DNA and RNA Modification Promoted by $[Co(H_2O)_6]Cl_2$ and $KHSO_5$: Guanine Selectivity, Temperature Dependence, and Mechanism

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Abstract: Reaction of a single-stranded oligodeoxynucleotide or a 17-base hairpin-forming oligodeoxynucleotide with $CoCl_2$ and $KHSO_5$ produced guanine-specific cleavage after piperidine treatment. The observed reactivity is shown to be nearly twice that obtained for NiCR (CR = 2,12-dimethyl-3,7,11,17-tetraazabicyclo[11.3.1]heptadeca-1(17),2,11,13,15-pentaene) under equivalent conditions, although NiCR displays a slightly higher degree of selectivity for unpaired guanine residues. Cobalt-induced DNA modification was catalytic with respect to the metal complex and was observed at temperatures up to 80 °C, conditions under which NiCR was ineffective. Mechanistic studies of the cobalt-mediated reaction suggest that $SO_4^{\bullet-}$ is responsible for guanine oxidation. Reaction with $tRNA^{Phe}$ induced aniline·HOAc-labile (pH 4.5) lesions also at accessible guanine sites. The high reactivities of G20 and G34 are consistent with attack of $SO_4^{\bullet-}$ on the π face of the guanine heterocycle as opposed to recognition of G N7 as proposed for NiCR. $CoCl_2$ should become an extremely attractive probe of nucleic acid structure since it induces base-specific and conformation-specific cleavage of DNA under a much wider variety of experimental conditions than NiCR, acts with a different mode of guanine selectivity than do nickel complexes, and is commercially available.

Introduction

Metal complexes that act as probes of DNA and RNA structure are a topic of continued pursuit.[1−3] The ability to probe nucleic acid structures under a wide variety of conditions that might include elevated temperatures and a broad range in ionic strength is of particular interest. For example, temperature-dependent studies have recently provided insight concerning RNA folding of ribozymes and RNA pseudoknots.[4−6] While reagents such as kethoxal,[4,5] diethylpyrocarbonate,[4−6] and permanganate[7] have shown some success at high temperature, the use of chemical or enzymatic probes under these conditions raises questions concerning reagent concentration, reactivity, and specificity and the possibility of enzyme denaturation. Our previous studies led to the development of a macrocyclic nickel complex, NiCR,[8] as a highly sensitive and predictable probe for accessible guanine residues found in a variety of DNA and RNA structures[9−13] in which there is a strong correlation between the extent of guanine modification and the exposure of N7.[9,12] While the guanine-specific modification of DNA and RNA has been extended to a variety of nickel(II) complexes,[14−16] the utility of other transition metal complexes[15] or unusual reaction conditions had only been briefly explored in our laboratories. Cobalt(II), by virtue of its similar binding characteristics to nickel(II), might also be expected to participate in guanine-specific chemistry.

Considerable evidence points to direct ligation between a cobalt ion and guanine N7 including a crystal structure of a cobalt(II) GMP complex,[17] facilitation of B → Z transitions in poly-[d(GC)] which could be attributed to enhanced exposure of G N7 in the Z-form,[18] corroboration of this latter idea by the crystal structure of $[Co(H_2O)_5]^{2+}$ ligated to G N7 in Z-DNA,[19] and a recent report of binding of $[Co(NH_3)_5(H_2O)]^{3+}$ to guanine in a Z-form oligodeoxynucleotide by loss of a labile water molecule.[20] Furthermore, cobalt complexes have found ap-

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plication in DNA chemistry in the form of a cobalt bleomycin analog,[21] $Co(NH_3)_6^{3+}$,[22−24] cobalt(III) polypyridines,[23−27] $[Co^{III}(cyclam)(H_2O)Me]^{2+}$,[28] cobalt(III) desferal,[29,30] and a cobalt(III) porphyrin[31] using photochemical activation or an oxidant such as iodosylbenzene, H_2O_2 or O_2. Often guanines were the preferred site of reaction in duplex DNA, although the reactivity was attenuated in some cases by sequence and helicity. Furthermore, guanines were indiscriminately modified when $[Co(H_2O)_6]^{2+}$ was used to promote a DNA reaction with sulfite and O_2, and Gs, Cs, and Ts were susceptible to reaction when H_2O_2 was the oxidant.[32]

Our current investigation reveals that cobalt(II) complexes, used in conjunction with $KHSO_5$, lead to guanine-specific reaction under conditions that include high temperature and catalytic metal concentrations. Furthermore, the specificity of reaction with unpaired guanine residues as found, for example, in hairpin loops is only somewhat less than that of NiCR. The reagent is also applicable to the study of guanine exposure in RNA. Importantly, this technique uses an "over-the-counter" metal catalyst, namely, $CoCl_2$.

Results and Discussion

General Observations. For initial DNA modification studies, the single-stranded oligodeoxynucleotide [5′-^{32}P]-d(ATAGTCTAGATCTGATAT), **1**, was used. The reaction at 20 °C of **1** (3 μM), $CoCl_2$ (3 μM), and $KHSO_5$ (50 μM) for 10 min followed by treatment with piperidine revealed strand scission corresponding to reactions occurring at all guanine residues (Figure 1, lane 2). This observed guanine-specific cleavage is analogous to that observed for NiCR and $KHSO_5$ (Figure 1, lane 3), although densitometry revealed that the extent of reaction for $CoCl_2$ (57%) was nearly twice that observed for NiCR (35%) under equivalent conditions. The higher reactivity of $CoCl_2$ allows its application to conditions of high temperature or low reagent concentration. For example, reactions conducted using 3 μM $CoCl_2$ and only 10 μM $KHSO_5$ revealed an overall DNA cleavage activity of 15% (Figure 1, lane 4). The ability to employ $KHSO_5$ concentrations of $\leq$10 μM is of particular importance for studies using temperatures above 50 °C at which nonspecific cleavage occurs from high concentrations of the oxidant alone. When $KHSO_5$ remained at 50 μM, it was found that cobalt-mediated DNA cleavage was very active (26% cleavage) even at concentrations of $CoCl_2$ as low as 100 nM (Figure 1, lane 6). Furthermore, the activity of $CoCl_2$ at 100 nM concentration is catalytic in nature, yielding 8 turnovers in a period of 30 min. The lack of significant DNA cleavage from analogous reactions employing NiCR concentrations less than 500 nM or oxidant concentrations less than 50 μM (Figure 1, lanes 5 and 7) is consistent with the previously reported data for NiCR-promoted DNA modification.[33]

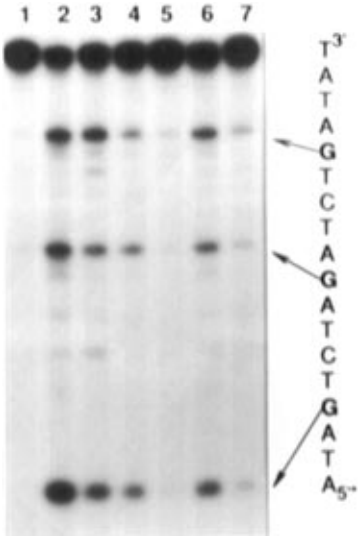

Figure 1. Autoradiogram of denaturing polyacrylamide gel (20%) of modification of **1** by $CoCl_2$ and NiCR. All reaction mixtures were dialyzed and then treated with 60 μL of 0.2 M piperidine for 30 min at 90 °C. Lane 1: control lane with 50 μM $KHSO_5$. Lane 2: 3 μM $CoCl_2$ and 50 μM $KHSO_5$. Lane 3: 3 μM NiCR and 50 μM $KHSO_5$. Lane 4: 3 μM $CoCl_2$ and 10 μM $KHSO_5$. Lane 5: 3 μM NiCR and 10 μM $KHSO_5$. Lane 6: 100 nM $CoCl_2$ and 50 μM $KHSO_5$. Lane 7: 100 nM NiCR and 50 μM $KHSO_5$.

$CoCl_2$-Induced Conformational Specificity. The guanine conformational specificity of $CoCl_2$ was investigated through the use of the hairpin-forming oligodeoxynucleotide [5′-^{32}P]-d(AGTCTATGGGTTAGACT), **2**, which was chosen because it contains both unpaired guanine residues in the loop region, G8−G10, as well as typical Watson−Crick base-paired guanine residues in the stem region, G2 and G14. A quantitative comparison of the conformation-specific cleavage of **2** employing $CoCl_2$ vs NiCR is shown in Figure 2a. In previous studies conducted with **2** and NiCR,[11] the three guanine residues located in the loop region were highly susceptible to modification, while the stem guanines were on the average 10-fold less reactive. While $CoCl_2$ also displayed a high preference for modification of guanine residues located in loop regions, the average selectivity over Watson−Crick base-paired guanine was only a factor of 4.

Temperature-Induced Conformational Changes. Although NiCR displayed a greater sensitivity toward guanine-specific cleavage of DNA, the fact that $CoCl_2$ functions under conditions of low $KHSO_5$ concentration makes it a candidate for probing temperature-induced DNA (or RNA) conformational changes. Such studies were conducted with **2** at temperatures above and below its reported T_m value of 49 °C.[11] It was anticipated that at temperatures above its T_m the reactivity of $CoCl_2$ toward G2 and G14 would increase owing to the loss of base-stacking and Watson−Crick base-pairing. When **2** was treated with $CoCl_2$ and $KHSO_5$ at 80 °C (Figure 2b), the relative reactivity of G14 increased by a factor of 2.5, while the relative reactivity of G2 near the helix terminus increased by a factor of 3.5. While the overall extent of cleavage of **2** induced by $CoCl_2$ at 80 °C vs 20 °C remained at ∼20%, the average selectivity toward loop vs stem guanine residues was quite small, a factor of only 1.4. The enhanced reactivity of G2 and G14 is

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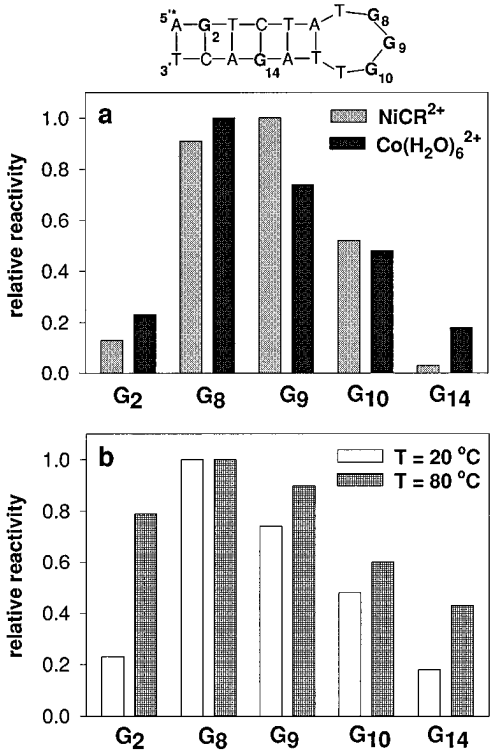

Figure 2. Comparative reactivity of a hairpin DNA with $KHSO_5$ mediated by $CoCl_2$ vs NiCR at 20 °C (a) and by $CoCl_2$ at 20 °C vs 80 °C (b). [5′-^{32}P]-**2** (3 μM) was allowed to react with 50 μM $KHSO_5$ + 3 μM NiCR at 20 °C or 10 μM $KHSO_5$ + 3 μM $CoCl_2$ at 20 or 80 °C for 10 min followed by piperidine treatment.[14] Autoradiograms were quantified by scanning densitometry. Relative reactivity represents the extent that an individual guanine residue was modified relative to the most reactive residue. Errors are estimated to be ±10% of the reported values.

consistent with the conformational denaturation anticipated for **2** at 80 °C. Thus, $CoCl_2$ is an effective probe for temperatures up to at least 80 °C while the activity of NiCR appears to diminish greatly around 50 °C (data not shown).

Sulfate Radical-Induced DNA Damage. The nature of the species responsible for the observed guanine modification may be related to the production of reactive radicals formed upon interaction of $KHSO_5$ with $[Co(H_2O)_6]^{2+}$. Marsh and Edwards have demonstrated that the decomposition of $KHSO_5$ by catalytic amounts of $CoCl_2$ leads to the production of sulfate radical, $SO_4^{\bullet-}$,[34] a species that has recently been shown to oxidize guanosine[35,36] and to modify duplex DNA with a preference for guanine.[37] In order to determine if the production of sulfate radicals was responsible for the observed reactivity of cobalt-catalyzed DNA modification, the reactivity of $SO_4^{\bullet-}$ generated independent of Co^{2+} was examined with oligodeoxynucleotides. The modification of single-stranded [5′-^{32}P]-d(ATATCAGATCTAGACTAT), **3**, induced by the photolysis of persulfate ($S_2O_8^{2-}$), a process known to yield sulfate radical,[36] revealed that strand cleavage (following piperidine treatment) occurred predominantly at guanine residues. In contrast to reactions of hydroxyl radical,[38] no DNA strand scission was observed without piperidine treatment. Furthermore, the reaction of the hairpin **2** with sulfate radical ($S_2O_8^{2-}/h\nu$) revealed that the three guanine residues located in the loop region (G8, G9, and G10) were highly modified, while the stem guanines (G2 and G14) were on the average 6-fold less reactive. This result is nearly identical to that obtained from the analogous experiment conducted with $CoCl_2$, where the preference for modification at guanine residues in the loop vs in the stem region was a factor of 4. Consequently, $SO_4^{\bullet-}$ is strongly implicated as the DNA damaging agent in the cobalt-mediated reaction. While sulfate radical is a common product of the decomposition of $KHSO_5$, especially by $[Co(H_2O)_6]^{2+}$, other radical species such as hydroxyl radical and peroxomonosulfate radical have also been postulated as products of metal-catalyzed reactions of $KHSO_5$.[39−42]

Table 1. Quenching Data for DNA Oxidations[a]

reagent	additive	% change in DNA cleavage[b]
photolysis of $K_2S_2O_8$	25 mM ethanol	−80
	50 mM ethanol	−84
	100 mM ethanol	−90
	25 mM *tert*-butyl alcohol	+21
	50 mM *tert*-butyl alcohol	+17
	100 mM *tert*-butyl alcohol	−08
$CoCl_2$[c] + $KHSO_5$[d]	25 mM ethanol	−89
	50 mM ethanol	−97
	100 mM ethanol	−95
	25 mM *tert*-butyl alcohol	+05
	50 mM *tert*-butyl alcohol	+05
	100 mM *tert*-butyl alcohol	−01
NiCR[c] + $KHSO_5$[d]	25 mM ethanol	−12
	50 mM ethanol	−24
	100 mM ethanol	−48
	25 mM *tert*-butyl alcohol	+09
	50 mM *tert*-butyl alcohol	+03
	100 mM *tert*-butyl alcohol	+03

[a] All reactions were conducted with the single-stranded oligodeoxynucleotide, **4**, d(ATATCAGATCTAGACTAT) in 3 μM concentration and were piperidine treated for strand scission. [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] [NiCR] and $[CoCl_2] = 3$ μM. [d] $[KHSO_5] = 50$ μM.

In order to gain further evidence for sulfur oxyradicals vs hydroxyl radical in our observed DNA modification, alcohol quenching studies were performed, and the results are summarized in Table 1. It has been previously shown that while sulfate radical reacts with alcohols containing an α-hydrogen, peroxomonosulfate radical ($SO_5^{\bullet-}$) is relatively inert toward alcohols.[43] In addition, although *tert*-butyl alcohol is an effective quenching agent for hydroxyl radical it reacts approximately 1000-fold slower with sulfate radical.[43,44] Accordingly, 25 mM ethanol was added to reactions of the single-stranded oligonucleotide **3**, $CoCl_2$, and HSO_5^-, and the observed oxidation of DNA was reduced by 89%. The use of either 50 or 100 mM ethanol in comparable experiments led to ≥95% reduction in the extent of DNA modification. Analogous experiments conducted with *tert*-butyl alcohol led to essentially no reduction in reactivity, in fact using 25 and 50 mM *tert*-

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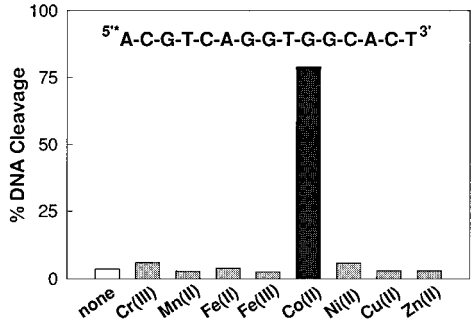

Figure 3. Comparative reactivity of a single-stranded DNA sequence induced by various transition metal salts. [5′-^{32}P]-**3** (3 μM) was allowed to react with 60 μM $KHSO_5$ and 3 μM of either $CrCl_3$, $Mn(H_2O)_6Cl_2$, $(NH_4)_2[Fe(H_2O)_6](SO_4)_2$, $FeCl_3$, $[Co(H_2O)_6]Cl_2$, $[Ni(H_2O)_6]Cl_2$, $Cu(H_2O)_2Cl_2$, or $ZnCl_2$ at 20 °C for 30 min. Autoradiograms were quantified by scanning densitometry in which the concentration ratio of cleavage products to total strand is reported.

Scheme 1

$$[Co^{II}(H_2O)_5L]^{2+} + HSO_5^- \longrightarrow [Co^{III}(H_2O)_4(OH)L]^{2+} + SO_4^{\bullet -} + 2H^+$$

L = H_2O or G N7 of DNA

$$[Ni^{II}CR]^{2+} + HSO_5^- \longrightarrow [Ni^{III}CR(L)(SO_4)]^{2+} + OH^-$$

L = G N7 of DNA

butyl alcohol resulted in a slight increase in reactivity (5%), possibly due to a solvent effect. Quenching experiments conducted using photolytically produced sulfate radical revealed that an 80% reduction in the extent of modification was obtained with the addition of 25 mM ethanol. The use of 25 mM *tert*-butyl alcohol resulted in a moderate increase (21%) in the extent of sulfate radical-mediated DNA modification. While the observed quenching data for $CoCl_2$-induced DNA modification appear to be consistent with the production of a highly diffusible sulfate radical ($SO_4^{\bullet -}$) rather than peroxomonosulfate radical ($SO_5^{\bullet -}$) or hydroxyl radical ($HO^{\bullet}$), it is interesting to note that the results obtained for NiCR are quite different. Specifically, when 25 mM ethanol was added to a comparable NiCR-induced DNA oxidation, the extent of modification decreased only 12%, while the addition of 100 mM ethanol resulted in a 48% reduction. These data may suggest that while reactions involving $CoCl_2$ produce a highly diffusible sulfate radical, reactions employing NiCR may involve an intermediate in which nickel is ligated to $SO_4^{\bullet -}$, effectively producing a caged sulfate radical which may be shielded from reaction with ethanol (Scheme 1). The extent to which cobalt is coordinated to G N7 prior to guanine oxidation is yet to be determined but is not a prerequisite for conformational specificity.

Metal and Ligand Dependence of DNA Modification. To investigate further the metal dependency of the observed DNA chemistry, the modification of a single-stranded oligodeoxynucleotide [5′-^{32}P]-d(ACGTCAGGTGGCACT), **4**, by $KHSO_5$ in the presence of various first-row transition metal salts was examined (Figure 3). Only $CoCl_2$ displayed significant reactivity when compared to the background level of oxidation, which is consistent with the observation that Co^{2+} is the most effective transition metal ion for catalytic decomposition of $KHSO_5$.[45] The simple metal salt $CoCl_2$ exists as $[Co(H_2O)_6]^{2+}$ in aqueous solutions, and its higher oxidation state, cobalt(III), is extremely unstable at pH 7. The reactivity of other cobalt(II) complexes[8] (Figure 4) was investigated with [5′-^{32}P]-d(ATATCAGATCTAGACTAT), **3**, and studies revealed that the macrocyclic complexes $[CoCT](ClO_4)_2$ and $[Co(DAPBH)(H_2O)(NO_3)]NO_3$ displayed the same reactivity as $[Co(H_2O)_6]^{2+}$. In contrast,

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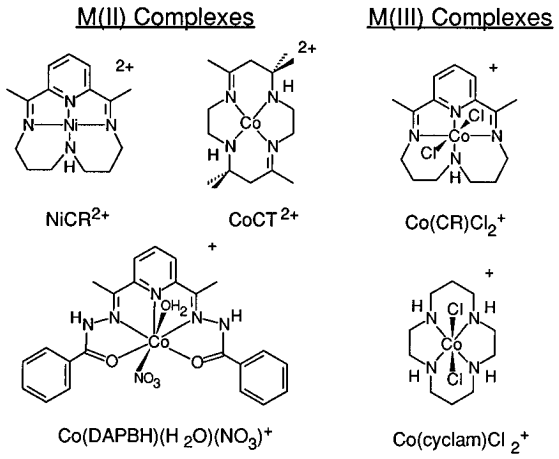

Figure 4. Macrocyclic nickel and cobalt complexes used in DNA and RNA studies.

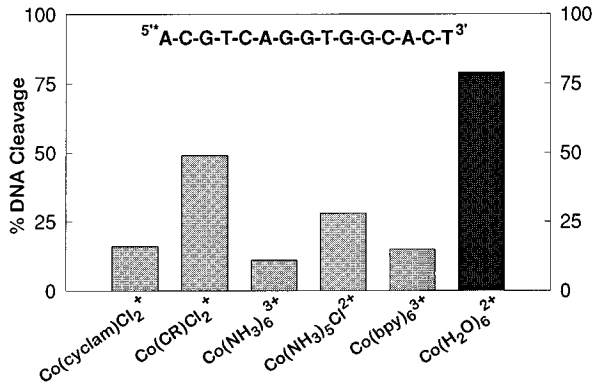

Figure 5. Relative reactivities of $Co(H_2O)_6^{2+}$ and various cobalt(III) complexes in the HSO_5^- oxidation of the single-stranded oligodeoxynucleotide **4**. All reactions employed 3 μM metal complex, 60 μM $KHSO_5$, and 3 μM **4** and were piperidine treated. Reactivities are reported as percent of total guanine reaction vs total strand concentration.

when cobalt(III) coordination complexes[8] were employed in an analogous study with **4**, the resulting order of reactivity leading to DNA cleavage was $[Co(H_2O)_6]Cl_2 \gg [Co(CR)Cl_2]Cl > [Co(NH_3)_5Cl]Cl_2 > [Co(cyclam)Cl_2]Cl > [Co(bpy)_3](ClO_4)_3 > [Co(NH_3)_6](ClO_4)_3$ (Figure 5). This observed trend for cobalt is in sharp contrast to the case of nickel, where $[Ni(H_2O)_6]^{2+}$ is inactive in our assay but the presence of tetraazamacrocyclic ligands activates nickel to promote DNA modification.[10,14,15] The inability of ligands to affect the reactivity for cobalt(II) may be related to the known lability of cobalt(II) complexes,[46] leading to the formation of a significant concentration of $[Co(H_2O)_6]^{2+}$ that acts as the DNA modification agent. The surprising inactivation by ligands associated with cobalt(III) complexes may be explained by the fact that these complexes do not have sufficiently high redox potentials to act as oxidants of HSO_5^-. A cobalt(III) species possessing a high reduction potential is crucial in the catalytic decomposition of HSO_5^-, for which it has been proposed that HSO_5^- is oxidized to peroxomonosulfate radical, $SO_5^{\bullet -}$ (eqs 1−6, from Edwards and co-workers[34,47]). The potential associated with oxidation of HSO_5^- to peroxomonosulfate radical has been estimated at 1.1 V (vs NHE),[43] while the reported cobalt(III) complexes possess $Co^{III/II}$ reduction potentials $\ll$1.0 V (vs NHE).

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$$Co^{2+} + H_2O \rightleftarrows CoOH^+ + H^+ \quad (1)$$

$$CoOH^+ + HSO_5^- \rightarrow Co^{III}O^+ + H_2O + SO_4^{\bullet -} \quad (2)$$

$$Co^{III}O^+ + 2H^+ \rightleftarrows Co^{3+} + H_2O \quad (3)$$

$$Co^{3+} + HSO_5^- \rightarrow Co^{2+} + H^+ + SO_5^{\bullet -} \quad (4)$$

$$2SO_5^{\bullet -} \rightleftarrows {}^-O_3SOOOOSO_3^- \quad (5)$$

$${}^-O_3SOOOOSO_3^- \rightarrow O_2 + 2SO_4^{\bullet -} \quad (6)$$

$CoCl_2$-Induced Modification of RNA. The conformational specificity of $CoCl_2$ toward RNA was investigated with the well-characterized RNA substrate, $tRNA^{Phe}$ (Figure 6). Native [5′-^{32}P]$tRNA^{Phe}$ was oxidized with $CoCl_2$ and $KHSO_5$ and subsequently treated with aniline·HOAc (pH 4.5) to produce strand scission at G10,[48] G18, G19, G20, and G34 predominantly (Table 2). While the sites of modification are consistent with those previously observed in the presence of NiCR,[12] the relative reactivities of G18, G19, G20, and G34 are significantly different. For NiCR the order of reactivity associated with $tRNA^{Phe}$ reaction was G19 ≫ G18 ∼ G20 ∼ G34, and this trend closely parallels the exposure and local electrostatics of guanine N7s.[12] Inspection of the crystal structure[49] of $tRNA^{Phe}$ (Figure 7) further demonstrated that the accessibility of guanine N7 determines the extent of reactivity induced by NiCR, since only G19 is well positioned with its N7 lone pair directed outward from the surface of the molecule. These observations support a mechanism in which the determining factor of guanine modification with NiCR is the ligation of nickel to N7,[9] possibly at the nickel(III) stage where hexacoordination is favored. In this case, one of the other ligands might be $SO_4^{\bullet -}$. This is also consistent with radical quenching studies that suggest that reaction between NiCR, $KHSO_5$, and DNA generates a caged sulfate radical. On the other hand, the trend in reactivity observed for $CoCl_2$ with $tRNA^{Phe}$ was G34 > G20 > G18 ∼ G19. Since the reactivity trend with $CoCl_2$ does not correlate well with the surface accessibility of N7, the ligation of Co^{2+} to guanine, via N7, may not be a crucial factor in the observed modification pattern. Once again, examination of the crystal structure (Figure 7) reveals that while G19 has the greatest surface accessibility of N7, G34 appears to have high exposure of the purine ring, which lies parallel to the surface of the molecule. Furthermore, G20 also has an accessible purine ring, while G18 and G19 appear more shielded. This correlation between purine ring surface exposure and reactivity toward $CoCl_2$ is consistent with the reactive species being a freely diffusible sulfate radical, since radical attack on guanine is expected to have a dependency on the surface exposure of the entire guanine heterocycle and not on exposure of a particular ligating atom. Studies with deoxynucleosides show that $SO_4^{\bullet -}$ reacts about 10-fold faster with guanosine than with any other base, and that H$^\bullet$ abstraction from deoxyribose is 100-fold slower than deoxyguanosine reaction.[43] Consistent with those studies, guanines are the preferred site of reaction with the $CoCl_2/HSO_5^-$ system, and no direct strand scission arising from hydrogen atom abstraction from the ribose unit is observed.

Chemical modification of $tRNA^{Phe}$ was also carried out in the absence of Mg^{2+} in which the RNA structure is partially denatured. For both NiCR and $CoCl_2$-induced oxidations, more G sites (*e.g.* G15, G22, G24, G26) become reactive under these conditions (Table 2), and the relative reactivities of these sites are more equal. These observations are entirely consistent with

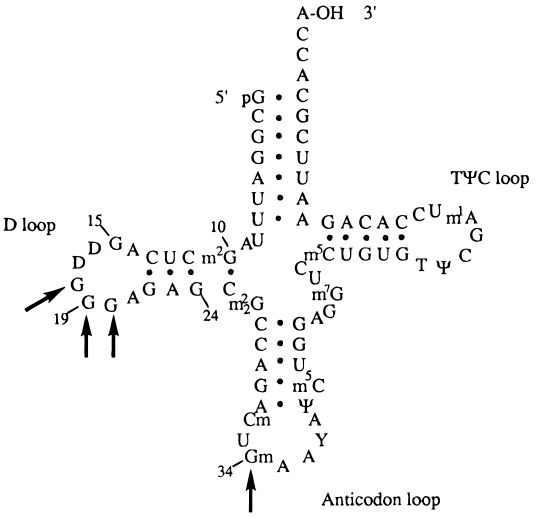

Figure 6. Secondary structure of $tRNA^{Phe}$. Arrows indicate principal reaction sites for NiCR and $CoCl_2$-mediated oxidations of the native form.

Table 2. Relative Reactivity of Individual Guanines in $tRNA^{Phe}$ for NiCR and $CoCl_2$-Promoted Oxidation Reactions[a]

	NiCR		$CoCl_2$	
reaction site	$-Mg^{2+}$	$+Mg^{2+}$	$-Mg^{2+}$	$+Mg^{2+}$
G34	1.3	0.2		6.9
G26	0.6		2.3	
G24	0.3			
G22			0.4	
G20	1.0	0.2	0.6	3.2
G19	1.0	1.0	1.0	1.0
G18	0.5	0.3	2.0	1.7
G15	0.6			

[a] Reaction conditions: [NiCR] and [$CoCl_2$] = 3 μM; [$KHSO_5$] = 100 μM; [NaCl] = 100 mM; [NaPi] 10 mM (pH 7.0). G19 was chosen as the reference for relative reactivities within each experiment.

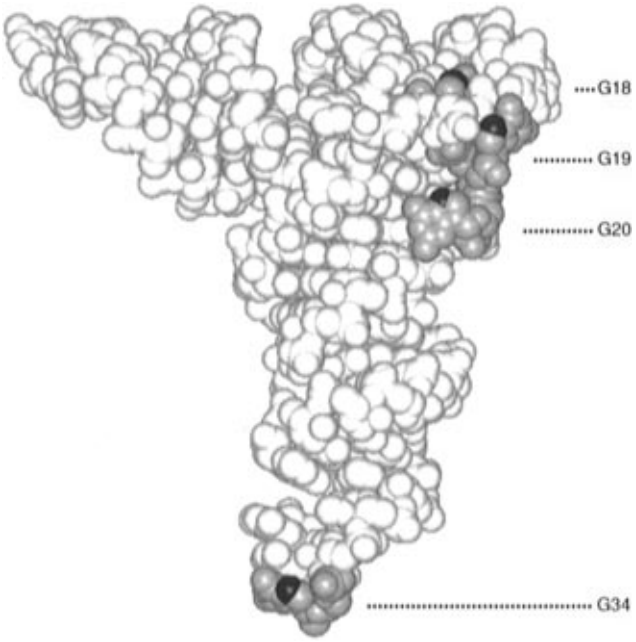

Figure 7. Three-dimensional structure of $tRNA^{Phe}$ and location of reactive guanine residues. The refined crystallographic coordinates[49] were modeled with the Biosym software package. G18, G19, G20, and G34 are shown in gray, and the positions of N7 are shown in black.

(48) Modification of N2-methyl G10 was observed to occur spontaneously in the presence of $KHSO_5$.

(49) Westhof, E.; Dumas, P.; Moras, D. *Acta Crystallogr.* **1988**, *A44*, 112−123.

the proposed mechanisms since both the purine face and N7 will become more exposed to reagents upon denaturation.

Conclusions

Modification of DNA employing $CoCl_2$ and $KHSO_5$ has been shown to be catalytic in metal ion, and specific for guanine residues with a preference for those guanines exposed in single-stranded sequences. Overall, the site specificity of $CoCl_2$ vs NiCR differs in two ways. $CoCl_2$ is more reactive and slightly less selective than NiCR allowing its application to more demanding reaction conditions. Importantly, the chemical mechanism and mode of guanine recognition appear to differ between $CoCl_2$ and NiCR. While NiCR recognizes exposure of N7, $CoCl_2$ is sensitive to the exposure of the face of the purine ring. The difference in the mode of molecular recognition is attributed to the proposed mechanisms in which free $SO_4^{\bullet-}$ (cobalt-induced) vs metal-coordinated $SO_4^{\bullet-}$ (nickel-induced) reactive intermediates are responsible for guanine oxidation. This argument is substantially strengthened by the observed similarities between DNA reactions of photolytically generated $SO_4^{\bullet-}$ and the cobalt/HSO_5^- system, and both are in contrast to the NiCR-mediated DNA chemistry.

Together, these reagents provide a complementary set of probes for the local structure of guanine in folded nucleic acids. Furthermore, the ability to use reduced concentrations of $KHSO_5$ and elevated temperatures allows $CoCl_2$ to probe temperature-induced conformational changes. While it is true that photolytically generated $SO_4^{\bullet-}$ is an equivalent reagent to $CoCl_2$/HSO_5^- at room temperature, the latter is more accurately and reproducibly dosed in most laboratory settings than is $S_2O_8^{2-}$/$h\nu$. This assay may find utility in mapping temperature-dependent RNA folding,[4−6] and drug−DNA or protein−DNA interactions.

Experimental Section

Materials. The oligodeoxynucleotides d(ATAGTCTAGATCTGATAT), d(AGTCTATGGGTTAGACT), d(ACGTCAGGTGGCACT), and d(ATATCAGATCTAGACTAT) were purchased from Oligos Etc. Inc. and purified to homogeneity under strongly denaturing conditions (pH 12) using anion exchange chromatography (Mono Q, Pharmacia).[11] $tRNA^{Phe}$ was obtained from Sigma and prepared as previously described.[12] T4 kinase was purchased from BRL, and γ-[^{32}P]-ATP (3000 Ci/mmol) was purchased from Amersham. Potassium monopersulfate (Oxone) and potassium persulfate were purchased from Aldrich Chemical Co. $CoCl_2 \cdot (H_2O)_6$ was purchased from Fisher Scientific. All aqueous solutions utilized purified water (Nanopure, Sybron/Barnsted) and reagents of the highest commercial quality. Chemicals used for the synthesis of cobalt and nickel complexes were of reagent grade and used without further purification.

Caution! While we have used perchlorate as a counterion with a number of nickel and cobalt complexes 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. These complexes, as well as any other perchlorate salt, should only be handled in small quantities.

The following complexes were prepared by standard procedures: $[Ni(CR)](ClO_4)_2$,[50] $[Co(CR)Cl_2]Cl$,[51] $[Co(cyclam)Cl_2]Cl$,[52] $[CoCT](ClO_4)_2$,[53] and $[Co(DAPBH)(H_2O)(NO_3)](NO_3)$.[54] $[Co(NH_3)_5Cl]Cl_2$, $[Co(bpy)_3](ClO_4)_3$, and $[Co(NH_3)_6](ClO_4)_3$ were a gift of Prof. A. Haim (Stony Brook).

Metal-Dependent Modification of DNA. DNA experiments were conducted as previously described.[14] Reaction mixtures (50 μL) contained 3 μM unlabeled oligodeoxynucleotide, 2 nCi labeled oligodeoxynucleotide, 100 nM or 3 μM concentrations of the desired metal complex, 10 to 60 μM $KHSO_5$, 100 mM NaCl, and 10 mM sodium phosphate (pH 7.0).

Sulfate Radical Modification of DNA. Reaction mixture (50 μL) containing 3 μM unlabeled oligodeoxynucleotide, 2 nCi labeled oligodeoxynucleotide, 1 mM K_2SO_8, 100 mM NaCl, and 10 mM sodium phosphate (pH 7.0) were illuminated with a 254-nm (6 W) UV lamp (UVP) at a distance of 12 cm for 10 min. Samples were rotated with the use of a motorized carousel to ensure uniform radiation. Sample workup was conducted as for the metal-dependent reactions described above.

Metal-Dependent Modification of RNA. RNA experiments were conducted as previously described.[12] Reaction mixtures contained 12 μg of carrier tRNA, 10 nCi labeled tRNA, 3 μM either NiCR or $CoCl_2$, 100 μM $KHSO_5$, 0 to 50 mM $MgCl_2$, 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 either a Enprotech scanner or a Beckman DU 650 spectrophotometer.

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Supporting Information Available: Autoradiogram of NiCR and $CoCl_2$-mediated reactions of $tRNA^{Phe}$ (1 page). This material is contained in many libraries on microfiche, immediately follows this article on the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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