Ligand Effects Associated with the Intrinsic Selectivity of DNA Oxidation Promoted by Nickel(II) Macrocyclic Complexes

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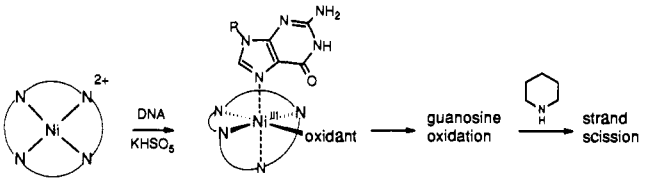
Abstract: Nickel(II)-promoted oxidation of accessible guanine residues in deoxyoligonucleotides using $KHSO_5$ as oxidant was found to be highly dependent upon the macrocyclic ligand employed. Systematic structural variations of the ligands Me_2[14]py-dieneN_4 (CR) and [14]aneN_4 (cyclam) have provided information about the importance of ring size, degree of unsaturation, steric bulk, redox potential, in-plane ligand field strength, and conformational flexibility in determining DNA reactivity with a 17-base hairpin oligonucleotide Reactions were monitored by analyzing DNA fragments produced after alkaline-induced strand scission. Certain nickel(II) complexes, such as $NiTMPP^{4+}$ (TMPP = tetra(*N*-methylpyridyl)porphyrin), $Ni(phen)_3^{2+}$, and $Ni(phen)_2^{2+}$, were unreactive. Tetraazamacrocycles providing strong in-plane coordination by amine, imine, or pyridine donors gave the optimum characteristics for DNA reactivity as long as the complex was sufficiently flexible to adapt to DNA binding. These features now define the important criteria for design of nickel-based reagents as structural probes of nucleic acids.

Introduction

Site-specific recognition and cleavage of DNA using coordination compounds continues to be the subject of considerable interest in our laboratories and elsewhere.[1-4] Nickel(II) complexes draw particular attention due to the environmental toxicity and carcinogenic nature of certain nickel(II) compounds[5,6] and the chemotherapeutic properties of other group VIII transition metal complexes.[7-10] Nickel toxicity appears to involve oxidative chemistry of chromatin resulting in depurination of DNA, DNA strand breaks, and DNA–protein cross-links.[5,11-14] Recently, the square planar nickel(II) complex NiGGH tethered to a DNA-binding protein fragment was shown to induce site-specific cleavage of duplex DNA through the use of monoperoxyphthalic acid (MMPP) as oxidant followed by alkaline treatment to give strand scission.[15] The site-specific strand modification near the binding domain of the metalloprotein was attributed to formation of a nondiffusible high valent nickel species.

Our current studies focus on the intrinsic reactivity of a series of simple nickel(II) square planar complexes which, in the presence of potassium monopersulfate ($KHSO_5$) or MMPP, induce guanine-specific modification of single-stranded oligonucleotides leading to strand scission after treatment with piperidine.[1] For duplex DNA, the Schiff base complex [Ni(Me_2[14]py-diene-N_4)]$(ClO_4)_2$ (**5**) displayed high conformational specificity for freely accessible guanine residues such as those found in oligonucleotides containing mispaired, bulged, looped, or terminal guanosine residues.[2] Strong dependence on the nature of the nickel complex employed was observed, and initial studies suggested the important factors to be (i) the availability of vacant coordination sites, (ii) the overall charge of the coordination complex, and (iii) the redox potential associated with the Ni(III)/(II) redox couple.

Scheme I



Upon the basis of these observations, the specificity for accessible guanine residues and the well documented preference for nickel(II) ions to bind to N7 of guanine,[16] a mechanism has been postulated involving direct ligation of nickel to guanine followed by base oxidation (Scheme I).[1,2] The key intermediate proposed is an octahedral nickel(III) species in which the two additional ligands are guanine and an oxidant, possibly monopersulfate. It is attractive to propose cis coordination of guanine and the oxidant since the subsequent reductive elimination of these groups would seem facile and might result in the formation of an alkaline-labile site such as 8-oxoguanine.

In order to further explore the structural and mechanistic aspects associated with the highly site- and conformation-specific cleavage of DNA a systematic study of ligand effects was undertaken. Such studies, as reported herein, are consistent with the mechanism proposed in Scheme I. Importantly, the studies now allow us to predict the necessary features of nickel complexes for the design of structural probes or therapeutic agents that would entail oxidative modification of nucleic acids. The macrocyclic ligands used are shown in Figure 1. These complexes represent modification of the ring size, degree of unsaturation, steric bulk,

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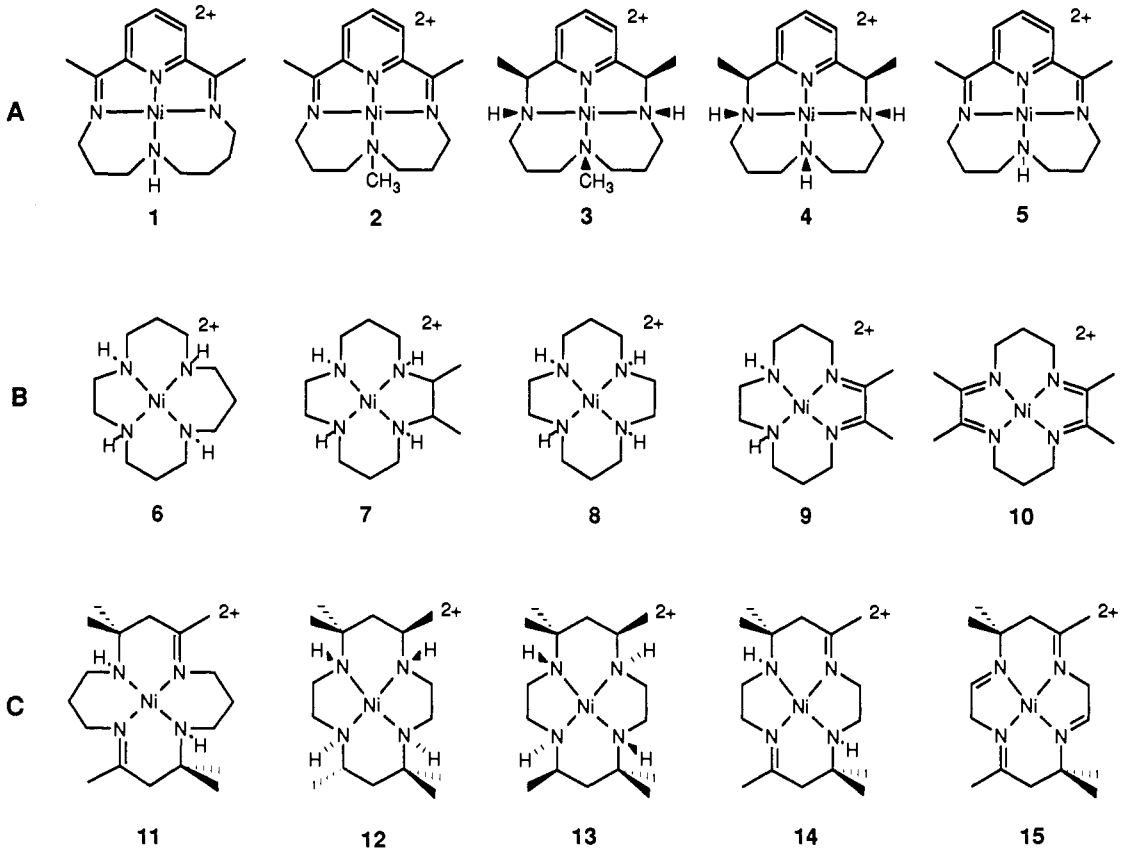

Figure 1. Nickel(II) macrocyclic complexes used in the study of ligand effects associated with the oxidation of DNA.

redox potential, and conformational flexibility of the ligands Me_2[14]py-dieneN$_4$ (CR) and [14]aneN$_4$ (cyclam), which were previously shown to induce guanosine-specific oxidation of oligonucleotides.[1,2]

Experimental Section

1. Materials. The oligonucleotide, d(AGTCTATGGGTTAGACT), which has been previously shown to form a unimolecular hairpin containing five unpaired bases (-TGGGT-) in the loop,[2] was purchased from Clontech Laboratories, Inc., and then purified to homogeneity under strongly denaturing conditions (pH 12) using anion exchange chromatography (Mono Q, Pharmacia).[2] T4 kinase was purchased from BRL, and γ-[^{32}P]-ATP (3000 Ci/mmol) was purchased from Amersham. Potassium monopersulfate (Oxone) was purchased from Aldrich Chemical Co. All aqueous solutions utilized purified water (Nanopure, Sybron/Barnsted) and reagents of the highest commercial quality. Chemicals used for the synthesis of the nickel(II) complexes were of reagent grade and used without further purification. The following nickel(II) complexes were prepared by standard procedures: [Ni(Me_2[15]py-dieneN$_4$](ClO$_4$)$_2$ (**1**),[17] [Ni(Me_3[14]py-dieneN$_4$](ClO$_4$)$_2$ (**2**),[18] [Ni(α-*meso*-Me_2[14]py-aneN$_4$](ClO$_4$)$_2$ (**4**),[19] [Ni(Me_2[14]py-dieneN$_4$](ClO$_4$)$_2$ (**5**),[19] [Ni[15]aneN$_4$](ClO$_4$)$_2$ (**6**),[20] [Ni(Me_2[14]aneN$_4$](ClO$_4$)$_2$ (**7**),[20] [Ni[14]aneN$_4$](ClO$_4$)$_2$ (**8**),[21] [Ni(Me_2[14]1,3-dieneN$_4$)](ZnCl$_4$) (**9**),[22] [Ni(Me_4[14]1,3,8,10-tetraeneN$_4$)](ZnCl$_4$) (**10**),[22] [Ni(Me_6[16]4,12-dieneN$_4$](ClO$_4$)$_2$ (**11**),[23] [Ni(*meso*-Me_6[14]aneN$_4$](ClO$_4$)$_2$ (**12**),[22] [Ni(α-*rac*-Me_6[14]aneN$_4$](ClO$_4$)$_2$ (**13**),[22] [Ni(Me_6[14]4,11-dieneN$_4$](ClO$_4$)$_2$ (**14**),[24] [Ni(Me_6[14]1,4,8,11-tetraeneN$_4$)](ClO$_4$)$_2$ (**15**).[25] The complex [Ni(α-*meso*-Me_3[14]py-aneN$_4$](ClO$_4$)$_2$ (**3**) was prepared by the same procedure used for the synthesis of [Ni(α-*meso*-Me_2[14]py-aneN$_4$]-(ClO$_4$)$_2$ (**4**).

2. Electrochemical Experiments. Cyclic voltammetric experiments were carried out at room temperature using a 0.3 M Na_2HPO_4 (pH 2.0) aqueous buffer. A glassy carbon working electrode or a gold working electrode, a platinum auxiliary electrode, and a Ag/AgCl reference electrode (all from Bioanalytical Systems) were used for electrochemical experiments. Prior to use, the working electrode was polished for 1 min using a 27/8 in. polishing cloth (Buehler Ltd.) and polishing alumina (Bioanalytical Systems), followed by sonication in water for 30 s. Voltammetric experiments were conducted with a BAS 100B electrochemical analyzer.

3. Preparation of Oligonucleotides. The concentrations of the oligonucleotide stock solutions were determined from the absorbance at 260 nm and the corresponding extinction coefficient (ϵ) estimated from the sum of the nucleotide absorptivities as affected by the adjacent bases.[2] The oligonucleotide sequence was labeled at the 5′ terminus with [^{32}P] using T4 kinase and γ-[^{32}P]ATP.[1,2]

4. DNA Cleavage Experiments. The reaction mixtures (100 μL) contained 3 μM (strand concentration) unlabeled oligonucleotide, 2 nCi labeled oligonucleotide, 3 μM of the desired nickel(II) complex, 60 μM $KHSO_5$, 100 mM NaCl, and 10 mM potassium phosphate (pH 7.0). This mixture was maintained at ambient conditions and quenched after 30 min with 20 mM Na_2SO_3. Samples were then individually dialyzed against 1 mM EDTA pH 8 (2 × 3 h) and water (1 × 12 h), lyophilized, treated with 60 μL 0.2 M piperidine for 30 min at 90 °C, lyophilized, treated with 20 μL water, lyophilized again, and then resuspended in 80% formamide containing 0.1% xylene cyanole and bromophenol blue. The DNA product fragments were analyzed using 20% polyacylamide gel electrophoresis under denaturing conditions (7 M urea) and identified by autoradiography using Kodak X-Omat AR5 film. Quantification of the DNA product fragments was accomplished using an Intertechnique Liquid Scintillation Spectrometer (Model SL30) by Cerenkov counting of the resulting dried polyacrylamide gels. Percentage of DNA oxidation was calculated from the ratio of radioactivity above background associated with the oxidation of G_8, G_9, and G_{10} versus the total radioactivity.

Results and Discussion

General Observations. In previous work, the ability of nickel(II) complexes to promote the oxidation of DNA was observed to be highly ligand dependent.[1] Certain complexes were found to be unreactive, notably those with a coordination number of 6 (coordinatively saturated) and those derived from anionic ligands such that the charge on the nickel(II) complex was neutral or

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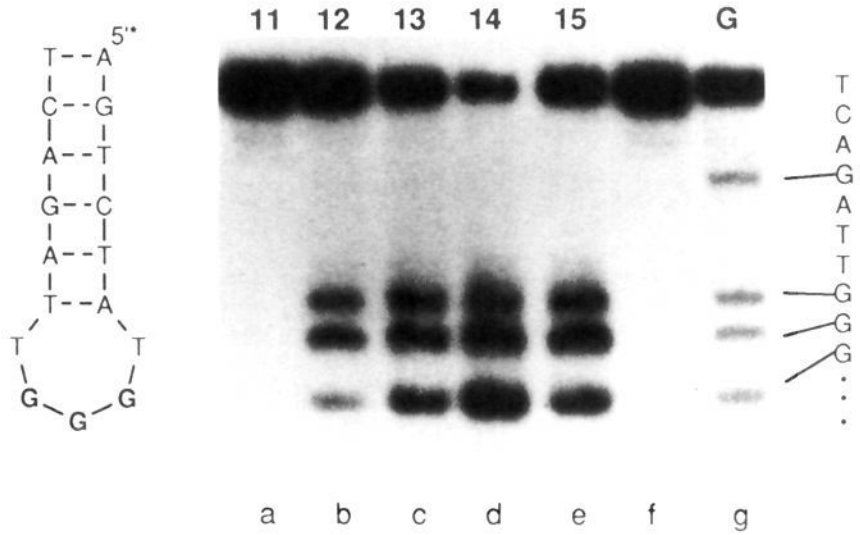

Figure 2. Characterization of hairpin DNA oxidation by complexes **11–15**. The 5′-end labeled hairpin oligonucleotide was allowed to react with $KHSO_5$ in the presence of various nickel complexes followed by treatment with piperidine as described in the Experimental Section. After gel electrophoresis and autoradiography, the following lanes were visualized. Lanes a–e: complexes **11–15**, respectively. Lane f: control lane with no nickel complex, $KHSO_5$, or Na_2SO_3. Lane g: Maxam–Gilbert sequencing lane to locate G's.[48]

Table I. Spectroscopic, Electrochemical, and DNA Reactivity Data for Nickel(II) Macrocyclic Complexes

complex	% DNA oxidation[a]	$E_{1/2}$[b] (V)	ΔE_p[c] (mV)	(Dq^{xy})[d] (cm^{-1})
$[Ni(Me_2[15]py\text{-}dieneN_4)](ClO_4)_2$ (**1**)	3 ± 1	1.25[e]	198	1188[h]
$[Ni(Me_3[14]py\text{-}dieneN_4)](ClO_4)_2$ (**2**)	5 ± 1	1.12	85	
$[Ni(\alpha\text{-}meso\text{-}Me_3[14]py\text{-}aneN_4)](ClO_4)_2$ (**3**)	8 ± 1	0.89	76	
$[Ni(\alpha\text{-}meso\text{-}Me_2[14]py\text{-}aneN_4)](ClO_4)_2$ (**4**)	42 ± 2	0.78	83	1398
$[Ni(Me_2[14]py\text{-}dieneN_4)](ClO_4)_2$ (**5**)	46 ± 1	0.98	91	1866
$[Ni([15]aneN_4)](ClO_4)_2$ (**6**)	1 ± 1	0.85	96	1202
$[Ni(Me_2[14]aneN_4)](ClO_4)_2$ (**7**)	11 ± 1	0.63	90	1414
$[Ni([14]aneN_4)](ClO_4)_2$ (**8**)	6 ± 1	0.58	86	1418
$[Ni(Me_2[14]1,3\text{-}dieneN_4)](ZnCl_4)$ (**9**)	17 ± 1	0.79	95	1553
$[Ni(Me_4[14]1,3,8,10\text{-}tetraeneN_4)](ZnCl_4)$ (**10**)	23 ± 1	1.04	80	1767
$[Ni(Me_6[16]4,12\text{-}dieneN_4)](ClO_4)_2$ (**11**)	0 ± 1	*f*		1223
$[Ni(meso\text{-}Me_6[14]aneN_4)](ClO_4)_2$ (**12**)	26 ± 2	0.78	113	1399
$[Ni(\alpha\text{-}rac\text{-}Me_6[14]aneN_4)](ClO_4)_2$ (**13**)	51 ± 3	0.89	360	1399[h]
$[Ni(Me_6[14]4,11\text{-}dieneN_4)](ClO_4)_2$ (**14**)	73 ± 1	0.92[g]	497	1569
$[Ni(Me_6[14]1,4,8,11\text{-}tetraeneN_4)](ClO_4)_2$ (**15**)	52 ± 1	1.08	110	1799[h]

[a] Percentage of DNA oxidation was calculated from the ratio of radioactivity above background associated with the oxidation of G_8, G_9, and G_{10} versus the total radioactivity. Each value represents the mean ± the standard error of three independent experiments. [b] Conditions: 0.3 M NaH_2PO_4 supporting electrolyte, pH 2.0; glassy carbon working electrode; Ag/AgCl reference electrode; scan rate = 100 mV/s. $E_{1/2} = (E_{p,a} + E_{p,c})/2$. [c] ΔE_p = peak separation. [d] Dq^{xy} values were obtained from ref 20. [e] Scan rate = 1 V/s. [f] $E_{1/2} > 1.3$ V. [g] Gold working electrode. [h] Dq^{xy} values were estimated from comparison to similar complexes.

negative. Others showed varying reactivity. Specifically, the reactions involving complexes **5**, **8**, or **9** displayed guanosine specific oxidation of DNA. The extent of reactivity was shown to increase as follows, **8** < **9** < **5**, and was thought to be correlated to the $Ni^{III/II}$ redox potential. Potassium monopersulfate, $KHSO_5$, is a strong oxidant in water with a potential near 2 V,[26] sufficiently high to cause oxidation of essentially all Ni^{II} complexes. The dependence of the DNA reaction on redox potential was consistent with the notion that a Ni^{III} species acts as the oxidant, and those complexes with higher potentials represent the less stable, or more reactive, Ni^{III} complexes.

In the present studies, the hairpin oligonucleotide d(AGTCT-ATGGGTTAGACT) was chosen for study since the three G residues of the loop, G_{8-10}, were found to be highly reactive toward nickel(II) complexes while G_2 and G_{14}, normally present as GC pairs, were unreactive.[2] This substrate allowed evaluation of both the reactivity and the specificity of the nickel complexes. If the specificity for unpaired G's changed from one nickel complex to the next, it would signify a change in mechanism. Figure 2 shows a schematic diagram of the hairpin and a typical autoradiogram obtained from treatment of the oligonucleotide with a subset of the nickel complexes (**11–15**) and monopersulfate followed by reaction with piperidine to induce strand scission. The complexes **1–15** represent the systematic modification of various ligand parameters, and their preparation, coordination chemistry, and properties have already been well-described by Busch, Barefield, and others.[19,27-34] This series of complexes showed a range of reactivities with DNA. In all cases, the high selectivity for reaction at only the accessible G's of the loop region was retained. We therefore assume that all of the nickel complexes are operating by approximately the same mechanism. Also, among the three G residues of the loop, reactivity was similar within a few percent. The percent DNA oxidation at the G_{8-10} sites is given for all complexes in Table I.

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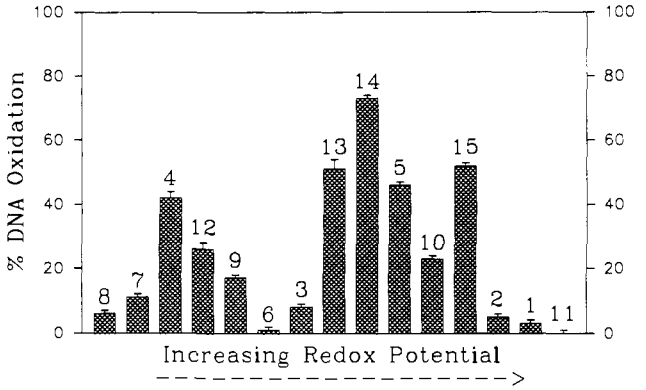

Figure 3. Percentage of DNA oxidation promoted by complexes **1–15** as a function of redox potential. Error bars represent the standard error of three independent experiments.

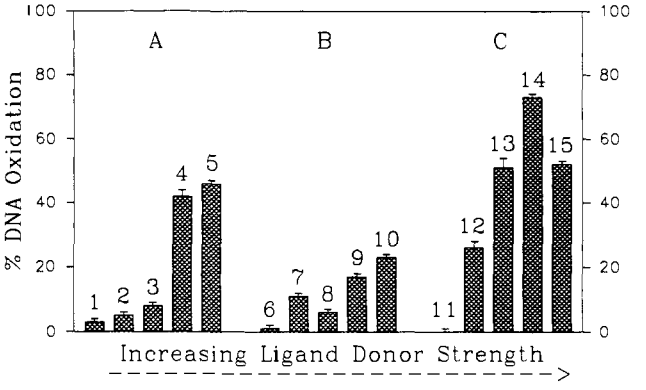

Figure 4. Percentage of DNA oxidation promoted by complexes **1–15** as a function of ligand donor strength grouped by series of related structures. Error bars represent the standard error of three independent experiments.

Redox Potential. Early indications suggested the importance of the $Ni^{III/II}$ redox couple in DNA reactivity with $KHSO_5$.[1,2] If the reduction potential were the predominant factor in determining the extent of reactivity, one would anticipate good correlation between the two. The $Ni^{III/II}$ potentials of complexes **1–15** were measured under aqueous conditions at pH 2 where the cyclic voltammograms showed good reversibility and where H_2O allows a substantially wide solvent window. The $E_{1/2}$ values vs Ag/AgCl are shown in Table I. The oxidation of square planar nickel(II) complexes yields solvent-coordinated octahedral nickel(III) species and therefore their stability is highly solvent dependent. Still, the redox couples obtained in aqueous solution show trends similar to those previously reported in CH_3CN.[35] When the percentages of DNA oxidation promoted by complexes **1–15** are arranged in order of increasing redox potential (Figure 3), the correlation is poor. However, trends can be discerned. Up to a point, generally higher reactivity is observed for complexes with high $E_{1/2}$ values (**13–15**) compared to those with lower values (**7** and **8**). When the reactivity of complexes possessing similar ligand donor strength and ligand flexibility are compared, such as **4**, **7**, and **8**, the extent of DNA oxidation does increase regularly with increasing potential. We conclude, therefore, that redox potential is a factor in determining reactivity, but that other factors overwhelm this trend. Both ligand donor strength and flexibility vs rigidity must also be considered.

Ligand Donor Strength. Direct ligation of a nickel species to guanine is supported by the observation of high selectivity for only accessible guanine sites in duplex DNA. Furthermore, complexes without vacant coordination sites, such as high spin, octahedral nickel(II) complexes, failed to promote DNA oxidation.[1] To further test the importance of vacant coordination sites, systematic variation of the ligand field strengths (Dq^{xy}) associated with complexes **1–15** was investigated. Busch demonstrated for nickel(II) complexes that as the ligand donor strengths of four coordinate macrocyclic ligands increase, the tendency to form an octahedral complex decreases.[20] At first sight, this principle seems contradictory to the proposed mechanism that requires axial binding of DNA. However, the key point may be the ability of axial ligands to exchange after the nickel(III) oxidation state has been reached. If the same trend holds true for nickel(III) macrocyclic complexes as for nickel(II), complexes with a strong x–y ligand field should have more labile z (axial) ligands. An excellent demonstration of this is found in a recent example by Collins and co-workers who reported a square planar nickel(III) complex derived from an tetraamido ligand.[36] The complex showed little tendency for axial ligation. In the present case, this axial ligand lability would permit exchange of water, chloride, or phosphate ligands for a binding site on DNA such as guanine. Comparison of the Dq^{xy} values for complexes **1–15** allows direct determination of the importance of vacant coordination sites in nickel(II) or exchangeable ligand sites in nickel(III) during the oxidation of DNA.

Values of Dq^{xy}, where available, are listed in Table I, and percentage of DNA oxidation by complexes **1–15** is plotted in Figure 4 in series of increasing ligand donor strength. The complexes are grouped into three structural families, A, B, and C. Within each family, a general trend is observed correlating in-plane ligand field strength with reactivity. Complexes **1–5** represent incremental modification of the Schiff base complex **5** (Figure 4, series A). Upon the basis of the observations of Busch and Barefield,[20,32] the ligand donor strength of these complexes is expected to increase in the order, **1** ~ **2** ~ **3** < **4** < **5**, and the extent of DNA oxidation nicely correlates with this trend. A higher donor strength is typically observed for smaller ring sizes, increased unsaturation in the ring, and a lack of additional *N*-alkyl groups that cause a lengthening of the Ni–N bond.[20,32]

A similar observation is seen for a series of substituted cyclam ligands ([14]aneN_4), complexes **6–10** (Figure 4, series B). One exception in series B is the cyclam complex itself, **8**, whose donor strength is similar to **7** but whose $E_{1/2}$ is 50 mV lower. This lower $E_{1/2}$ value likely accounts for the somewhat lower reactivity. The extent of DNA reactivity for the hexamethylcyclam series, complexes **11–15**, also followed the trend of increasing ligand donor strength (Figure 4, series C) with the exception of complex **15**. The tetraimine ligand **15** is likely to have a higher ligand field strength than the analogous diimine complex, although Dq^{xy} has not been measured. The reduced reactivity might be attributed to the higher rigidity of the macrocyclic ligand making the complex less conformationally adaptable for DNA binding. A detailed discussion of the importance of ligand flexibility is presented below. Another unexpected result in the C series is seen in comparing *meso*- and *dl*-hexamethylcyclam complexes **12** and **13**. The ligand field strengths of the two epimeric complexes should be identical; however, their redox potentials and DNA reactivities differ. This difference can also be ascribed to conformational properties.

Overall, the trend of increased in-plane ligand strength correlating with increased DNA reactivity is observed for the three families of compounds. A mechanism in which the square planar nickel(II) complex is oxidized to an octahedral nickel(III) complex followed by displacement of an auxiliary ligand by guanine is consistent with the data obtained. Further support for the oxidation of nickel(II) prior to binding comes from electrochemical studies of $[Ni(cyclam)](ClO_4)_2$, **8**, in the reaction medium (pH 7) in the presence and absence of 5'-guanosine monophosphate. A cathodic shift of 90 mV was observed in the cyclic voltammogram of **8** in the presence of GMP. Using Bard's analysis,[37] a ratio of binding constants of the Ni(III) complex compared to the Ni(II) complex is estimated to be 30:1. From a similar

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experiment using calf thymus DNA in tris buffer (pH 7), the ratio of binding constants, $K_a(Ni^{III}):K_a(Ni^{II})$, is estimated to be >200:1.

Conformational Flexibility. Two potential roles for conformational flexibility of the nickel complex during DNA oxidation may be envisioned. First, binding of the nickel ion to DNA may require a slight distortion of the macrocyclic ring to relieve unfavorable steric interactions. Alternatively, more dramatic folding of the ring to a cis geometry may be required, as is depicted for the key intermediate in Scheme I. The importance of ligand flexibility was investigated using complexes **11**–**15**. Upon the basis of ligand donor strength, the anticipated order of reactivity for complexes **11**–**15** was **11** < **12** = **13** < **14** < **15**. The observed reactivity (Figure 3, series C) followed the order **11** < **12** < **13** < **15** < **14**. As mentioned, complexes **12** and **13**, which are diastereoisomers of each other, should possess similar ligand donor strengths and reactivity. The increased reactivity of complex **13** (51%) as compared to complex **12** (26%) may be associated with the differences in conformational flexibility between these two complexes. Upon the basis of extensive structural and reactivity studies, complex **13** has been shown to be capable of folding its macrocyclic ligand leaving vacant two cis coordination sites, while complex **12** cannot assume such an unstrained conformation because of steric constraints.[30,38,39] In addition, **13** readily forms cis complexes with oxalate, whereas **12** can only form highly strained cis complexes due to steric encumbrance of the methyl groups in the meso isomer.[38,39] The decreased reactivity of complex **15** (52%) as compared to complex **14** (73%) may also be attributed to differences in conformational flexibility. Specifically, the tetraene ligand of complex **15** inhibits any distortion of the macrocyclic ligand from its trans orientation, while the diene ligand present in complex **14** is known to exist in a cis conformation.[31] Additionally, ligand flexibility may be used to explain the fact that even though the ligand donor strength of complex **5** is much greater than that of complex **4** the extent of reactivity is comparable (Figure 3, series A). Again, the increased rigidity of **5** is due to the increase in unsaturation compared to the reduced form **4**.[19]

Inactive Nickel(II) Complexes. In addition to the polyazamacrocyclic nickel(II) complexes depicted in Figure 1, nickel(II) complexes containing porphyrin, phenanthroline, and acyclic polyamine ligands were also investigated as possible DNA oxidation reagents. In particular, $NiTMPP^{4+}$ (where TMPP = tetra(*N*-methylpyridyl)porphyrin) failed to induce detectable DNA oxidation, in contrast to complexes containing iron, cobalt, or manganese.[40] This observation may be related to an inappropriate ring size for stabilizing nickel(III),[41] the lack of sufficient conformational flexibility,[41] and the presence of ligand rather than metal-centered oxidation.[42] Since TMPP is known to bind intercalatively to DNA, the lack of $NiTMPP^{4+}$ reactivity may also result from the lack of direct metal–DNA binding. The absence of DNA reactivity that we observe for other nickel complexes containing intercalatively binding phenanthroline ligands,[43] such as $Ni(phen)_3^{2+}$ [44] and $Ni(phen)_2^{2+}$,[45] further supports the hypothesis that the specific coordination environment of nickel is important in DNA binding and subsequent reaction. The inability of nickel complexes containing acyclic polyamine ligands such as trisaminoethylamine (tren)[1] to promote the oxidation of DNA confirms the macrocyclic effect in providing a relatively strong ligand field. Finally, although nickel complexes containing ligands such as acetylacetone (acac) and bis(salicylaldehyde)ethylenediimine (salen) have been shown to be potent olefin epoxidation catalysts[46] and may react with DNA, meaningful reactivity studies were not possible because of their insolubility under physiological conditions.

Conclusions

Neutral tetraazamacrocyclic ligands significantly enhanced the reactivity of nickel(II) in promoting DNA oxidation using $KHSO_5$. The most important feature of the complex is high in-plane donor strength such that a stable, square planar complex with a relatively high $Ni^{III/II}$ reduction potential is obtained. Structurally, such complexes are obtained using 14-membered macrocycles with nitrogens present as pyridine or imine groups in preference to secondary or tertiary amines. However, a high degree of unsaturation in the ring also leads to conformational rigidity which may be detrimental to formation of the key intermediate bound to DNA.

The results of a systematic investigation of ligand effects support a proposed mechanism in which the square planar nickel(II) complex is oxidized to a square pyramidal or octahedral nickel(III) complex possessing sufficiently labile axial sites to bind to N7 of guanine. Accessibility of the guanine residue plays a role, since an octahedral nickel complex bound directly to guanine cannot form in the major groove. Only when the Watson–Crick B helix is deformed by unpairing or unstacking may the nickel species react with G.[2] In addition, flexibility of the macrocyclic ring is important, potentially to allow formation of a cis coordinated complex bringing guanine and a bound oxidant into adjacent positions around the metal ion. These features now define the important criteria for design of nickel complexes in DNA binding and reactivity.

Several questions remained to be answered. The precise nature of Ni^{III}–DNA binding is under investigation. The identity of the oxidant bound to nickel is not known, although we speculate that it is a second molecule of HSO_5^-. The products of DNA oxidation and subsequent reaction with piperidine have yet to be defined, though the work of Kasprzak and Hernandez[14] with $[Ni(H_2O)_6]^{2+}/H_2O_2$ has demonstrated that 8-oxoguanine is a major product of DNA oxidation, and Kouchakdjian et al.[47] suggested that 8-oxoguanine can be an alkaline-labile site. These questions are the subject of current research efforts.

Acknowledgment. Support of this work through a Seed Grant from the Stony Brook Center for Biotechnology sponsored by the New York State Science and Technology Foundation (to S.E.R. and C.J.B.) and a grant (to C.J.B.) from the National Science Foundation (CHE-9006684) is gratefully acknowledged.

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