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Oxidative DNA damage mediated by metal–peptide complexes

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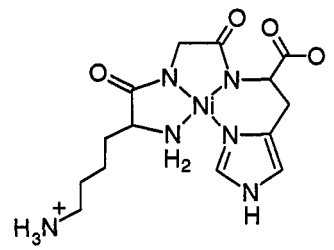
Abstract: Peptides and proteins are able to form coordination complexes with nickel(II) and copper(II) through ligation of deprotonated amide nitrogens, the amino terminus, and the side chains of such residues as histidine and the amino terminus. These complexes are reactive with either O_2 alone or O_2 in the presence of other oxidants or reductants to generate reactive intermediates capable of DNA damage. Both DNA strand scission and cross-linking have been observed.

INTRODUCTION

Oxidative damage to DNA can lead to transcriptional errors, mutagenesis and strand scission. Guanine is the nucleobase most sensitive to oxidation because of its low redox potential and its ability to bind redox active transition metals capable of catalyzing oxidative processes. In cells, metal ions such as Ni^{2+} and Cu^{2+} are primarily complexed with proteins in which the N-terminus, N-deprotonated amides, histidine imidazoles and cysteine thiolates provide the principal ligating groups.(1) Other cellular components such as O_2, SO_3^{2-}, NO and $O_2^{-\bullet}$ can conspire to form reactive oxidants that, in the presence of nickel and copper peptides, yield DNA-damaging agents.(2)

Nickel ion binding to peptides has been recently reviewed.(3) In serum, nickel is most often associated with amino acids such as histidine or with a protein such as albumin.(4) The K_d of Ni^{2+} with histidine is on the order of 10^{-9} M, representing about a 10-fold weaker binding constant than Cu^{2+}.(4) Nickel also exhibits quite slow exchange kinetics compared to other metal ions, (10^3 slower than Zn^{2+} and about 10^5 slower than Cu^{2+}) so that its complexes may have a substantial lifetime in vivo, even in the presence of physiologically higher concentrations of Cu^{2+}. The albumin binding site for nickel ions is believed to be the N-terminal peptide Asp-Ala-His.(5) Indeed, tripeptides in general are good ligands for formation of square planar nickel(II) complexes, and those with a histidine residue as the third amino acid in the sequence form particularly strong chelates. Another example is the square planar complex formed by coordination of Ni^{2+} to lysylglycylhistidine (NH_2-KGH-CO_2H), or Ni^{II}KGH.



Ni KGH

Studies with a copper tripeptide, $[Cu^{II}GGH]^-$, suggested that it displayed some anti-tumor activity, but little information has appeared beyond the initial report.(6) Early work in our labs showed that neither $[CuGGH]^-$ nor $[NiGGH]^-$ had any redox activity with oligodeoxynucleotides in the presence of strong oxidants, likely due to the -1 charge on the complex and the consequent electrostatic repulsion of nucleic acids.(7) With this information as background, we initiated studies to enhance the DNA reactivity of nickel peptides through modification of the charge and types of ligands surrounding the metal ion.

The inorganic chemistry of nickel and copper peptides has been studied in detail, and much is known about the coordination properties and the kinetics of O_2 reaction of these metal complexes.(8) In particular, $[Ni^{II}GGH]^-$ has been well studied, and its reactivity with oxidants led to applications in a number of labs to DNA cleavage chemistry. Very recently, Long and coworkers examined the DNA reaction of simple nickel tripeptide complexes with peracid oxidants and found modest site-specificity.(9) Mack and Dervan showed that appendage of a 52-amino acid helix-turn-helix from Hin recombinase to the C-terminus of $Ni^{II}GGH$ led to site-specific cleavage of a thymine residue in the target DNA duplex.(10) Perphthalate was used as oxidant, and an alkaline work-up was required for strand scission. In conceptually related work, Sugiura and coworkers showed that a NiGGH-three zinc finger (aa's 529-696 of transcription factor Sp1) bioconjugate led to site-specific cleavage at a cytosine residue of the target duplex DNA.(11) In both of these latter cases, the appended protein dominates the molecular recognition event, rather than the intrinsic selectivity of nickel complexes for unpaired guanines(12), and reaction occurred on duplex DNA at the site closest to the metal complex. However, in neither case did these bioconjugates take advantage of the reactivity of nickel peptides with O_2. They were prevented from doing so by appendage of a protein fragment on the C-terminus of $Ni^{II}GGH$. Studies described herein show that DNA cleavage is possible with dioxygen, the physiologically most relevant oxidant, if the peptide ligand includes a free carboxylate terminus.

RESULTS AND DISCUSSION

Preparation and characterization of nickel(II) peptides

Nickel complexes that carry an overall negative charge appear to have low reactivity with DNA.(7) Consequently, nickel peptide complexes were designed to incorporate lysine or arginine residues whose side chains would bear a positive charge at neutral pH. The nickel peptide complexes prepared for this study are shown in Table 1. In studies of O_2 reactivity, it was of interest to compare peptides bearing a free carboxylate terminus (GGGK, KGH, RGH, RRH) to those with a carboxamide terminus (GGGK-NH_2, GGGR-NH_2, KGH-NH_2). The peptides were synthesized by solid phase methods using Fmoc protection and either a *p*-benzyloxybenzyl alcohol resin or a 2,4-dimethoxybenzhydrylamine resin for preparation of carboxylate vs. carboxamide terminal peptides, respectively.(13,14) Nickel(II) complexes of the peptides were prepared by incubation of the peptide ligand with $Ni(OAc)_2$ in an aqueous pH 7.5 solution. All of the resulting yellow complexes showed similar electronic spectra (pH 7.5) and $Ni^{III/II}$ reduction potentials (10 mM KP_i, 100 mM NaCl, pH 7) indicative of square planar complexation as shown in Table 1.

Table 1. Characterization of square-planar nickel(II) peptide complexes.

Peptide ligand	$E_{1/2}$ (mV vs. SCE)	λ_{max} (nm)	ϵ ($M^{-1}cm^{-1}$)
GGGK-NH_2	670	416	131
GGHR-NH_2	737	420	105
KGH-NH_2	731	422	77
GGGK	611	420	105
KGH	686	420	73
RGH	671	420	84
RRH	720	420	94

Reactivity with dioxygen

All of the nickel peptide complexes with carboxamide termini were stable indefinitely upon exposure to air. On the other hand, those with free carboxylate termini underwent aerobic oxidation as seen by an increase in an absorbance band at 310 nm. The O_2 reaction was faster for peptide complexes of the type XXH compared to GGGK. As shown in Fig. 1, the oxidation of NiKGH is complete in a few hours while the oxidation of NiGGGK was only partially complete in 24-48 hours. No change in the absorbance spectra of the carboxamide-terminal peptide complexes was observed over a period of days.

The results confirm that the carboxylate group plays a role in aerobic oxidation of the complex. Indeed, a recent report indicates that the ultimate product of $[NiGGH]^-$ autoxidation is replacement of the carboxylate with a hydroxyl group (*e.g.* Eqn. 1).(15) Taken together, these results suggest that ligand oxidation is a requirement for O_2 uptake by the complex. We propose that the role of the carboxylate group is to donate an electron to a transiently formed nickel(III) species (Eqn. 1). The resulting carboxyl radical could undergo rapid decarboxylation leading to a carbon radical that would be highly reactive with dioxygen. Loss of superoxide, facilitated by the adjacent nitrogen, would lead to an N-acyl iminium group that would give the crystallographically characterized product after attack of water. Further studies are needed to support this mechanism. In particular, an oxygen labeling study should confirm whether or not the hydroxyl group is derived from water.

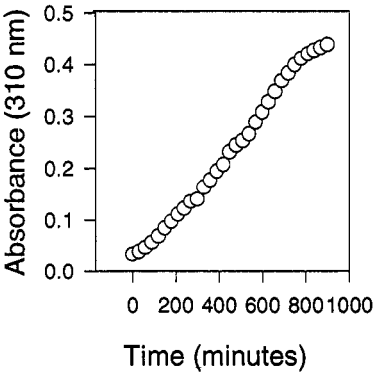
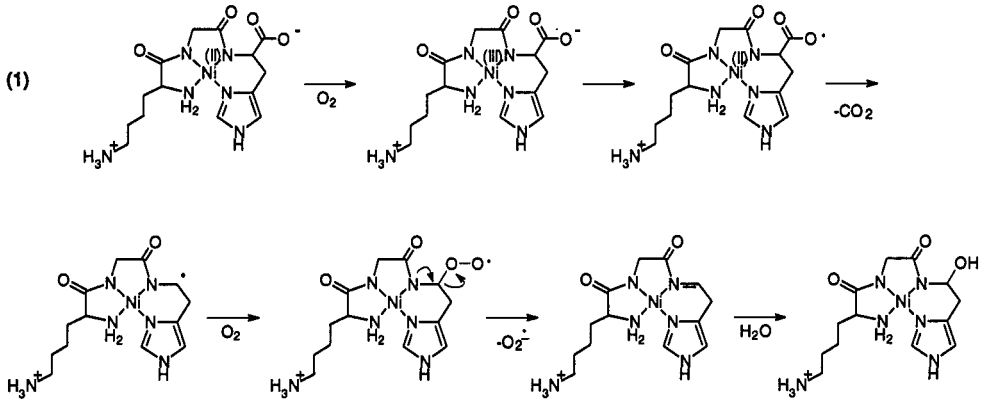


Fig. 1. Aerobic oxidation of NiKGH monitored at 310 nm.



Reactivity with DNA

Initial studies of DNA modification were carried out with a supercoiled plasmid since plasmid nicking is a sensitive assay of chemical reactivity. As shown in Fig. 2A, $Ni^{II}KGH$ effected DNA strand scission using ambient oxygen as oxidant (lane 2), while little or no reaction was observed in the absence of O_2 (lanes 3-4). Using 2 mM $Ni^{II}KGH$, the conversion to nicked, form II plasmid was essentially complete in 40 min. at room temperature (Fig. 2B). The nickel(II) complex of KGH carries a net charge of 0 at pH 7.5, and thus it is not expected to have a high binding affinity for DNA. On the other hand, use of a singly positively charged complex, $[Ni^{II}RRH]^+$, resulted in efficient nicking at a ten-fold lower concentration (Fig. 2C). Whereas all of the complexes that were O_2 reactive led to some degree of DNA modification, those bearing carboxamide C-termini (Table 1) showed no evidence of plasmid nicking in the presence of O_2 alone.

DNA modification studies were also carried out with oligonucleotide substrates. In this case, little evidence of strand scission was observed when O_2 alone was used as oxidant; however, some indications of DNA alkylation have been seen, warranting further study. In contrast, all of the nickel complexes are reactive when strong oxidants such as potassium monoperoxysulfate, $KHSO_5$, or magnesium monoperoxyphthalate, MMPP, are used. Guanine-specific modification was observed with $[Ni^{II}KGH\text{-}NH_2]^+$ and $KHSO_5$. Oxidation of the guanine nucleobase is implied by the fact that a piperidine work-up is required for strand scission and that guanine is the most easily oxidized base.

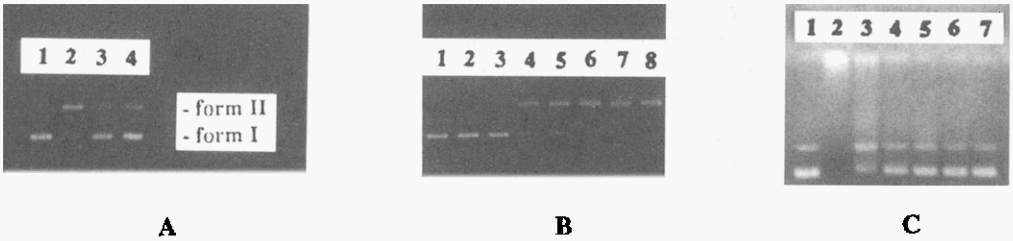


Fig. 2. Aerobic oxidation of plasmid DNA leading to conversion of form I closed supercoiled DNA to form II nicked DNA. Reactions were carried out using 15 μM (per base pair) pBR322, tris-HCl buffer (10 mM + 5 mM NaCl) at pH 7.5, 23°C for 60 min. A. Reactions with NiKGH. Lane 1: control (no NiKGH). Lane 2: 2 mM NiKGH, aerobic. Lanes 3-4: 2 mM NiKGH, anaerobic. B. Time-dependent plasmid nicking using 2 mM NiKGH. Lane 1: control (no NiKGH). Lanes 2-8: 0, 10, 20, 30, 40, 50, 60 min., respectively. C. Plasmid nicking with $[NiRRH]^+$. Lane 1: control (no $[NiRRH]^+$). Lanes 2-4: 200, 100 and 50 μM $[NiRRH]^+$, respectively. Lanes 5-7: 200, 100, and 50 μM RRH peptide, respectively.

SUMMARY & PERSPECTIVES

Nickel is a known carcinogen, particularly under circumstances where particulate nickel compounds enter cells by phagocytosis. Proteins or peptides serve as ligands for Ni^{2+} in the cellular medium. Such coordination helps to stabilize the nickel(III) oxidation state, which may play a key role in the observed DNA strand scission and DNA-protein cross-links. The work described herein demonstrates that oxidative DNA damage can result from exposure of nickel peptide complexes to ambient oxygen when the peptide has a free carboxylate terminus near the nickel binding site. Whether the DNA damage is in the form of strand scission or cross-linking is the subject of current investigation; evidence for guanine radical cations is seen. In the event of binding to a protein (*i.e.* carboxamide species), stronger oxidants such as HSO_5^- are needed to effect DNA damage. Given the ability of metal complexes to catalyze formation of HSO_5^- from autoxidation of sulfite(18), this constitutes an additional pathway for biomimetic DNA oxidation.(2)

Acknowledgements

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