# Guanine-Specific Binding by Bleomycin–Nickel(III) Complex and Its Reactivity for Guanine-Quartet Telomeric DNA†

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ABSTRACT: Bleomycin–Ni(III) [BLM–Ni(III)] complex, generated from oxidation of the corresponding BLM–Ni(II) complex with Ir(IV) or oxone, binds to single-stranded DNA and causes strand scission at specific guanine sites. In the telomeric-like oligodeoxynucleotide $d(T_2G_4)_4$, the cleavage by the BLM–Ni(III) complex occurs preferentially at positions G-9 and G-15 in loop regions. By contrast, this Ni(III) complex does not induce cleavage of G-quartet $d(G_4T_4G_4)_2$, which contains no loop guanines, ESR evidence supports that binding of the N-7 position of guanine occurs axially to the Ni(III) center in BLM. The present results provide interesting insights into Ni–BLM chemistry.

In the presence of certain metal ions, such as iron, cobalt, manganese, and vanadium, BLM[1] antibiotics are well-known to cleave specifically double-stranded DNA at 5′ → 3′ G-C and G-T sequences (Sugiura et al., 1985; Chang & Meares, 1984; Ehrenfeld et al., 1984; Burger et al., 1984; Kuwahara et al., 1985). The DNA cutting of these metallobleomycins is induced by reducing agents, hydrogen peroxide, or ultraviolet light. Recently, we proposed that metallobleomycin binds in the minor groove of B-DNA and that the 2-amino group of the guanine base adjacent to the 5′-side of the cleaved pyrimidine base is one key element of specific 5′-guanine–pyrimidine recognition by the BLM–metal complex (Kuwahara & Sugiura, 1988).

Although Ni(III) has been considered to be a relatively rare oxidation state, the Ni(III) center of hydrogenases appears to play an important role in the activation of hydrogen by these exzymes (Whitehead et al., 1991). It is of special interest to investigate the chemical reactivity of Ni(III). Recently, Chen et al. (1991, 1992) reported that some Ni(II) cyclams selectively modify guanine bases in the presence of oxone and that these nickel complexes can detect guanine conformation-specifically in DNA, such as mismatches and bulges. Here, we report guanine-specific binding and cleavage by a trivalent nickel complex of BLM. The BLM–Ni(III) complex obtained by Ir(IV) oxidation (Sugiura & Mino, 1979) bound preferentially guanine residues of single-stranded DNA and then induced DNA cutting at modification sites of the DNA by treatment with piperidine. The specific binding of the BLM–Ni(III) complex toward guanine-quartet telomeric DNA has also been investigated. In addition, ESR spectroscopy clearly indicated the presence of BLM–Ni(III)–9-ethylguanine adduct complex. The present DNA interaction and cutting features of the BLM–Ni(III) complex are substantially different from those of other metallobleomycins demonstrated previously. The results provide interesting insights into Ni–BLM chemistry.

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[1] Abbreviations: BLM, bleomycin; Ir(IV), sodium hexachloroiridate hexahydrate ($Na_2IrCl_6·6H_2O$); oxone, potassium peroxymonosulfate ($2KHSO_5·KHSO_4·K_2SO_4$); DMS, dimethyl sulfate; HPLC, high-performance liquid chromatography; $Na_2EDTA$, ethylenediaminetetraacetic acid, disodium salt; ESR, electron spin resonance.

## MATERIALS AND METHODS

*Materials.* The BLM antibiotic peplomycin (terminal amine = $NH(CH_2)_3NHCH(CH_3)C_6H_5$), provided kindly by Nippon Kayaku, was used in these experiments. Sodium hexachloroiridate(IV) and oxone were purchased from Alfa and Sigma, respectively. All other chemicals used were of commercial reagent grade. Fully deionized water was used throughout the experiments, and labware was acid-washed and thoroughly rinsed to avoid contamination by foreign metal ions. The 1:1 BLM–Ni(II) complex was prepared by mixing a small excess of the metal ion with the antibiotic (BLM: Ni(II) = 1.0:1.2) in Tris-HCl buffer (pH 7.5). The excess nickel was removed by ion-exchange column chromatography.

*Electronic Spin Resonance Characteristics of the BLM–Ni(II)–Ir(IV) System.* The 1:1 BLM–Ni(II) complex and the 1:1:1 BLM–Ni(II)–9-ethylguanine adduct complex were oxidized by Ir(IV), and then the reaction solutions were immediately frozen in liquid nitrogen. X-band ESR spectra of magnetically dilute aqueous glasses containing the Ni(III) complex species (≤1 mM) were measured at 77 K by using a JES-FE-3X spectrometer operating at 100-kHz magnetic field modulation. Conditions of ESR spectroscopy were as follows: modulation amplitude, 5.0 G; microwave power, 10 mW; time constant, 0.01 s. The $g$ values were determined by taking manganese ($g = 1.981$) as a standard, and the magnetic fields were calculated by the splitting of Mn(II) in MgO ($\Delta H_{3-4} = 86.9$ G).

*Sequence-Specific Binding Analysis.* DNA binding and cleavage of the BLM–Ni(III) complex were determined by polyacrylamide gel electrophoresis, using pIBI SV DNA. After treatment with *Nco*I, the 5′-end or 3′-end was labeled with $^{32}P$ and then the DNA fragment was treated with restriction endonuclease *Eco*RI. The labeled DNA samples included 20 mM Tris-HCl buffer (pH 7.5) and 0.4 μg of calf thymus DNA. After incubation at 90 °C for 1 min, the samples were chilled with ice and were reacted with 10 μM BLM–Ni(II) complex and 100 μM Ir(IV) (or 100 μM oxone). The reaction was carried out at 20 °C for 30 min. After ethanol precipitation, the sample was treated with 0.2 M piperidine at 90 °C for 30 min. The samples were subjected to electrophoresis, together with Maxam–Gilbert (Maxam & Gilbert, 1989) sequencing fragments, on a 10% polyacrylamide/7 M urea slab gel.

Deoxyoligonucleotides were synthesized by an Applied Biosystems Model 391 PCR-MATE EP DNA synthesizer

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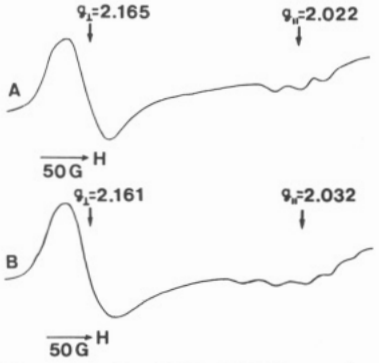


FIGURE 1: ESR spectra for BLM–Ni(III) complex (A) and its 9-ethylguanine adduct complex (B) at 77 K.

and purified by HPLC. Sequence binding analysis was performed by 20% denaturing gel electrophoresis. The nickel oxidation reaction and piperidine treatment were carried out as described above. After alkaline treatment, the samples were dried under vaccum in a Speed-Vac, resuspended in 30 $\mu$L of water, and dried twice more. The reaction samples were then dissolved in 5 $\mu$L of deionized formamide and loaded onto a denaturing 20% polyacrylamide gel.

*Sequence Analysis of Telomeric DNAs $d(T_2G_4)_4$ and $d(G_4T_4G_4)_2$.* The synthesized deoxyoligonucleotide d(TTGGGG)$_4$ or d(G$_4$T$_4$G$_4$)$_2$ was purified by electrophoresis on a denaturing 15% polyacrylamide gel. The end-labeled deoxyoligonucleotides were recovered from the gel by soaking the gel slice in 0.5 M $NH_4OAc$ and then loading the solution onto a Sep-Pak $C_{18}$ cartridge.

The G-quartet form of d(T$_2$G$_4$)$_4$ or d(G$_4$T$_4$G$_4$)$_2$ was detected by native gel electrophoresis, which was performed in a 12% polyacrylamide gel [29:1 ratio of acrylamide to bis(acrylamide)], using 0.5× TBE buffer (44.5 mM Tris–borate and 1.25 mM $Na_2EDTA$, pH 8). The gel electrophoresis was run at 4 °C at 8 V/cm and 10 W in running buffer containing 50 mM salt. Prior to electrophoresis, the DNA sample, dissolved in 5 $\mu$L of TE buffer (10 mM Tris-HCl and 1 mM $Na_2EDTA$, pH 8) plus 50 mM $Na^+$, was heated at 95 °C for 2 min, cooled to room temperature, and mixed with 1 $\mu$L of 30% glycerol containing marker dyes. The electrophoresis was carried out until the bromophenol blue marker migrated 16 cm.

The G-specific cleavage by DMS was performed by using a modification of the standard procedure. The deoxyoligonucleotide was dissolved in 19 $\mu$L of TE buffer, and 1 $\mu$L of fresh 1% DMS solution was added. After 10 min at 20 °C, the reaction was stopped by adding 20 $\mu$L of 0.4 M piperidine. For methylation protection for the G-quartet, the samples containing 50 mM NaCl were heated at 95 °C for 2 min in TE buffer before the reaction and equilibrated at 0 °C for 5 min. The DMS cleavage was carried out by adding 1 $\mu$L of fresh 6% DMS solution. The G-quartet cleavage reaction was performed after heating (95 °C) and then ice-cooling the sample containing 50 mM NaCl. After the piperidine treatment, the samples were dissolved in 5 $\mu$L of deinoized dye and loaded onto a 20% polyacrylamide gel.

## RESULTS

*ESR Evidence for Ni(III) Complex and Its Guanine Adduct with Bleomycin.* When ESR-negative 1:1 BLM–Ni(II) complex and its 9-ethylguanine adduct complex were treated with Ir(IV), the ESR spectra shown in Figure 1 were clearly detected at 77 K. These ESR features are consistent with Ni(III)(d$^7$) in a tetragonal geometry ($g_\parallel > g_\perp$) rather than

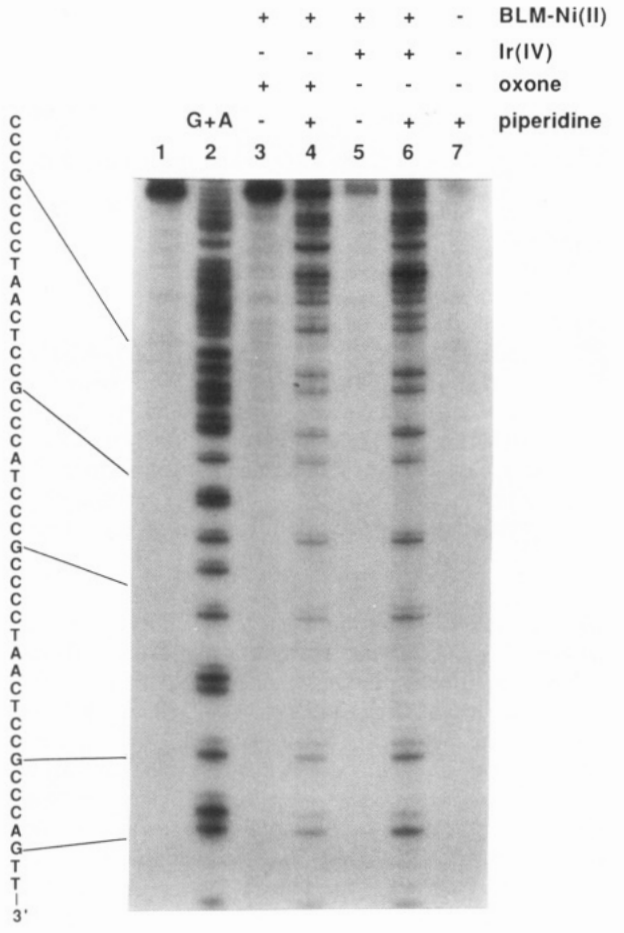


FIGURE 2: Autoradiogram for sequence analysis of pIBI SV DNA reacted with BLM–Ni(II) complex plus Ir(IV) (or oxone). The reaction mixtures contained 20 mM Tris-HCl buffer (pH 7.5), the 3′-labeled DNA fragment, 0.4 $\mu$g of carrier calf thymus DNA, and the following additions: lanes 3 and 4, BLM–Ni(II) complex (10 $\mu$M) plus oxone (100 $\mu$M), followed by alkaline treatment in lane 4; lanes 5 and 6, BLM–Ni(II) complex (10 $\mu$M) plus Ir(IV) (100 $\mu$M), followed by alkaline treatment in lane 6. Lane 7 presents intact pIBI SV DNA with alkaline treatment. Lanes 1 and 2 show intact DNA and the Maxam–Gilbert reaction for G+A, respectively.

a square-planar geometry ($g_\perp > g_\parallel$). It is known that tetragonally distorted octahedral geometry is the most common in the Ni(III) state. Indeed, three-line hyperfine splittings of the BLM–Ni(III) complex (Figure 1, A) reveal a species in which one nitrogen nucleus ($^{14}$N, $I = 1$) is bound in an axial position. In the latter adduct complex (Figure 1, B), evident alteration from three to five lines in the hyperfine splitting of the $g_\parallel$ region strongly indicates that the nitrogen donor of 9-ethylguanine, presumably guanine N-7, binds to an additional coordination site of the BLM–Ni(III) complex. The additional axial nitrogen donor is presumably the N-7 nitrogen of 9-ethylguanine, because the addition of 7-methylguanine did not give a similar ESR change after treatment with Ir(IV). The estimated ESR parameters were as follows: the BLM–Ni(III) complex, $g_\parallel = 2.165$, $g_\perp = 2.022$, $A^N = 24.0$ G; its 9-ethylguanine adduct complex, $g_\parallel = 2.165$, $g_\perp = 2.032$, $A^N = 21.2$ G. As for the 1:1 BLM–Ni(II) complex, the ESR parameters that differ from our previous values (Sugiura & Mino, 1979) are probably due to the experimental conditions (present conditions, BLM:Ni(II) = 1.0:1.2; previous conditions, BLM:Ni(II) = 1.2:1.0). We presume that excess BLM binds to six positions of the Ni(III) site under the previous conditions. If axial coordination of two nitrogens toward Ni(III) sites are magnetically equivalent, the ratio of the relative amplitudes of the five-line pattern should be 1:2:3:2:1. Taking

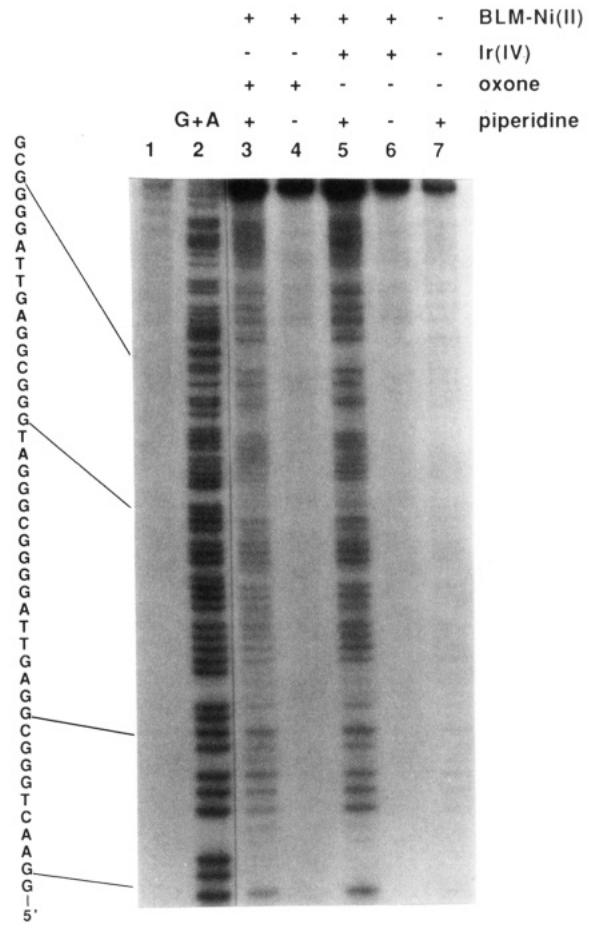


FIGURE 3: Autoradiogram for sequence analysis of pIBI SV DNA reacted with BLM–Ni(II) complex plus Ir(IV) (or oxone). The experimental conditions were the same as those of Figure 2, except that 5′-labeled DNA was used in place of the 3′-labeled DNA.

into account the estimated nitrogen hyperfine splitting ($A^N$ = 21.2 G) and the applied modulation amplitude (5.0 G), therefore, the present ESR spectrum of the BLM–Ni(III)–9-ethylguanine complex may suggest that two axial nitrogens (probably the α-amino and purine N-7 nitrogen groups) are not completely identical. Under the same experimental conditions, the glycylglycylhistidine–Ni(III)–$(NH_3)_2$ and (mercaptoacetyl)histidylhistidine–Ni(III)–*N*-methylimidazole adduct complexes, in which axial coordination of two equivalent nitrogen groups is expected, gave five-line ratios of 1:2:3:2:1 (Sugiura & Mino, 1979).

*Reaction of pIBI SV DNA Fragment (235 bp) with BLM–Ni(III) Complex Species.* Figures 2 and 3 show the sequence analyses of pIBI SV DNA (*Nco*I–*Eco*RI fragment) reacted with 10 μM BLM–Ni(II) complex plus 100 μM Ir(IV) (lanes 5 and 6 in both figures). In both the 3′- and 5′-$^{32}$P-labeled fragments, the denatured DNA samples were prepared by heating at 90 °C for 2 min. The 1:1 BLM–Ni(II) complex oxidized by Ir(IV) formed the corresponding Ni(III) species as clearly shown in the ESR experiment. When the DNA samples were subsequently treated with 0.2 M piperidine, the evident cutting bands were observed, and these bands corresponded well to guanine bases of the Maxam–Gilbert reactions. We also used oxone as an oxidant and obtained results close to those with Ir(IV) (see lanes 3 and 4 in both the figures). Similar guanine-specific cleavage induced by the BLM–Ni(III) complex was also detected for denatured *Sal*I–*Sph*I (90 bp), *Sal*I–*Bam*HI (270 bp), and *Bst*NI–*Hae*III (101 bp) fragments of pBR322 DNA. It is of interest that the present guanine-specific binding and cleavage of the BLM-Ni(III) complex are distinct from those of other metal complexes of BLM reported previously.

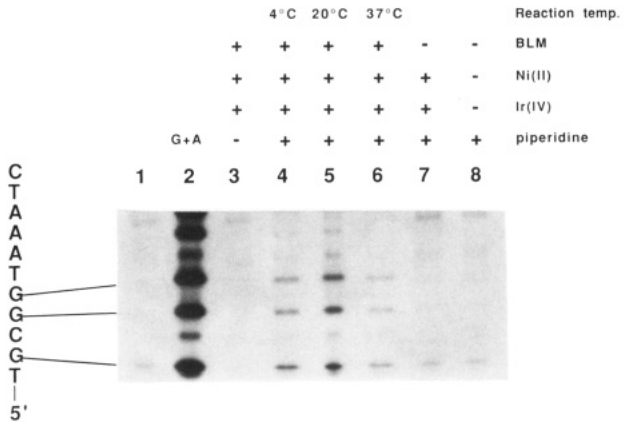


FIGURE 4: Autoradiogram for sequence analysis of deoxyoligonucleotide 5′-TGCGGTAAATC reacted with BLM–Ni(II) complex plus Ir(IV). Lane 1, intact deoxyoligonucleotide; lane 2, Maxam–Gilbert G+A reaction; lane 3, deoxyoligonucleotide plus the BLM–Ni(II)–Ir(IV) system; lanes 4–6, piperidine treatment of lane 3 at reaction temperatures of 4 °C (lane 4), 20 °C (lane 5), and 37 °C (lane 6). Lanes 7 and 8 show the controls, piperidine treatment of Ni(II)–Ir(IV)–DNA and intact deoxyoligonucleotide.

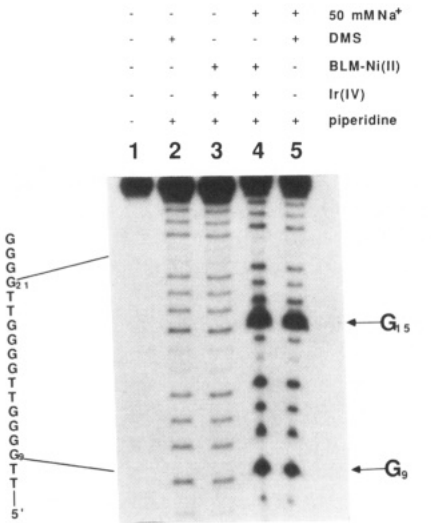


FIGURE 5: Autoradiogram for sequence analysis of telomeric deoxyoligonucleotide $d(T_2G_4)_4$ reacted with BLM–Ni(II) complex plus Ir(IV). Lane 1, intact deoxyoligonucleotide $d(T_2G_4)_4$; lane 2, G-specific cleavage for $d(T_2G_4)_4$ by DMS; lane 3, piperidine treatment after reaction of deoxyoligonucleotide $d(T_2G_4)_4$ plus the BLM–Ni(II)–Ir(IV) system; lane 4, piperidine treatment after reaction of G-quartet $d(T_2G_4)_4$ plus the BLM–Ni(II)–Ir(IV) system; lane 5, G-specific cleavage for G-quartet $d(T_2G_4)_4$ by DMS.

*Reaction of Deoxyoligonucleotide 5′-TGCGGTAAATC with BLM–Ni(III) Complex Species.* Figure 4 presents the sequence analyses of single-stranded deoxyoligonucleotide reacted with the BLM–Ni(II) complex plus Ir(IV). Using 20% polyacrylamide gel electrophoresis, we found that the DNA sample treated with piperidine gives predominant cleavage at guanine sites (lanes 4–6 in Figure 4). Therefore, the reaction of the BLM–Ni(III) complex was substantially the same between the long DNA chain and the short deoxyoligonucleotide. The results strongly indicate specific binding of the BLM–Ni(III) complex species to guanine bases. At different reaction temperatures (4 and 37 °C), this reaction showed no alterations of the DNA cleavage sites.

*Reactions of Telomeric DNAs $d(TTGGGG)_4$ and $d(G_4T_4G_4)_2$ with BLM–Ni(III) Complex Species.* The formation of telomeric-like G-quartets was checked by gel

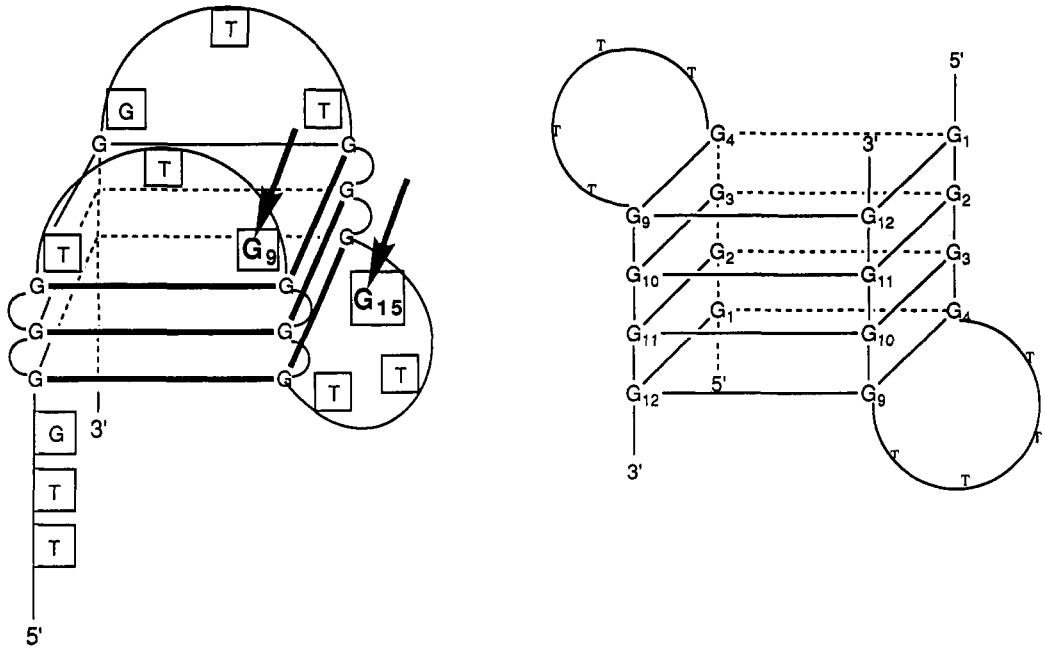


FIGURE 6: Preferential base sites of G-quartet $d(T_2G_4)_4$ (left) and $d(G_4T_4G_4)_2$ models (right) attacked by BLM–Ni(III) complex species.

electrophoresis as reported previously (Williamson et al., 1989). Figure 5 shows the sequence analysis of $d(T_2G_4)_4$ treated with piperidine after reaction with the BLM–Ni(II) complex plus Ir(IV). Lanes 3 and 4 present the results for deoxyoligonucleotide $d(T_2G_4)_4$ and G-quartet $d(T_2G_4)_4$, respectively. The reaction of the BLM–Ni(III) complex was also guanine-specific for telomeric-like G-quartet $d(T_2G_4)_4$ as well as for single-stranded oligonucleotide $d(T_2G_4)_4$. Of special interest is the fact that $G_9$ and $G_{15}$ sites were remarkably activated in the cleavage of G-quartet $d(T_2G_4)_4$ (lane 4). A similar phenomenon was clearly observed in the methylation of G-quartet $d(T_2G_4)_4$ using DMS (lane 5). By contrast, telomeric $d(G_4T_4G_4)_2$ formed under 50 mM $Na^+$ gave no cleavage due to the BLM–Ni(III) complex. Certainly, the BLM–Ni(III) complex cleaved at all G bases for the normal deoxyoligonucleotide $d(G_4T_4G_4)_2$.

## DISCUSSION

*Specific Binding and Cleaving of Single-Stranded Guanine Residues by BLM–Ni(III) Complex.* Several BLM–metal complexes are known to cleave double-stranded DNA in the presence of reductants or $H_2O_2$. In this study, we found guanine-specific binding and cleavage by the BLM–Ni(III) complex toward single-stranded DNA. The base-stacking and Watson–Crick base-pairing of normal DNA are not essential in the modification reaction by the BLM–Ni(III) complex. The mechanism of this reaction is primarily due to guanine binding of the BLM–Ni(III) complex. Presumably, BLM coordinates Ni(III) by its β-aminoalanine–pyrimidine–β-hydroxyhistidine region like other transition-metal ions (Sugiura et al., 1985). The ESR evidence indicates that the sixth axial coordination position of the Ni(III) site is the N-7 nitrogen of guanine residues of DNA.

*Reaction of Telomeric DNA with BLM–Ni(III) Complex.* In 50 mM $Na^+$, deoxyoligonucleotide $d(T_2G_4)_4$ forms a stable folding conformation. In the reaction of the BLM–Ni(III) complex with G-quartet $d(T_2G_4)_4$, it is interesting that the cleavage of the $G_9$ and $G_{15}$ sites was remarkably activated. DMS also gave similar results. The deoxyoligonucleotide $d(TTGGGG)_4$ is a telomeric sequence from *Tetrahymena*. Cech et al. (1989) have also reported that $d(T_2G_4)_4$ forms a four-helix in the presence of $Na^+$ (Williamson et al., 1989). In a four-stranded form of DNA with guanine self-pairing, each guanine residue serves as both the donor and acceptor in adjacent G·G Hoogsteen base pairs (Gellert et al., 1962). They propose a model for $d(T_2G_4)_4$ that three G-quartets are stacked over each other and connected by loops of d(GTT). In the G-quartet $d(T_2G_4)_4$ model, the $G_9$ and $G_{15}$ guanine residues situate just in the loop sites. Therefore, this result reveals that the loop $G_9$ and $G_{15}$ guanine sites are preferentially attacked by the BLM–Ni(III) complex (Figure 6). We also investigated the reaction of the BLM–Ni(III) complex toward G-quartet DNA $d(G_4T_4G_4)_2$. Recently, the structure of the sequence $d(G_4T_4G_4)_2$ from the 3′-overhang of *Oxytricha* telomere was clarified by X-ray crystallography (Kang et al., 1992). This telomeric sequence forms a complex containing two $d(G_4T_4G_4)$ molecules held together by cyclic hydrogen bonding of four guanines, and two thymine tracts form loops at opposite ends of the molecule. Indeed, the BLM–Ni(III) complex binds to single-stranded $d(G_4T_4G_4)$, but does not cleave any guanine sites for this telomeric DNA (data not shown). In the G-quartet $d(G_4T_4G_4)_2$ model, no loop guanines exist (Figure 6). Our study is the first time that noble reactions of the BLM–Ni(III) complex toward telomeric DNAs have been demonstrated. It is suggested that G-quartet telomeric DNA plays a key role in cell meiosis (Sen & Gilbert, 1988). Telomeres containing telomeric DNA also take an important part in maintaining the stability and integrity of chromosomes (Blackburn & Szostak, 1984; Blackburn, 1991; Zakian, 1989). The present reactivity of the BLM–Ni(III) complex may be useful in development of new pharmaceutical agents and chemical reagents for studying DNA local structure. In addition, the results make a significant contribution to our knowledge of BLM derivative binding to DNA.

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