A Highly Sensitive Probe for Guanine N7 in Folded Structures of RNA: Application to tRNA^{Phe} and *Tetrahymena* Group I Intron†

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ABSTRACT: A nickel complex has been shown to promote conformation-specific oxidation of guanosine in polynucleotide RNA. In all cases, reaction was strictly dependent on the solvent exposure and surface properties of guanine N7. Modification of native tRNA^{Phe} (yeast) was detected at G18, G19, G20, and Gm34 and concurred with predictions based on its crystal structure. Additional guanine derivatives became exposed to oxidation only after the tRNA unfolded in the absence of Mg^{2+}. Reaction of the *Tetrahymena* group I intron RNA (L-21 *Sca*I) also compared favorably to its three-dimensional model by appropriately identifying guanosine residues in hairpin loops, duplex termini, and the essential cofactor binding site. These results complemented prior data generated by hydroxyl radical, and in combination they served to distinguish the solvent accessibility of sugar backbone and base positions in guanosine residues. Most importantly, this nickel complex exhibited greater selectivity than either dimethyl sulfate or RNase T1 for characterizing tRNA^{Phe} and intron RNA.

Structure determination persists as a central goal in the study of RNA since the biological activity of nontranslated RNA is intimately related to its three-dimensional folding. Consequently, routine sequencing protocols have been crucial but insufficient tools for relating RNA structure and function. Methods for predicting and characterizing polynucleotide conformation are also required, and standard approaches have begun to emerge. While X-ray crystallography and nuclear magnetic resonance can provide the most detailed information on nucleic acid structure, their application to RNA has remained limited. The great majority of experimental data derive instead from chemical and enzymatic modification studies [reviewed in Ehresmann et al. (1987) and Knapp (1989)]. By these methods, the accessible and inaccessible residues of a folded macromolecule may be distinguished and regions of secondary structure identified.

Each chemical reagent and enzyme applied to the problem of nucleic acid structure present a particular set of advantages and disadvantages (Ehresmann et al., 1987). For example, chemical reagents are usually small enough to approach most of their intended targets but may be too small to possess significant conformational selectivity. Conversely, enzymes are typically very selective but may be too large to gain access to all of their potential targets. Accordingly, thorough investigation of polynucleotide structure relies on the use of a complementary and overlapping series of structural probes.

The highest resolution map of RNA folding may be obtained only after the individual functional domains of each nucleotide are examined. In the case of guanosine, the relative reactivity of its ribose group indicates the accessibility of the backbone. The availability of its N1 and 2-amino groups for modification provides information on its state of base pairing. Another principal position, N7 of guanosine, may participate in a variety of noncanonical associations including Hoogsteen, G-U, and G-G base pairs as well as base triplets and quartets. Traditional reagents are limited in their ability to characterize the steric and electronic environment of this position. Alkylation of N7 by dimethyl sulfate is insensitive to nucleic acid secondary structure (Peattie & Gilbert, 1980), and strand hydrolysis by RNase T1 requires simultaneous access to the O6 and N1 groups (Heinemann & Saenger, 1985).

A variety of transition metal complexes have become effective probes for DNA and RNA structure through unique mechanisms of recognition and reactivity (Tullius, 1989; Pyle & Barton, 1990; Chow et al., 1992; Chow & Barton, 1992). Recently, our laboratories have developed a metal-based reagent that is highly specific for G residues in oligodeoxynucleotides (Chen et al., 1991, 1992). The illustrated nickel complex (NiCR,[1] **1**) and related structures (Muller et al., 1992) uniquely oxidize mismatched, bulged, looped, or otherwise accessible G residues in the presence of $KHSO_5$. Although the specificity of this complex has been suggested to derive from interactions with guanine N7, only the most general correlations between solvent exposure of guanosine and reactivity could be drawn from the initial studies. The activity and specificity of this complex have now been applied to the more complex targets of polynucleotide RNA. Our results illustrate its significant utility in current problems of nucleic acid folding. Investigations with tRNA^{Phe} demonstrate that NiCR is a highly effective probe of guanine N7, and in turn application of the nickel complex to the *Tetrahymena* group I intron provides new information on the active site of this ribozyme.

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[1] Abbreviations: ASIF, accessible surface integrated field; MMPP, magnesium monoperoxyphthalate; NiCR, (2,12-dimethyl-3,7,11,17-tetraazabicyclo[11.3.1]heptadeca-1(17),2,11,13,15-pentaenato)nickel(II) perchlorate.

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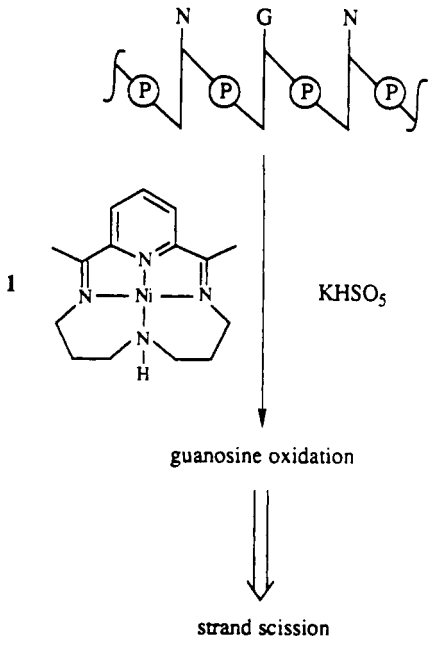

MATERIALS AND METHODS

Materials. All buffer solutions were made with purified water that had been autoclaved after treatment with diethyl pyrocarbonate. Magnesium monoperoxyphthalate (MMPP)[1] and $KHSO_5$ (in the form $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$, oxone) were purchased from Aldrich. All other reagents and enzymes were obtained in the highest grade commercially available and used without further purification. NiCR [(2,12-dimethyl-3,7,11,17-tetraazabicyclo[11.3.1]heptadeca-1(17),2,11,13,15-pentaenato)nickel(II) perchlorate] was prepared according to published procedures (Karn & Busch, 1966).

Preparation of tRNA. Commercial tRNA samples (Sigma) were dissolved in a solution of 0.3 M NaOAc, 10 mM Tris·HCl, pH 7.5, 10 mM EDTA, and 0.5% SDS, extracted with phenol–chloroform, and precipitated in ethanol prior to all subsequent manipulations. $tRNA^{Phe}$ (yeast) was 5′-dephosphorylated under standard conditions using alkaline phosphatase (calf intestinal) and rephosphorylated to form [5′-^{32}P]$tRNA^{Phe}$ in the presence of T4 polynucleotide kinase, [γ-^{32}P]ATP (3000 Ci/mmol), and RNasin (5 units/20 μL reaction volume) (Zaug et al., 1988). The radiolabeled product was purified by denaturing gel electrophoresis (20% polyacrylamide, 8 M urea) and recovered by crushing and soaking gel fragments containing the desired material in a solution of 0.5 M NH_4OAc, 0.1% SDS, and 0.1 mM EDTA. This mixture was gently shaken at 37 °C for 4 h and centrifuged. The supernatant was collected and [5′-^{32}P]$tRNA^{Phe}$ was precipitated with ethanol. The purity of each sample was analyzed by electrophoresis (20% polyacrylamide, 8 M urea). Aliquots of this RNA (10 nCi) were renatured for the reaction below in a final volume of 20 μL containing carrier tRNA (2 μg), 10 mM $MgCl_2$, 100 mM NaCl, and 10 mM potassium phosphate, pH 7.0. This solution was heated to 50 °C for 5 min and allowed to cool slowly to ambient temperature (1.5 h) before further use. Semidenatured samples were prepared similarly by heating (50 °C, 5 min) and then immediately cooling (4 °C) analogous solutions of tRNA in the absence $MgCl_2$.

Preparation of Tetrahymena Intron RNA. The L-21 *Sca*I form of the *Tetrahymena* intron was transcribed by T7 RNA polymerase from linear pT7L-21 DNA as described previously (Zaug et al., 1988). The purified RNA was dephosphorylated and 5′-^{32}P-end-labeled according to the procedures for [5′-^{32}P]$tRNA^{Phe}$. Electrophoretic separation was accomplished using 6% polyacrylamide and 8 M urea. To renature the radiolabeled RNA, 10-nCi aliquots were added to 20 μL containing carrier tRNA (2 μg), 10 mM Tris·HCl, pH 7.0, and 10 mM $MgCl_2$. This mixture was heated to 50 °C for 15 min and then immediately cooled to 4 °C. Denatured samples were prepared under equivalent conditions except 0.1 mM EDTA was substituted for $MgCl_2$.

Nickel-Dependent Modification of RNA. NiCR (**1**) (3 μM) and an oxidant, either $KHSO_5$ (100–200 μM) or MMPP (300 μM), were added to aliquots of RNA (20 μL) and incubated at ambient temperature for 30 min. Reactions were quenched by adding a solution (180 μL) of 3 μg of carrier tRNA 0.3 M NaOAc, 10 mM Tris·HCl, pH 7.5, 10 mM EDTA, and 0.5% SDS, extracted with phenol–chloroform (200 μL), and precipitated with ethanol overnight at −20 °C. The precipitated RNA was subsequently redissolved in 1 M aniline acetate (pH 4.5, 20 μL) and incubated at 60 °C for 20 min in the dark (Peattie, 1979). Samples were next frozen at −80 °C and lyophilized. The dried samples were repeatedly dissolved in water and lyophilized in order to remove the aniline acetate completely. Samples were analyzed by denaturing gel electrophoresis and detected by autoradiography.

Sequencing Reactions of RNA. RNA samples were sequenced and treated with RNases (T1, U2, and *Phy*M) and alkaline conditions using standard protocols (Donis-Keller et al., 1977; Donis-Keller, 1980). Hydroxyl radical mapping of RNA was performed according to published procedures (Latham & Cech, 1989). The electrophoretic conditions used to analyze the products of these reactions are described in Figures 1 and 3.

RESULTS

Modification of tRNA Is Base Specific. Reaction between NiCR, $KHSO_5$ (or MMPP), and [5′-^{32}P]$tRNA^{Phe}$ did not produce spontaneous fragmentation of the polynucleotide backbone. Instead, oxidative modification was detected after standard treatment with aniline acetate, pH 4.5 (Peattie, 1979). Under these conditions, RNA strand scission was induced at sites of nucleoside oxidation, and the resulting scission products were identified through comparison to standards formed by enzymatic sequencing (Figure 1). This analysis demonstrated that only guanine and its derivatives were subject to oxidation by NiCR. Electrophoretic mobilities of the fragments formed by sequencing and NiCR were similar but not identical since their respective treatments produced different 3′ termini. Ribonuclease digestion generated the faster migrating species containing 3′-phosphate whereas aniline acetate incubation lead to the formation of a 3′ protonated Schiff's base that retarded migration (Ehresmann et al., 1987).

Many reactive sites were exposed to the nickel reagent when the native structure of $tRNA^{Phe}$ was disrupted by the absence of Mg^{2+}. Approximately uniform reaction was observed at G15, G18, G19, G20, G24, N2,N2-dimethyl G26, and O2′-methyl G34 (lane 4, Figure 1). Further electrophoretic analysis indicated similar reaction at Y37, G43, G45, G53, and G57. Residues such as G22 and G30 remained inert to oxidation under these conditions and thus probably retain their base-paired and helical structure (Figure 2). Modification of N2-methyl G10 was found to be independent of NiCR and occurred spontaneously in the presence of $KHSO_5$. This nonspecific oxidation was prevented by substituting $KHSO_5$ with a weaker oxidant such as MMPP (lane 2, Figure 1).

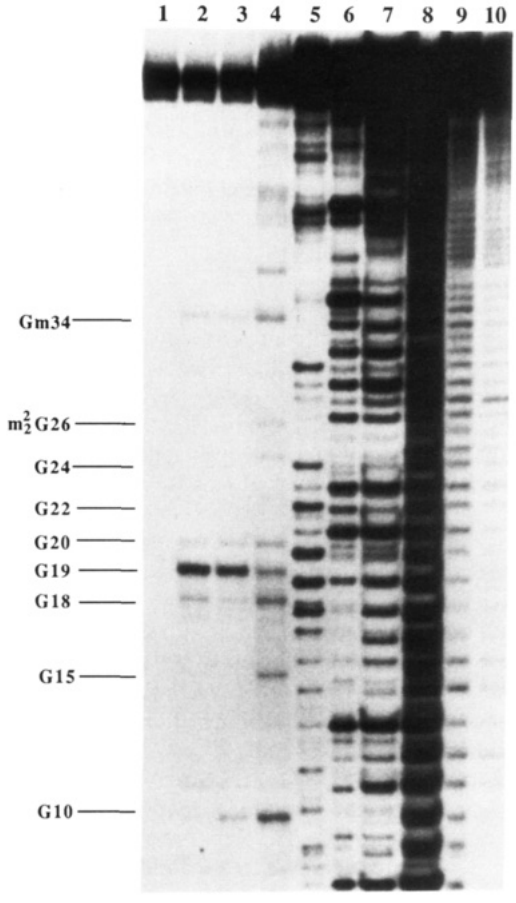

FIGURE 1: Specific cleavage of tRNA^{Phe} (yeast) by NiCR and other reagents. 5′-^{32}P-labeled tRNA (10 nCi and 2 μM nucleotide using carrier tRNA per lane) was treated as described below and analyzed by gel electrophoresis (20% polyacrylamide, 8 M urea) and autoradiography. Native RNA^{Phe} was subjected to aniline acetate treatment after incubation (30 min) with no additional reagents (lane 1), with NiCR (3 μM) and MMPP (300 μM) (lane 2), or with NiCR (3 μM) and $KHSO_5$ (100 μM) (lane 3). Semidenatured tRNA^{Phe} (no Mg^{2+} present) was also incubated with NiCR (3 μM) and $KHSO_5$ (200 μM) for 30 min and then subjected to aniline acetate (lane 4). RNA sequencing was performed with RNase T1 (lane 5), RNase U2 (lane 6), RNase *Phy*M (lane 7), and alkaline hydrolysis (lane 8). Further characterization included modification of native (lane 9) and semidenatured (lane 10) tRNA^{Phe} with hydroxyl radical.

Modification of tRNA Is Conformation Specific. In contrast to the numerous sites of reaction detected above, few residues in the native conformation of tRNA^{Phe} were accessible to NiCR. In the presence of 10 mM Mg^{2+}, the RNA tertiary structure inhibited oxidation at all sites except for G19 (lane 3, Figure 1). Modification at this site now dominated the pattern of recognition. Three other residues (G18, G20, and O2′-methyl G34) sustained weak modification in the native structure of tRNA^{Phe}, and their level of reactivity was well below that of G19. Other sites available for modification in the denatured RNA became inert upon polynucleotide folding (Figure 2). Since these results could be anticipated from the crystal structure of this RNA (see Discussion), the phosphate buffer used in this section appeared not to inhibit the Mg^{2+}-dependent formation of native tRNA^{Phe}. The alternative use of Tris·HCl (pH 7) moderately inhibited the NiCR reaction perhaps through its coordination to NiCR. However, this did not preclude the use of Tris·HCl in our examination of intron RNA (see below).

Modification of Intron RNA Is Also Conformation Specific. Reactivity of *Tetrahymena* intron RNA (L-21 *Sca*I) was also significantly affected by its folded structure. In the absence

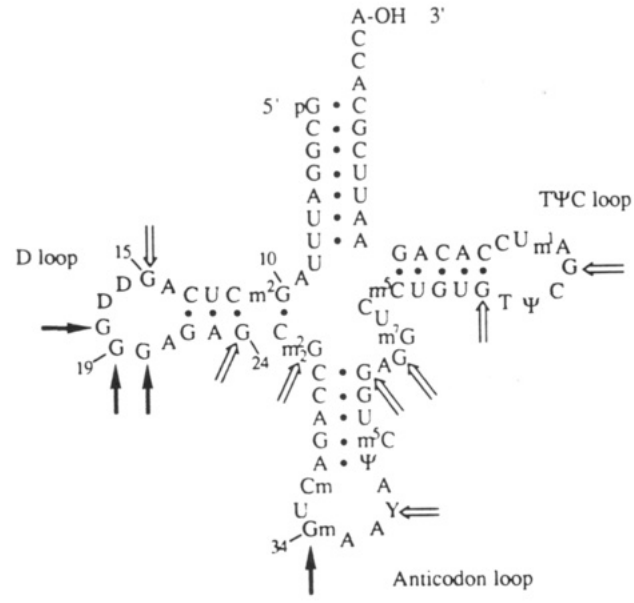

FIGURE 2: Sequence and secondary structure of tRNA^{Phe}. Solid arrows (→) indicate sites of NiCR reaction observed for both native ($+Mg^{2+}$) and denatured ($-Mg^{2+}$) tRNA. Open arrows (⇒) indicate sites of reaction observed for only the denatured tRNA.

of Mg^{2+}, the tertiary conformation of the intron is not maintained (Celander & Cech, 1991), and consequently many nucleotides become accessible to chemical and enzymatic reagents. Strand scission induced by NiCR under these conditions occurred predominantly at G residues, and the resulting fragments were formed in approximately equal amounts (lane 2, Figure 3). A great number of guanosines also remained inert and inaccessible to NiCR due to the RNA secondary structure that persists independent of Mg^{2+} (Inoue & Cech, 1985; Jaeger et al., 1990). Formation of the fully folded conformation is thought to require the presence of more than 0.75 mM Mg^{2+} (Celander & Cech, 1991), and consistent with this, the profile of NiCR modification was unaffected by the addition of only 0.1 mM Mg^{2+}.

In the presence of 1.0–10 mM Mg^{2+}, the reactivity of the RNA changed dramatically. Modification of G288 was strongly enhanced (lane 3, Figure 3). Slight activation was also observed at G188 and G264. Otherwise, modification was either suppressed (for example, G169 and G257) or completely inhibited (for example, G176, G212, and G215). A total of 11 major sites of reaction were observed with NiCR, and all are generally consistent with the current model of the intron structure (Michel & Westhof, 1990). Seven of these sites are located in loops and unpaired sequences outside of the catalytic core (Figure 4). The other unpaired site (G303) is part of the core but may become accessible to NiCR through a possible equilibrium or heterogeneity of nucleotide structure of Wang & Cech, 1992). In the natural transcript, the region surrounding this residue is thought to interact with P1, the helix formed between the exon and internal guide sequences (Pyle et al., 1992). These interactions are not present in the L-21 *Sca*I RNA used in this report. The remaining target sites for NiCR are G-C pairs, and no reaction was detected at G-U pairs within the intron structure.

DISCUSSION

Reactivity of NiCR Correlates to the Three-Dimensional Structure of tRNA. Commercially available yeast tRNA^{Phe} served as the prototypical RNA for our initial investigations. This species has been frequently chosen to characterize new

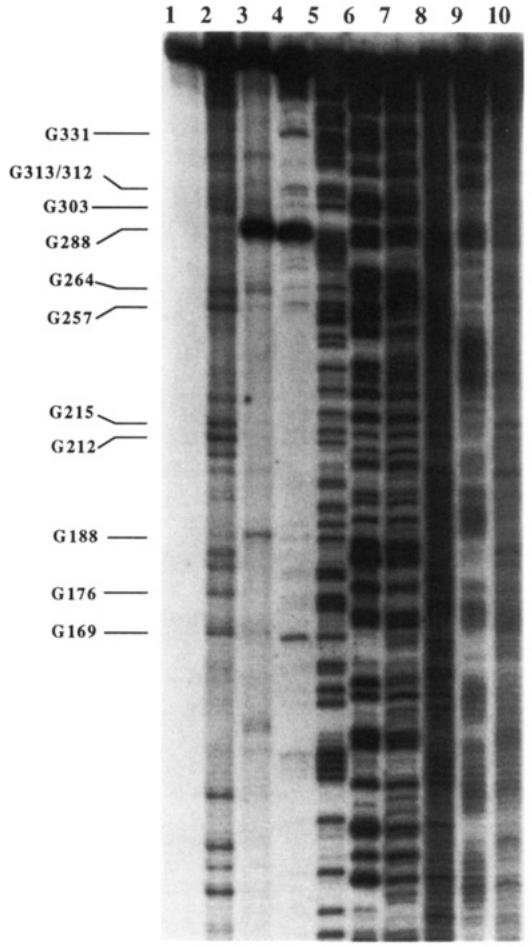

FIGURE 3: Specific cleavage of *Tetrahymena* intron by NiCR and other reagents. 5′-^{32}P-labeled RNA (10 nCi of L-21 *Sca*I and 2 μM nucleotide using carrier tRNA per lane) was treated as described below and analyzed by gel electrophoresis (6% polyacrylamide, 8 M urea) and autoradiography. As a control, the intron was treated solely with aniline acetate (lane 1). Denatured ($-Mg^{2+}$) (lane 2) and native ($+Mg^{2+}$) (lane 3) introns were incubated at room temperature for 30 min with NiCR (3 μM) and $KHSO_5$ (200 μM) and then treated with aniline acetate as described under Materials and Methods. Native intron RNA was also incubated at 4 °C for 10 min with RNase T1 (0.01 unit) in the presence of 200 mM NaCl, 5 mM $MgCl_2$, 10 mM Tris·HCl, pH 7.6, and carrier tRNA (2.5 μg) (lane 4). RNA sequencing under standard denaturing conditions utilized RNases T1 (lane 5), U2 (lane 6), and *Phy*M (lane 7) and alkaline hydrolysis (lane 8). Further characterization included modification of the native (lane 9) and denatured (lane 10) intron with hydroxyl radical.

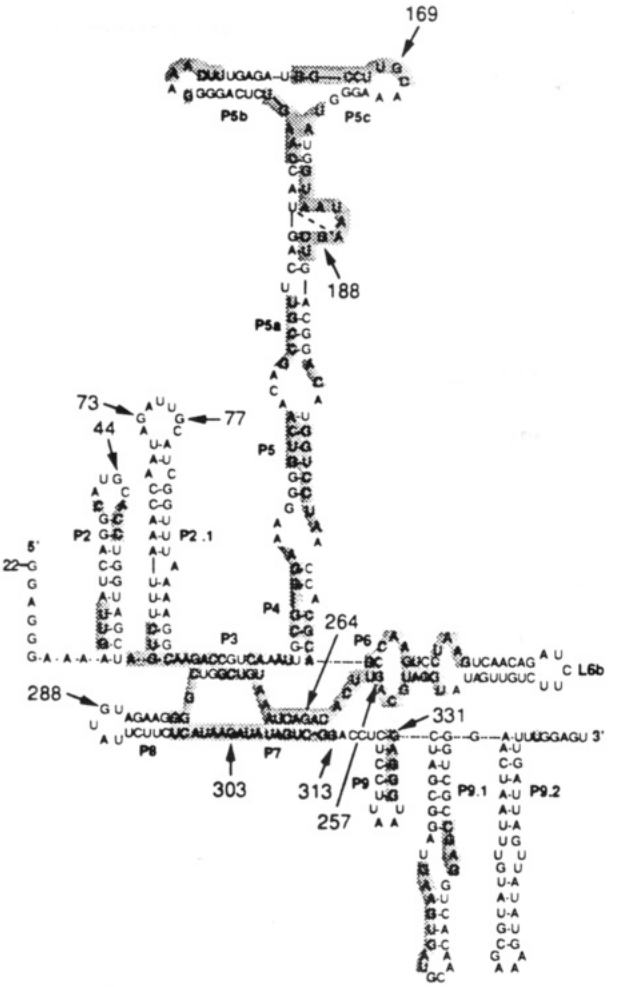

FIGURE 4: Structure vs reactivity of L-21 *Sca*I RNA. Sites of modification by NiCR are indicated with arrows to the model of intron secondary structure (Michel & Westhof, 1990). Data for nucleotides beyond A334 were not determined in this study. Shaded sequences were protected from strand scission by hydroxyl radical as originally reported (Latham & Cech, 1989), subsequently revised (Heuer et al., 1991; Murphy & Cech, 1993), and finally adapted with permission (Murphy & Cech, 1993).

conformation-specific reagents since correlations are easily made between modification sites and structure (Latham & Cech, 1989; Chow et al., 1992). The folding pattern of this RNA has been solved by crystallography (Kim et al., 1974; Robertus et al., 1974) and has been the subject of many further experimental and theoretical investigations (Murakawa et al., 1989; Carter et al., 1990; Holbrook & Kim, 1983; Lavery & Pullman, 1984; Furois-Corbin & Pullman, 1985). In addition, natural tRNA contains an array of modified bases that futher test the specificity of new reagents.

Solvent-accessible guanine derivatives were the sole targets of oxidation by NiCR. Although many of these residues were modified in the unfolded $tRNA^{Phe}$, only four sites maintained reactivity in the native structure. Visual inspection of the tRNA crystal structure (Figure 5) reveals that few of the 22 guanosines (highlighted in magenta) present highly exposed N7 surfaces (yellow). Most of these groups are shielded within the major groove of the helixes. Two of the most reactive residues, Gm34 and G19, provide notable exceptions since they both terminate helical regions. While G19 also participates in a Watson–Crick base pair with C56, its reactivity was predicted from oligodeoxynucleotide studies previously demonstrating the modification of a guanosine base pair at the end of a duplex (Chen et al., 1992).

The conformational specificity of NiCR is most precisely established for polynucleotide RNA by comparing the sites of reaction to the calculated surface properties of $tRNA^{Phe}$. In this analysis, the relative modification of G residues corresponded exactly to the published availability of the guanine N7 positions on the basis of surface and electrostatic parameters (Lavery & Pullman, 1984) (Table I). Alternative data derived solely from accessible surface area and probe radii of 1–4 Å were not sufficient to account for the reactivity of G residues with either dimethyl sulfate (Holbrook & Kim, 1983; Lavery & Pullman, 1984) or NiCR. Only the accessible surface integrated field index (ASIF; Lavery & Pullman, 1984) correctly anticipated the results of NiCR. Moreover, modification based on NiCR conformed to this index even better than modification based on dimethyl sulfate since G20, an expected target of reaction, was oxidized by NiCR but was inexplicably inert to dimethyl sulfate (Peattie & Gilbert, 1980). Equivalent computational analysis of the N1, 2-amino, and

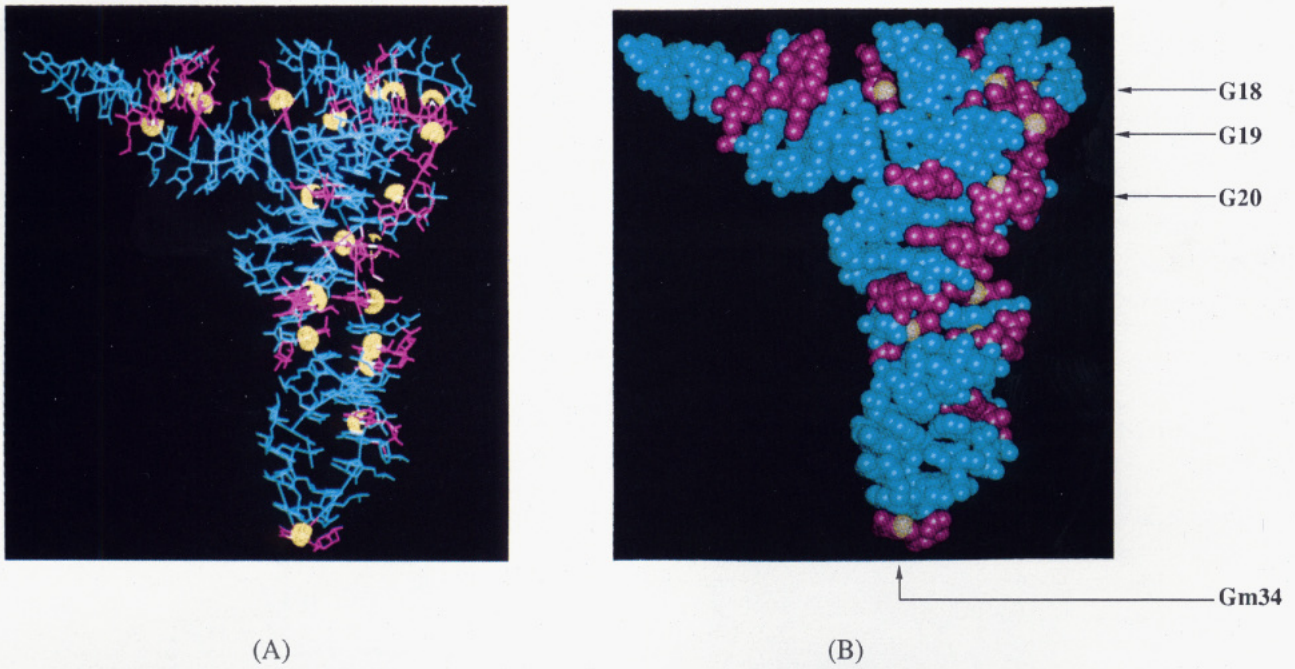

FIGURE 5: Three-dimensional structure of tRNA^{Phe} and location of reactive guanosine residues. The refined crystallographic coordinates of tRNA^{Phe} (Westhof et al., 1988) were modeled with the SYBYL software package. (A) All guanine derivatives are shown in magenta for this skeletal diagram, and the van der Waals radius of each guanine N7 is marked by a yellow stippled surface. (B) The N7 positions of the bases modified by NiCR are indicated by arrows to a space-filling representation of equivalent view.

Table I: Theoretical and Experimental Reactivity of G Residues in Native tRNA^{Phe}

residue[a]	ASIF[b]	modification by[c]	
		dimethyl sulfate	NiCR
G19	−26	++	++
G18	−21	++	+
O2′-methyl G34	−20	++	+
G1	−13	+	nd[d]
G20	−11	0	+
G45	−5.5	++	0
G65	−3.7	+	0
G30	−2.6	++	0

[a] G residues with ASIF values of greater than −2.0 are not listed. [b] Accessible surface integrated field was calculated by Lavery and Pullman (1984) in units of V·Å. A higher reactivity is suggested by a more negative value. [c] Relative reactivities are indicated by (++) very reactive, (+) moderately reactive, and (0) not reactive. Dimethyl sulfate data derive from Peattie and Gilbert (1980) and Lavery and Pullman (1984). [d] Not determined in this report.

phosphate oxygen moieties of G residues (Lavery & Pullman, 1984) did not reveal any relationship to the action of NiCR. The overall utility of NiCR may therefore be judged on the basis of its highly predictable pattern of modification.

A number of metal salts have been shown by crystallography to interact with tRNA^{Phe} but none mimic the selectivity of NiCR. Magnesium and certain lanthanides such as samarium closely associate with the phosphate oxygens rather than the bases of RNA (Jack et al., 1977; Saenger, 1984). These ions are not redox active, and they do not bind to all of the residues modified by NiCR. Alternatively, platinum and lead interact strongly and directly with nucleotide bases (Jack et al., 1977; Brown et al., 1985), but again these do not bind near G19, the site of greatest reactivity with NiCR. The redox-active metals manganese, cobalt, and osmium also associate directly with nucleotide bases of tRNA^{Phe} (Jack et al., 1977), and yet none of the metals exhibit a binding specificity that would anticipate the observed pattern of NiCR reaction. Equivalent data on the binding of nickel and its complexes are not currently available. For DNA, nickel is known to bind selectively to guanine N7 sites that are highly accessible to solvent as in Z-form helixes (Taboury et al., 1984). For tRNA^{Phe}, NiCR appears to promote an oxidative process that is similarly dependent on the accessibility of guanine N7.

The activity presented by NiCR is complementary to that of other modification reagents including those based on metal complexes (Pyle & Barton, 1990). For example, Fe·EDTA is used to generate hydroxyl radical for examining the sugar backbone of DNA and RNA (Dixon et al., 1991; Latham & Cech, 1989; Celander & Cech, 1990). When this method was applied to native tRNA^{Phe}, residues including G18 G19, and G20 were shown to be partially protected. In contrast, these same residues were the major sites of modification by NiCR. Such results complement each other and together suggest that guanine N7 is forced out onto the surface of the folded structure whereas the ribose group is held within its interior. Such interpretation is consistent with the crystal structure of tRNA^{Phe} (Kim et al., 1974; Robertus et al., 1974). On the basis of the excellent correlation between the reactivity of NiCR and the conformation of RNA in this example, the use of NiCR was extended to a current problem in RNA folding.

NiCR-Identified Guanine N7 Positions on the Surface of Tetrahymena Intron RNA. Investigations on RNA structure and folding have intensified since the discovery of catalytic RNA [reviewed in Cech (1990) and Pace and Smith (1990)]. The activity of *Tetrahymena* group I intron tRNA, for example, requires the intron sequence to adopt a discrete conformation with a defined surface and active site (Latham & Cech, 1989; Michel & Westhof, 1990). This structure has been analyzed using a variety of chemical and biochemical methods, and a detailed model of its organized core has been proposed (Michel & Westhof, 1990). Sufficient data are therefore available to assess subsequent application of NiCR and determine its comparative utility for macromolecules more complex than tRNA. The target of this and many previous modification studies has been the L-21 *Sca*I derivative of the

Tetrahymena intron. This sequence lacks the 3′ and 5′ termini required for self-splicing but is fully active as an endonuclease in the presence of Mg^{2+} and a guanosine cofactor (Cech, 1990).

The conformational specificity of NiCR observed from reaction with intron RNA is reminiscent of that determined with $tRNA^{Phe}$ and again appears to correlate with the availability of guanine N7. As expected, the pattern of reaction did not coincide with the backbone oxidation induced by hydroxyl radical, and in the case of intron RNA, most residues modified by NiCR were inert to hydroxy radical (lane 9 and 10, Figure 3; Latham & Cech, 1989, Figure 4). These data consequently suggest that the N7 positions of G169, G188, G257, G264, G303, G312/313, and G331 are solvent accessible even though their corresponding ribose groups are solvent protected. Likewise, the pattern of NiCR-induced modification appears more dependent on the overall tertiary structure than on any local secondary structure. NiCR reacted with only eight of the many G residues that are depicted as single-stranded in the predicted secondary structure (Figure 4). Furthermore, three guanosines (G257, G264, and G331) thought to be base-paired and helical were still found to interact with NiCR.

Specific modification of only three individual G-C pairs in L-21 *Sca*I affirms the most salient and useful feature of NiCR. In each case, reaction could have been anticipated by the unusually high exposure of guanine N7, as suggested by the three-dimensional model (Michel & Westhof, 1990). Both G257 and G331 participate in base pairs that terminate helical regions (P6 and P9, respectively) and do not seem to stack coaxially onto other duplex structures. Finally, the most intriguing result of this investigation is the selective oxidation of G264 by NiCR. This guanosine would not ordinarily be expected to react since it resides within the P7 helix and is base-paired with C311. However, it is also considered to play a unique role in the intron active site by binding to the catalytically essential guanine cofactor through its 2-amino and N7 groups (Michel et al., 1989). This region of P7 might then be partially distorted to facilitate recognition of GTP or, in this study, NiCR by G264. Modification at this site could have alternatively resulted from a reversible denaturation of P7, but this is not likely since another G residue in this helix (G309) did not react with NiCR under equivalent conditions (Figure 3).

The beneficial properties of NiCR are also evident through a comparison to other guanine-specific reagents (Table II). Each site of modification by NiCR was consistent with the current model of intron structure. In contrast, dimethyl sulfate exhibited only a limited selectivity for the accessible guanine N7 positions in L-21 *Sca*I RNA (Inoue & Cech, 1985). The published pattern of RNase T1 reaction is similar to that of NiCR but includes extra sites in the predicted helical regions of P2.1 (G91 and G92) and P3 (G100) (Cech et al., 1983; Inoue & Cech, 1985).

Application of RNase T1 is also subject to other ambiguities. The backbone hydrolysis catalyzed by this enzyme may permit strand dissociation and partial denaturation of the target structure that in turn could expose extraneous sites to reaction (Ehresmann et al., 1987). This event may explain our unexpected detection of sites throughout P3 and P7 that were sensitive to RNase T1 (lane 4, Figure 3). Finally, RNase T1 may be unable to modify potential substrates such as G188 that are likely unpaired but inaccessible to a reagent as large as an enzyme. NiCR is not likely to produce such anomalous results since it is significantly smaller than RNase T1 and does not produce spontaneous strand scission.

Table II: Reactivity of Selected G Residues in Intron RNA (L-21 *Sca*I)

residue[a]	modification by[b]		
	dimethyl sulfate	RNase T1	NiCR
G44	0	+	+
G58	+	0	0
G73	+	+	+
G77	0	+	+
G91	0	+	0
G92	0	+	0
G100	+	+	0
G117	++	0	0
G118	++	0	0
G119	++	0	0
G169	++	+	+
G188	0	0	+
G200	++	0	0
G234	++	0	0
G257	0	0	+
G264	0	0	+
G272	++	0	0
G285	++	0	0
G288	++	+	++
G303	+	+	+
G313	+	+	+
G331	0	0	+

[a] Only G residues that were subject to modification by RNase T1, NiCR, or dimethyl sulfate (partial list) are included in this table. The residues indicated in bold type are modified by NiCR only. [b] Relative reactivities are indicated by (++) very reactive, (+) moderately reactive, and (0) not reactive. Dimethyl sulfate data derive from Inoue and Cech (1985) and Kim and Cech (1987). RNase T1 data derive from Cech et al. (1983) and Inoue and Cech (1985).

NiCR has maintained a highly specific and predictable pattern of reactivity in all of our past studies on oligodeoxynucleotides (Chen et al., 1992; Muller et al., 1992) as well as in this current report on polynucleotide RNA. In each example, only G residues with highly exposed N7 positions were targets of oxidation. This selectivity became most apparent after our modification data were related to the calculated surface properties of $tRNA^{Phe}$ and the proposed folding model of L-21 *Sca*I RNA. The pattern of recognition exhibited by NiCR also provides consistent evidence for a mechanism of action proposed previously and thought to involve a specific association between NiCR and guanine N7 (Muller et al., 1993). Most importantly, this study demonstrates the value of NiCR as a highly precise probe for nucleic acid structure.

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