mechanism of DNA damage by Ni(II)·Xaa-Xaa-His + $KHSO_5$, MMPP, *or* H_2O_2 when "low" ionic strength conditions are employed (10 mM sodium cacodylate, pH 7.5), we sought to determine if guanine nucleobase oxidation occurs as a result of (1) reaction conditions or (2) as a function of the activating oxidant (i.e., $KHSO_5$ vs MMPP/H_2O_2). Results of these experiments indicated that activation of Ni(II)·Lys-Gly-His with H_2O_2 or MMPP under "low" ionic strength conditions yielded the expected pattern of nucleotide damage also observed with $KHSO_5$ activation under these conditions (Supporting Information). In addition, treatment of aliquots of these reactions with piperidine/90 °C did not affect cleavage selectivity nor did it produce a detectable enhancement of guanine-based damage. In contrast to the above, however, attempts to mediate DNA damage with these activating reagents under "high" ionic strength conditions (100 mM NaCl, 10 mM phosphate) failed to produce comparable DNA strand scission even upon treatment with piperidine/90 °C. These data, in light of the observations illustrated in Figure 3, suggest that (1) guanine nucleobase oxidation occurs as a function of metallopeptide activation with $KHSO_5$ under conditions of excess $KHSO_5$ and relatively "high" ionic strength buffers (100 mM NaCl, 10 mM phosphate) and (2) deoxyribose-based strand scission can occur upon metallopeptide activation with all three activating reagents under relatively "low" ionic strength conditions as commonly employed in small molecule-DNA cleavage reactions.[16,19,20]

Given these data, Ni(II)·Xaa-Xaa-His + $KHSO_5$ appears to have the potential to form two "active" DNA-modifying species, one in common with those derived from metallopeptide activation with H_2O_2 or MMPP, and the other, most likely a "caged" sulfate radical as proposed previously.[9] In the case of activation with equimolar $KHSO_5$, the common activated species might predominate which, under low ionic strength conditions, is permitted to recognize and bind intimately with the DNA minor groove[1] resulting in abstraction of the C4′−H which is conspicuously located there. In comparison, under conditions of excess $KHSO_5$, it is possible that a metallopeptide-bound (caged) sulfate radical is formed in a greater abundance; under "high" ionic strength conditions this species is prevented from forming a tight complex with DNA subsequently limiting deoxyribose-based cleavage chemistry and facilitating the observation of collisionally derived guanine nucleobase oxidation events.

Proposed Mechanism of Formation of the Active Metallopeptide Responsible for Deoxyribose C4′−H Damage. A "common" metallopeptide-derived active species appears to be involved in C4′−H deoxyribose modification by metallopeptides activated with $KHSO_5$, MMPP, or H_2O_2. Given the characteristics observed, this "common" reactive species is proposed to be a high valent, peptide-bound Ni(III)-HO$^\bullet$ or Ni(IV)═O (i.e., a Ni-bound hydroxyl radical equivalent) which is likely generated from the heterolytic splitting of the oxygen−oxygen bond present in $KHSO_5$, MMPP, and H_2O_2[23] (Scheme 2). While the evidence presented here is not definitive proof, there are several further observations which support this proposal in addition to the previously presented mechanistic evidence for a common "activated" species. The first comes from the observed differences in the relative reactivities of the three activating reagents employed. The proposed mechanism for the generation of the active Ni intermediate involves an oxygen transfer from an oxygen donor to the metallopeptide. The facility of the heterolytic splitting of the oxygen−oxygen bond in the peroxidic compound is known to be determined by the acidity of the resulting leaving group;[24] the more acidic the leaving group, the more likely the peroxide functional group is to act as an oxygen atom donor to the metal center.[24c,d,25] Our observed order of reactivity toward DNA cleavage ($KHSO_5$ > MMPP ≫ H_2O_2) parallels very closely the order of the acidity of the resulting products of heterolytic oxygen−oxygen bond splitting (H_2SO_4 > $COOHC_6H_4COOH$ ≫ H_2O).

Additional support for the above mechanism comes from a spectral transition resulting from metallopeptide oxidation. A UV−vis absorption shift from 425 nm to a more intense peak at 375 nm is observed when $KHSO_5$ is added to a neutral Ni(II)·Lys-Gly-His solution in the absence of a DNA substrate. This shift is indicative of a structural change of the metal complex from square-planar to an oxidized higher valent Ni(III) or Ni(IV) species of octahedral geometry.[26] In addition to the above, indirect support can be derived also from mechanistic studies of Ni(II)-catalyzed alkene epoxidation reactions. Ni(II) complexes are known catalysts in alkene epoxidation reactions; a ligand-bound Ni(III)-HO$^\bullet$ or Ni(IV)═O has been suggested to be the active intermediate in these reactions.[27] In addition, Ni(II)·Gly-Gly-His in the presence of iodosobenzene was found to catalyze alkene epoxidation.[5c] The similarities in structure and chemistry between the metallopep-

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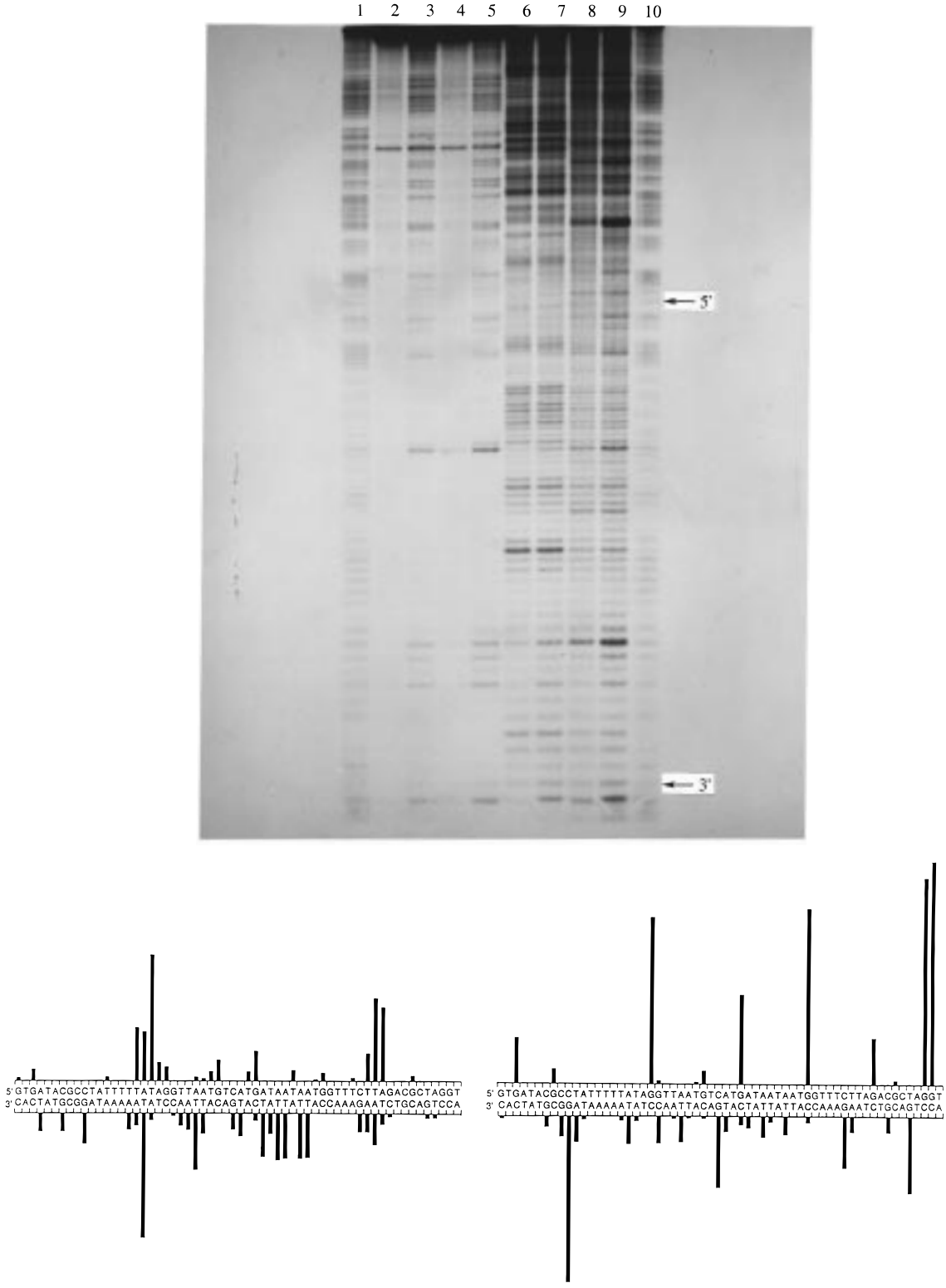
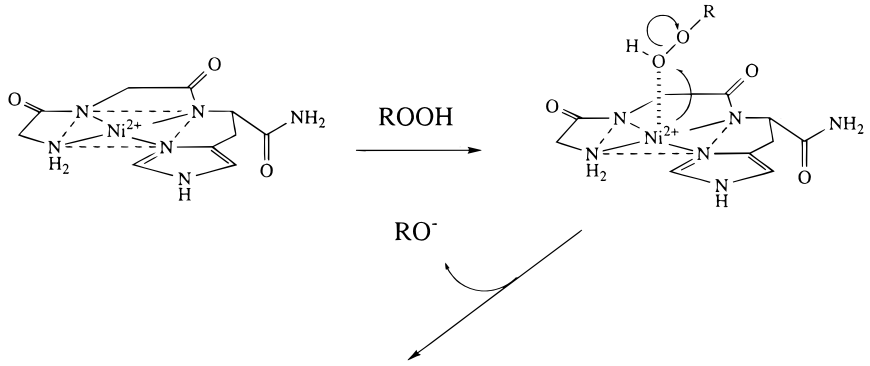


Figure 3. (top) Autoradiogram of a high-resolution denaturing polyacrylamide gel illustrating the condition-dependent modification of a 3′-^{32}P end-labeled restriction fragment from pB*R*322 DNA by $KHSO_5$-activated Ni(II)·Xaa-Xaa-His. Arrows represent the extreme 5′- and 3′-nucleotides of the sequence shown in the lower strand of the histogram (see bottom). Lane 1, Maxam−Gilbert G+A reaction; Lanes 2 and 3, intact DNA, −/+ piperidine treatment, respectively; Lanes 4 and 5, reaction controls, 15 μM $Ni(OAc)_2$, 15 μM oxone, −/+ piperidine treatment, respectively; Lanes 6 and 7, 15 μM Ni(II)·Lys-Gly-His, 15 μM oxone, 10 mM cacodylate buffer, pH 7.5, −/+ piperidine treatment, respectively; Lanes 8 and 9, 15 μM Ni(II)·Lys-Gly-His, 100 μM oxone, 100 mM NaCl, 10 mM phosphate buffer, pH 7.0, −/+ piperidine treatment, respectively; Lane 10, Maxam−Gilbert G+A reaction. (bottom) Histogram generated from the densitometric analysis of the piperidine-treated reactions from the autoradiogram shown above (lanes 7 and 9) along with data derived from cleavage of the complementary strand (autoradiogram not shown). The histogram on the left shows the cleavage patterns produced by Ni(II)·Lys-Gly-His under "low" ionic strength conditions (deoxyribose modification) while that on the right shows cleavage resulting from "high" ionic strength conditions resulting in guanine oxidation.[9] Background cleavage of controls resulting from piperidine treatment was subtracted from results shown in the histogram.

Scheme 2. Proposed Mechanism of Ni(II)·Xaa-Xaa-His Activation by $KHSO_5$, MMPP, or H_2O_2 through Ni-Promoted Heterolytic Cleavage of the Peroxide/Peracid Oxygen−Oxygen Bond[a]



[a] R = $-SO_3^-$ (oxone), $-COC_6H_4COO^-$ (MMPP), or −H (H_2O_2).

tides and macrocyclic Ni(II) complexes[27] suggest that a reactive intermediate similar to that involved in alkene epoxidation is likely formed from activation of the metallopeptides with $KHSO_5$, MMPP, and H_2O_2.

Summary and Conclusions. The findings presented herein define the deoxyribose-centered mechanism of DNA strand scission induced by Ni(II)·Xaa-Xaa-His metallopeptides and confirm that the oxidizing species responsible resides in the DNA minor groove in close proximity to the C4′−H. In addition, given the sequence- and complex-dependent mechanistic results obtained, indicating a partitioning of multiple pathways to product formation, the suggestion[1] that Ni(II)·Xaa-Xaa-His metallopeptides are sensitive to structural differences in the minor groove is supported. Further, these findings indicate that Ni(II)·Xaa-Xaa-His metallopeptides are capable of modeling the recognition functionalities of site-selective antitumor agents and can exhibit a sensitivity to the presence of ambient dioxygen. Given the mechanistic similarities these metallopeptides exhibit to the chemistry of Fe(II)·bleomycin, the notion that some form of activated metal−oxygen species is responsible for the initial abstraction of the C4′−H is likely; this hypothesis is directly supported by the studies contained herein which suggest the formation of a hydroxyl radical-bound metallopeptide derived from a Ni-promoted heterolytic splitting of the oxygen−oxygen bond of $KHSO_5$, MMPP, or H_2O_2. Further, the versatility of $KHSO_5$ as a supporting reagent in these systems is demonstrated by delineation of conditions under which deoxyribose *or* nucleobase oxidation chemistries can be *selected*. Overall, these studies indicate that Ni(II)·Xaa-Xaa-His metallopeptides are uniquely poised to increase our understanding of nucleic acid recognition and modification.

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Supporting Information Available: Autoradiograms of the results of 5′- and 3′-termini analyses, the selectivity of DNA cleavage mediated by $KHSO_5$-, MMPP-, and H_2O_2-activated metallopeptides, a comparison of DNA cleavage selectivity by metallopeptides activated with MMPP and H_2O_2 under conditions of varied ionic strength, and a table of the results of the effect of radical scavengers on DNA cleavage are available (6 pages). See any current masthead page for ordering and Internet access information.

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