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DNA Damage from Sulfite Autoxidation Catalyzed by a Nickel(II) Peptide

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Abstract: Guanine-specific modification of both single- and double-stranded oligodeoxynucleotides via the autoxidation of sulfite is shown to be catalyzed by $[NiCR]^{2+}$ (where CR = 2,12-dimethyl-3,7,11,17-tetraazabicyclo[11.3.1]-heptadeca-1(17),2,11,13,15-pentaene) and $[NiKGH\text{-}NH_2]^{+}$ (where KGH = lysylglycylhistidine). In the latter case, the nickel complex is proposed to act as a catalyst in three separate steps of sulfur oxide chemistry. Oxidative damage of guanines led to strand scission after piperidine treatment. The observed reactivity represents the first demonstration of DNA damage by sulfite and nickel(II) complexes. Importantly, these reactions were conducted using sulfite concentrations relevant to levels known to be cytotoxic. Mechanistic studies suggest the importance of both monoperoxysulfate and sulfate radical anion in the observed DNA damage. Evidence for formation of guanine radical cation as the initial product of DNA oxidation was found by comparison of the sequence dependence of guanine reactivity in a duplex restriction fragment. These studies underscore a role for sulfite in nickel toxicity and suggest a new method of site-specific oxidation with bioconjugates using sulfite rather than highly reactive oxidants such as monoperoxysulfate.

Introduction

Human exposure to sulfite results from inhalation of SO_2, largely from industrial emissions,[1−3] and ingestion of SO_3^{2-} (or HSO_3^-) as a preservative in food, alcoholic beverages, or drugs.[2,4] Sulfite can also undergo autoxidation to a reactive peracid, monoperoxysulfate (HSO_5^-). In fact, 35% of the sulfur content of remote marine clouds has been reported to be in the form of monoperoxysulfate produced from sulfite oxidation.[5] Toxic effects associated with sulfite[6] include asthma, mutagenic or comutagenic effects, and the ability to act as a co-carcinogen.[2,7] While the mechanistic details of sulfite toxicity are not fully understood, several studies have implicated sulfur oxy radicals ($SO_3^{\bullet-}$, $SO_4^{\bullet-}$, or $SO_5^{\bullet-}$) as potential oxidants of cell membranes, proteins, and DNA.[1−3] Generation of these radicals from oxidation of sulfite can be catalyzed by enzymes[8] or transition metal complexes,[3,9−13] or can be uncatalyzed.[3,14] Recently, it was observed that micromolar concentrations of $CoCl_2$, $CuCl_2$, and $Cr_2O_7^{2-}$ in the presence of sulfite (500 μM

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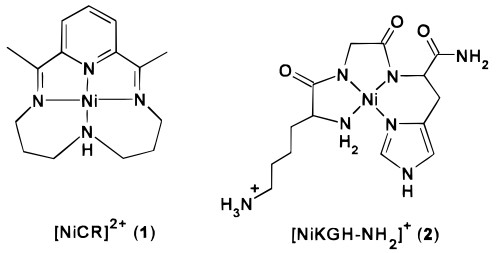
to 2 mM) induced DNA damage.[15,16] In addition, the autoxidation of sulfite by copper and nickel peptides as well as copper-dependent monooxygenases has been shown to involve the formation of $SO_3^{\bullet-}$.[17−19] On the other hand, our previous work on guanine-specific oxidation of DNA using $[NiCR]^{2+}$ (**1**)[20] or $CoCl_2$ and $KHSO_5$ has implicated $SO_4^{\bullet-}$ as the reactive DNA-oxidizing species.[21]

Proteins are a principal chelator of Ni^{2+} *in vivo*. In serum, the N-terminal tripeptides Asp-Ala-His of human serum albumin[22] and Val-Ile-His from desangiotensinogen[23a] have been identified as nickel binding sites. Nickel has also been shown to bind to the Cys-Ala-Ile-His sequence of histone H3, and the resulting complex promotes oxidative damage to DNA in the presence of H_2O_2.[23b,c] Despite their low overall concentrations in biological tissues, both nickel and chromium show very high accumulation in genetic material and are carcinogenic.[24] For nickel, this activity has been linked to DNA strand breaks, DNA−DNA cross-links and DNA−protein cross-links.[25] These lesions are thought to occur with nickel bound to biological ligands such as proteins and peptides since simple nickel salts are redox inactive.

The coordination features of nickel(II) bound to tripeptides whose third residue is histidine (XXH) have been investigated thoroughly, and this motif has been used to deliver a redox-active metal to DNA or proteins when tethered to a longer protein (or DNA) fragment.[26] Long and co-workers have investigated the intrinsic selectivity of $[NiKGH\text{-}NH_2]^+$ (**2**) as a mediator of DNA oxidation.[27]

Our present investigation reveals that $[NiKGH\text{-}NH_2]^+$ (**2**),[28,29] in the presence of ambient O_2 and 100 μM Na_2SO_3, results in guanine-specific modification of both single- and double-stranded DNA. Furthermore, HSO_5^- and $SO_4^{\bullet-}$ are implicated as important intermediates in this process leading to formation of guanine radical cation, a principal intermediate in oxidative damage to nucleic acids. Interestingly, this study represents the first observation of DNA damage by a nickel(II) complex in the presence of sulfite and dioxygen. These observations lend insight into the possible roles of nickel(II) and sulfite in mutagenicity and carcinogenicity.



Experimental Section

Materials. The oligodeoxynucleotide d(ATATCAGATCTAGACTAT) (**3**) was purchased from Oligos Etc., Inc., and purified to homogeneity under strongly denaturing conditions (pH 12) using anion exchange chromatography (Mono Q, Pharmacia).[30] Supercoiled pBR322 plasmid DNA was obtained from BRL. 3′-End-labeled restriction fragments were obtained by digestion with *Eco*RI restriction endonuclease and then treatment with α-[^{32}P]-dATP and the Klenow fragment of DNA polymerase I. A second digestion with *Rsa*I restriction endonuclease yielded a 167 and a 514 base pair fragment. The 167 base pair fragment was purified by 8% preparative nondenaturing gel electrophoresis and isolated by the crush and soak method. All enzymes were obtained from New England Biolabs. γ-[^{32}P]-ATP (3000 Ci/mmol) and α-[^{32}P]-dATP (3000 Ci/mmol) were purchased from Amersham, and radioactivity was quanitfied by scintillation counting. Protected amino acids were purchased from Novabiochem. Peptide syntheses were carried out by conventional solid-phase techniques,[31] purified by reverse-phase HPLC, and verified by ^{1}H NMR. Potassium monoperoxysulfate (Oxone) and sodium sulfite were purchased from Sigma and Fluka, respectively. All aqueous solutions utilized purified water (Nanopure, Sybron/Barnsted) and reagents of the highest commercial quality. Chemicals used for the synthesis of nickel complexes were of reagent grade and used without further purification.

Caution! While we have used perchlorate as a counterion in $[NiCR](ClO_4)_2$ without incident, perchlorate salts of metal complexes with organic ligands are potentially explosive. Care should be exercised when using a spatula or stirring rod to mechanically agitate any solid perchlorate. This complex, as well as any other perchlorate salt, should only be handled in small quantities.

The complex $[NiCR](ClO_4)_2$ was synthesized as previously described,[32] while $[NiKGH\text{-}NH_2]^+$ was formed *in situ* by the addition of 1 equiv of $Ni(CH_3CO_2)_2$ to an aqueous solution of $KGH\text{-}NH_2$ followed by the addition of 2 equiv of NaOH.

Metal-Dependent Modification of Oligodeoxynucleotides. DNA experiments were conducted as previously described.[32] Reaction mixtures (50 μL) contained 3 μM unlabeled oligodeoxynucleotide, 2 nCi 5′-end-labeled oligodeoxynucleotide, 10 μM of the desired nickel complex, 100−1000 μM of either $KHSO_5$ or $NaSO_3$, 100 mM NaCl, and 10 mM sodium phosphate (pH 7.0).

Metal-Dependent Modification of Restriction Fragments. DNA experiments were conducted as previously described.[32] Reaction mixtures (50 μL) containing 20 μM calf thymus DNA (base pair concentration), 9 nCi 3′-end-labeled restriction fragment, 10 μM of the desired nickel complex, 100 μM of $KHSO_5$ or 50−100 μM $NaSO_3$, 100 mM NaCl, and 10 mM sodium phosphate (pH 7.0).

Quantification of Nucleic Acid Product Fragments. The extent of reactivity was determined by densitometric analysis of the resulting autoradiograms with a Beckman DU 650 spectrophotometer.

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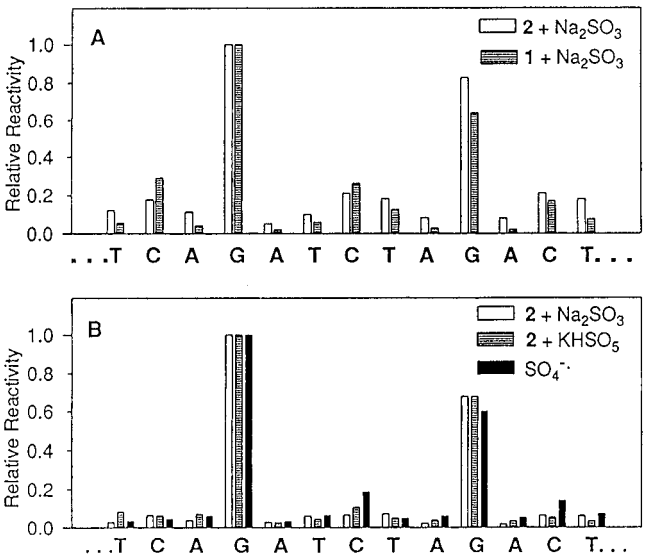

Figure 1. (A) Comparison of the reactivity of single-stranded DNA with Na_2SO_3 mediated by **2** (white) vs **1** (gray) at 25 °C. Reaction mixtures (50 μL) contained final concentrations of 3 μM **3** (see text for sequence), 2 nCi [5′-^{32}P]-**3**, 10 μM **2** or **1**, 1 mM Na_2SO_3, 100 mM NaCl, and 10 mM sodium phosphate (pH 7.0). After 1 h all reaction mixtures were quenched with 10 mM EDTA (pH 8), dialyzed, and then treated with 50 μL 1 M piperidine for 30 min at 90 °C. Autoradiograms were quantified by scanning densitometry. Relative reactivity represents the extent that an individual DNA base was modified relative to the most reactive residue. Errors are estimated to be ±10% of the reported values. (B) Comparison of the reactivity of single-stranded DNA using various oxidants: 10 μM **2** and 100 μM Na_2SO_3 (white), 10 μM **2** and 100 μM $KHSO_5$ (gray), or photolytically generated $SO_4^{-\bullet}$ (black).[21] Reactions involving nickel complexes were conducted as described in A.

Results

Oligodeoxynucleotides. For studies with single-stranded DNA, the oligodeoxynucleotide [5′-^{32}P]-d(ATATCAGATCTA-GACTAT) (**3**) was used. The reaction at 25 °C of **3** (3 μM), **2** (10 μM), and Na_2SO_3 (1 mM) for 1 h followed by treatment with piperidine[32] and subsequent analysis by denaturing gel electrophoresis revealed predominant strand cleavage occurring at guanine residues. Scanning densitometry showed that the extent of reaction under these conditions was 44% with an average selectivity for guanine over adenine, cytidine or thymine of 7:1 (Figure 1A). Analogous results were obtained for reactions employing **1** and Na_2SO_3 (Figure 1A), yielding an extent of reaction of 24% and the same guanine preference. Experiments conducted without piperidine treatment showed no evidence of strand scission under the conditions used here.[33]

Most importantly, aerobic reactions conducted using **2** (10 μM) and reduced Na_2SO_3 concentrations (100 μM) led to extensive strand scission (44%), although **1** was unreactive under the same conditions (Figure 1B). Interestingly, the formation of HSO_5^- from **2**-catalyzed sulfite autoxidation is, within experimental error, as efficient at DNA modification as the use of preformed HSO_5^-.

The observed selectivity of guanine in the nickel(II)/sulfite system is similar to that previously obtained for reactions involving **1** or $CoCl_2$ and $KHSO_5$ with single-stranded oligodeoxynucleotides.[21] These results have been correlated to the production of a caged sulfate radical $[NiCR(SO_4)]^{2+}$ or a highly diffusible, free sulfate radical ($SO_4^{-\bullet}$), respectively. In order to determine if $SO_4^{-\bullet}$ is produced from nickel-mediated sulfite oxidation, a comparison was first made between the reactivity of photolytically generated $SO_4^{-\bullet}$,[21] and that of **2** with either $KHSO_5$ or Na_2SO_3/O_2, again using **3**. The selectivities of the three systems were nearly identical, yielding the expected guanine-specific modification (Figure 1B). These results point to similar, but not necessarily identical, reactive intermediates.

(33) Liang *et al.*[27] have observed direct strand scission in a related system, but no quantification or comparison to alkaline-labile scission was reported.

Table 1. Quenching Data for DNA Oxidations[a]

reagent	additive	% change in DNA cleavage[b]
10 μM **2** + 100 μM Na_2SO_3	25 mM EtOH	-11
10 μM **2** + 100 μM Na_2SO_3	25 mM *t*BuOH	+04
10 μM **2** + 1 mM Na_2SO_3	25 mM EtOH	-16
10 μM **2** + 1 mM Na_2SO_3	25 mM *t*BuOH	+07
10 μM **2** + 100 μM $KHSO_5$	25 mM EtOH	-20
10 μM **2** + 100 μM $KHSO_5$	25 mM *t*BuOH	-09
10 μM **1** + 1 mM Na_2SO_3	25 mM EtOH	-87
10 μM **1** + 1 mM Na_2SO_3	25 mM *t*BuOH	+04
3 μM **1** + 50 μM $KHSO_5$[c]	25 mM EtOH	-12
3 μM **1** + 50 μM $KHSO_5$[c]	25 mM *t*BuOH	+09
photolysis of $K_2S_2O_8$[c]	25 mM EtOH	-80
photolysis of $K_2S_2O_8$[c]	25 mM *t*BuOH	+21

[a] All reactions were conducted with the single-stranded oligodeoxynucleotide **3**. Reaction mixtures (50 μL) contained final concentrations of 3 μM **3**, 2 nCi [5′-^{32}P]-**3**, 100 mM NaCl, and 10 mM sodium phosphate (pH 7.0). Reaction mixtures were quenched with 10 mM EDTA (pH 8), dialyzed, and then treated with 50 μL of 1 M piperidine for 30 min. at 90 °C. [b] % change in DNA cleavage is defined as the difference between the extent of reaction with and without additive. Errors are estimated to be ±10%. [c] From ref 21.

Alcohol-Quenching Studies. To gain additional evidence for the production of $SO_4^{-\bullet}$ and also to investigate the possible involvement of $SO_3^{-\bullet}$ and $SO_5^{-\bullet}$ as DNA-damaging species, alcohol-quenching studies were performed. While it has been shown that $SO_4^{-\bullet}$ reacts readily with ethanol, both $SO_3^{-\bullet}$ and $SO_5^{-\bullet}$ react 10000-fold slower.[1,34] Furthermore, *tert*-butyl alcohol reacts about 1000-fold faster with hydroxyl radical than with sulfur oxy radicals.[8,34] Accordingly, DNA experiments were conducted in the presence and absence of either ethanol or *tert*-butyl alcohol (Table 1). Consistent with previous observations involving **1** and $KHSO_5$,[21] the extent of reaction of **3** with **2** (10 μM) and $KHSO_5$ (100 μM) was reduced by only 20% in the presence of 25 mM ethanol. Similar results were obtained for reactions of **2** (10 μM) and Na_2SO_3 (100 μM and 1 mM), where 12% and 16% quenching of the DNA reaction were observed, respectively. These results are reminiscent of our previous observations for the oxidation of DNA by **1**/$KHSO_5$ and suggest an intermediate in which nickel is ligated to $SO_4^{-\bullet}$, effectively a caged sulfate radical.[21] In addition, the inability of *tert*-butyl alcohol to induce significant levels of quenching, the lack of direct strand scission, and the guanine-specific nature of the DNA oxidation suggest that hydroxyl radical is not produced, although the production of low levels of a caged hydroxyl radical, analogous to that proposed for the reaction of NiGGH and H_2O_2, cannot be ruled out.[35]

In sharp contrast, when 25 mM ethanol was added to DNA reactions containing **1** (10 μM) and Na_2SO_3 (1mM), nearly complete quenching was observed (87%). This result is similar to that obtained for the reaction of $CoCl_2/KHSO_5$ or photochemically generated $SO_4^{-\bullet}$, implying the production of freely diffusible $SO_4^{-\bullet}$, but opposite to the **1**/$KHSO_5$ results.[21]

Restriction Fragments. In order to examine the reactivity of guanines in duplex DNA and to gain further mechanistic

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Table 2. Sequence Dependence of Guanine Reactivity and Ionization Potentials[a]

sequence	relative reactivity **2**/HSO_5^-	relative reactivity **2**/SO_3^{2-}/O_2	relative reactivity DMS	ionization potential[b] (eV)
GG	4.3	5.3	1.1	7.28
GA	1.7	2.8	1.1	7.51
GT	1.1	1.1	1	7.69
GC	1	1	1.2	7.68

[a] Relative reactivities are averages of G sites found in a 167 bp restriction fragment from pBR322 as shown in Figure 2. HSO_5^- and SO_3^{2-} concentrations are given in the Experimental Section. [b] From ref 36a.

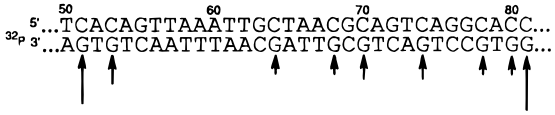

Figure 2. A portion of the sequence of the 167 bp restriction fragment from pBR322 used for comparison of G reactivity using **2** and SO_3^{2-}. The lower strand was 3′ end labeled and analyzed. Arrows indicate the relative reactivities of various sites. Quantitative data is given in Table 2.

information, studies were carried out with a 167bp restriction fragment from the plasmid pBR322 that was 3′ end labeled with ^{32}P. Reaction conditions were chosen to give ideally a single modification per strand (<40% strand scission) so that the relative reactivities of individual nucleobases could be compared. Accordingly, concentrations of 100 μM HSO_5^- or 50−100 μM SO_3^{2-} and 10 μM **2** were used. Guanine modification was also observed using even lower concentrations of Na_2SO_3 (25 μM), but band intensities were too weak to accurately quantify. A specific region of the restriction fragment (50−81, Figure 2) was chosen for analysis because of the variety of guanine sequences contained within a relatively small section, although analysis of various regions of both strands provided similar, but less complete, results.

Again, guanines were always the site of oxidation leading to piperidine-mediated strand scission; however, the reactivity of individual guanines depended upon local sequence, specifically upon the 3′ flanking base (Table 2, Figure 2). Guanines followed by a 3′ purine nucleotide in duplex DNA were more reactive than those flanked by a 3′ pyrimidine. Oxidations using **2** with HSO_5^- vs SO_3^{2-}/O_2 gave similar results, again suggesting the formation of the same reactive intermediate. On average, the reactivity of GG:GA:GT:GC was 5:2:1:1 for these oxidants. In contrast, N7 alkylation of guanines with dimethyl sulfate led to nearly equal reactivity of all guanines in the sequence. The reactivity pattern of guanines in these nickel peptide-mediated oxidations closely parallels the sequence dependence of guanine ionization potentials as discussed by Saito *et al*.[36a,b] and the qualitative reports of GG reactivity in other oxidation systems.[36b−f] Other nickel complexes mimic this behavior with duplex DNA (unpublished results); however, 5′-d[GG]-3′ sequences in *single*-stranded oligonucleotides do not show hyperreactivity at the 5′ Gs,[30] confirming that reactivity is affected by base stacking.

Oxygen Dependence. While the formation of $[Ni^{III}KGH\text{-}NH_2]^{2+}$ is implied in our observed reaction, no direct oxidation of **2** in the presence of only O_2 has been detected (Figure 3a). Furthermore, in the absence of O_2, the addition of 1 molar equiv of Na_2SO_3 to an aqueous solution of **2** (pH 7) resulted in no spectral changes associated with the d−d absorption band centered at 417 nm. However, similar experiments conducted by bubbling O_2 through a solution of 100 μM **2** and 100 μM Na_2SO_3 resulted in formation of an intense band centered at 367 nm, indicative of a Ni(III) peptide complex (Figure 3c).[37] When the concentration of **2** was held constant at 100 μM and the concentration of sulfite was increased up to 10-fold, the same band at 367 nm appeared, but more slowly, and its intensity was slightly diminished. Similar observations were made for the autoxidation of sulfite by copper(II) tetraglycine and O_2 at pH 9.2.[15] Furthermore, the addition of excess H_2O_2 to solutions containing **2** resulted in the formation of an intense absorbance at 367 nm. Therefore, it is reasonable to postulate that in the presence of O_2 and sulfite, **2** is being oxidized to $[Ni^{III}KGH\text{-}NH_2]^{2+}$.

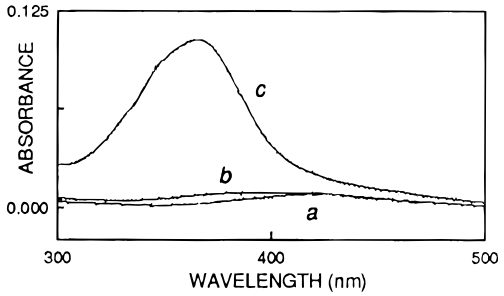

Figure 3. Electronic spectra of 100 μM aqueous $[NiKGH\text{-}NH_2]^+$ (**2**), pH 7, 10 mM sodium phosphate + 100 mM NaCl buffer: (a) in the presence of air, (b) anaerobic, with 100 μM Na_2SO_3 added, and (c) as in b and exposed to dioxygen for 5 min.

DNA modification by **2**-catalyzed sulfite autoxidation was also observed to be highly O_2 dependent, while DNA oxidations involving preformed HSO_5^- were much less so. Using 10 μM **2** and 100 μM either SO_3^{2-} or HSO_5^-, piperidine-labile strand scission of **3** occurred to the extent of 44% and 42% respectively under ambient dioxygen. Bubbling dioxygen through the reaction mixtures gave approximately the same result. On the other hand, when the same reactions were carried out under a nitrogen atmosphere, the sulfite-mediated strand scission was decreased 7-fold while the effect on the HSO_5^- reaction was negligible.

Discussion

The reaction of $[NiKGH\text{-}NH_2]^+$ with SO_3^{2-}/O_2 represents the lowest reported concentration of sulfite at which DNA damage has been observed for a transition metal-promoted reaction and is therefore particularly relevant in the area of sulfite toxicity where cellular damage has been observed at levels as a low as 100 μM.[2] Recently, Kawanishi *et al*. observed guanine-specific modification of duplex DNA in the presence of $CoCl_2$, sulfite (1−2 mM), and O_2. This observation was postulated to result from the formation of $SO_4^{\bullet-}$.[15] On the other hand, more random cleavage of DNA resulted from a reaction with $CuCl_2$, sulfite (1−2 mM), and O_2, and this was suggested to be due to the oxidation of DNA by $SO_3^{\bullet-}$.[15] While these combined observations suggest that $SO_4^{\bullet-}$ rather than $SO_3^{\bullet-}$ is responsible for the observed DNA damage involving **2** and sulfite, other radical species ($SO_5^{\bullet-}$ and $HO^{\bullet}$) need also to be considered.[3] For example, Coichev and van Eldik have postulated eqs 1−7, in addition to other initiation, chain propagation, and termination

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steps, for the autoxidation of sulfite by cobalt(II) in the presence of azide.[9]

$$M^{III} + SO_3^{2-} \rightarrow M^{II} + SO_3^{\bullet -} \qquad (E(SO_3^{\bullet -}/SO_3^{2-}) \approx 0.39\ V)^{34,38} \quad (1)$$

$$SO_3^{\bullet -} + O_2 \rightarrow SO_5^{\bullet -} \qquad (k = 1.5 \times 10^9\ M^{-1}\ s^{-1})^{34,39} \quad (2)$$

$$M^{II} + SO_5^{\bullet -} \rightarrow M^{III} + SO_5^{2-} \qquad (E(SO_5^{\bullet -}/SO_5^{2-}) \approx 0.86\ V)^{34,38} \quad (3)$$

$$SO_5^{2-} + H^+ \rightleftharpoons HSO_5^- \qquad (pK_a = 9.4) \quad (4)$$

$$M^{II} + HSO_5^- \rightarrow M^{III} + SO_4^{\bullet -} + OH^- \quad (5)$$

or

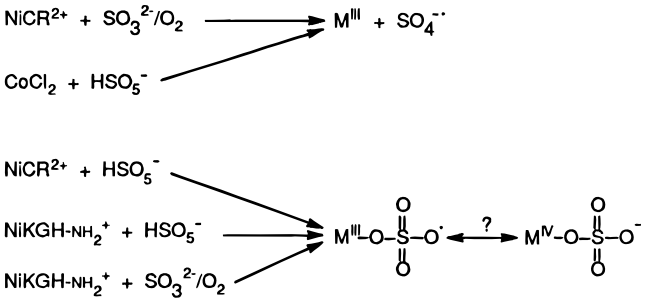
$$M^{II} + HSO_5^- \rightarrow M^{III} + SO_4^{2-} + {}^{\bullet}OH \quad (6)$$

$$2SO_5^{\bullet -} \rightarrow 2SO_4^{\bullet -} + O_2 \qquad (k = 6 \times 10^8\ M^{-1}\ s^{-1})^{40} \quad (7)$$

Of the four possible radical species in eqs 1−7, all but $SO_4^{\bullet -}$ can reasonably be eliminated as important DNA-damaging agents in the present studies. In this regard, the results of alcohol-quenching studies are instrumental. Evidence against the participation hydroxyl radicals is found in the difference in quenching data for ethanol vs *tert*-butyl alcohol (since ${}^{\bullet}OH$ would have been quenched by both) and in the lack of direct strand scission in the absence of piperidine.[33] Sulfite radical, $SO_3^{\bullet -}$, is an unlikely agent for DNA damage because its lifetime in the presence of dioxygen is short,[34] its potential (∼0.39 V vs SCE) for one-electron oxidation of guanine (0.90−1.0 V)[41] is too low, its second-order rate constants for oxidation of the four deoxynucleosides are all about the same ($10^6\ M^{-1}\ s^{-1}$),[34,42] and its rate constant for reaction with ethanol is low ($k \leq 2 \times 10^3\ M^{-1}\ s^{-1}$).[43] The properties of the monoperoxysulfite radical, $SO_5^{\bullet -}$, are less well known, but its high reactivity toward dimerization to yield $SO_4^{\bullet -}$ (eq 7),[40,44] its lower reduction potental (∼0.86 V) compared to guanine, and its lower reactivity with ethanol ($k < 10^3\ M^{-1}\ s^{-1}$)[43] make it less viable as the principal DNA-damaging agent. Sulfate radical, $SO_4^{\bullet -}$, either free or metal coordinated, is most consistent with the data. It is known to react more rapidly with ethanol compared to *tert*-butyl alcohol ($k = 1.6 \times 10^7$ vs $4 \times 10^5\ M^{-1}\ s^{-1}$).[34] In addition, it reacts preferentially with deoxyguanosine compared to other deoxynucleosides or deoxyribose alone ($k_G = 2.3 \times 10^9$, $k_A = 3.7 \times 10^8$, $k_C = 2.5 \times 10^8$, $k_T \leq 2 \times 10^8$, $k_{dr} = 3.8 \times 10^7\ M^{-1}\ s^{-1}$).[34,45] These rate constant data are consistent with the observed specificity of the nickel-catalyzed reactions for guanine and with the lack of direct strand scission arising from deoxyribose oxidation.

Alcohol-quenching studies provided insight into further subtleties regarding the nature of the sulfate radical involved.

Scheme 1



We previously reported that the oxidation of guanine using HSO_5^- involved slightly different intermediates for catalysis by $CoCl_2$ vs NiCR (**1**) (Scheme 1). Use of $CoCl_2$ led to an ethanol-quenchable intermediate while the species produced with **1** was barely affected by ethanol. This difference was proposed to be due to the formation of a free sulfate radical in the former case compared to a metal-bound species in the latter case, suggesting that the reactive intermediate formed with $[NiKGH\text{-}NH_2]^+$ (**2**) using either HSO_5^- or SO_3^{2-} is again a metal-bound species since it is not ethanol quenchable. Curiously, sulfite autoxidation catalyzed by $[NiCR]^{2+}$ (**1**) produces a different species than when preformed HSO_5^- is used as oxidant; the reactive intermediate formed from SO_3^{2-}/O_2 is quenched by ethanol while that from HSO_5^- is not. This apparent discrepancy between reactions involving SO_3^{2-}/O_2 vs HSO_5^- and **2** vs **1** can be explained by examination of eqs 1−7. On the basis of redox potentials, it is reasonable that both $[Ni^{III}KGH\text{-}NH_2]^{2+}$ ($Ni^{III/II} = 0.73$ V)[38,46] and $[Ni^{III}CR]^{3+}$ ($Ni^{III/II} = 0.98$ V)[32,38] oxidize SO_3^{2-} to $SO_3^{\bullet -}$ ($SO_3^{\bullet -}/SO_3^{2-} \sim 0.39$ V, eq 1). Once $SO_3^{\bullet -}$ is formed, it most likely reacts rapidly with O_2 to form $SO_5^{\bullet -}$ according to eq 2. For reactions involving **2**, the $SO_5^{\bullet -}$ formed ($SO_5^{\bullet -}/SO_5^{2-} \sim 0.86$ V) can oxidize **2**, yielding $[Ni^{III}\text{-}KGH\text{-}NH_2]^{2+}$ and HSO_5^- (eqs 3 and 4). Finally, HSO_5^- oxidizes **2** to form the caged radical species, $[Ni^{III}KGH\text{-}NH_2(SO_4)]^+$, which is only modestly quenched by ethanol. On the other hand, the oxidation of **1** by $SO_5^{\bullet -}$ (eq 3) is not expected to compete favorably (on the basis of redox potentials of **1** and $SO_5^{\bullet -}$) with the dimerization of $SO_5^{\bullet -}$ to produce 2 equiv of $SO_4^{\bullet -}$ and O_2 (eq 7). Therefore, for **2** the production of a caged sulfate radical can be envisioned as taking place through the *in situ* formation of HSO_5^-while for **1**, a free (and ethanol-quenchable) $SO_4^{\bullet -}$ is generated via dimerization and degradation of $SO_5^{\bullet -}$. Overall, the role of **2** in Scheme 1 is to catalyze three different steps (eqs 1, 3, and 5) in sulfite autoxidation to monoperoxysulfate and its subsequent decomposition.

The $nickel^{III}-(SO_4^{\bullet -})$ species proposed in Scheme 1 could also be formulated as a $nickel^{IV}-(SO_4^{2-})$ complex. Spectroscopic studies of **2** in the presence of SO_3^{2-} and O_2 show the formation of a charge-transfer band at 367 nm that is similar to the range (305−355 nm) reported for electrochemically generated nickel(III) peptide.[37] Additionally, oxidation of **2** with H_2O_2 generated the same absorption at 367 nm. While a nickel(IV) species cannot be ruled out as an intermediate (Scheme 1), whatever long-lived nickel species are formed have the spectroscopic characteristics of nickel(III).

The studies of O_2 dependence corroborate this overall mechanistic hypothesis. Sulfite autoxidation necessarily requires dioxygen in the second step of the mechanism (eq 2). In the present work, no DNA modification and no spectral changes in **2** are observed in the absence of O_2. On the other hand, metal-catalyzed decomposition of HSO_5^- does not require O_2

(38) Redox potentials are reported vs SCE.
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(eq 5), and accordingly, dioxygen has little effect on these reactions. Other oxidations believed to form guanine radical cation are also O_2 independent.[47] A complicating issue is the point that dioxygen may affect the type of guanine oxidation products formed—8-oxo-deoxyguanosine from reaction of guanine radical cation with H_2O[48] vs an oxazolone from reaction with O_2.[49] Nevertheless, both of these guanine modifications create piperidine-labile sites.

Evidence for formation of guanine radical cation as the initial intermediate in nickel-catalyzed oxidation was obtained from studies of a restriction fragment. In previous studies,[32] we have observed high reactivity of accessible guanines located in single-stranded regions (bulges, loops, etc.) but little reactivity of normal G−C pairs in a B-form duplex.[30] However, when no exposed guanine sites are available for reaction with nickel complexes, as in the 167 bp restriction fragment, duplex Gs are found to be reactive. A small section of this duplex fragment allowed comparison of guanines having different 3′ neighboring bases. Saito and co-workers have reported the dependence of guanine's ionization potential on sequence in duplex DNA and found the trend in potentials to be 5′-GGG-3′ < 5′-GG-3′ < 5′-GA-3′ < 5′-GC-3′ ∼ 5′-GT-3′.[36b] Therefore, one should expect to observe a sequence dependence upon guanine oxidation if (a) the reaction involves formation of a guanine radical cation and (b) the reaction occurs on stacked bases in duplex DNA. The sulfate radical has previously been shown to produce guanine radical cation.[50] The relative reactivities of guanines shown in Figure 2 and Table 2 are consistent with radical cation formation as the major event leading to guanine modification. Whether or not the 5′-Gs of high reactivity represent the initial sites of reaction or are formed from hole migration cannot be determined from these experiments.[36f,47]

The use of the nickel tripeptide motif as a means of introducing a redox active species into a bioconjugate has seen increasing application in protein−DNA chemistry.[26] DNA cleavage has then been site specifically effected by addition of a strong oxidant, usually a peracid. The new findings reported here suggest that the requirement for a peracid oxidant that might cause other nonspecific oxidative damage to biopolymers can be avoided by instead recruiting sulfite autoxidation. In this case, the strong oxidant, monoperoxysulfate, could be catalytically generated *in situ* by the nickel peptide complex at the site where it is needed.

In summary, the first example of DNA damage by the autoxidation of sulfite using a nickel(II) complex has been demonstrated. Complex **2** actually participates as a catalyst in three separate steps of sulfur oxide chemistry. The use of the biologically relevant tripeptide Lys-Gly-His-amide and sulfite concentrations applicable to previously reported toxicity levels suggests this system may be relevant to the toxicity observed by both nickel(II) and sulfite. Furthermore, it has been shown that $SO_4^{\bullet-}$ and guanine radical cations are involved in the observed DNA damage, which supports previous claims of the role of sulfite in biological toxicity.

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