Nickel and Cobalt Reagents Promote Selective Oxidation of Z-DNA†

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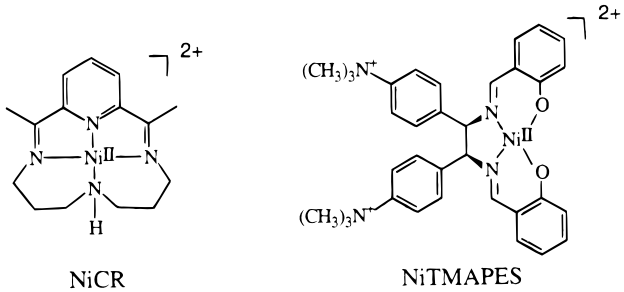
ABSTRACT: The structural characteristics of Z-DNA were used to challenge the selectivity of guanine oxidation promoted by nickel and cobalt reagents. Base pairing and stacking within all helical structures studied previously had hindered access to guanine and limited its reaction. However, the Z-helix uniquely retains high exposure of guanine N7. This exposure was sufficient to direct oxidation specifically to a plasmid insert -$(CG)_{13}$AATT$(CG)_{13}$- that adopted a Z-conformation under native supercoiling. An alternative insert -$(CG)_7$- retained its B-conformation and demonstrated the expected lack of reactivity. For a nickel salen complex made from a particularly bulky ligand, preferential reaction shifted to the junctions within the Z-DNA insert as is common for large reagents. Inactivation of the nickel reagents by high-salt concentrations prevented parallel investigations of Z-DNA, formed by oligonucleotides. However, the activity of Co^{2+} was minimally affected by salt and consequently confirmed the high reactivity of 5′-p$(CG)_4$ in its Z-conformation. These reagents may now be applied to a broad array of targets, since their structural specificity remains predictable for both complex and helical assemblies of nucleic acids.

Chemical and enzymatic probes of nucleic acid structure offer a rapid and convenient method for characterizing polynucleotide folding, dynamics, and stability (*1*−*4*). This approach also serves as an important complement to alternative techniques based on genetic (*5*) and physical analysis (*6*). Whether reagents have a narrow or broad target specificity, their utility is primarily a function of consistent and predictable reactivity. For example, hydroxyl radical has become the method of choice for characterizing the general accessibility of the phosphoribose backbone (*7*) despite its propensity for reacting at numerous sites within each nucleotide (*8*−*10*). Information on specific functional groups within nucleotides relies on reagents that are selective for those individual groups. Transition metal salts and complexes have contributed prominently to reagents expressing a range of specificities (*7*, *11*) as illustrated by Fe•EDTA (*7*), copper phenanthroline (*12*), oxoruthenium complexes (*13*), and a variety of manganese complexes (*14*, *15*).

Our laboratories have developed a series of probes based on nickel and cobalt that help to describe the structural environment of guanine (*16*−*19*). Representative species such as (2,12-dimethyl-3,7,11,17-tetraazabicyclo[11.3.1]-heptadeca-1(17),2,11,13,15-pentaenato)-nickel(II) (NiCR)[1] and Co^{2+} promote guanine oxidation in the presence of peracids such as $KHSO_5$ or magnesium monoperoxyphthalate (MMPP) (*17*, *20*). Initial studies based on oligonucleotides revealed a high selectivity for unpaired guanines in hairpin loops and bulges as well as base-paired guanines at helical termini (*17*, *21*). Later investigation with $tRNA^{phe}$ demonstrated a direct correlation between modification of guanine induced by NiCR and the steric and electrostatic environment of its N7 (*22*). This relationship has held constant through a number of recent applications (*18*, *23*−*28*). Mechanistic studies have suggested that the specificity originates from a direct interaction between guanine N7 and a transient Ni(III)−peracid complex (*29*, *30*) (Scheme 1).



NiCR NiTMAPES

Although guanine N7 lies on the surface of the major groove, its recognition by NiCR is apparently inhibited by the steric constraints of B-helical DNA and A-helical RNA. However, the solvent accessibility of guanine N7 increases greatly upon formation of Z-helical DNA (*31*), and consequently, this structure was considered a potential target of NiCR. The helix diameter of Z-DNA is narrower, and its major groove is much shallower than the counterparts in B-DNA (*32*). More importantly, guanine N7 is thrust out to the edge of Z-DNA (Figure 1) sufficiently for metal coordination (*33*, *34*). While simple nickel salts are able to bind guanine N7 in B-helical DNA (*35*), they promote and

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[1] Abbreviations: DEPC, diethypyrocarbonate; 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; NiTMAPES, [N, N′-bis(salicylaldehyde)-*meso*-1,2-bis(4-trimethylaminophenyl)ethylenediimino]nickel(II).

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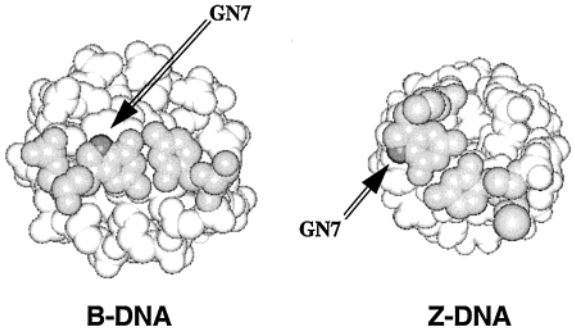
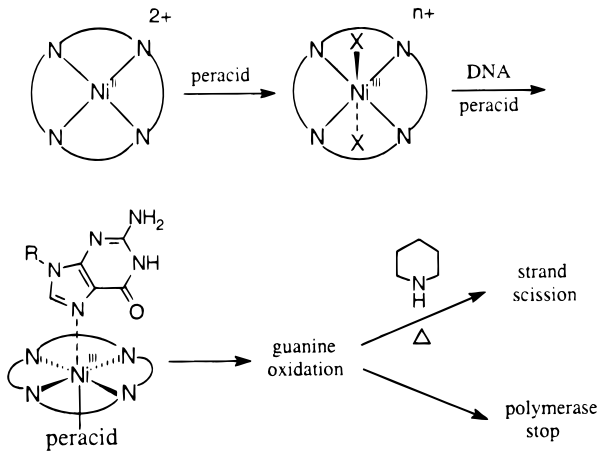


FIGURE 1: Top views of B- and Z-helical conformations. The terminal G−C base pairs are shaded for clarity, and the guanine N7 positions are further darkened for emphasis.

Scheme 1



preferentially stabilize formation of the alternative Z-helical conformation (*36*, *37*). Selective coordination between macrocyclic complexes of nickel and guanine N7 of Z-DNA, but not B-DNA, has also been suggested by recent circular dichroism studies (*30*). If such recognition is possible, then the macrocyclic nickel reagents should react with stacked and base-paired guanines in Z-DNA but equivalent residues in B-DNA should remain relatively inert.

Nickel salen derivatives such as [*N*,*N*′-bis(salicylaldehyde)-*meso*-1,2-bis(4-trimethylaminophenyl)ethylenediimino]nickel-(II) (NiTMAPES) and certain cobalt salts have provided complementary and related activities to NiCR. Under oxidative conditions, NiTMAPES conjugates to solvent accessible guanine residues and creates strong termination sites for DNA polymerase in primer extension assays (*38*, *39*). The cobalt salts provide an alternative method for selective oxidation of accessible guanine residues, although their reactivity is not solely dependent on the exposure of guanine N7 (*17*). Still, the cobalt reagent maintains activity under high ionic strength (see below) and high temperature (*17*) that are not compatible with the nickel complexes. All three probes, NiCR, NiTMAPES, and Co^{2+}, were consequently examined with duplex polynucleotides and oligonucleotides capable of forming Z-helical conformations as a further challenge to their predicted specificity.

MATERIALS AND METHODS

Reagents. NiCR [(2,12-dimethyl-3,7,11,17-tetraazacyclo-[11.3.1]heptadeca-1(17),2,11,13,15-pentaenato)nickel(II) perchlorate] and NiTMAPES [(*N*,*N*′-bis(salicylaldehyde)-*meso*-1,2-bis(4-trimethylaminophenyl)ethylenediimino)nickel(II) perchlorate] were prepared according to published procedures (*40*, *41*). Oligonucleotides were purchased from Oligos Etc., Inc and purified to homogeneity under strongly denaturing conditions (pH 12) using anion exchange chromatography (Mono Q, Pharmacia) (*20*). Commercially available materials, such as magnesium monoperoxyphthalate (MMPP), $CoCl_2$, $KHSO_5$, and piperidine, were used without purification. All buffers were made from purified water (Nanopure, Sybron/Barnsted) and reagents of the highest available quality.

General Methods. DNA reaction profiles were quantified by either autoradiography and densitometry (Enprotech) or phosphoimagery (Molecular Dynamics). Per nucleotide reactivities were normalized with respect to that of G4354. Ethidium bromide concentration was determined at 460 nm (4.22 $mM^{-1}cm^{-1}$) and 287 nm (53.9 $mM^{-1}cm^{-1}$) (*42*). Circular dichroism (CD) spectra were collected at 4 °C, using a Jasco J-20 A spectropolarimeter. Oligonucleotides (both reaction targets and sequencing primers) were radiolabeled with γ-[^{32}P]-ATP (3000 Ci/mmol, Amersham) and T4 polynucleotide kinase.

Plasmid Preparation. pBR322 derivatives, alternatively containing -TGAGATC$(CG)_{13}$AATT$(CG)_{13}$GATCTCAAT- (pRW1561) or -TGAGATC$(CG)_7$AAT- (pRW1567) at the unique EcoR1 site, were kindly provided by Dr. Robert Wells (University of Texas) (*43*). These and the parent plasmid pBR322 were maintained in *Escherichia coli* DH5α and purified using a Qiagen plasmid kit. The native superhelical density of pBR322 was altered by addition of ethidium bromide and topoisomerase I as described previously (*44*). The resulting topoisomers were resolved by agarose (1.5%) gel electrophoresis in the presence of chloroquine, and linking numbers were determined by the band-counting method of Keller (*42*). Plasmids were stored in Tris (10 mM pH 7) and EDTA (1 mM) but precipitated from ethanol prior to reaction.

Oxidative Modification of Plasmids. Various concentrations of the terminal oxidant MMPP and metal reagent (nickel complex or cobalt salt) were incubated with plasmid DNA (20 ng), potassium phosphate (5 mM pH 7), and 100 mM NaCl (total volume 100 μL) at 25 °C. After 30 min, the reaction was quenched by addition of 2 mM EDTA (2 μL) and β-mercaptoethanol (2 μL). DNA was isolated by precipitation from ethanol and then characterized by primer extension using Vent^R DNA polymerase (New England Biolabs) with either 5′-[^{32}P]-dGTGCCACCTGACGTCTAAGA (for analysis of the EcoR1 site and insert) or 5′-[^{32}P]-dCACGTTCGCTCGCGTATCGGTG (for analysis of pBR322 nucleotides 1440−1460). Extension products were separated by denaturing polyacrylamide (8%) gel electrophoresis and identified with respect to dideoxynucleotide sequencing (Vent^R sequencing kit, New England Biolabs).

Cobalt-Dependent Oxidation of Oligonucleotides. Reaction mixtures (50 μL) containing 6 μM oligonucleotide (2 nCi), 3 M $NaClO_4$, and 10 mM sodium phosphate (pH 7) were incubated for 1 h at 4 °C prior to addition of 6 μM $CoCl_2 \cdot (H_2O)_6$ and 100 μM $KHSO_5$. These mixtures were maintained at 4 °C and quenched after 30 min by addition of 2 mM HEPES and 10 mM EDTA (pH 7). Each incubation was then individually dialyzed and lyophilized as described previously (*17*). Samples were treated with piperidine (0.2 M, 90 °C,

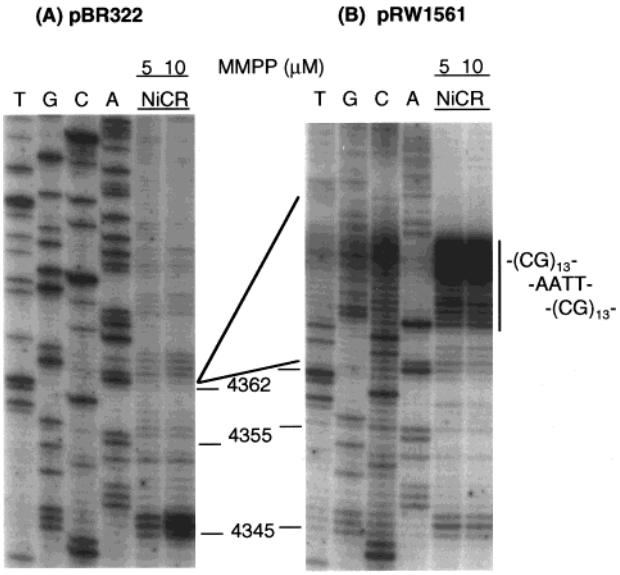


FIGURE 2: NiCR-dependent modification of duplex DNA in B- and Z-helical forms. (A) pBR322 (native superhelical density) was alternatively sequenced in the presence of dideoxynucleotides and subjected to primer extension after its oxidization by NiCR (3 μM) and MMPP (as indicated). (B) pRW1561 (native superhelical density) containing a Z-helical insert (....$(CG)_{13}$AATT$(CG)_{13}$...) at the EcoR1 site of pBR322 was examined under equivalent conditions.

30 min) prior to separation by denaturing polyacrylamide gel electrophoresis (25%).

RESULTS AND DISCUSSION

NiCR Exhibits Minimal Reactivity with B-Helical DNA. The full extent of NiCR's specificity toward duplex DNA could not be determined from our earliest investigations since the ratio of target to nontarget sites was not particularly large. Consequently, the intrinsic reactivity of sequences surrounding the EcoR1 site of pBR322 (covalently closed, native superhelical density) was measured to establish a baseline level of modification of B-helical polynucleotides. Under standard conditions, NiCR/MMPP produced no detectable guanine oxidation that inhibited primer extension by DNA polymerase except at a cluster of three contiguous guanines (pBR322 nt numbers 4345−4347) (Figure 2A). Such a cluster of guanines lowers their ionization potential and facilitates their oxidation relative to isolated guanines (*45*, *46*). This effect is most evident in the absence of extrahelical guanine residues that routinely dominate NiCR-mediated oxidation (*30*, *47*).

A Plasmid Insert Forming Z-DNA is Selectively Modified by NiCR. Two pBR322 derivatives first constructed by the Wells laboratory for their study of Z-DNA (*43*) have now been reexamined with NiCR to demonstrate the inserts' predicted high reactivity. Z-helical structures are typically less stable than their B-helical counterparts, but a number of factors influence the dynamic equilibrium between B- and Z-DNA (*48*). Alternating purine−pyrimidine sequences, and especially, $(CG)_n$ form Z-DNA most readily. Highly negative supercoiling, multivalent metal cations (such as Mg^{2+}, Mn^{2+}, Co^{2+}, and Ni^{2+}) and high ionic strength (*48*, *49*) also promote formation of Z-DNA. pBR322 contains about −33 superhelical turns (*44*) as isolated, but this not sufficient to stabilize the Z-form of most inserts. In contrast, the sequence -$(CG)_{13}$AATT$(CG)_{13}$- contained within pRW1561 adopted a Z-helical structure under native superhelical density (*43*). The isolated plasmid existed in two equal populations that only differed by their linking numbers. One was consistent with the insert in its B-form, and the other was consistent with the insert in its Z-form. Chemical and enzymatic analysis of this and related inserts also supported the presence of the Z-form as opposed to alternatives conformations such as cruciform or H-DNA (*43*, *50*).

Redundant and guanine-rich sequences often cause premature termination of primer-based sequencing, but this was not evident with the insert of pRW1561 (Figure 2B). Sequencing beyond the insert was ambiguous, but extension was still efficient and confirmed the absence of preexisting lesions (see lanes A and T). In a complementary effort, sequencing and reaction data was also obtained by primer extension of the alternative strand (See Supporting Information). Only the region forming Z-DNA was subject to strong termination in both strands (Figure 2B), and this result required the combined presence of NiCR and MMPP as expected from all previous studies (*16*). Under these conditions, the insert reacted 15- to 20-fold more efficiently than the neighboring B-helical regions. Oxidation of the triplet G (nt 4345−4347) was also detected but corresponded to a 6-fold weaker target of modification than the Z-forming DNA. Therefore, NiCR-dependent oxidation retains its sensitivity to guanine N7 accessibility even for fully complementary duplex DNA.

Most chemical reagents that have been used previously to identify regions of Z-DNA express hyperreactivity with the B−Z junction more often than with either the B- or Z-helical regions. For example, OsO_4, NH_2OH, dimethyl sulfate, and bromoacetaldehyde preferentially modify nucleotides at B−Z and Z−Z junctions (*50*, *51*). Another common reagent, hydroxyl radical, reacts equally with both helical forms and their junctions (*52*, *53*). Diethypyrocarbonate (DEPC) is the only reagent to date that has been routinely used for detecting Z-DNA, and it too reacts according to guanine N7 exposure (*51*, *54*). Interestingly, a bleomycin−Ni(III) complex selectively oxidized a Z-helical insert as well, but no data on target recognition or reaction mechanism have yet been described (*55*).

Inserts of -$(CG)_n$- that Form B-DNA are not Modified by NiCR. The repetitive nature of the -CG- insert alone is not responsible for the hyperreactivity observed with pRW1561. A short insert such as -$(CG)_7$- (pRW1567) does not form Z-DNA under physiological supercoiling (*43*) and does not express high reactivity with NiCR (Figure 3). Only the junctions of this insert appear to oxidize as readily as the guanine triplet (nt 4345−4347). This modest reactivity may indicate that a minor population of bulges are formed by base pair slippage within the redundant -CG- track (*56*). The guanine triplet along with G4354 also provide convenient standards when contrasting the reactivity of $(CG)_n$ sequences forming B- and Z-helical conformations (Figures 2 and 3). By this analysis, Z-DNA was 20-fold more reactive than B-DNA per nucleotide. Another obvious control for differences in reactivity might have contrasted the modification of -$(CG)_{13}$AATT$(CG)_{13}$- in its superhelical and relaxed forms. Unfortunately, this insert established non-B, non-Z structures in the absence of supercoiling (see below) that precluded its use in such a comparison.

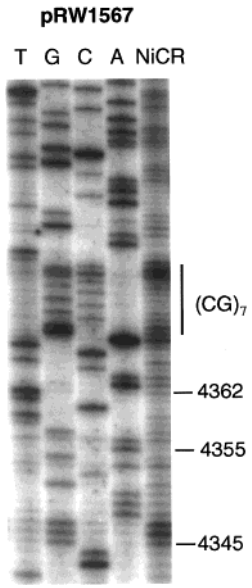


FIGURE 3: NiCR-dependent modification of plasmid DNA with a B-helical insert -$(CG)_7$-. pRW1567 (native superhelical density) was alternatively sequenced in the presence of dideoxynucleotides and subjected to primer extension after its oxidization by NiCR (3 μM) and MMPP (5 μM).

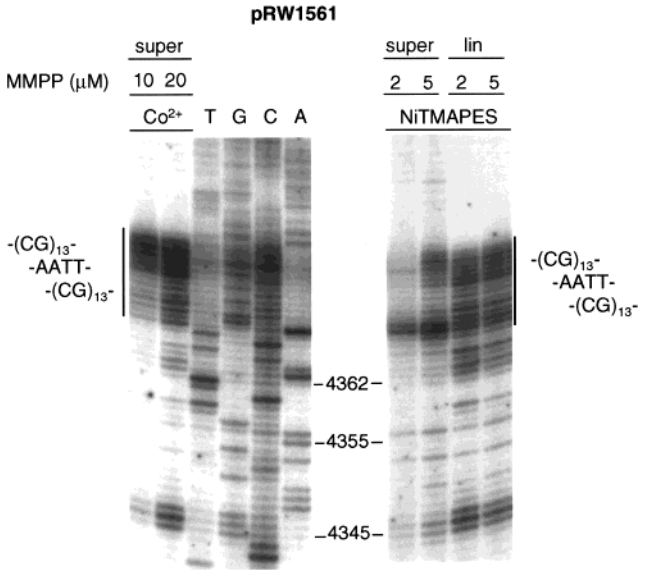


FIGURE 4: Co(II)- and NiTMAPES-dependent modification of plasmid DNA with a Z-helical insert. pRW1561 in its native superhelical (super) or relaxed (lin) forms was alternatively sequenced in the presence of dideoxynucleotides and subjected to primer extension after its oxidization by MMPP (as indicated) and either Co^{2+} (3 μM) or NiTMAPES (3 μM).

Cobalt and NiTMAPES also React Selectively at the Z-Helical Insert. Both cobalt complexes such as CoCR and simple salts such as $CoCl_2$ additionally promote guanine oxidation in the presence of peracids, and again, the reaction is dependent on the conformation of guanine (*17*). However, the selectivity of the cobalt reagents is not strictly based on the environment surrounding guanine N7. Exposure of guanine's aromatic face as well as its N7 position determine its targets of oxidation (*17*). Overall, the specificity for extrahelical guanines is lower for reactions based on cobalt versus nickel (*17*, *18*). This trend has continued for the plasmid insert of Z-DNA as described below.

The Z-helical insert -$(CG)_{13}$AATT$(CG)_{13}$- of supercoiled plasmid pRW1561 remained the most significant target of oxidation in the presence of $CoCl_2$ and MMPP (Figure 4). The specificity for this region relative to the guanine triplet or an isolated guanine (nt 4354) is approximately 50% lower than that for NiCR when comparing reactivity per nucleotide. A background oxidation of duplex DNA promoted by $CoCl_2$ was also apparent under high concentrations of MMPP (20 vs 10 μM). This cobalt salt still provided an important complement to NiCR despite its abridged selectivity. Unlike NiCR, Co^{2+} continues to promote guanine oxidation under a number of extreme conditions (see below) (*17*).

An alternative nickel complex, NiTMAPES, capable of ligand−guanine coupling, was also examined with the Z-helical insert -$(CG)_{13}$AATT$(CG)_{13}$-. Although this complex generally targets unpaired guanines in DNA and RNA, its ability to coordinate guanine N7 has not yet been determined (*38*, *39*, *41*, *57*). Interestingly, NiTMAPES did not mimic the specificity of NiCR for the plasmid insert forming Z-DNA. In the presence of a low concentration of MMPP (2 μM), NiTMAPES expressed a specificity for the junction of B- and Z-helical structures formed by the supercoiled plasmid (Figure 4, super). Reaction of Z-DNA was only observed when the concentration of MMPP was raised to $\geq$5 μM. Under these conditions, a low background level of modification was also evident. Perhaps the exposure of guanine afforded by Z-DNA is still not sufficient for recognition by the bulky TMAPES ligand.

A variety of compounds containing multicyclic aromatic substituents such as tris(4,7-diphenyl-1,10-phenanthroline)-Co(III) (58), dynemicin (*59*), and psoralen (*60*) have also demonstrated selectivity for reaction at B−Z junctions. This could in part reflect preferential association at these sites as previously described for ethidium bromide (*61*). The specificity of NiTMAPES may then result from its diminished ability to interact directly with guanine. N7 (relative to NiCR) as well as the enhanced ability of the helical junction to accommodate the aromatic substituents of the ligand. These characteristics could limit reaction of this nickel complex to only the most highly accessibility guanines presented by native structures and dynamic regions that easily adapt to the demands of the large ligand. In contrast, the smaller reagent NiCR promotes guanine oxidation without perturbing the native equilibrium of dynamic structures (*30*).

NiTMAPES-dependent reactivity at the junction of the -$(CG)_{13}$AATT$(CG)_{13}$- insert decreased after the plasmid pRW1561 was linearized with Bam H1 to remove the native superhelicity (Figure 4, lin). Under these conditions, the insert would not form the Z-helix nor the concomitant B−Z junction (*43*), and consequently an unreactive B-helical structure was expected. However, the entire insert and surrounding regions of DNA including the guanine triplet (nt 4345−4347) exhibited greater reactivity after plasmid linearization. Thus, the loss of topological constraints allowed the sequence to form nonhelical structures, whereas supercoiling actually limited the conformational disorder to the B/Z junction. The conformational heterogeneity of the linearized plasmid prevented its use as an accurate control for B-helical DNA.

NiCR Does Not Affect the Equilibrium Between B and Z-Helical DNA. Preliminary data based on nucleotides and oligonucleotides had previously suggested that the resting

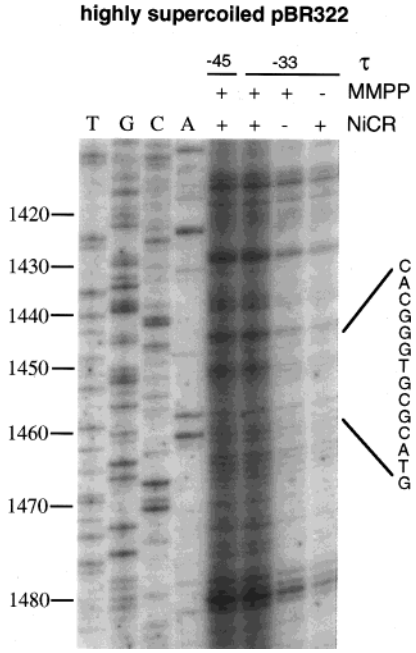


FIGURE 5: Reactivity of a site within pBR322 that bound anti-Z-DNA antibodies. pBR322 under enhanced and native superhelicity (−45 and −33 turns, respectively) was alternatively sequenced in the presence of dideoxynucleotides and subjected to primer extension after its oxidization by NiCR (3 μM) and MMPP (20 μM) as indicated.

state complex Ni(II)CR does not influence DNA structure (*30*). However, derivatives that mimic the transient Ni(III)-CR intermediate of reaction are capable of binding and stabilizing Z-DNA (*30*). This raised a concern that the probe might not only detect the presence of Z-DNA but also promote its formation. Similar arguments had been leveled against the use of antibodies for identifying Z-DNA (*44*, *62*). Under the influence of highly negative supercoiling (τ = −41), anti-Z-DNA antibodies are known to bind selectively to a 14-nucleotide sequence of pBR322 (nt 1447−1460) (*44*). Except for one guanine, this region alternates between pyrimidine and purine as expected for a sequence that might readily form Z-DNA. However, sequence alone cannot confirm the existence of a Z-helix. The conformational equilibrium could have shifted toward Z-DNA by its selective association with anti-Z-DNA antibodies.

This same region of pBR322 has now been characterized by its reactivity with the chemical probe NiCR as an alternative to antibody binding. None of the guanines between G1450 and G1460 exhibited any unusual reactivity under both native (τ = −33) or enhanced (τ = −45) superhelicity (Figure 5). Only background oxidation was observed from nucleotide 1480 to beyond nucleotide 1420. Hence, formation of Z-DNA in this region is unlikely in the absence of the anti-Z-DNA antibodies. More significantly, the conditions used for the NiCR-dependent oxidation of plasmid DNA did not detectably promote formation of Z-DNA. If selective oxidation of guanine had occurred, then the ability of this probe to detect Z-DNA could not easily have been distinguished from its potential ability to induce formation of Z-DNA.

The Hyperreactivity of Z-DNA Extends to Oligonucleotide Models. High ionic strength is commonly used as a necessary substitute of negative supercoiling for inducing a B- to

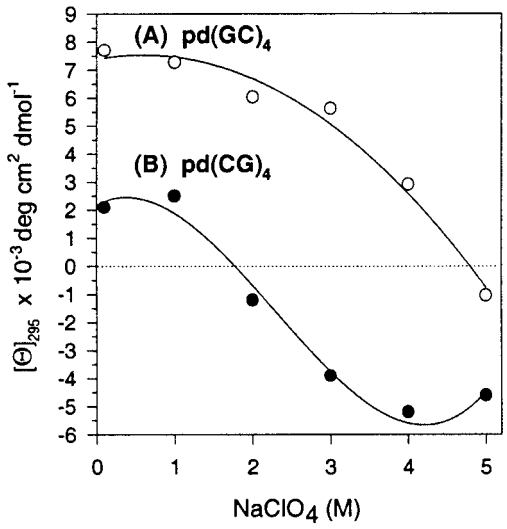


FIGURE 6: Circular dichroic spectra of (A) pd(GC)$_4$ and (B) pd(CG)$_4$ as a function of salt concentration. All samples contained 6 μM oligonucleotide, 10 mM sodium phosphate (pH 7), and 0.1−5.0 M $NaClO_4$, and measurements were performed at 4 °C.

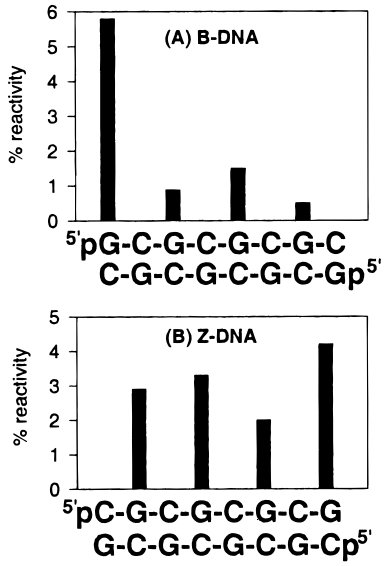


FIGURE 7: Co^{2+}-dependent oxidation of oligonucleotides forming (A) B-helical (5′-pd(GC)$_4$) and (B) Z-helical (5′-pd(CG)$_4$) structures. Oligonucleotides (6 μM) were treated with $CoCl_2$ (6 μM) and $KHSO_5$ (100 μM) in the presence of 3 M $NaClO_4$ and 10 mM sodium phosphate (pH 7) at 4 °C. The extent of reaction at each G was calculated relative to the total reacted and unreacted DNA.

Z-DNA conversion in oligonucleotides (*48*). However, the reactivity of NiCR was completely quenched by salt concentrations (0.5 M $NaClO_4$), well below that necessary for stabilizing a Z-helix. Furthermore, reaction could not be restored by replacing MMPP with the stronger oxidant $KHSO_5$ or using [5′-^{32}P]-d(ACGTCAGGTGGCACT), a single-stranded and fully accessible oligonucleotide, as a target. In contrast, cobalt-dependent oxidation of this same sequence was inhibited by less than 50% in the presence of as much as 4 M $NaClO_4$, and consequently, only the cobalt reagent was suitable for comparing the reactivity of B- and Z-helical oligonucleotides.

Additional variables associated with ionic strength were minimized in this study by examining two related sequences d(CG)$_4$ and d(GC)$_4$ under a single salt concentration rather than relying on a single sequence d(CG)$_4$ under a wide range

of salt concentrations. Selection of these oligonucleotides was based on a reported correlation between the sequence of short repeats containing C/G and an ability to form Z-DNA (*63*, *64*). Both $(CG)_n$ and $(GC)_n$ (where $n = 3$ to 5) exist as B-DNA in the presence of modest salt concentrations, but $(CG)_n$ alone converts to Z-DNA in the presences of 5 M salt. Since oligonucleotide modification routinely involves 5′-[^{32}P]-labeled targets, the conformational properties of the 5′-phosphorylated derivatives of $(CG)_4$ and $(GC)_4$ have also now been characterized by circular dichroism as a function of salt. Again, the (GC) derivative remained primarily in its B-form when the concentration of $NaClO_4$ was ≤ 3 M, whereas the (CG) derivative converted to its Z-form when the concentration of $NaClO_4$ increased above 2 M (Figure 6). A salt concentration of 3 M was consequently chosen for the reaction described below.

For B-DNA, base stacking even more than base pairing protects guanine residues from oxidation induced by Co^{2+} (*17*). This specificity is preserved at high ionic strength. The 5′-terminal guanine of B-helical $p(GC)_4$ is modified 4- and 12-fold more readily than the central guanines or the penultimate 3′-terminal guanine, respectively (Figure 7A). Similarly, the terminal guanine of Z-helical $p(CG)_4$ is also the most accessible and reactive residue (Figure 7B). However, the other guanines within the Z-DNA are significantly more reactive than their counterparts in B-DNA. The overall efficiency of oxidizing the central duplex region of Z-DNA increased 4-fold and approached that of the most accessible terminal guanine. Since the cobalt reagent is not strictly dependent on the environment of guanine N7, its specificity may additionally reflect the greater exposure of guanine C8 and possibly O6 in this conformation relative to B-DNA (*31*).

Conclusion. The utility of NiCR as a structural probe has now been extended by determining its specificity toward superhelical DNA. Individual guanine residues within long tracts of B-helical DNA were not significant targets of oxidation in the presence of NiCR and a peracid, although a single -GGG- was subject to modification due in part to its enhanced sensitivity to oxidation (*45*−*47*). Guanine modification was overwhelmingly directed with a 15- to 20-fold greater frequency to a plasmid insert forming Z-DNA rather than a related insert forming B-DNA. The unique structure of the Z-helix increases the exposure of guanine N7 sufficiently for recognition by the nickel-based intermediate proposed in the mechanism of oxidation by NiCR (Scheme 1). Of equal importance, this transient nickel−guanine interaction did not detectably perturb the conformational equilibrium of its target in contrast to the action of anti-Z-DNA antibodies. Alternative reagents NiTMAPES and $CoCl_2$ both expressed specificities that are complementary to NiCR. The bulky ligand of NiTMAPES likely helped to direct its reaction to the most disordered and accessible regions of duplex DNA. Since the cobalt salt was unaffected by ionic strength, it was most useful for detecting Z-DNA in oligonucleotides requiring the presence of high salt concentrations.

ACKNOWLEDGMENT

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SUPPORTING INFORMATION AVAILABLE

Sequence and reaction data are provided on the polynucleotide complementary to that characterized in Figure 2. This material is available free of charge via the Internet at http://pubs.acs.org.

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