Sequence-specific Changes in the Metal Site of Ferric Bleomycin Induced by the Binding of DNA*

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Joseph W. Sam‡§, Satoshi Takahashi¶, Istvan Lippai**, Jack Peisach†‡‡‡, and Denis L. Rousseau**

From ‡‡‡ AT&T Bell Laboratories, Murray Hill, New Jersey 07974 and the Departments of **Physiology and Biophysics and ‡‡‡Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, New York 10461-1602

The binding of the iron complex of the antineoplastic glycopeptide bleomycin A– (Fe-BLM) to calf thymus DNA and the self-complementary oligonucleotides d(CGCGCG) and d(ATATAT) has been studied using optical, EPR, and resonance Raman spectroscopies. An increase in the intensity of the bands at 365 and 384 nm is observed in the optical spectrum of Fe(III)-BLM when the drug binds to either oligonucleotide. However, in the absence of phosphate, this increase is observed only with d(CGCGCG) and not with d(ATATAT). In addition, the 

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† Supported by NIGMS, NIH, Grant T32 GM07288. Present address: Dept. of Radiology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

‡ Present address: The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-01, Japan.

§ To whom correspondence should be addressed.

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first experimental evidence of DNA sequence-specific alterations in the metal binding site of Fe(III)-BLM and further lends credence to the view that the metal binding site is responsible for sequence recognition related to specificity of cleavage.

**EXPERIMENTAL PROCEDURES**

**Sample Preparation**—Bleomycin A$_2$ was purified from bleomycin sulfate (Blenoxane; generously supplied by Bristol-Myers Co., Syracuse, NY) over a Source S fast protein liquid chromatography column (Amersham Pharmacia Biotech) as described previously (29). The purity of the BLM fractions was confirmed by mass spectrometry (30). The ferric complex of the drug was prepared by the addition of Fe(II)(NH$_4$)(SO$_4$)$_2$ to 1.1 equivalents of BLM followed by at least 10 equivalents of buffer to bring the pH to 7.5. Fe(II)-BLM was formed by the anaerobic addition of excess (2–5 equivalents) Na$_2$S$_2$O$_4$ to a septum-stopped Raman cell containing Fe(III)-BLM. The CO complex was produced from Fe(II)-BLM by anaerobically flushing the Raman cell with CO for several minutes. Isotopically labeled chemicals were obtained from Aldrich (D$_2$O, 99.9%) and ICON (Mt. Marion, NY; H$_2$-D$_2$O, 97%, and $^{13}$C-$^18$O, 99%). Final isotope enrichments for BLM solutions were at least 90%. Calf thymus DNA (Boehringer Mannheim) was purified by ethanol precipitation and centrifugal dialysis (Centricon-3, Amicon) and sheared by sonication and pipetting. DNA oligonucleotides were synthesized at the DNA synthesis facility of the Albert Einstein College of Medicine and purified by ethanol precipitation and centrifugal dialysis. The BLM fractions were further verified by mass spectrometry (30). The integrity of the samples used for the Raman spectra was confirmed by optical spectra (350–500 nm) taken before and after each Raman measurement using a DW2000 spectrophotometer (SLM-Aminco) as described previously (29). The purity of the BLM fractions was confirmed by mass spectrometry (30).

**RESULTS**

**Optical Spectra**—UV and visible spectra were acquired using a modified Cary 14DS spectrophotometer (Ariv Associates, Lakewood, NJ). The integrity of the samples used for the Raman spectra was verified by optical spectra (350–500 nm) taken before and after each Raman measurement using a DW2000 spectrophotometer (SLM-Aminco, Urbana, IL).

**EPR Spectroscopy**—EPR spectroscopy was conducted at 77 K on an X-band Varian E-112 spectrometer equipped with a Systron-Donner frequency counter, a Varian NMR gaussmeter, and a rectangular TE101 cavity. Peak widths at $g_{\text{max}}$ were measured at half-peak height with ±1 G accuracy.

**Raman Spectroscopy**—A krypton ion laser (Spectra Physics, Mountain View, CA) was employed to excite samples contained in quartz cells that were rotated at 1000 rpm at room temperature. Rayleigh scattered light was removed by a holographic filter (Kaiser, Ann Arbor, MI). Raman scattered light was dispersed by a single polychromator (SPEX, Metuchen, NJ) equipped with a 1200 groove/mm grating and detected by a cooled CCD camera (Princeton Instruments, Princeton, NJ). The spectral line width was approximately 5 cm$^{-1}$. Typically, eight consecutive measurements of 1-min duration, using 10 milliwatt of laser power at 406.7 nm, were taken and added to yield a Raman spectrum. Cosmic ray-induced spikes were removed before averaging by a computer software routine. Raman shifts were calibrated using neat indene and/or laser fluorescence emission lines as frequency standards, providing absolute accuracy of ±2 cm$^{-1}$.

Fig. 2 displays the optical spectrum from 290 to 525 nm of Fe(III)-BLM as it is titrated with calf thymus DNA; the bands at 365 and 384 nm seen for Fe(III)-BLM (Fig. 2, inset, trace D) have not been assigned but may result from charge transfer transitions between the iron atom and its various ligands, since charge transfer bands have been observed in the presence of DNA (14). When Fe(III)-BLM (Fig. 2, inset, trace D) binds to ct-DNA, there is a slight increase in the intensity of these bands (trace A) as well as a red shift to 370 and 387 nm, respectively. This latter effect of DNA binding may be due to broadening of the large underlying peak at 291 nm, which has been ascribed to the bithiazole π-π$^*$ transitions (31, 32). These changes are also observed when Fe(III)-BLM binds to either d(CGCGCG) (trace B) or d(ATAATAT) (trace C). Note that the concentrations of the drug (150 μM) and oligomers (250 μM) are much lower than those used in the Raman experiments, and further, that increases (to ~300 μM) in the concentration of either oligomer had no effect on the optical spectra (not shown).
for 

DNA. That one obtains a width with DNA and a reduction in width at half-peak height in 5 m M Tris, pH 7.5, to a final drug:DNA ratio of one molecule of Fe(III)-BLM.

a single conformer is recognized by this method at the same g value. In the presence of d(CGCGCG) or ct-DNA, only clearly shows more than one component, confirming the multiplicity of 

shoulders at 365 and 384 nm increase. The drug to DNA, the peak at 291 nm decreases in intensity, and the are the difference spectra versus 

induced by binding DNA is small. In addition, the structure of the sequence of the bound oligonucleotide.

Continuous Wave EPR Spectra

Albertini and Garnier-Suillerot (21) have reported that when Fe(III)-BLM binds to ct-DNA, the only change is an increase in the rhombicity of its EPR spectrum, manifested as a shift in g_{max} from 2.40 to 2.43. This is a peculiar result, since the value of g_{max} for Fe(III)-BLM has previously been measured as 2.43 by Sugiura (33) and 2.45 by Burger et al. (34). Traces A and B of Fig. 3 display the X-band EPR spectrum of free, low spin Fe(III)-BLM in Tris buffer. The value of g_{max} for this species is 2.43, in good agreement with the results of Sugiura (33). Furthermore, when Fe(III)-BLM binds to d(CGCGCG) or ct-DNA, there is no shift in any of the features in the derivative EPR spectrum (Fig. 3, traces D and E); however, there is a sizable decrease in the width (at half-peak height) of the g = 2.43 feature. Less narrowing is seen at other features of the spectrum (a reduction of g_{mid} from 24 to 22 gauss in peak-to-trough width with DNA and a reduction in width at half-peak height for g_{min} from 41 to 31 gauss with DNA). That one obtains a nearly identical EPR spectrum, but with narrowing of the spectral features, suggests that the change in the metal site induced by binding DNA is small. In addition, the structure of the metal binding site of the d(CGCGCG)-bound form of the drug is more homogeneous; i.e. the iron drug complex without DNA can assume multiple conformers in frozen solution as judged from the asymmetry of the g = 2.43 feature. The apparent narrowing is then a consequence of the reduction of the number of conformers upon binding to d(CGCGCG) or DNA. The EPR spectrum of Fe(III)-BLM bound to ct-DNA (Fig. 3, trace E) is essentially the same as that of the d(CGCGCG)-bound form (Fig. 3, trace D). This is consistent with the early observation that Fe-BLM preferentially cleaves CG sequences.

The second derivative of the EPR absorption at the g = 2.43 feature clearly shows more than one component, confirming the multiplicity of conformers of the drug. In the presence of d(CGCGCG) or ct-DNA, only a single conformer is recognized by this method at the same g value.

**FIG. 2.** The effect of DNA binding on the optical spectrum of Fe(III)-BLM. Calf thymus DNA was added to a solution of Fe(III)-BLM in 5 mM Tris, pH 7.5, to a final drug:DNA ratio of one molecule of Fe-BLM (150 μM) to five base pairs of DNA (750 μM/base). Traces A–E display the optical spectrum of Fe(III)-BLM bound to calf thymus DNA (−), d(CGCGCG) (●), and d(ATATAT) (○), d(CGCGCG) (●), and d(ATATAT) (○). Trace D is the spectrum of DNA-free Fe(III)-BLM (−−−).

**FIG. 3.** X-band EPR spectra of Fe(III)-BLM (traces A and B) and Fe(III)-BLM bound to d(ATATAT) (trace C), d(CGCGCG) (trace D), and calf thymus DNA (trace E). Traces B–E are expanded views of the g = 2.43 feature. Peak widths in gauss, as measured at half-peak height, are indicated. All samples were in 20 mM Tris, pH 7.5. Spectra were recorded with a modulation amplitude of 5 gauss.

In contrast, the spectrum of the d(ATATAT)-bound form of the drug displays no shifts and minimal narrowing of the g = 2.43 feature (Fig. 3, trace C). These data demonstrate that there are structural differences in the iron site of the drug depending on the sequence of the bound oligonucleotide.

**Raman Spectra**

**High Frequency Region**—Fig. 4, trace A, displays the nonresonance Raman spectrum of metal-free BLM. The spectrum has a strong line at 1540 cm⁻¹, which has been assigned to the bithiazole moiety (35). This line at the same frequency is present in the Raman spectrum of Fe(III)-BLM (Fig. 4, trace B) but at a slightly lower frequency in the spectrum of d(CGCGCG)-bound Fe(III)-BLM (Fig. 4, trace C). This shift is reproducible and shows that the conformation of the bithiazole group changes slightly upon DNA binding. Fig. 4, trace D, displays the nonresonance Raman spectrum of d(CGCGCG), which is essentially identical to previously published spectra and demonstrates that the oligomer adopts a B-helix conformation (36).

Since the resonance Raman lines from the iron complexes of BLM are weak and overlapped by the nonresonant lines from metal-free BLM and from the oligomer, all subsequent Raman spectra are difference spectra obtained by subtracting the nonresonant components. Fig. 5, trace A, shows the 1200–1700 cm⁻¹ region of the Raman spectrum of Fe(III)-BLM after subtracting the metal-free components of the spectrum, so as to minimize the bithiazole peak near 1540 cm⁻¹. Likewise, trace A' in Fig. 5 was obtained by subtracting the spectra of metal-free BLM (Fig. 4, trace A) and d(CGCGCG) (Fig. 4, trace D).
from the resonance Raman spectrum of Fe(III)-BLM bound to d(CGCCCG) (Fig. 4, trace C) so as to minimize the difference pattern between 1510 and 1590 cm⁻¹; this difference pattern is caused by a small shift of the bithiazole line to a lower frequency induced by the binding of d(CGCCCG). Similar difference spectra obtained between the free and the d(CGCCCG)-bound drug in either H₂¹⁸O (traces B and B', respectively) and D₂O (traces C and C', respectively) are also presented in Fig. 5.

The spectrum of DNA-free Fe(III)-BLM in H₂O (Fig. 5, trace A) contains resonance-enhanced lines at 1611 and 1478 cm⁻¹, assigned as the amide I and II modes of the β-hydroxyhistidyl amide (Fig. 1B), as well as lines at 1390 and 1372 cm⁻¹, proposed as internal modes of the pyrimidine ring (13, 14). The frequencies and proposed assignments of the various resonance-enhanced Raman lines referred to in this paper have been compiled in Table I. When the drug is bound to d(CGCCCG), the amide modes are narrowed and shifted significantly, and new peaks appear at 1405, 1367, and 1352 cm⁻¹ (Fig. 5, trace A'); these new lines are resonance-enhanced and are not present in the spectrum of d(CGCCCG) alone (Fig. 4, trace D). The narrowing of the β-hydroxyhistidyl amide modes indicates an increase in the homogeneity of these motions and is consistent with the metal binding site adopting a more rigid, unique structure when the drug is bound to d(CGCCCG), as inferred from the above EPR spectra (Fig. 3).

With D₂O as the solvent, the Raman lines between 1250 and 1450 cm⁻¹ show complex deuterium shifts (Fig. 5, traces C and C'), while the data for the samples in H₂¹⁸O (Fig. 5, traces B and B') are identical with that for samples in H₂O (Fig. 5, traces A and A'). At present, the lines in the D₂O spectra cannot be correlated definitively with those in the H₂O spectra. However, since there is a weak shoulder at 1405 cm⁻¹ in the spectra of the DNA-free form of the drug (Fig. 5, traces A and B), one can speculate that the "new" line at 1405 cm⁻¹ in the spectra of the d(CGCCCG)-bound Fe(III)-BLM (Fig. 5, traces A' and B') represents the same vibrational mode but is observed with greater intensity when the drug is bound to d(CGCCCG). Interestingly, this line is absent from the D₂O spectra (Fig. 5, traces C and C'), presumably due to a large deuterium shift to an obscured position in the spectrum. Similarly, the doublet appearing at 1367 and 1352 cm⁻¹ in the spectra of Fe(III)-BLM bound to d(CGCCCG) (Fig. 5, traces A' and B') might be interpreted as arising from the broad line at 1372 cm⁻¹ in the spectra of the DNA-free form (Fig. 5, traces A and B), which is shifted and split by the binding of d(CGCCCG). We have previously (13) assigned the line at 1372 cm⁻¹ as a pyrimidine ring mode; thus, we propose that the spectral features between 1350 and 1420 cm⁻¹ represent internal modes of the pyrimidine ring, which displays a different splitting pattern when the drug interacts with the d(CGCCCG) oligomer.

Low Frequency Region—Fig. 6 contains the 480–820 cm⁻¹ region of the Raman spectra of DNA-free and d(CGCCCG)-bound Fe(III)-BLM. The lines at 784 and 682 cm⁻¹ for samples in H₂O (Fig. 6, trace A') and H₂¹⁸O (Fig. 6, trace B') are due to nonresonant DNA modes. The deuterium shifts of these modes are identical to those for the drug-free d(CGCCCG) solution (data not shown). The peak at 561 cm⁻¹ in trace A has been assigned (13) as the Fe-OH stretching mode of free Fe(III)-BLM and shifts to 556 and 541 cm⁻¹ in D₂O and H₂¹⁸O, respectively (Fig. 6, traces B and C). When the drug binds d(CGCCCG), the line at 561 cm⁻¹ is significantly narrowed and shifts to 558 cm⁻¹ (Fig. 6, trace A'). Also, line narrowing is observed when the drug binds d(CGCCCG) in H₂¹⁸O or D₂O. Interestingly, when D₂O is employed as the solvent, this mode displays a much larger (9 cm⁻¹) low frequency shift upon binding d(CGCCCG). Last, it should be noted that the assignments of all of the observed resonance Raman lines are the same for both DNA-free and d(CGCCCG)-bound Fe(III)-BLM and are consistent with the presence of the β-hydroxyhistidyl amide, pyrimidine, and hydroxide moieties as ligands to the iron atom. These results together with the optical absorption and EPR data strongly suggest that although there are conformational changes in the metal binding site when Fe(III)-BLM binds
d(CG) Recognition by Ferric Bleomycin

* Table I

| Proposed assignment                          | DNA-free Fe(III)-BLM (in H$_2$O/H$_2$O/D$_2$O) | d(CGCGCG)-bound Fe(III)-BLM (in H$_2$O/H$_2$O/D$_2$O) | d(ATATAT)-bound Fe(III)-BLM in H$_2$O |
|---------------------------------------------|-----------------------------------------------|--------------------------------------------------|--------------------------------------|
| Anhydrate (C = O stretch) of the β-hydroxyhistidyl amide | 1611/1611/1612 22 | 1614/1614/1616 17 | 1613 NA* |
| Amide II (C–N stretch) of the β-hydroxyhistidyl amide | 1478/1478/1480 22 | 1475/1475/1475 15 | 1482 23 |
| Pyrimidine ring mode                         | 1390/1390/1391 NA | 1390/1390/1390 NA | 1389 NA |
| Pyrimidine ring mode                         | 1372/1372/1375 NA | 1367/1367/1374 NA | 1372 NA |
| Pyrimidine ring mode                         | NO NO NO | 1405/1405/NO NO | NO NO |
| Pyrimidine ring mode                         | NO NO NO | 1332/1354/1358 NA | NO NO |
| Fe(III)-OH stretch                           | 561/541/556 34 | 558/540/547 20 | 560 24 |

* Average of the three readings obtained for H$_2$O, H$_2$O/D$_2$O, and D$_2$O solutions.

* NA, not assessed due to spectral overlap or noise.

* NO, not observed.

![Graph](image-url) Low frequency region of the resonance Raman spectra of free Fe(III)-BLM (traces A, B, and C) and d(CGCGCG)-bound Fe(III)-BLM (traces A’, B’, and C’) in H$_2$O (traces A and A’), and D$_2$O (traces C and C’) and H$_2$O/D$_2$O (traces B and B’). No subtractions of the nonresonant spectra were made.

![Graph](image-url) Low and high frequency regions of the resonance Raman spectra of d(ATATAT) (trace A), d(ATATAT)-bound Fe(III)-BLM (1:1 double-stranded oligomer-drug) (trace B), and free Fe(III)-BLM (trace D). Trace C is the difference spectrum obtained by subtracting trace A from trace B.

d(CGCGCG), the identities of the iron ligand remain the same.

**Binding to d(ATATAT)—**As mentioned above, d(CGCGCG) and d(ATATAT) have an identical effect on the optical spectrum of Fe(III)-BLM in the absence of phosphate, but the EPR spectrum of low spin Fe(III)-BLM is altered only when the drug binds the d(CGCGCG) oligomer. The resonance Raman spectra of Fe(III)-BLM bound to d(ATATAT) are presented in Fig. 7. Trace C has been corrected by subtracting the nonresonant Raman spectra of Fe(III)-BLM (Fig. 7, trace B) from the spectrum obtained when the drug is bound to this oligomer (Fig. 7, trace C). It should be noted that the nonresonant bithiazole mode at 1538 cm$^{-1}$ is not shifted when the drug is bound to d(ATATAT) (Fig. 7, traces C and D). Indeed, the DNA-free (Fig. 7, trace D) and d(ATATAT)-bound (Fig. 7, trace C) spectra are nearly identical. Although these data might lead one to conclude that Fe(III)-BLM does not form a complex with d(ATATAT), we contend that Fe(III)-BLM does in fact bind d(ATATAT). For example, there are identical changes in the optical spectrum of the drug in the presence of ct-DNA, d(CGCGCG), or d(ATATAT) (Fig. 2); further evidence for the binding of Fe(III)-BLM to d(ATATAT) is provided under “Discussion.” Therefore, the observation of changes in the resonance Raman and continuous wave EPR spectra of Fe(III)-BLM induced by the addition of d(CGCGCG) but not by the addition of d(ATATAT) provides the first direct evidence for sequence-specific alterations in the structure of the metal-binding site of Fe-BLM.

**Fe(II)-BLM and CO-Fe(II)-BLM—**We have obtained the resonance Raman spectra of Fe(II)-BLM and CO-Fe(II)-BLM bound to d(CGCGCG) and d(ATATAT) (data not shown). The spectra in the presence of DNA are essentially identical with those of the DNA-free form. Of particular interest is that the modes of the iron-bound CO of CO-Fe(II)-BLM are unchanged (±1 cm$^{-1}$) when the drug is bound to d(CGCGCG) or d(ATATAT).

**The Effect of Phosphate—**Fe(III)-BLM in neutral phosphate solution forms a high spin complex (28). Fig. 8, trace C, displays the optical spectrum of free Fe(III)-BLM in phosphate buffer; the absence of the two bands at 365 and 384 nm seen in Tris buffer (Fig. 2) is apparent. Not surprisingly, the Raman spectrum of this species shows no resonance-enhanced lines (not
shown). However, upon adding d(CGCGCG), the optical bands reappear in the spectrum (Fig. 8, trace A), and the resulting Raman spectrum is identical to that of the d(CGCGCG)-bound form in Tris buffer. Furthermore, the Raman spectra of the d(CGCGCG)-bound forms of Fe(II)-BLM and CO-Fe(II)-BLM in phosphate buffer are identical to the same species in Tris buffer. However, upon adding d(ATATAT) to a solution of phosphate-buffered Fe(III)-BLM, one does not see a reappearance of the 365 and 384 nm bands (Fig. 8, trace B). The simplest explanation of these data is that phosphate binds to free Fe(III)-BLM, causing the changes in its optical spectrum and spin state, and d(CGCGCG) is capable of displacing the phosphate, whereas d(ATATAT) is not. It should be stressed, however, that there is no direct evidence that phosphate binds to the iron atom in Fe(III)-BLM.

**DISCUSSION**

We have confirmed that Fe(III)-BLM forms complexes with d(CGCGCG) and d(ATATAT) by demonstrating an increase in the intensity of the absorption bands at 365 and 384 nm of the drug when bound to either of the oligonucleotides. The binding of Fe-BLM to these oligomers was not surprising, since several reports have demonstrated binding of the drug to both CG and AT sequences (3, 37, 38, 39) as well as to several short (4–6-base pair) oligomers (38, 40). Binding constants for the drug-DNA complexes in these reports are in the $10^5$ to $10^6$ M$^{-1}$ range (3, 37, 41). Although the binding constants of Fe-BLM for d(CGCGCG) and d(ATATAT) under the conditions of the present study are not known, the optical spectroscopic results presented in Fig. 2 demonstrate that the drug is bound in both instances. However, EPR spectroscopy shows narrowing of the $g_{\text{max}} = 2.43$ feature when Fe(III)-BLM forms complexes with d(CGCGCG) but not with d(ATATAT). Furthermore, the resonance Raman spectra of d(CGCGCG)-bound Fe(III)-BLM indicate that there are structural alterations of the drug's metal binding site that are not observed for d(ATATAT)-bound Fe(III)-BLM. We therefore conclude that while the identities of the iron ligands are not changed, their conformations are altered by binding to d(CGCGCG) and not to d(ATATAT). The absence of changes in the EPR and the resonance Raman spectra in the d(ATATAT)-bound Fe(III)-BLM complex in contrast to the intensity changes seen in the optical spectrum indicates that these spectroscopies are probing different properties of the complexes. The changes in the intensity in the optical spectra may reflect only excited state variations and are therefore silent in the ground state EPR and resonance Raman measurements. Firm assignment of the optical transitions should help to clarify this point.

These results demonstrate that the metal binding site of Fe(III)-BLM undergoes sequence-specific conformational changes when the drug binds to DNA. The narrowing of the $g_{\text{max}}$ EPR spectral feature suggests that a preferred conformer, rather than multiple conformers, of the drug is produced by ct-DNA or d(CGCGCG) binding and not with d(ATATAT). Similarly, the EPR spectrum of activated BLM suggests the presence of multiple conformers (see Fig. 3 of Ref. 34), while upon the addition of DNA, only a single conformer is observed. These conformational changes implicate specific regions of the drug as essential for the interaction between the metal binding site of BLM and CG sequences of double-stranded DNA, as elaborated below.

**Changes at the β-Hydroxyhistidyl Amide**—When the drug binds to d(CGCGCG), the resonance Raman lines assigned as the amide I (C=O stretching) and II (C–N stretching) vibrations of the β-hydroxyhistidyl amide show significant narrowing and shifts to higher and lower frequencies, respectively. The frequency shift of the amide II line is greater than that of the amide I transition, suggesting that the structural perturbation is centered on the nitrogen atom. One possible explanation for these results is that the changes in the amide lines result from a more confined conformation of the linker region caused by the binding with d(CGCGCG). The deuterium shift pattern of the amide I and II modes are different before and after the DNA binding; although the DNA-free form shows small high frequency shifts of both lines, the d(CGCGCG)-bound form of the drug exhibits a high frequency shift in only the amide I mode. This suggests that an alteration in the hydrogen bonding structure of the β-hydroxyhistidyl amide occurs when the drug binds d(CGCGCG).

**Changes at the Pyrimidine**—When Fe(III)-BLM binds d(CGCGCG), three new lines appear in the resonance Raman spectrum at 1405, 1367, and 1352 cm$^{-1}$, and several lines between 1350 and 1420 cm$^{-1}$ shift in frequency (Fig. 5). The lines in this region of the spectrum of DNA-free Fe-BLM have been proposed (13, 14) to represent internal vibrations of the pyrimidine ring. Since the binding of DNA induces only a slight increase in the intensity of the optical absorption bands that are likely to be iron-pyrimidine charge transfer transitions of Fe(III)-BLM, it appears that the new lines in the resonance Raman spectra of the d(CGCGCG)-bound form of the drug represent slight changes in the internal vibrational modes of the pyrimidine group and are not novel vibrational modes from other ligands. The sequence-specific changes in the resonance Raman spectra further demonstrate that the alterations of the pyrimidine group occur only when the drug binds d(CGCGCG) and not d(ATATAT). The 4-NH$_2$ group of the pyrimidine ring is a probable site for the structural modification, since the Raman lines show deuterium shifts that can only be explained by the involvement of exchangeable protons. These observations strongly suggest the direct involvement of the pyrimidine in DNA sequence recognition. Indeed, Boger et al. (42) have shown that dimethylating the 4-NH$_2$ group of pyrimidine both diminishes DNA cleavage efficiency of deglycobleomycin A$_2$ and, more importantly, causes a loss of cleavage selectivity.

**Changes at the Axial Hydroxide**—The Fe(III)-OH stretching vibration displays different isotope shift patterns in the DNA-free and d(CGCGCG)-bound states; these are summarized in Table II along with the values for several other heme-hydroxide
termined using NMR spectroscopy. Manderville et al. (20, 21) studied the structure of DNA-bound Zn- and Co-BLM and concluded that the metal binding site is not in direct contact with DNA. In contrast, Wu et al. (20, 26) report, based on an NMR study, that hydrogen bonding occurs between the pyrimidine ligand of HOO-Co(III)-BLM and the dG-3 of d(CCAG-TACG) and demonstrates that HOO-Co(III)-BLM, as reported by Wu et al. (20, 26) is a better model than Zn(II)-BLM for Fe(III)-BLM and, presumably, for activated BLM, HOO-Fe(III)-BLM (30).

In their NMR study of DNA-bound HOO-Co(III)-BLM, Wu et al. (19, 20) indicate that the 4-NH$_2$ group and N-3 of the pyrimidine ring of the drug hydrogen bond with N-3 and the 2-amino group, respectively, of the guanine residue in the drug’s cleavage site. Based on our EPR investigation of Fe(III)-BLM, the reduction in the number of conformers that the iron drug complex can achieve with d(CCGCGG) or DNA, but not with d(ATATAT) (Fig. 3), is indicative of the formation of a rigid hydrogen-bonded structure, in accord with the results obtained by Wu et al. (19, 20). Since d(ATATAT) lacks the 2-NH$_2$ group, its hydrogen bonding capabilities are reduced.

Our Raman results show that the binding of d(CCGCGG) induces an alteration in the internal vibrations of the pyrimidine residue and, thus, support the contention by Wu et al. that the pyrimidine residue is essential for DNA sequence recognition by BLM. It should be noted, however, that there are several minor differences between their model and the present data. First, the proximal oxygen atom of HOO-Co(III)-BLM has been shown (19, 20) to form strong hydrogen bonds with the amide hydrogens of the threonine and methylvalerate residues in the linker region of BLM in both the free and DNA-bound states. The present Raman result shows significant broadening of the β-hydroxyhistidyl amide vibrational modes in the DNA-free form, suggesting that the linker moiety of Fe(III)-BLM does not assume such a well-defined structure. Second, the hydroxide ligand of Fe(III)-BLM complexed with DNA shows vibrational characteristics, suggesting that this ligand is isolated and not involved in a hydrogen bond network, such as that observed for HOO-Co(III)-BLM by Wu et al. (19, 20). These discrepancies may be due to differences in the metal ion, iron versus cobalt, or the axial ligand, OH versus OOH, employed in the two studies; further investigation of the extent of the similarity between the ferric and cobaltic complexes of the drug is clearly warranted.

**CONCLUSION**

Optical, EPR, and resonance Raman spectroscopy have been used to demonstrate that there is no change in identities of the ligands to iron when Fe-BLM binds DNA. However, the conformations of the ligands are altered when Fe(III)-BLM binds d(CCGCGG) but not when the drug binds d(ATATAT). These changes suggest that 1) the metal-binding site assumes a preferred conformation when bound to DNA and 2) changes in the vibrational modes of the pyrimidine ring are consistent with a recently proposed model (20) in which the pyrimidine group hydrogen bonds with the guanine in the conserved CG cleavage site. Last, as suggested by Wu et al. (19, 20), it would be of considerable interest to repeat these experiments using synthetic BLMs that have modified pyrimidines, such as prepared by Boger et al. (42), noting that an important benefit of the experimental methods employed in this article is that one can study Fe-BLM, which is presumed to be the biologically relevant complex of the drug.

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