Recognition of Guanine Structure in Nucleic Acids by Nickel Complexes

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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$_2$S$_3$) 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 nucleapse was created by appending a nickel–peptide complex (Ni–GHH) 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.

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
the Ni$^{III}$ oxidation state, had been well characterized,\(^\text{(10)}\) 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.\(^\text{(11)}\) 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,\(^\text{(12)}\) 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,\(^\text{(13)}\) or more recently, (2) analysis by primer extension assay, which has been demonstrated for RNA in collaboration with S. Woodson.\(^\text{(13)}\) 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).\(^\text{(14)}\) 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$_9$ 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$^{\text{Phe}}$.

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

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$^{\text{Phe}}$ has been subjected to numerous studies of chemical and enzymatic modification,\(^\text{(15)}\) and its crystal structure has been solved.\(^\text{(16)}\) Of the 23 guanosine derivatives of tRNA$^{\text{Phe}}$, half of

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Recognition of Guanine Structure  

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\textsuperscript{Phe} shows that a few of the guanine residues (Figure 3A, red) are located in hairpin loops or as part of an extended hairpin structure. A closer look at the region surrounding G\textsubscript{19} reveals why this is the case. N7 of G\textsubscript{19} lies on a surface convex to the molecule so that its N7, though exposed on the surface, is not well oriented for binding. G\textsubscript{57} is also located in this region, but its reactivity with NiCR can be explained by other factors.

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\textsuperscript{Phe} according to an "accessible surface integrated field index" (ASIF) in V-A\textsuperscript{-1} that took into account both the exposed surface area of N7 and its electrostatic potential as influenced by neighboring groups. 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\textsubscript{54}, 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\textsubscript{18}. Also in accord with expectations, we found that denatured tRNA\textsuperscript{Phe} (obtained in the absence of Mg\textsuperscript{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\textsuperscript{Phe} tertiary structure and its reactivity with NiCR/KHSO\textsubscript{4}: (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 nonspecific dimethyl sulfate and the much larger enzyme probe RNase T1. 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.

Other members (and their target sites) include DEPC (A N7), CMCT (U N3, G NU), kethoxal (G N1, N2), KMnO\textsubscript{4}/OsO\textsubscript{4} (T C5,6), Fe-EDTA/H\textsubscript{2}O\textsubscript{2}, and a variety of nucleases. Collectively these provide a detailed picture of all nucleosides and their varying degrees of exposure to solvent.

### Application to a Ribozyme

Having confirmed the predictability of G recognition by NiCR using the well-characterized tRNA\textsuperscript{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 ScuI 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\textsuperscript{2+} and guanosine cofactors. 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. A model of its organized core has emerged.

Reaction of L-21 ScuI with NiCR/KHSO\textsubscript{4} located 11 reactive G sites between nucleotides 30 and 334 (Figure 4). The major reaction site was found to be G\textsubscript{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\textsubscript{19} of tRNA\textsuperscript{Phe}, the

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<th>Table 1. Comparison of Experimentally and Theoretically Determined Relative Reactivity of Selected Guanine Residues in tRNA\textsuperscript{Phe}</th>
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<td>rel reactivity\textsuperscript{a} (exptl)</td>
<td>ASIF\textsuperscript{b} (V-A\textsuperscript{-1}) (calcd)</td>
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<tr>
<td>G\textsubscript{19}</td>
<td>2.2</td>
<td>-21</td>
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<td>G\textsubscript{18}</td>
<td>5.1</td>
<td>-26</td>
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<td>G\textsubscript{50}</td>
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<td>G\textsubscript{57}</td>
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\textsuperscript{a} Only guanines in the northeast quadrant as shown in Figure 3B are compared. For a complete discussion, see ref 18. \textsuperscript{b} Determined as average relative intensity of fragment bands from gel electrophoresis and autoradiography from three separate experiments. \textsuperscript{c} Reference 19.

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 \(G_{513})\). 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-NH₂ 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 (\(G_{303}\)) was unreactive.

The general agreement between the predicted structure of the L-21 Sco 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) nickel to guanine N7 as a logical conclusion from the presence of KHSO₅ 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.\(^{29}\)

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 polynuclear macrocycles provide a strong in-plane ligand field (14-membered rings with a preference for imine > secondary amine > tertiary amine) 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 KHSO₅ 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

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A Switch from Oxidation to Alkylation

Because of its utility as an olefin epoxidation catalyst,\textsuperscript{26} \textit{Ni}\textsuperscript{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.\textsuperscript{31} It was anticipated that 3b would show essentially identical behavior toward KHSO\textsubscript{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 of Tetrahymena group I intron. The G_{261}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^{II}/Ni oxidation state with this ligand. 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^+ (eq 2). In the absence of other reactants, the Ni^{II}L^+ 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.

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. 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. 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-

(33) Muller, J. G.; Paikoff, S. J.; Rokita, S. E.; Burrows, C. J. Manuscript in preparation.
Recognize of Guanine Structure

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 O2-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 O2. However, a particularly appealing system has been described by Kimura and co-workers. The pentaaazamacrocyclic nickel complexes 6a and 6b were found to take up 1 equiv of O2 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, followed self-oxidation to give the C15 hydroxides, 7. 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.

The 15-benzyl-15-fluoro macrocycle was synthesized, and its nickel complex 6d was prepared. 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 O2 reactivity of the nickel complexes depends upon subtle changes in coordination geometry around NiII, making it 5-coordinate and reactive in some cases, and 6-coordinate and unreactive in others.

Preliminary analysis of the reaction between nickel—O2 adducts and DNA has relied on the very sensitive assay of plasmid scission. 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. 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 O2, 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.

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