Photoinduced DNA Cleavage Reactions by Designed Analogues of Co(III)−Bleomycin: The Metalated Core Is the Primary Determinant of Sequence Specificity

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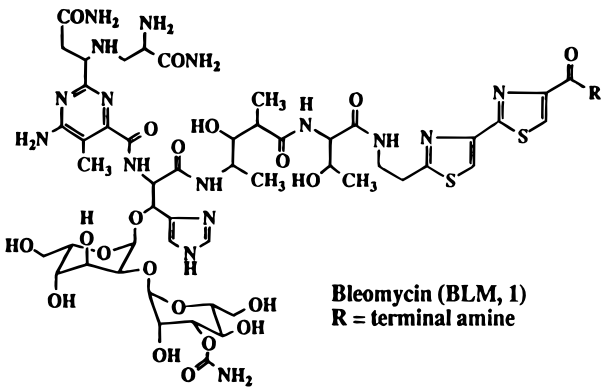
A 2,4′-bithiazole group has been covalently attached to the Co(III) complex of a designed ligand PMAH that mimics the metal-binding locus of the antitumor drug bleomycin (BLM). The deprotonated PMA^- ligand binds Co(III) via five nitrogens located in primary and secondary amines, a pyrimidine and an imidazole ring, and a peptide moiety. The 2,4′-bithiazole group is tethered to the $[Co(PMA)]^{2+}$ unit via an imidazole that is connected to the bithiazole moiety with a $(CH_2)_3$ spacer. The structure of this hybrid analogue, namely, $[Co(PMA)(Bit)]Cl_2$ (**7**, Bit = 2′-methyl-2,4′-bithiazole-4-carboxamido-*N*′-(3-propyl)imidazole) has been established by spectroscopic techniques. **7** promotes photocleavage of DNA at micromolar concentrations. Unlike simpler analogues like $[Co(PMA)(H_2O)]^{2+}$ and $[Co(PMA)Cl)]^+$ which induce random DNA cleavage upon UV illumination, **7** exhibits sequence specificity in the DNA photocleavage reaction. Intriguing is to note that **7** exhibits the same 5′GG-N3′ sequence preference as another hybrid analogue $[Co(PMA)(Int\text{-}A)]Cl_2$ (**6**, Int-A = acridine-9-carboxamido-*N*′-(3-propyl)imidazole) that contains an acridine moiety as the DNA-binding group. The observed sequence specificity of **6** and **7** therefore does not reflect the sequence preferences of the DNA-binding groups (acridine and bithiazole). The results indicate that the metalated core of the hybrid analogues, i.e., the $[Co(PMA)]^{2+}$ unit is the key factor in determining their sequence specificity.

Introduction

The Bleomycin (BLM, **1**) family of glycopeptide antitumor antibiotics is used in combination chemotherapy against several types of cancer.[1] BLM contains at least two functional domains: the metal-binding region and the DNA-binding locus. The bithiazole moiety of the DNA-binding domain of BLM either intercalates into the base stacks or occupies the minor groove while the positively charged amine tail of BLM allows electrostatic association(s) of the drug with the negatively charged backbone of the DNA helix.[2,3] Binding of metal ions by BLM occurs at the β-aminoalaninamide−pyrimidine−β-hydroxyhistidine portion of the antibiotic.



Aerobic oxidation of a solution containing Co(II) salts and BLM affords several Co(III) chelates of the drug that are often referred to as ORANGE, BROWN, and GREEN Co^{III}−BLM.[4−7] Spectroscopic studies have indicated that the cobalt(III) chelates of BLM most possibly employ five N donor centers located in the primary and secondary amines, the pyrimidine and the imidazole ring, and the peptide moiety adjacent to the pyrimidine.[8,9] The kinetically inert (low spin, d^6 system) Co^{III}−BLMs bind to DNA quite strongly ($10^6-10^7\ M^{-1}$)[10] and do not cause any oxidative DNA damage like the Fe−BLMs.[2,3] However, Co^{III}−BLMs cleave DNA when illuminated with UV[10,12] or visible[13] light. Co^{III}−BLMs photocleave DNA preferentially at pyrimidine residues located at the 3′ side of guanines.[10]

To date, there has been no X-ray cystallographic information available on the metallobleomycins (M−BLMs). As part of our synthetic analogue approach toward elucidation of the structures and mechanism(s) of the photoinduced DNA strand scission reactions of the Co^{III}−BLMs, we have reported the structures and spectroscopic properties of three Co(III) complexes of the designed ligand PMAH (**2**, H is the dissociable amide H) which resembles the proposed metal-binding domain of BLM.[14] The three complexes $[Co(PMA)(N\text{-}MeIm)]^{2+}$ (**3**) (N-MeIm = *N*-methylimidazole), $[Co(PMA)(H_2O)]^{2+}$ (**4**), and $[Co(PMA)(Cl)]^+$ (**5**) mimic the spectroscopic properties of the ORANGE, BROWN, and GREEN Co^{III}−BLM respectively and photocleave DNA when illuminated with UV light. Results of the EPR and gel electrophoretic studies performed under aerobic and anaerobic conditions demonstrate that (i) these positively charged complexes bind to DNA electrostatically, (ii) UV

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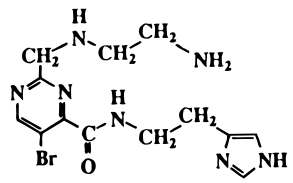
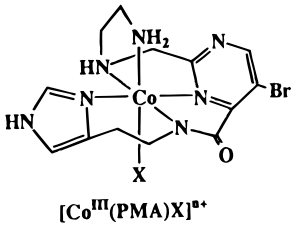
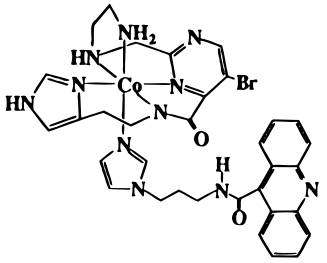


PMAH (2)

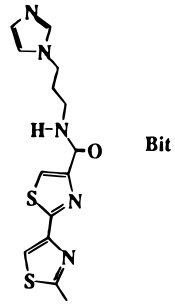


$[Co^{III}(PMA)X]^{n+}$

X = N-MeIm, n = 2 (3)
X = H_2O, n = 2 (4)
X = Cl^-, n = 1 (5)



$[Co(PMA)(Int\text{-}A)]^{2+}$ (6)



Bit

irradiation gives rise to C/N-based radical(s) on the ligand framework of these complexes, and (iii) in aqueous solution, the C/N-based radical(s) rapidly produces hydroxyl radicals in the vicinity of the DNA helix which in turn induce DNA strand scission. This mechanism is supported by the fact that the process of DNA photocleavage by the Co^{III}−BLMs as well as the analogues (**3**−**5**) is indifferent to the presence of dioxygen in the incubation medium (the •OH radical is derived from water).[10,14]

Since the model complexes (**3**−**5**) bind to DNA solely through electrostatic interactions, they do not photocleave DNA as efficiently as the Co^{III}−BLMs which exhibit much higher affinities toward DNA due to both electrostatic and intercalative interactions. This hypothesis is supported by the hybrid molecule $[Co(PMA)(Int\text{-}A)]Cl_2$ (**6**) (Int-A = acridine-9-carboxamido-*N*′-(3-propyl)imidazole) that we reported recently.[15] The presence of the tethered acridine in **6** makes it a strong DNA-binding agent (binding constant > 10^6 M^{-1}), and **6** induces DNA photocleavage at micromolar concentrations much like the Co^{III}−BLMs.[16] Most important of all, **6** exhibits sequence specificity and inflicts photodamage at 5′GG-N3′ sites. Quite in contrast, the simpler analogues (**3**−**5**) as well as Int-A alone do not show any sequence specificity.

It has been argued that the metalated portions of the M−BLMs are key factors in determining the sequence specificities of the DNA cleavage reactions.[17,18] We have shown previously that, in the case of the iron chelates of PMAH, the $[Fe(PMA)]^{n+}$ units are sufficient to warrant sequence specificity in oxidative DNA cleavage reactions and the observed specificity is very similar to that of the Fe−BLMs.[19] The sequence specificity of **6** therefore could arise either from the combined effect of the $[Co(PMA)]^{2+}$ moiety and Int-A or from the $[Co(PMA)]^{2+}$ moiety alone with Int-A acting just as an anchor (i.e., assisting the $[Co(PMA)]^{2+}$ unit to stay close to the DNA helix). To address this issue further, we have now isolated another designed hybrid $[Co(PMA)(Bit)]Cl_2$ (**7**) where Bit = 2′-methyl-2,4′-bithiazole-4-carboxamido-*N*′-(3-propyl)imidazole. This new analogue contains Bit, a 2,4′-bithiazole unit with no cationic charge (unlike BLM), as the DNA-binding group. Bit is not a classical intercalator like acridine and binds to DNA only by partial insertion of a thiazole ring which wedges in between the bases to create bends.[20a] Reported in this account are the synthesis and the spectroscopic properties of **7** together with the results of the photoinduced DNA cleavage reactions. Despite the presence of two distinctly different DNA-binding groups, both **6** and **7** induce photocleavage at the same 5′GG-N3′ sites. It is thus evident that the $[Co(PMA)]^{2+}$ moiety is responsible for the chemistry as well as the sequence specificity. The DNA-binding groups (Int-A and Bit) are, however, necessary to allow more contact between the $[Co(PMA)]^{2+}$ unit and DNA; without them, $[Co(PMA)]^{2+}$ induces random DNA cleavage.

Experimental Section

$[Co(PMA)(H_2O)](ClO_4)_2$ (**4**), $[Co(PMA)(Int\text{-}A)]Cl_2$ (**6**), and the self-complimentary oligonucleotide duplex $[d(GATCCGGATC)]_2$ (**8**) were synthesized by following published procedures.[14−16] Plasmid DNA (pBR322), bacterial alkaline phosphatase, and the restriction enzymes Xma III and Nar I were purchased from Bethesda Research Laboratories. T4 polynucleotide kinase was purchased from the U. S. Biochemical Corporation. The enzymatic reactions were performed according to the protocols provided by the suppliers. [γ-^{32}P]ATP (specific activity 3000 Ci/mmol) was procured from Amersham. Long Ranger Gel solution was obtained from AT Biochemicals. Nanopure water was used throughout. Reagents for the DNA synthesis and the C18 Sep-Pak columns were purchased from Millipore. 100% and 99.8% D_2O were purchased from the Aldrich chemical company.

2′-Methyl-2,4′-bithiazole-4-carboxamido-*N*′-(3-propyl)imidazole (Bit). A mixture of 2′-methyl-2,4′-bithiazole-4-carboxylic acid[20b] (0.17 g, 0.75 mmol) and thionyl chloride (excess) was heated to reflux for 3 h. The unreacted thionyl chloride was then removed under vacuum. The resulting brown residue was dissolved in 10 mL of chloroform and reacted with a chloroform solution (10 mL) of 1-(3-aminopropyl)-imidazole (0.18 mL, 1.54 mmol) at room temperature. Upon completion of the reaction (followed by TLC), the reaction mixture was filtered and the mother liquor containing Bit was evaporated to dryness. The residue was redissolved in methanol and purified on a silica gel column with methanol as the eluent.

$[Co(PMA)(Bit)]Cl_2$ (7). A batch of 0.28 g (0.43 mmol) of $[Co(PMA)(H_2O)](ClO_4)_2$ was dissolved in 25 mL of acetone and heated to reflux for 1 h. To the brown green solution was then added a solution of 0.16 g (0.47 mmol) of Bit in 20 mL of acetone dropwise over 5 h. The resulting mixture was heated to reflux for 12 h. The orange solution thus obtained was filtered, and the filtrate was evaporated to dryness. The solid was redissolved in 5 mL of water, loaded on a SP-C50-120 Sephadex column, and eluted with a KCl gradient from 0.05 to 0.1 M. The first orange band was collected, and the solution was evaporated to dryness. The product $[Co(PMA)(Bit)]Cl_2$ (**7**) was desalted by extracting the residue with ethanol. The yield of **7** was 66% based on $[Co(PMA)(H_2O)](ClO_4)_2$. Selected IR bands (KBr pellet, cm^{-1}): 3422 (s), 3073 (s), 2893 (s), 1654 (s), 1617 (ν_{co} ,vs), 1556 (s), 1458 (m), 1163 (m), 1089 (s), 623 (m).

Preparation of Radiolabeled DNA Fragments. pBR322 DNA was treated with the restriction endonuclease Nar I. Dephosphorylation of the 5′ end was accomplished with the use of bacterial alkaline phosphatase. Phosphorylation of the free 5′-OH ends with [γ-^{32}P]-ATP was performed with T4 polynucleotide kinase. The labeled restriction fragments were then digested with the restriction endonuclease Xma III. The labeled DNA fragments thus produced were separated on a preparative 5% non-denaturing polyacrylamide gel, and

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Table 1. Spectral Data for [Co(PMA)(Bit)]Cl_2 (**7**)

Electronic Absorption (DMSO): λ_{max}, nm (ϵ, M^{-1} cm^{-1})
520 sh (165), 350 sh (2,050), 312 sh (11,000), 296 (16,400)

^{1}H NMR (500 MHz, 298 K, D_2O): δ from TSP
2.045, 2.414, 2.653, 2.799, 3.255, 3.268, 3.536, 4.068, 6.212, 7.101, 7.247, 7.280, 8.012, 8.151, 8.405, 9.380

^{13}C NMR (300 MHz, 303 K, $(CD_3)_2SO$): δ from TMS
18.665, 25.743, 29.936, 35.386, 42.049, 45.384, 55.292, 57. 455, 115.443, 117.103, 117.575, 122.149, 123.991, 127.057, 137.422, 139.556, 139.556, 147.220, 150.583, 156.958, 160.518, 161.685, 164.841, 167.346, 167.535, 171.155

^{13}C NMR (500 MHz, 298 K, D_2O): δ from TSP
22.460, 29.890, 33.630, 40.987, 45.333, 46.693, 50.918, 60.509, 121.139, 122.839, 122.979, 127.355, 128.958, 131.969, 142.118, 142.677, 143.235, 150.932, 153.028, 161.349, 166.520, 166.800, 171.134, 172.951, 174.069, 175.578

the 266-bp and the 391-bp fragments were excised from the gel. The DNA was extracted from the gel by electroelution. The sequences of both fragments were determined by the method of Maxam and Gilbert[21] and were verified by comparison with the published sequence of pBR322. Autoradiography was carried out at −70 °C on Kodak XAR-5 film with an intensifying screen.

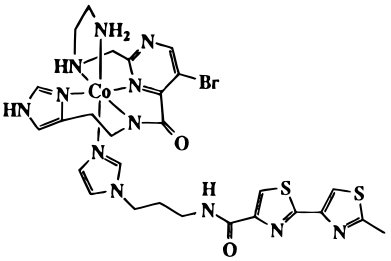
DNA Cleavage Experiments. The reaction mixtures (25 μL total volume) contained approximately 5 fmol (approximately 4000 cpm) of the 391- or 266-bp end-labeled DNA duplex and 1 μg of cold carrier ΦX174 DNA in 25 mM Tris−borate buffer (190 μM EDTA, pH 8). Following the addition of the cobalt complexes, the samples were placed behind a Corning 5950 filter (cutoff at 290 nm) and irradiated with a UV-transilluminator (UVP-TM-36, λ_{max} = 302 nm) for 3 h. The distance between the UV light and the samples was 5 cm. After the irradiation, the samples were heated at 90 °C for 10 min, quick-chilled on ice, and loaded on 12% denaturing (7 M urea) polyacrylamide Long Ranger gel. The samples were electrophoresed at 1500 V for 3 h.

NMR Studies. ^{1}H-NMR spectra were obtained at 25 °C on a Varian 500 MHz Unity Plus Spectrometer interfaced with a Sun OS 4.1.3 computer. TSP (sodium 3-(trimethylsilyl)propionate-2,2,3,3-d_4) was used as the internal standard, and the HOD signal was suppressed with low-power presaturation irradiation to avoid dynamic range problems. The parameters for the COSY and the phase-sensitive NOESY experiments have already been published by this group.[16]

Other Physical Measurements. Absorption spectra were obtained with a Perkin-Elmer Lamda-9 spectrophotometer. Infrared spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. A BRL H-5 horizontal gel apparatus was used for the agarose gel (1%) electrophoreses. Photographs were taken with a Polaroid MP-4 system following ethidium bromide staining.

Results

Structure of [Co(PMA)(Bit)]Cl_2 (7). The structure of this analogue (shown below) has been established on the basis of its spectral parameters (Table 1) along with the published structures of [Co(PMA)(Int-A)]Cl_2 (**6**) and [Co(PMA)(N-MeIm)]$(NO_3)_2$ (**3**). The presence of the CoN_6 chromophore in **7** is evidenced by an absorption at 520 nm of moderate intensity (ϵ = 165 M^{-1} cm^{-1}) while the bithiazole moiety exhibits its characteristic absorption at 296 nm (ϵ = 16 400 M^{-1} cm^{-1}). Further support comes from the ^{1}H and ^{13}C NMR spectra of **7** in $(CD_3)_2SO$ and D_2O. The ^{1}H NMR spectrum of **7** in D_2O is shown in Figure 1 along with the numbering scheme. Assignments of the various peaks have been accomplished with the aid of ^{1}H−^{1}H COSY and ^{13}C−^{1}H COSY spectra (data not shown). Coordination of the Bit fragment to the $[Co(PMA)]^{2+}$ moiety via the nitrogen atom of the imidazole is confirmed by the ^{13}C chemical shifts of the C14, C15, and C16 of the imidazole ring [^{13}C chemical shifts (ppm from TMS, $(CD_3)_2$-SO) of the coordinated (a) N-MeIm in **3**, C14, 124.12; C15, 127.43; C16, 139.72; (b) Int-A in **6**, C14, 122.34; C15, 128.99; C16, 139.50; (c) Bit in **7**, C14, 122.15; C15, 127.06; C16, 139.56].



[Co(PMA)(Bit)]$^{2+}$ (7)

As shown in Figure 1, the pyrimidine H9 proton and the two imidazole protons H1 and H2 of the $[Co(PMA)]^{2+}$ moiety of **7** resonate at 9.380, 8.405, and 7.280 ppm, respectively. These peaks are good probes for demonstrating electrostatic interaction between the $[Co(PMA)]^{2+}$ moiety and the DNA backbone (vide infra). The methyl group on the bithiazole ring that gives rise to the sharp peak at 2.799 ppm is an excellent indicator of binding of the bithiazole unit to DNA. Peaks for the two bithiazole protons, H22 and H25, also shift upon binding of the bithiazole end of **7** to DNA (vide infra). Discussions on the remaining peaks can be found in our earlier accounts.[14−16]

DNA Strand Scission of Covalently Closed Circular (ccc) Supercoiled ΦX174 DNA. When a mixture of ccc supercoiled (form I) ΦX174 DNA and 50 μM of **7** in 25 mM Tris−borate/190 μM EDTA (pH 8) buffer was irradiated with UV light (290 nm) for 3 h, extensive DNA strand cleavage was observed (Figure 2, lane 2). No DNA cleavage was observed in the dark. Bit alone did not induce strand cleavage at 50 μM concentration. Significant DNA cleavage was observed with Bit at 500 μM (Figure 2, lane 4). [Co(PMA)(N-MeIm)]$(NO_3)_2$ (**3**) inflicted comparable DNA damage only at millimolar concentrations (Figure 2, lane 3). It is therefore evident that the combined effect of the $[Co(PMA)]^{2+}$ moiety and Bit allows **7** to bind to DNA and cause photocleavage at micromolar concentrations.

We have previously demonstrated that the positively charged $[Co(PMA)]^{n+}$ moiety in **3**−**5** binds to DNA through electrostatic interaction.[14] Such binding is weak and is readily inhibited under high-salt conditions. Though the present analogue **7** binds to DNA much more strongly due to additional bithiazole−DNA interactions, the extent of DNA photocleavage by **7** is noticeably altered in the presence of a high concentration of salt. This is shown in Figure 3. The extent of DNA photodamage is very low (lane 5) when the DNA buffer contained 100 mM Na^+ (as Na_2SO_4). Clearly, the added Na^+ ions prohibit electrostatic binding of the $[Co(PMA)]^{2+}$ portion of **7** to the phosphate backbone of DNA (negatively charged) and cause diminution of the extent of DNA photodamage.

Sequence Specific DNA Photocleavage by [Co(PMA)(Bit)]Cl_2 (7). The results of the DNA photocleavage reactions with the 391-bp fragment are shown in Figure 4. The first four lanes contain products of the Maxam−Gilbert sequencing reactions.

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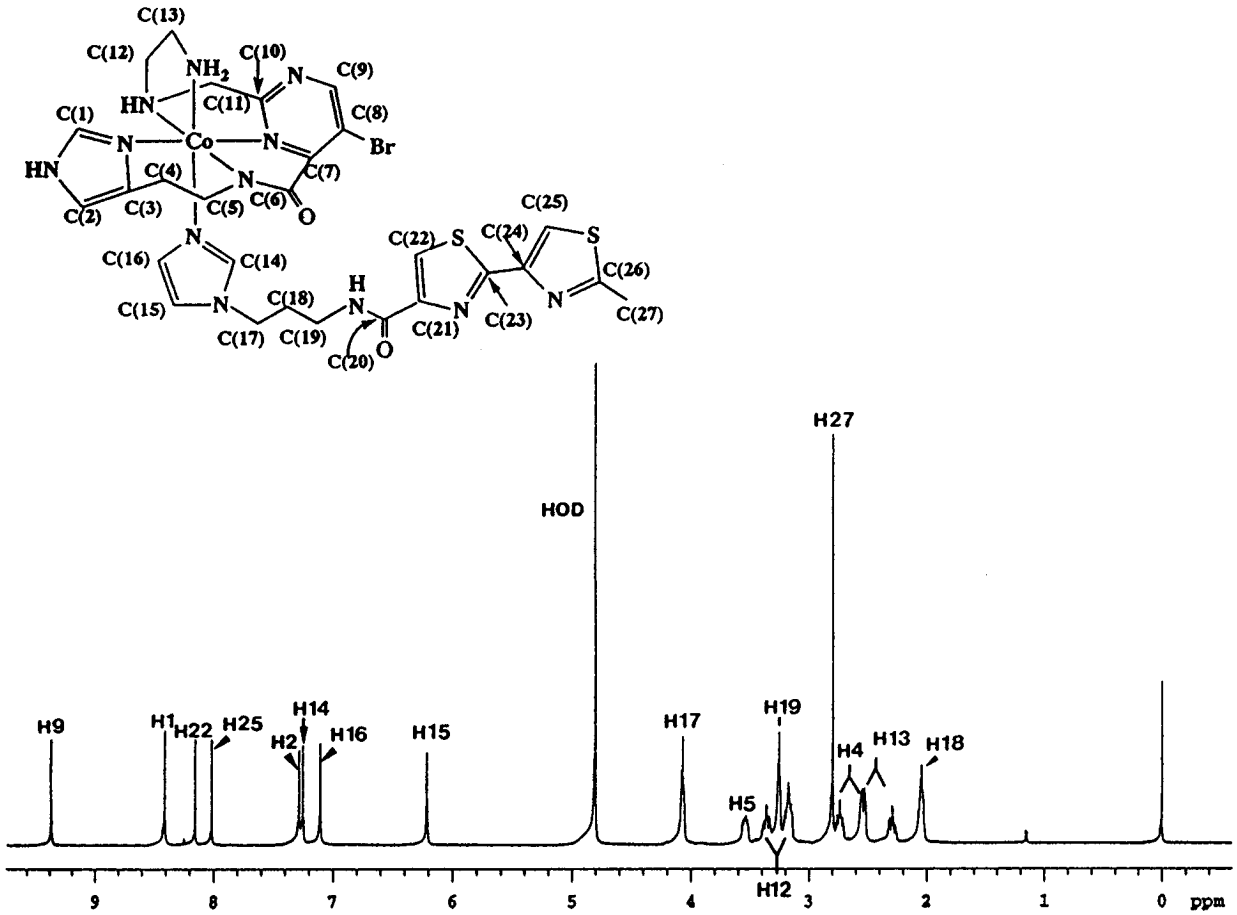

Figure 1. ^{1}H NMR spectrum (500 MHz, 298 K) of [Co(PMA)(Bit)]Cl_2 (**7**) in D_2O. The signal assignments are shown. The structure of **7**, as discerned from its spectral parameters, is shown at the top with the atom-labeling scheme.

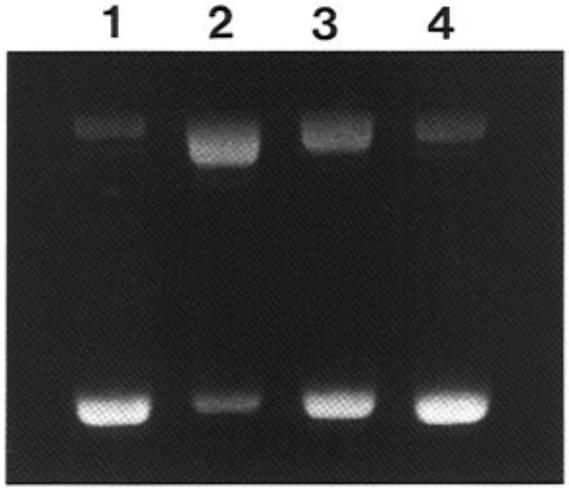

Figure 2. Photoinduced DNA cleavage by [Co(PMA)(Bit)]Cl_2 (**7**), [Co(PMA)(N-MeIm)]$(NO_3)_2$ (**3**), and Bit. Reactions were performed in 25 mM Tris−borate, 190 μM EDTA, pH = 8 buffer. Each reaction mixture contained 1 μg of ΦX174 plasmid DNA in a total reaction volume of 25 μL and was illuminated for 3 h at 290 nm. Lane 1, DNA alone; lane 2, DNA + 50 μM **7**; lane 3, DNA + 500 μM **3**; lane 4, DNA + 500 μM Bit.

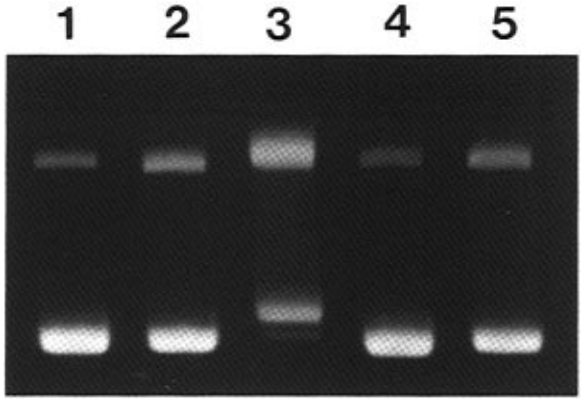

Figure 3. Effect of cation on the photoinduced DNA cleavage by [Co(PMA)(Bit)]Cl_2 (**7**). Reactions were performed in 25 mM Tris−borate, 190 μM EDTA, pH = 8 buffer. Each reaction mixture contained 1 μg of ΦX174 plasmid DNA in a total reaction volume of 25 μL and was illuminated for 3 h at 290 nm. Lane 1, DNA alone; lane 2, DNA + 500 μM [Co(PMA)(H_2O)]$(NO_3)_2$ (**4**); lane 3, DNA + 50 μM **7**; lane 4, DNA + 500 μM Bit; lane 5, DNA + 50 μM **7** + 100 mM Na^+ (as Na_2SO_4).

Lane 5 displays the products of the photocleavage reaction by [Co(PMA)(Int-A)]Cl_2 (**6**) while lane 6 exhibits the same by [Co(PMA)(Bit)]Cl_2 (**7**). It is quite evident that, under similar conditions (3 h of irradiation at 290 nm in the presence of 30 μM cobalt complex), the two hybrid molecules afford identical photoproducts and major cleavage occurs at the 5′GG-N3′ sites. The acridine-containing analogue **6**, however, inflicts more damage to DNA (darker bands). Analogous results have also been obtained with the 266-bp fragment.

Association of [Co(PMA)(Bit)]Cl_2 (7) with the Designed Decamer [d(GATCCGGATC)]$_2$ (8). The technique of high-field NMR spectroscopy has been utilized to establish the mode of DNA binding by **7**. A designed oligonucleotide duplex [d(GATCCGGATC)]$_2$ (**8**) that contains a GG-N site has been employed in such studies. Assignments of all the nonexchangeable protons of this decamer have recently been reported by this group.[16] In D_2O solution at 25 °C, addition of **7** to **8** did

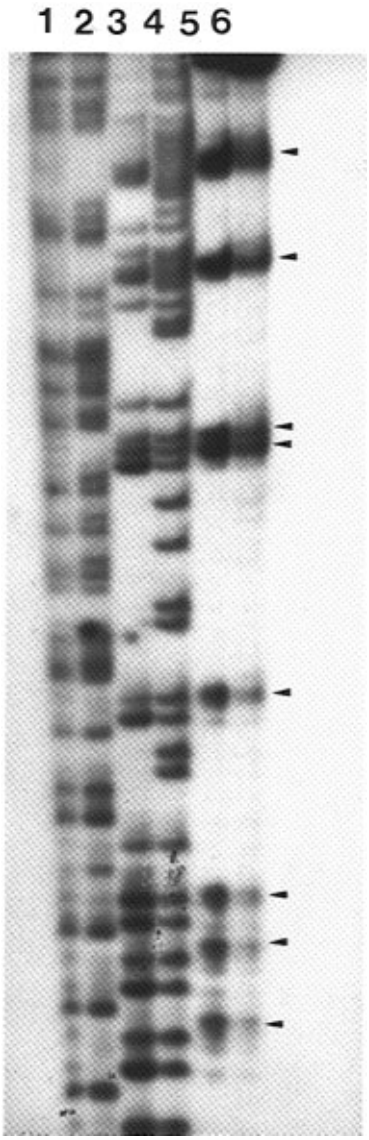

Figure 4. Sequence-dependent photocleavage of the 5′ end-labeled 391-bp restriction fragment of pBR322 by [Co(PMA)(Int-A)]Cl_2 (**6**) and [Co(PMA)(Bit)]Cl_2 (**7**). Lanes 1−4 contain the products of the Maxam−Gilbert sequencing reactions (C, C + T, G, and G + A, respectively). Lanes 5 and 6 contain the products of the reactions of approximately 5 fmol of the 5′ end-labeled double stranded restriction fragment (in a total volume of 25 μL of 25 mM Tris−borate, 190 μM EDTA, pH 8 buffer) with the following compounds: lane 5, 30 μM **6**; lane 6, 30 μM **7**. The reaction mixtures contained 1 μg of ΦX174 DNA as carrier and were irradiated at 290 nm for 3 h. The major cleavage sites are indicated with arrows.

not cause significant broadening of the resonances corresponding to the sugar and base protons of **8**. This indicated that the spectra were in the fast exchange regime. The aromatic (top panel) and the sugar region (bottom panel) of the ^{1}H NMR spectrum of the **7**:**8** (1:1) complex are shown in the Figure 5. In the **7**:**8** (1:1) complex, small downfield shifts are noted for the pyrimidine H9 (9.387 ppm as compared to 9.380 ppm in free **7**) and the imidazole H1 peaks (8.444 ppm as compared to 8.405 ppm in free **7**). Similar downfield shifts, albeit of greater magnitudes, have been observed for these protons in the **6**:**8** (1:1) complex.[16] The downfield shifts observed with H9 and H1 indicate significant interaction between the $[Co(PMA)]^{2+}$ moiety of **7** and the duplex **8**. It is interesting to note that the imidazole H1 appears as a doublet (8.453 and 8.435 ppm) in the **7**:**8** (1:1) complex. The H9 resonance also exhibits a broad peak at the high-field side which sharpens up at higher

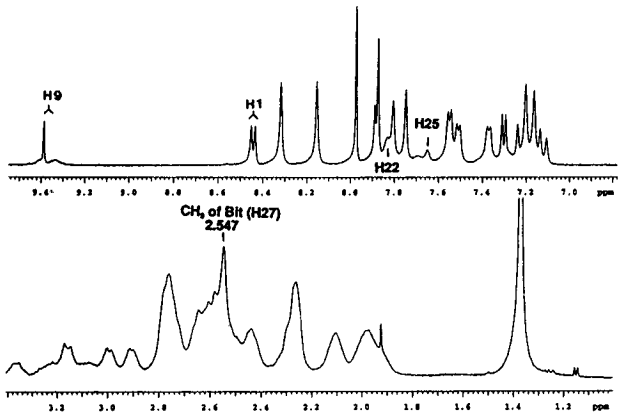

Figure 5. ^{1}H NMR (500 MHz) spectrum of the **7**:**8** (1:1) complex in D_2O phosphate buffer (pH 7.1) at 298 K. The binding of the bithiazole group of **7** to the duplex **8** is evident by the broadening and upfield shift of H22 and H25 as well as the methyl group of Bit. The doubling of the imidazole H1 and the pyrimidine H9 of the PMA^- ligand frame due to enantiomeric interactions between the duplex and the Δ and Λ isomers of the $[Co(PMA)]^{2+}$ unit is also indicated.

temperatures (at 60 °C, two distinct peaks are observed).[22] These doubling effects arise due to chiral discrimination between the Λ and Δ isomers of the $[Co(PMA)]^{2+}$ unit by **8**. Similar enantioselective interactions between the $[Co(PMA)]^{2+}$ moiety of **6** and DNA have been reported.[16]

Binding of the bithiazole moiety of **7** to **8** results in significant upfield shifts for H22 and H25. As indicated in the top panel of Figure 5, in the **7**:**8** (1:1) complex, the peaks corresponding to H22 and H25 appear at 7.820 and 7.648 ppm as compared to 8.151 and 8.012 ppm, respectively, in free **7**. Also, the resonance for the bithiazole methyl group shifts from 2.799 ppm in free **7** to 2.547 ppm in **7**:**8** (1:1) complex. Upfield shifts (of similar magnitude) of these bithiazole peaks have been observed in bleomycin−DNA adducts.[23−25]

Discussion

The three synthetic analogues $[Co(PMA)(N\text{-}MeIm)]^{2+}$ (**3**), $[Co(PMA)(H_2O)]^{2+}$ (**4**), and $[Co(PMA)(Cl)]^{+}$ (**5**), like the Co^{III}−BLMs, induce DNA strand scission under UV illumination. Spectroscopic evidence suggests that exposure to UV light generates a C/N-based radical on the ligand framework of these model complexes.[14] In aqueous buffer, this ligand-based radical collapses rapidly into •OH radicals. The DNA photodamage by **3**−**5** is therefore a result of the production of •OH radicals in the vicinity of the DNA helix by the bound metal complexes. This unusual reactivity of the $[Co(PMA)]^{2+}$ unit is worth further exploration in connection to its use in the design of new DNA photocleaving agents.

It is important to note that only moderate *random* DNA cleavage is observed when DNA is illuminated with submillimolar concentrations of **3**−**5**. This random photocleavage stems from weak DNA binding by the chiral complexes **3**−**5**.

(22) The DNA melting curve of the **7**:**8** (1:1) complex, as followed by the ^{1}H NMR peak of the thymine methyl groups, indicates that the duplex is only partially melted at 60 °C. Thus a considerable extent of interaction exists between the cobalt complex **7** and the oligonucleotide duplex (**8**) even at 60 °C. This fact allows observation of the two chiral forms of **7** (one with the $[Co(PMA)]^{2+}$ moiety bound to the duplex and one with the same moiety free) in the form of doublings of H9 and H1 protons (supporting information).

(23) Manderville, R. A.; Ellena, J. F.; Hecht, S. M. *J. Am. Chem. Soc.* **1994**, *116*, 10851.
(24) Wu, W.; Vanderwall, D. E.; Stubbe, J.; Kozarich, J. W.; Turner, C. J. *J. Am. Chem. Soc.* **1994**, *116*, 10843.
(25) Urata, H.; Ueda, Y.; Usami, Y.; Akagi, M. *J. Am. Chem. Soc.* **1993**, *115*, 7135.

Recognition of distinct features of base sequences by small metal complexes is noted only when the chiral metal complexes have high affinity for DNA.[26] The positively-charged complexes **3**−**5**, however, bind to DNA solely through electrostatic interactions (weak and nonspecific binding) and hence fail to recognize DNA conformations at specific sequences (a rapid on-and-off situation). The behaviors of the hybrid analogue $[Co(PMA)(Int\text{-}A)]Cl_2$ (**6**) support this hypothesis. **6** not only binds to DNA very strongly (binding constant $> 10^6\ M^{-1}$) and promotes DNA photocleavage at micromolar concentrations but also exhibits sequence specificity (5′GG-N3′).[16] It is quite obvious that the tethered acridine, a strong intercalator, is responsible for the enhanced DNA affinity of **6**. However, the observed sequence specificity of **6** could arise either from the acridine portion or from the $[Co(PMA)]^{2+}$ unit which, in **6**-DNA adducts, stays strongly associated with the helix for a longer period of time due to additional interaction between the tethered acridine moiety and the DNA base stack. Clearly, it is difficult to predict the origin of the observed preference for the 5′GG-N3′ sequence on the basis of the results of DNA photocleavage by **6** alone.

To determine whether the $[Co(PMA)]^{2+}$ unit itself exhibits any sequence specificity or not, one needs to attach a moderately strong DNA-binding group to the $[Co(PMA)]^{2+}$ unit and investigate the sequence specificity of the conjugate analogue. Such a strategy will allow the $[Co(PMA)]^{2+}$ unit to stay associated with DNA for enough time to exhibit its intrinsic sequence specificity without overriding interference(s) from the DNA-binding group. In the present work, one such hybrid analogue has been synthesized. This analogue, namely, $[Co(PMA)(Bit)]Cl_2$ (**7**) contains a 2,4′-bithiazole as the DNA-binding group. The DNA affinity for 2,4′-bithiazoles without any positively-charged substituents is quite moderate compared to acridines, which are strong intercalators.[20,27] The structure of the cation of **7**, as discerned from its spectral parameters, is shown in Figure 1. The octahedral coordination geometry around cobalt is evidenced by the shoulder at 520 nm ($\epsilon = 165\ M^{-1}\ cm^{-1}$) in the electronic absorption spectrum.[28] The bithiazole moiety of **7** exhibits its characteristic absorption band at 296 nm ($\epsilon = 16\,400\ M^{-1}\ cm^{-1}$). The ^1H and ^{13}C NMR spectra of **7** establish its structure in an unambiguous manner.

Results of fluorescence quenching experiments suggest that the DNA-binding constant of **7** lies in the range $10^4-10^5\ M^{-1}$. Although this value is appreciably higher than the DNA-binding constants of **3**−**5** ($10^3-10^4\ M^{-1}$), it is no way close to the binding constant of **6** ($>10^6\ M^{-1}$). Quite expectedly, **7** induces photodamage to plasmid DNA in micromolar concentrations (lane 2, Figure 2) while simpler analogues like **3** inflict a similar extent of photocleavage in millimolar concentrations (lane 3, Figure 2). Since micromolar concentrations of Bit do not photocleave DNA to any significant extent (lane 4, Figure 2), the enhanced photoactivity of **7** must be related to the $[Co(PMA)]^{2+}$ portion of the molecule. This in turn indicates that bithiazole−DNA interactions in the **7**−DNA adduct do assist the $[Co(PMA)]^{2+}$ unit to stay close to the helix for a longer period of time and inflict more photodamage. The extent of DNA photodamage by **7** is, however, lower than that by **6**, a fact in line with the values of the DNA-binding constants of the two hybrid analogues.

We have previously reported that the extent of DNA photodamage by **3**−**5** can be modulated by the amounts of cations like Na^+ in the incubation medium.[14] Increasing amounts of Na^+ in the reaction mixture disrupt electrostatic binding to DNA by **3**−**5** and result in steady diminution of DNA photocleavage. With simpler analogues like **3**−**5** as the photocleaving agent, complete protection of DNA is noted with 100 mM Na^+. In contrast, the presence of acridine permits **6** to remain associated with the DNA under such "high-salt" conditions and hence its photocleaving capacity is barely altered.[15] The behavior of **7** is just inbetween. When the incubation medium contains 100 mM Na^+ (lane 5, Figure 3), **7** exhibits very low (nevertheless noticeable) DNA photodamage activity (compare lane 3 with lane 5, Figure 3). Collectively, these results confirm that the covalently attached bithiazole does enhance the DNA affinity of **7** to a moderate extent and assist the $[Co(PMA)]^{2+}$ unit of **7** to cause significant DNA photodamage.

To determine the sequence selectivity (if any) of **7**, we analyzed the photocleavage products of reactions between **7** and radiolabled 266-bp and 391-bp restriction fragments of pBR322 plasmid DNA by gel electrophoretic techniques. As shown in Figure 4, both **6** and **7** preferentially photocleave DNA at 5′GG-N3′ sites (lane 5 and lane 6, respectively) with **6** being more active (darker bands). It is intriguing to note that **6** and **7** exhibit identical sequence specificity. If the tethered acridine or bithiazole were responsible for the sequence recognition, then the cleavage patterns should have been quite different. Previous studies have shown that acridines bind most favorably to GC regions of DNA.[29,30] However, the cleavage pattern of **6** does not indicate preference for the GC sequences (lane 5, Figure 4). Studies by Hecht and co-workers[17] have demonstrated that EDTA-bithiazole conjugates, in the presence of Fe^{2+} and dithiothreitol, promote *random* DNA cleavage. This suggests that bithiazole does not impart any sequence preference to the conjugate molecules. The 5′GG-N3′ sequence specificity of **6** and **7** is therefore not dictated by the acridine or the bithiazole moiety—it is the $[Co(PMA)]^{2+}$ unit that is responsible for the sequence specificity of the hybrid analogues **6** and **7**. Studies on oxidative damage of DNA by Fe−BLM analogues have indicated that the metalated core is the primary determinant of the sequence selectivity.[17−19] The present results strongly suggest that the metalated portions of the synthetic analogues of Co^{III}−BLMs also dictate their sequence selectivity in light-induced DNA cleavage reactions.

In the present work, we have also explored the mode of binding of **7** to the 5′GG-N3′ regions of DNA. A self-complementary oligonucleotide duplex $[d(GATCCGGATC)_2]$ (**8**) with a built-in GG-N site has been employed in such a pursuit. The ^1H NMR spectrum of the **7**:**8** (1:1) complex displays upfield shifts of the H22 and H25 of the bithiazole moiety of **7** upon complexation (Figure 5). Also, the peak for the methyl substituent on the bithiazole of **7** is broadened and shifted downfield in the ^1H NMR spectrum of the **7**:**8** (1:1) complex. Both these observations indicate that the bithiazole group of **7** does intercalate into the designed oligonucleotide duplex **8**. However, the NOESY spectra of the **7**:**8** (1:1) complex at two different temperatures did not reveal any intermolecular NOE cross peaks. This latter fact does not

(26) (a) Barton, J. K. *Science* **1986**, *233*, 727. (b) Barton, J. K. *Chem. Eng. News* **1988**, Sept 26, 30.

(27) BLM carries a 2,4′-bithiazole in its DNA-binding domain although the ring system is connected to positively-charged amine tails that results in stronger DNA binding.

(28) $[Co(PMA)(Int\text{-}A)]Cl_2$ (**6**) exhibits the same band at 520 nm ($\epsilon = 140\ M^{-1}\ cm^{-1}$).[15]

(29) Cieplak, P.; Rao, S. N.; Grootenhuis, P. D.; Kollman, P. A. *Biopolymers* **1990**, *29*, 717.

(30) Muller, W.; Crothers, D. M. *Eur. J. Biochem.* **1975**, *54*, 267.

(31) With the **6**:**8** (1:1) complex, strong intermolecular NOE cross peaks between acridine protons and specific nonexchangeable base protons and sugar protons of **8** are observed. [16] Clearly, the acridine ring of **6** intercalates into the base stack of **8** in a classical manner.

support a classical mode of intercalation of the bithiazole group into the base stack of **8**.[23,31,32] Similar nonclassical intercalative behavior of the bithiazole group has been noted for a Zn^{II}−$BLMA_5$−[d(CGCTAGCG)]$_2$ complex.[23] The ^{1}H NMR spectrum of the **7**:**8** (1:1) complex also indicates considerable interaction between the $[Co(PMA)]^{2+}$ unit of **7** and the duplex **8**. For example, the pyrimidine H9 and the imidazole H1 peaks of the PMA^- ligand framework shift downfield upon complexation of **7** with **8**. Also, these peaks appear as doublets in the NMR spectrum of the **7**:**8** (1:1) complex (Figure 5, top panel). As discussed in our earlier account,[16] such doubling of peaks results from diastereomeric complexes formed upon association of the Λ and Δ isomers of the $[Co(PMA)]^{2+}$ unit of **7** with **8**. NMR resolution of the enantioselective interactions between the chiral portion of **7** and DNA indicates that the $[Co(PMA)]^{2+}$ unit remains associated with the helix for a reasonable amount of time.[33,34] Although the present NMR data are not sufficient to establish (a) the precise location of the analogue **7** on the duplex **8** and (b) the three-dimensional structure of the **7**:**8** (1:1) complex, the results suggest that the bithiazole group enhances binding of **7** to DNA presumably via groove interactions and places the $[Co(PMA)]^{2+}$ unit close to the DNA helix for a reasonable amount of time to cause sequence specific photodamage.

(32) Wu et al. have reported intermolecular NOEs between bithiazole protons and base protons in NOESY spectra of the GREEN Co^{III}−$BLMA_2$−[d(CCAGGCCTGG)]$_2$ adduct.[24]

(33) No doubling of peaks is observed when simpler analogues like **3**−**5** are added to **8**. These complexes most possibly do not stay close to the helix for long time (rapid on-and-off situation), and hence the diastereomeric Λ-DNA and Δ-DNA complexes are not resolved in the NMR spectra of the analogue−DNA adducts.

(34) Pyle, A. M.; Rehmann, J. P.; Meshoyrer, R.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1989**, *111*, 3051.

Conclusion

Covalent attachment of acridine or bithiazole to the $[Co(PMA)]^{2+}$ unit gives rise to hybrid analogues $[Co(PMA)(Int\text{-}A)]^{2+}$ (**6**) and $[Co(PMA)(Bit)]^{2+}$ (**7**) which promote DNA photodamage at micromolar concentrations much like the Co^{III}−BLMs. These conjugate complexes exhibit sequence specificity in the DNA photocleavage reactions. The metalated core of these analogues is the primary determinant of the observed sequence specificity.

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Supporting Information Available: NMR traces (of the aromatic region) of the titration of **7** with **8** showing chiral discrimination of the two enantiomers of **7** upon duplex binding (Figure S1) and the NMR spectrum of the **7**:**8** (1:1) complex at 60 °C showing considerable extent of interaction between **7** and **8** and doubling of the H9 and H1 peaks due to chiral discrimination of the enantiomers of **7** by **8** (Figure S2) (2 pages). Ordering information is given on any current masthead page.

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