Recognition of Guanine Structure in Nucleic Acids by Nickel Complexes

CYNTHIA J. BURROWS*,† AND STEVEN E. ROKITA*

Department of Chemistry, University at Stony Brook, Stony Brook, New York 11794-3400

Received December 21, 1993

Structure determines function. So saith the central dogma of biological chemistry. How then does one determine structure? For proteins and nucleic acids, the primary structure, or linear order of monomers comprising the polymer, is routinely obtained by modern sequencing methods. The secondary structure of helices and loops can be predicted to a certain extent by examination aided by computer programs. However, the key information, that of the tertiary folded structure of the biopolymer, requires a combination of sophisticated methods and good fortune. X-ray crystallographic determination of structure provides the most detailed information but requires the highest amount of good fortune in obtaining suitable crystals. NMR spectroscopy is a powerful technique, but the method becomes arduous with large biopolymers. One then resorts to classical methods of chemical and enzymatic modification to probe the molecule's surface features and to deduce its folding patterns.

The toolbox of reagents for determination of nucleic acid structure includes a limited set of enzymes and an ever-expanding set of synthetic reagents as chemists create new compounds designed to interact in specific ways with DNA or RNA.[1] For reasons wholly unrelated to such goals,[2] we initiated an investigation of the oxidative chemistry of nickel(II) coordination compounds such as **1**–**3** (Figure 1) with nucleic acids. In fact, considerable evidence in the existing literature already predicted a rich and varied chemistry of nickel with DNA: (1) Nickel is carcinogenic.[4] Particulate nickel compounds (such as Ni_2S_3) enter a cell by phagocytosis, find their way to the nucleus, and cause oxidative DNA strand breaks, DNA–DNA cross-links and DNA–protein cross-links.[5] The mechanism by which this occurs is of considerable interest to toxicologists investigating carcinogenesis as well as to chemists designing DNA-targeted drugs. (2) Ni^{2+} is known to bind well to guanosine via the N7 nitrogen of the purine's imidazole ring (*vide infra*).[6] Such binding is thought to be responsible for the fact that submillimolar concentrations of Ni^{2+} will induce a conformational change in DNA from the right-handed B helical form to a left-handed Z helical form.[7] (3) A site-specific nuclease was created by appending a nickel–peptide complex (Ni–GGH) onto the N-terminus of the DNA recognition fragment of a protein (Hin recombinase).[8] Taken together, these results suggested that nickel(II) coordination compounds would display interesting binding and oxidative reactivity with nucleic acids, and this is indeed the case.

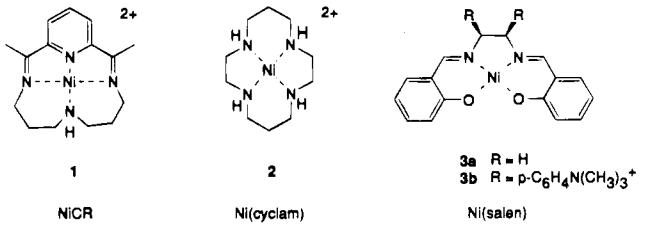


Figure 1. Nickel complexes used for catalytic oxidation.

Recognition of Guanine in Oligodeoxynucleotides

Nickel(II) complexes of 13–14-membered tetradentate macrocycles are typically square planar diamagnetic compounds. Common examples include NiCR (**1**), first described by Karn and Busch in 1966,[9] and Ni(cyclam) (**2**), whose chemistry and derivatives are described in literally hundreds of papers. While their physical properties, including an ability to stabilize

Cynthia Burrows was born in Minnesota in 1953 and moved to Colorado, where she did her undergraduate work in chemistry at the University of Colorado. She received the Ph.D. degree in 1982 from Cornell University working with Professor B. K. Carpenter on Claisen rearrangements and was then an NSF-CNRS postdoctoral fellow in Strasbourg, France, in the laboratory of Professor J. M. Lehn. She joined the faculty at the University at Stony Brook in 1983 and, beginning in January 1995, will be Professor of Chemistry at the University of Utah. Her research endeavors include the use of transition metal complexes as catalysts in bioorganic and biomimetic chemistry and the observation of triplet phenomena.

Steven Rokita was born in Northern California in 1957 and obtained a B.S. in chemistry at the University of California at Berkeley. He received a Ph.D. in 1983 from Massachusetts Institute of Technology, where he worked with Professor Christopher T. Walsh on fluorinated citrate analogues as probes for mechanistic enzymology and flavin-dependent models for the light-requiring repair of DNA *in vivo*. After being an NIH postdoctoral fellow in the laboratory of the late Professor E. T. Kaiser at Rockefeller University, he joined the faculty at the University at Stony Brook in 1986 and is now Associate Professor. His research interests range from enzyme catalysis to sequence and structure-dependent reactivity of nucleic acids.

† Beginning January 1995, address correspondence to Department of Chemistry, University of Utah, Salt Lake City, UT 84112.

(1) For leading reviews, see: (a) Sigman, D. S.; Mazumder, A.; Perrin, D. M. *Chem. Rev.* **1993**, *93*, 2295–2316. (b) Price, M. A.; Tullius, T. D. *Methods Enzymol.* **1992**, *212*, 194–219. (c) Lilley, D. M. *Methods Enzymol.* **1992**, *212*, 133–319. (d) Pyle, A. M.; Barton, J. K. In *Progress in Inorganic Chemistry: Bioinorganic Chemistry*; Lippard, S. J., Ed.; Wiley: New York, 1990; Vol. 38, pp 413–474. (e) Nielsen, P. E. *J. Mol. Recognit.* **1990**, *3*, 1–25. (f) Knapp, G. *Methods Enzymol.* **1989**, *180*, 192–215. (g) Dervan, P. B. In *Nucleic Acids and Molecular Biology*; Eckstein, F., Lilley, D. M. J., Eds.; Springer-Verlag: Berlin, 1988; Vol. 2, pp 49–64. (h) Ehresmann, C.; Baudin, F.; Mougel, M.; Romby, P.; Ebel, J.-P.; Ehresmann, B. *Nucleic Acids Res.* **1987**, *22*, 9109–9128.

(2) Curiosity about the mechanism of nickel-catalyzed olefin epoxidation[3] prompted us to use DNA as a probe for diffusible intermediates.

(3) (a) Kinneary, J. F.; Albert, J. S.; Burrows, C. J. *J. Am. Chem. Soc.* **1988**, *110*, 6124–6129. (b) Wagler, T. R.; Fang, Y.; Burrows, C. J. *J. Org. Chem.* **1989**, *54*, 1584–1589. (c) Yoon, H.; Wagler, T. R.; O'Connor, K. J.; Burrows, C. J. *J. Am. Chem. Soc.* **1990**, *112*, 4568–4570.

(4) (a) Costa, M. *Annu. Rev. Pharmacol. Toxicol.* **1991**, *31*, 321–327. (b) Costa, M.; Heck, J. D. *Met. Ions Biol. Syst.* **1986**, *20*, 259–278. (c) Nieboer, E.; Maxwell, R. I.; Rossetto, R. E.; Stafford, A. R.; Stetsko, P. I. In *Frontiers in Bioinorganic Chemistry*; Xavier, A. V., Ed.; VCH: New York, 1986; pp 142–151.

(5) (a) Kasprzak, K. S. *Chem. Res. Toxicol.* **1991**, *4*, 604–615. (b) Ciccarelli, R. B.; Wetterhahn, K. E. *Cancer Res.* **1984**, *44*, 3892–3897. (c) Ciccarelli, R. B.; Wetterhahn, K. E. *Cancer Res.* **1982**, *42*, 3544–3549. (d) Ciccarelli, R. B.; Hampton, R. H.; Jennette, K. W. *Cancer Lett.* **1981**, *12*, 349–354.

(6) Adam, S.; Bourtayre, P.; Liquier, J.; Taillandier, E. *Nucleic Acids Res.* **1986**, *14*, 3501–3513.

(7) Taboury, J. A.; Bourtayre, P.; Liquier, J.; Taillandier, E. *Nucleic Acids Res.* **1984**, *12*, 4247–4257.

(8) Mack, D. P.; Dervan, P. B. *Biochemistry* **1992**, *31*, 9399–9405.

(9) Karn, J. L.; Busch, D. H. *Nature* **1966**, *211*, 160–162.

0001-4842/94/0127-0295$04.50/0

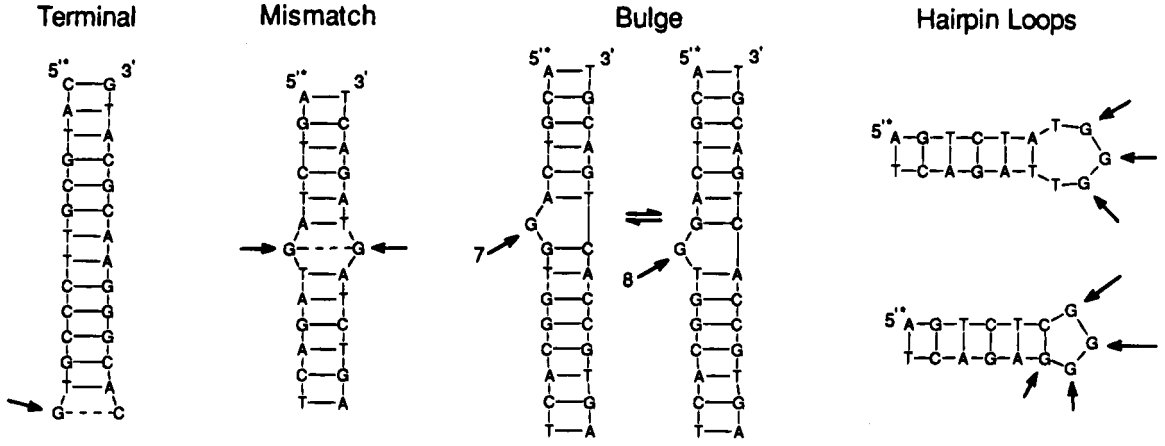


Figure 2. Synthetic oligodeoxynucleotides used in reaction with NiCR/HSO_5^-. Arrows indicate sites of modification observed for oligos labeled at the 5′ end with ^{32}P.

the Ni^{III} oxidation state, had been well characterized,[10] surprisingly little was known of their ability to catalyze oxidation of organic or biological substrates.

In our laboratories, both **1** and **2** were found to be effective agents for oxidation of guanine residues in nucleic acids. In an initial study with a single-stranded oligodeoxynucleotide, incubation with micromolar concentrations of a nickel complex and an oxidant such as potassium monopersulfate ($KHSO_5$) led to equal modification of every guanine residue.[11] Electrophoresis indicated that the oxidation reaction itself did not cause strand scission nor any major change in the size or charge of the oligomer. However, the oxidative damage could be visualized in either of two ways: (1) treatment with piperidine,[12] which promotes depurination of modified guanines and ultimately leads to hydrolytic cleavage of the sugar–phosphate backbone at that site, such lesions being readily viewed by electrophoresis/autoradiography,[11] or more recently, (2) analysis by primer extension assay, which has been demonstrated for RNA in collaboration with S. Woodson.[13] In this case, polymerase activity is halted at sites of base modification, and again this can be detected by gel electrophoresis and autoradiography.

While every G was modified in a single-stranded oligodeoxynucleotide, only a terminal G residue was reactive in a duplex structure in which every base was paired in a normal Watson–Crick scheme. Apparently, only G's that are particularly accessible, such as those at the end of a helix, are able to interact with nickel complexes or oxidant. To further test this hypothesis, a series of duplex oligomers was studied; each oligo contained guanine residues in a noncanonical structure such as a G-G mismatch, G bulge, or hairpin loop (Figure 2).[14] In each case, complexes **1** and **2** recognized the unpaired G sites with high specificity compared to normal G-C base pairs or other bases. The particular sequence chosen for the G bulge study was one that could exist in two major conformations: either G_7 or G_8 of the 5′ end-labeled oligonucleotide could exist as an extrahelical bulged base while G_8 or G_7, respectively, is paired to C_8 of the opposite strand. Preferential reaction at G_7 was seen compared to G_8 (60:40), suggesting perhaps a higher equilibrium concentration of the G_7 bulge. Whether this equilibrium constant reflects only the DNA conformational change or an equilibrium of nickel-bound DNA conformers is now being tested with the aid of NMR studies.

These studies with short oligodeoxynucleotides whose structures could be designed with a significant level of confidence demonstrate that nickel complexes such as **1** and **2** react predictably in the presence of oxidant to modify only those guanine residues showing high accessibility to external reagents. Does reaction of G with nickel reagents then indicate the presence of an unusual DNA conformation in which the base is particularly exposed? In order to be completely confident that NiCR is a reliable probe of nucleic acid structure, it was necessary to study a nucleic acid whose tertiary structure had been thoroughly characterized by X-ray crystallography. The archetypical example is $tRNA^{Phe}$.

Correlation with the Tertiary Structure of $tRNA^{Phe}$

Whereas DNA strands routinely fold into a regular double helix of the B type, RNA adopts a double-helical structure of the A type as well as including many hairpin loops, triple helices, and a variety of other non-Watson–Crick interactions. As a result, most RNA tertiary structures are difficult, if not impossible, to predict in the absence of extensive chemical and structural data. Yeast $tRNA^{Phe}$ has been subjected to numerous studies of chemical and enzymatic modification,[15] and its crystal structure has been solved.[16] Of the 23 guanosine derivatives of $tRNA^{Phe}$, half of

(10) (a) Foulds, G. A. *Coord. Chem. Rev.* **1990**, *98*, 1–122. (b) Hancock, R. D.; Martell, A. E. *Comments Inorg. Chem.* **1988**, *6*, 237–284. (c) Lappin, A. G.; McAuley, A. *Adv. Inorg. Chem.* **1988**, *32*, 241–295. (d) Foulds, G. *Coord. Chem. Rev.* **1987**, *80*, 1–127. (e) Schröder, M. *Coord. Chem. Rev.* **1986**, *71*, 139–234. (f) Fabbrizzi, L. *Comments Inorg. Chem.* **1985**, *4*, 33–54. (g) Busch, D. H. *Acc. Chem. Res.* **1978**, *11*, 392–27.

(11) Chen, X.; Rokita, S. E.; Burrows, C. J. *J. Am. Chem. Soc.* **1991**, *113*, 5884–5886.

(12) Maxam, A. M.; Gilbert, W. *Methods Enzymol.* **1980**, *65*, 499–560.

(13) Woodson, S. A.; Muller, J. G.; Burrows, C. J.; Rokita, S. E. *Nucleic Acids Res.* **1993**, *21*, 5524–5525.

(14) Chen, X.; Burrows, C. J.; Rokita, S. E. *J. Am. Chem. Soc.* **1992**, *114*, 322–325.

(15) (a) Chow, C. S.; Behlen, L. S.; Uhlenbeck, O. C.; Barton, J. K. *Biochemistry* **1992**, *31*, 972–982. (b) Chow, C. S.; Barton, J. K. *Biochemistry* **1992**, *31*, 5423–5429. (c) Chow, C. S.; Barton, J. K. *J. Am. Chem. Soc.* **1990**, *112*, 2839–2841. (d) Behlen, S. L.; Sampson, J. R.; DiRenzo, A. B.; Uhlenbeck, O. C. *Biochemistry* **1990**, *29*, 2515–2523. (e) Latham, J. A.; Cech, T. R. *Science* **1989**, *245*, 276–282. (f) Peattie, D. A.; Gilbert, W. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 4679–4682.

them are located in hairpin loops or as part of an unusual pairing scheme such as a G-G or G-U mismatch (Figure 3). Not unexpectedly, the tertiary form of the molecule is compact, with many of the hydrophobic groups, such as guanine bases, oriented toward the interior of helices.

Inspection of the surface of $tRNA^{Phe}$ shows that a few of the guanine residues (Figure 3A, red) are oriented such that N7 of the imidazole ring (Figure 3A, yellow) is exposed to solvent. N7 was the anticipated recognition site since is the most basic nitrogen of guanine and the best transition metal binding site.[17] Reaction of yeast $tRNA^{Phe}$ in its native (folded) state with NiCR (**1**) and $KHSO_5$ revealed four reaction sites comprising G_{18}, G_{19}, G_{20}, and G_{34}, all of which were predicted to be reactive on the basis of the accessibility of N7 seen in the crystal structure.[18] G_{19} is by far the most reactive site, and G_{18} and G_{20} were progressively less reactive. A closer look at the region surrounding G_{19} (Figure 3B) reveals why this is the case. N7 of G_{19} lies on a convex surface with its lone pair oriented outward such that there is little hindrance to binding or reaction with external reagents. In comparison, N7 of G_{18} is somewhat more sterically encumbered and appears less reactive. Similarly, the purine of G_{20} lies parallel to the surface of the molecule so that its N7, though exposed on the surface, is not well oriented for binding. G_{57} is also located in this region, but its N7 is barely visible on the surface, thus explaining its failure to react.

It was gratifying to note that the pattern of reactivity observed with nickel complexes correlated well with computational studies. Lavery and Pullman reported their analysis of guanine N7's in $tRNA^{Phe}$ according to an "accessible surface integrated field index" (ASIF) in $V\text{-}A^{-1}$ that took into account both the exposed surface area of N7 and its electrostatic potential as influenced by neighboring groups.[19] In this analysis, a highly negative value of ASIF suggests greater nucleophilicity at N7. No direct comparison of ASIF with our observed relative reactivities is possible since the units differ, but there is good general agreement in the trends predicted and observed (Table 1). G_{34}, at the bottom of the anticodon loop, is also predicted to have moderate reactivity via N7 (data not included in Table 1), and indeed its reactivity is similar to that of G_{18}. Also in accord with expectations, we found that denatured $tRNA^{Phe}$ (obtained in the absence of Mg^{2+}) presented many more reaction sites to nickel reagents, and approximately uniform cleavage was observed at 12 different guanine residues.

Several conclusions can be drawn from the successful correlation between $tRNA^{Phe}$ tertiary structure and its reactivity with NiCR/$KHSO_5$: (i) The strong dependence upon the local environment of N7 of guanine is highly suggestive of a role for nickel–N7 binding during recognition of G's. (ii) The lack of reaction at bases (even guanines) adjacent to modification sites suggests that diffusible intermediates are not generated during the reaction. (iii) The excellent correlation between reactivity and structure further suggests that nickel complexes do not deform the RNA structure in the process of recognition and reaction. This problem is always a concern with structural probes that strongly interact with nucleic acids. (iv) NiCR is an excellent probe of RNA structure. It is intermediate in size between the only other probes of guanine N7, the small and relatively nonselective dimethyl sulfate[1h] and the much larger enzyme probe RNase T1.[1f] NiCR should now hold a unique position in the growing family of probes routinely used for mapping the secondary and tertiary structure of nucleic acids.[20] Other members (and their target sites) include DEPC (A N7), CMCT (U N3, G N1), kethoxal (G N1, N2), $KMnO_4/OsO_4$ (T C5,6), Fe-EDTA/H_2O_2, and a variety of nucleases.[1e,h] Collectively these provide a detailed picture of all nucleosides and their varying degrees of exposure to solvent.

Table 1. Comparison of Experimentally and Theoretically Determined Relative Reactivity of Selected[a] Guanine Residues in $tRNA^{Phe}$

	rel reactivity[b] (exptl)	ASIF[c] ($V\text{-}A^{-1}$) (calcd)
G_{18}	2.2	−21
G_{19}	5.1	−26
G_{20}	1	−11
G_{57}	0	0

[a] Only guanines in the northeast quadrant as shown in Figure 3B are compared. For a complete discussion, see ref 18. [b] Determined as average relative intensity of fragment bands from gel electrophoresis and autoradiography from three separate experiments. [c] Reference 19.

Application to a Ribozyme

Having confirmed the predictability of G recognition by NiCR using the well-characterized $tRNA^{Phe}$, we next turned our attention to an RNA structure that is currently the subject of much investigation, *Tetrahymena* group I intron RNA. The L-21 *Sca*I derivative of this ribozyme lacks the 3′ and 5′ termini necessary for self-splicing, but it is fully active as an endonuclease in the presence of Mg^{2+} and guanosine cofactors.[21] For activity, the intron must adopt a specific conformation with a well-defined surface and active site, and it therefore nicely exemplifies the interplay of structure and function. Its structure remains under investigation and has been analyzed by a variety of chemical and biochemical methods.[15e,22] A model of its organized core has emerged.[23]

Reaction of L-21 *Sca*I with NiCR/$KHSO_5$ located 11 reactive G sites between nucleotides 30 and 334 (Figure 4).[18] The major reaction site was found to be G_{288}, a site also modified strongly by dimethyl sulfate and RNase T1. It apparently lies in a hairpin loop at the end of the P8 helix, and this result with NiCR would further predict that, like G_{19} of $tRNA^{Phe}$, the

(16) (a) Kim, S. H.; Suddath, F. L.; Quigley, F. J.; McPherson, A.; Sussman, J. L.; Wang, A. H. J.; Seeman, N. C.; Rich, A. *Science* **1974**, *185*, 435–440. (b) Robertus, J. D.; Ladner, J. E.; Finch, J. T.; Rhodes, D.; Brown, R. S.; Clark, B. F. C.; Klug, A. *Nature* **1974**, *250*, 546–551.
(17) Martin, R. B. *Acc. Chem. Res.* **1985**, *18*, 32–38.
(18) Chen, X.; Woodson, S. J.; Burrows, C. J.; Rokita, S. E. *Biochemistry* **1993**, *32*, 7610–7616.
(19) Lavery, R.; Pullman, A. *Biophys. Chem.* **1985**, *19*, 171–181.
(20) For very recent applications of NiCR in other labs, see: (a) Wurdeman, R. L.; Douskey, M. C.; Gold, B. *Nucleic Acids Res.* **1993**, *21*, 4975–4980. (b) Butcher, S. E.; Burke, J. M. *J. Mol. Biol.*, in press.
(21) Cech, T. R. *Annu. Rev. Biochem.* **1990**, *59*, 259–271.
(22) (a) Wang, J.-F.; Cech, T. R. *J. Am. Chem. Soc.* **1994**, *116*, 4178–4182. (b) Banerjee, A. R.; Jaeger, J. A.; Turner, D. H. *Biochemistry* **1993**, *32*, 153–163. (c) Caprara, M. G.; Waring, R. B. *Biochemistry* **1993**, *32*, 3604–3610. (d) Wang, J.-F.; Cech, T. R. *Science* **1992**, *256*, 526–529. (e) Pyle, A. M.; Cech, T. R. *Nature* **1991**, *350*, 628–630. (f) Latham, J. A.; Cech, T. R. *Science* **1989**, *245*, 276–282. (g) Kim, S. H.; Cech, T. R. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8788–8792.
(23) Michel, F.; Westhof, E. *J. Mol. Biol.* **1990**, *216*, 585–610.

N7 lone pair is directed outward from a convex surface of the molecule. Most of the other reactive G's also reside in predicted loops (G_{44}, G_{73}, G_{77}, G_{169}, G_{188}, G_{303}, and G_{313}). The majority of sites modified by NiCR lie in regions protected from reaction with Fe-EDTA/H_2O_2, a reagent that is used to detect solvent-exposed sugar residues.[22] Certain sequences in which the sugar–phosphate backbone is exposed will tend to twist the corresponding bases into the center of helical regions, and *vice versa*. Thus, RNA folding leads to conformations in which the backbone is buried but the guanine bases may be exposed.

An intriguing result is found in the selective oxidation of G_{264} by NiCR/HSO_5^-. This guanine faces a relatively large cavity and participates in binding the guanosine cofactor *via* hydrogen bonds between N7 and O6 on G_{264} and N1 and the 2-NH_2 group of guanosine.[24] Modeling indicates that NiCR fits well in the cavity when guanosine is absent, and binding to N7 is unencumbered (Figure 5). Denaturation of this area cannot explain the specificity of G_{264} modification because another G residue in the P7 helix (G_{309}) was unreactive.

The general agreement between the predicted structure of the L-21 *Sca*I intron and the observed reactivity with NiCR provides additional confidence that nickel complexes can be employed in a predictable fashion to probe nucleic acid structure. While the action of many metal complexes may be rationalized after their application, few have exhibited the dependability necessary for routine use as structural probes. The Fe-EDTA and NiCR systems are two examples that have been proven reliable.

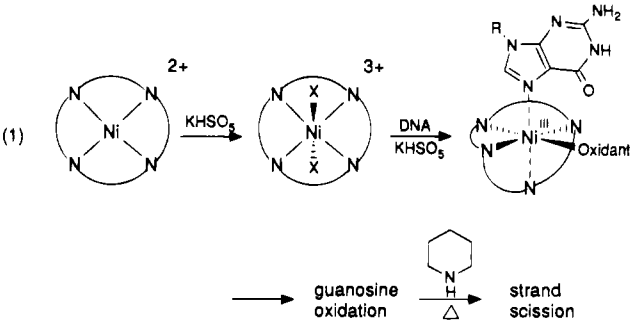
Mechanistic Investigations

How does the NiCR/HSO_5^- system work? Why do certain square planar nickel complexes interact with specific guanine residues of nucleic acids ultimately leading to oxidation and strand scission? A mechanistic picture is beginning to emerge after examination of metal, ligand, and oxidant dependence. Unfortunately, no mechanism can ever be proven correct; but certain ones can be ruled out.

It is tempting to suggest direct metal binding of nickel to guanine N7 as a logical conclusion from the excellent correlation between G-N7 accessibility and G reactivity. This concept is also consistent with the following experiments in our labs[25] and others: (i) Cyclic voltammetric studies of $[Ni(cyclam)]^{2+}$, **2**, with either GMP or calf thymus DNA show a shift in the $E_{1/2}$ of the $Ni^{III/II}$ redox couple toward lower potential, indicating a stabilization of Ni^{III} in the presence of nucleic acid ligands.[26] This suggests that Ni^{III} is the key oxidation state in recognition. (ii) Simple Ni^{2+} salts are known to convert poly d(GC) from the right-handed B form to a left-handed Z helix, and this appears to occur through N7 binding.[7] In our laboratories, a 70 μM concentration of $[Ni^{III}(cyclam)]^{3+}$ was sufficient to effect this conversion while higher concentrations of the Ni^{II} complex were ineffective. (iii) A crystal structure of a Ni^{II} ethylenediamine complex with GMP shows N7 as one of the ligands.[27]

The current mechanistic hypothesis calls for first oxidation of the nickel(II) complex to a nickel(III) complex by HSO_5^-. Nickel(III) coordination compounds are typically 6-coordinate, and therefore two more ligands may be added to the initial 4-coordinate square planar complex in the process of oxidation. This is where the macrocyclic ligand appears to play a key role.

Studies of ligand effects were carried out using analogues of **1** and **2** in which the ring size, donor atom type (imine, secondary amine, tertiary amine), and steric properties of the ligand were systematically varied.[26] It was found that polyazamacrocycles providing a strong in-plane ligand field (14-membered rings with a preference for imine > secondary amine > tertiary imine) without making the complex overly rigid (too many double bonds or quaternary carbons in the ring) demonstrated the highest reactivity with DNA. One interpretation of these results is that the initially formed nickel(III) complex most likely accepts H_2O, phosphate (from the buffer), or HSO_5^- as axial ligands, but that these ligands are exchangeable when the in-plane ligand field is sufficiently strong. This would allow N7 of a guanosine residue to be bound subsequently. (See eq 1.) If HSO_5^- and guanosine are in close proximity by virtue of binding to Ni^{III}, one can envision an oxidative degradation of the purine. Although this chemistry has yet to be fully characterized, a potential route is formation of 8-oxoguanine and related oxidation products that would then comprise the alkaline labile site.[28] 8-Oxoguanine could only be the initial oxidation product since it is not stable under the conditions of the reaction. In attempts to identify the products of DNA degradation, 8-oxodeoxyguanosine was found to rapidly degrade in the presence of $KHSO_5$ or magnesium monoperoxyphthalate (MMPP).[29] On the other hand, high-resolution gel electrophoresis confirms that the DNA products have clean 3′ phosphate ends and not phosphoglycolate groups as is found in some sugar oxidation reactions.[29]



Some mechanisms can be ruled out by the observation of a high dependence of the reaction on certain

(24) Michel, F.; Hanna, M.; Green, R.; Bartel, D. P.; Szostak, J. W. *Nature* **1989**, *342*, 391–395.
(25) Shih, H.-C.; Muller, J. G.; Burrows, C. J.; Rokita, S. E. Unpublished results.
(26) Muller, J. G.; Chen, X.; Dadiz, A. C.; Rokita, S. E.; Burrows, C. J. *J. Am. Chem. Soc.* **1992**, *114*, 6407–6411.
(27) De Meester, P. et al. *Biochim. Biophys. Acta* **1974**, *340*, 113–115.
(28) Kouchakdjian, M.; Bodepudi, V.; Sibutani, S.; Eisenberg, M.; Johnson, F.; Grollman, A. P. *Biochemistry* **1991**, *90*, 1403–1412.
(29) Rokita, S. E.; Zheng, P.; Tang, N.; Cheng, C.-C.; Yeh, R.-H.; Muller, J. G.; Burrows, C. J. In *Metals and Genetics*; Sarkar, B., Ed.; M. Dekker: New York, in press.
(30) Muller, J. G.; Chen, X.; Dadiz, A. C.; Rokita, S. E.; Burrows, C. J. *Pure Appl. Chem.* **1993**, *65*, 545–550.
(31) Muller, J. G.; Paikoff, S. J.; Rokita, S. E.; Burrows, C. J. *J. Inorg. Biochem.* **1994**, *52*, 199–206.

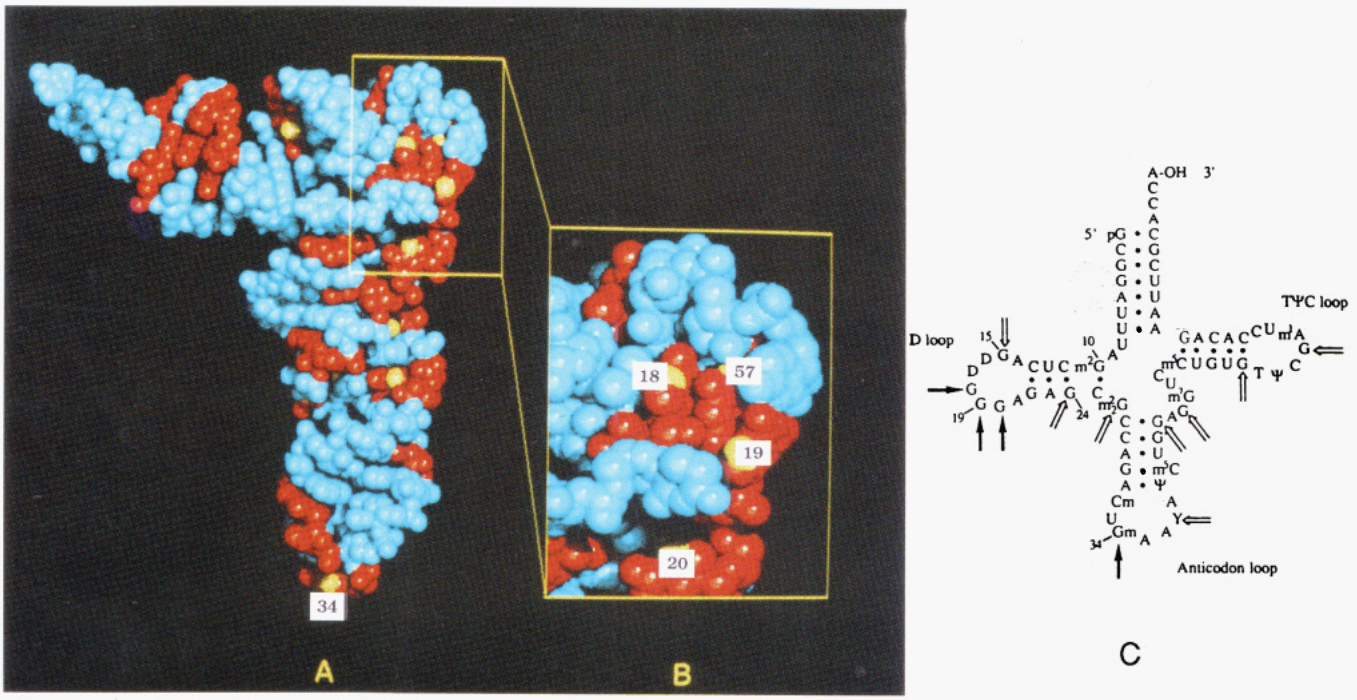


Figure 3. (A) Space-filling model of the crystallographically determined structure of yeast $tRNA^{Phe}$.[16] Guanosine and related nucleotides are shown in red, and N7 of each is highlighted in yellow. (B) Close-up of the northeast quadrant showing the area around G_{19}. (C) Secondary structure of $tRNA^{Phe}$. Solid (and open) arrows indicate sites of modification by NiCR/HSO_5^- in the presence (and absence) of Mg^{2+}.

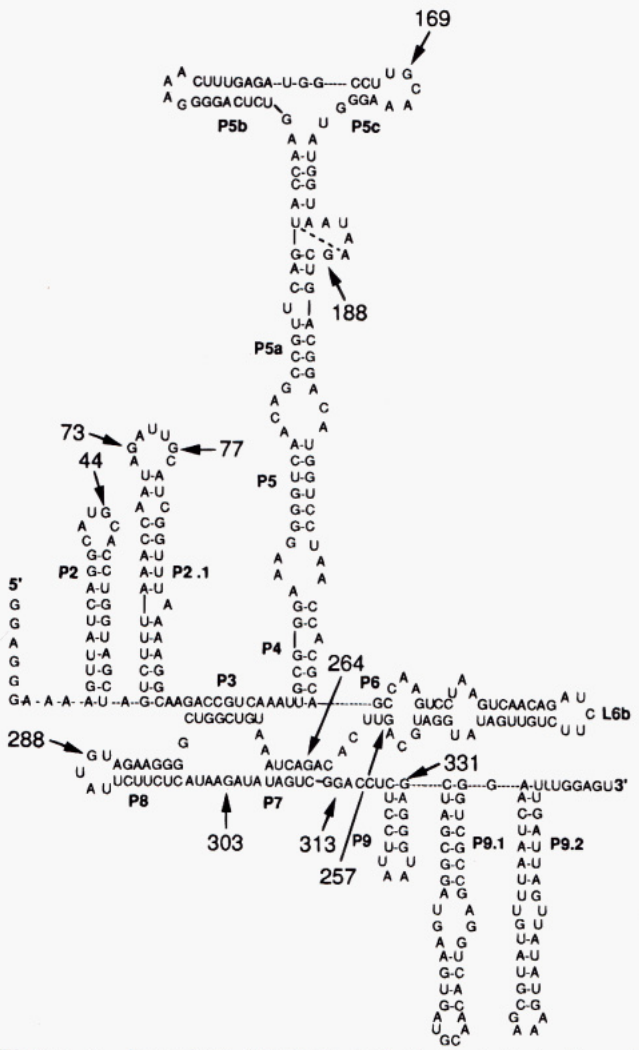


Figure 4. Secondary structure of L-21 *Sca*I derivative of *Tetrahymena* group I intron. Arrows point to sites of modification by NiCR/HSO_5^-.

oxidants. Only peracids such as HSO_5^- or MMPP were able to participate in nickel-catalyzed DNA modification. Other oxidants including $IrCl_6^{2-}$, $S_2O_8^{2-}$, and H_2O_2 were ineffective.[30] In the case of Ni(cyclam), **2**, the nickel(III) complex is sufficiently long-lived at pH 7 that it can be independently prepared and studied as an oxidant. In the absence of additional oxidants such as HSO_5^- or MMPP, the nickel(III) complex was incapable of effecting any detectable modification of DNA. Such a result rules out a simple electron transfer reaction in which a guanine radical cation would be formed. Taken together, the current mechanistic evidence supports the pathway proposed in eq 1. Studies of DNA binding and product analysis continue and are expected to provide further refinements in the mechanism.

A Switch from Oxidation to Alkylation

Because of its utility as an olefin epoxidation catalyst,[3c] Ni^{II}(salen) (**3a**, R = H) was chosen for further study with oligodeoxynucleotides. This charge-neutral metal complex is insoluble in water, and therefore, a derivative bearing tetraalkylammonium groups (**3b**, R = p-$C_6H_4N(CH_3)_3^+$) was prepared for study.[31] It was anticipated that **3b** would show essentially identical behavior toward $KHSO_5$ and DNA as **1** and **2**. Curiously, this was not the case. Alkaline-labile guanine modification was again observed at accessible G sites, but additional products were also observed that had slower electrophoretic mobility than the parent oligonucleotide. These products have either higher molecular weight, a decreased negative charge on the molecule, or both. Strong binding, most likely covalent, between the complex **3b** and the oligonucleotide would account for the fact that the adduct survives denaturing gel electrophoresis.

Important clues about the mode of action came from the study of complexes **4** and **5**. Complex **4** lacks the

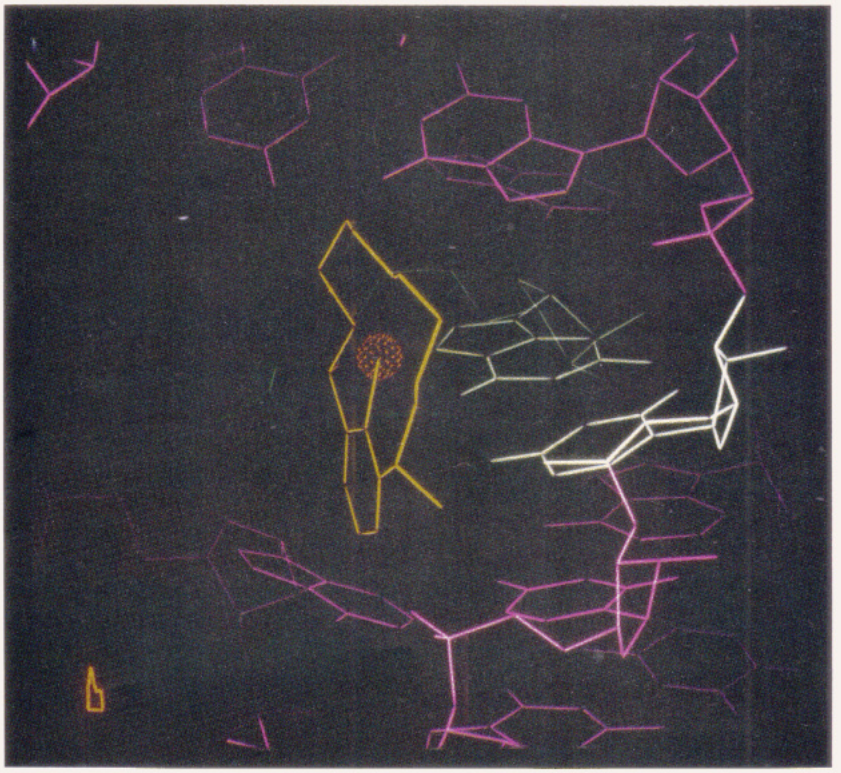
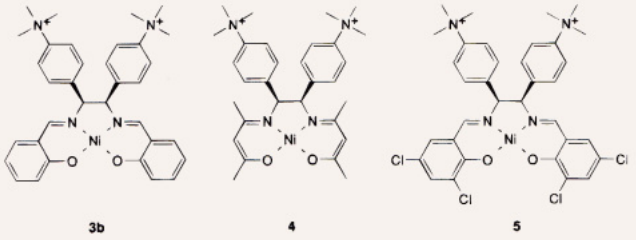
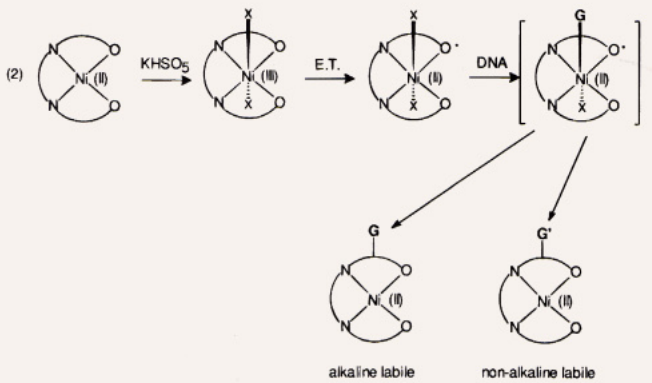


Figure 5. Computer model of NiCR docked in the active site[23] of *Tetrahymena* group I intron. The G_{264}–C_{311} pair is shown in white. NiCR (yellow) is positioned with the Ni center (red) approximately 2.0 Å from N7 of G_{264}.



phenol moieties of **3b** and was found to be ineffective for DNA modification. Cyclic voltammetric studies of **3a** and related compounds by Goldsby suggest that the irreversible behavior of the $Ni^{III/II}$ couple stems from the instability of the Ni^{III} oxidation state with this ligand.[32] In this case, Ni^{III} can accept an electron from the phenolate ligand, a good one-electron donor, yielding a Ni^{II} complex of a ligand radical cation, $L^{\bullet+}$ (eq 2). In the absence of other reactants, the $Ni^{II}L^{\bullet+}$ species may undergo polymerization *via* a phenol coupling reaction. In the presence of DNA, accessible guanines appear to be the target of the phenol radical leading to G alkylation, or rather, arylation in this case. A primer extension assay performed on *Tetrahymena* group I intron RNA confirmed that only G's, and no other bases, were the sites of modification.[13]

In order to gain further evidence for the involvement of phenol radicals as guanine arylating agents, a series of *o*- and *p*-substituted derivatives of **3b** were investigated. Substitution in these positions by either an electron donor (methyl) or an electron acceptor (chloro) group had similar effects in reducing the amount of DNA alkylation, and the *o*,*p*-dichloro derivative **5** showed no trace of alkylation.[33] Thus, it appears that



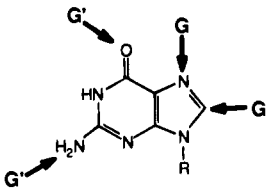
steric encumbrance in these positions of the phenolate group can effectively shut down the ligand–guanine coupling.

There are several possible sites of alkylation on the guanine base just as there is also more than one site of the salen ligand that may be the point of covalent attachment. In fact, at least two different products are obtained; one is alkaline labile, and the other is not. For alkaline-labile products, the possibilities are reaction at N7 or C8. Both are good candidates since N7 is quite nucleophilic and C8 is known to react with methyl radicals.[34] Non-alkaline-labile products would be expected from reaction at N3, O6, or the 2-amino group of guanine. The exact identity of this mixture of reaction products is under investigation.

The observed switch to G alkylation, as opposed to G oxidation, further demonstrates the versatility of nickel as a redox catalyst whose mode of operation and degree of reactivity can be regulated through ligand design. However, the reactions described thus far require the use of powerful oxidants such as mono-

(32) Goldsby, K. A. *J. Coord. Chem.* **1988**, *19*, 83–90.
(33) Muller, J. G.; Paikoff, S. J.; Rokita, S. E.; Burrows, C. J. Manuscript in preparation.

(34) Bichara, M.; Fuchs, R. P. P. *J. Mol. Biol.* **1985**, *183*, 341–351.

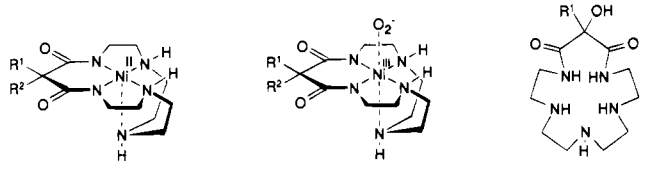


guanine alkylation sites

persulfate and are only applicable to *in vitro* studies such as those described for nucleic acid structure determination. Chemistry occurring *in vivo* between nickel complexes and nucleic acids requires the use of cellular reagents such as dioxygen.

Toward O_2-Dependent Modification of Nucleic Acids

Nickel compounds have two characteristics in common with leading antitumor drugs: (i) as with cisplatin, direct metal binding to N7 of guanine is possible, and (ii) like Fe-bleomycin, nickel complexes are able to catalyze oxidative damage to nucleic acids. In order to develop nickel-based reagents that demonstrate an activity compatible with cellular conditions, one might consider synthetic complexes capable of interaction with molecular oxygen. Few nickel complexes have been so fortunate. Most nickel(II) coordination compounds have potentials too high for reduction of O_2. However, a particularly appealing system has been described by Kimura and co-workers.[35] The pentaazamacrocyclic nickel complexes **6a** and **6b** were found to take up 1 equiv of O_2 in aqueous solution to give what appears to be a nickel(III)–superoxide complex. The lifetime of the complex is on the order of minutes to hours depending upon substituents, particularly at C15. Martell and collaborators contributed a significant observation when they found that the superoxide intermediates, **7**, underwent self-oxidation to give the C15 hydroxides, **8**.[36] It was then a small intellectual leap for us to propose that C15-*disubstituted* complexes would favor *intermolecular* oxidation, *e.g.*, of DNA, rather than self-destruction.



6 **7** **8**

a $R^1 = R^2 = H$

b $R^1 = CH_2C_6H_5$, $R^2 = H$

c $R^1 = F$, $R^2 = H$

d $R^1 = F$, $R^2 = CH_2C_6H_5$

The 15-benzyl-15-fluoro macrocycle was synthesized, and its nickel complex **6d** was prepared.[37] It does not react with oxygen! In our hands, only 15-*monosubstituted* macrocyclic complexes reacted with atmospheric oxygen. Investigation of a series of complexes bearing fluoro or alkyl substituents at C15, N7, or both C2 and C12 led to the conclusion that the O_2 reactivity of the nickel complexes depends upon subtle changes in coordination geometry around Ni^{II} making it 5-coordinate and reactive in some cases, and 6-coordinate and unreactive in others.[38]

Preliminary analysis of the reaction between nickel–O_2 adducts and DNA has relied on the very sensitive assay of plasmid scission.[37] Nearly complete scission of one strand (nicking) was observed with 120 μM **7c** in 45 min. The reaction is dependent upon the concentration of **7c**, but it is insensitive to the presence of hydroxyl radical scavengers. The reaction was significantly inhibited by superoxide dismutase, however, lending some support to the proposed superoxo intermediate. Interestingly, oligonucleotide studies showed that both adenine and guanine residues were oxidized, with a slight preference for adenine.[38] Although a relatively high concentration of nickel complex was employed in these experiments, it should be noted that **7c** is charge neutral and bears no particular groups that would have much affinity for DNA. Appropriate substitution in future derivatives may ultimately allow tailoring of the macrocycle for cellular transport, reaction with O_2, DNA binding, and subsequent oxidation. Such efforts have now been initiated.

Conclusions

Nickel is a remarkably versatile metal in biological chemistry. It is a necessary component of certain metalloproteins and is at the same time an environmental carcinogen causing DNA damage and protein–DNA cross-links. Among all of the transition metals, nickel is particularly well suited for structural recognition of and reaction with nucleic acids. This is largely the result of its rich coordination chemistry in which the bound organic ligands determine the number, type and orientation of additional bound ligands, the kinetics of ligand exchange, and the thermodynamics of redox processes. For the molecular biologist, nickel offers a tool for the study of nucleic acid structure in the form of tetraazamacrocyclic complexes. For the chemist, the diversity of its interactions with oxidants and the keen dependence upon the organic ligand entreat further exploration into its uses in organic and biological transformations.

We are grateful to the dedicated co-workers whose names appear in the references, to Prof. E. Westhof (Strasbourg) for providing Figure 5, to the Stony Brook Center for Biotechnology funded by the New York State Science and Technology Foundation for early support of our collaboration, and to the NSF (CHE-9006684 to C.J.B. and MCB9106957 to S.E.R.), the American Cancer Society (CN-72), and finally to the NIH (GM-47531) for continued support.

(35) Kimura, E.; Machida, R.; Kodama, M. *J. Am. Chem. Soc.* **1984**, *106*, 5497–5505.

(36) Chen, D.; Motekaitis, R.; Martell, A. E. *Inorg. Chem.* **1991**, *30*, 1396–1402.

(37) Cheng, C.-C.; Rokita, S. E.; Burrows, C. J. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 277–278.

(38) Cheng, C.-C. Ph.D. Dissertation, University at Stony Brook, 1993.