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Interaction of Bleomycin A_2 with Poly(deoxyadenylylthymidylic acid). A Proton Nuclear Magnetic Resonance Study of the Influence of Temperature, pH, and Ionic Strength†

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ABSTRACT: The binding of bleomycin A_2 to poly(deoxyadenylylthymidylic acid) [poly(dA-dT)] has been monitored by proton nuclear magnetic resonance spectroscopy. This study includes an analysis of the effects of temperature, ionic strength, and pH. Sites of drug–nucleic acid interaction have been delineated on the basis of chemical shift perturbations of drug and nucleic acid resonances. The data indicate that the binding of the antibiotic occurs with partial intercalation of the aromatic bithiazole group and immobilization of the cationic dimethylsulfonium group. This complex dissociates as the nucleic acid is denatured to the single-stranded form. The absence of significant pH effects suggests that the N terminus of bleomycin A_2, which contains the titratable groups, does not contribute to the interaction of the drug molecule with poly(dA-dT). The problems associated with assigning a specific geometry to the drug–nucleic acid complex are discussed.

The bleomycins (Bleo;[1] Figure 1), a group of glycopeptide antibiotics used clinically in the treatment of various neoplastic diseases, cause the degradation of DNA [see reviews in Hecht (1979)]. It is this action that is generally believed to be responsible for their biological activity. In vitro, the degradation requires the presence of iron(II), dioxygen, and a reducing agent and is accompanied by the generation of various oxygen radicals, which may or may not be the ultimate reactive species (Burger et al., 1981). The Bleo molecule possesses two functional portions. One part comprised of the cationic C terminus and the bithiazole moiety appears to be responsible for the association of the drug with the target DNA (Chien et al., 1977; Chen et al., 1980; Glickson et al., 1981; Sakai et al., 1981). The other portion, containing the pyrimidine and adjoining residues, appears to be responsible for binding the necessary metal cofactor. The two regions of bleomycin appear to be essentially independent of each other: The binding of Bleo-A_2 (the most common congener) to poly(deoxyadenylylthymidylic acid) [poly(dA-dT)] is not influenced by and does not affect the binding of various metal ions to Bleo (Glickson et al., 1981). Similarly, the metal-binding residues are not influenced by the binding of the distal portion of the molecule to poly(dA-dT), although the valerate and threonine residues, which bridge the two functionally distinct regions, exhibit perturbations due to the presence of either the metal ion or the nucleic acid (Glickson et al., 1981).

The bifunctional nature of the Bleo-A_2 molecule has permitted the separate study of the nucleic acid and metal ion binding sites. Earlier fluorescence and proton NMR studies by Chien et al. (1977) monitored the interaction of Bleo-A_2 with calf thymus DNA. Quenching of the bithiazole fluoresence by DNA indicated involvement of that chromophore in the association process. Proton NMR spectra of the complex showed that the two aromatic bithiazole hydrogens and the methyl hydrogens of the sulfonium group of Bleo-A_2 exhibit the greatest degree of preferential broadening in the presence of DNA. These groups (and hence the C-terminal dipeptide) appear to be most extensively immobilized when the drug is bound to the nucleic acid. A preliminary proton NMR study of the interaction of Bleo-A_2 with poly(dA-dT) was reported by Chen et al. (1980). The utility of poly(dA-dT) as a model DNA in NMR studies has been amply demonstrated (Patel, 1979; Patel & Canuel, 1977, 1978). The extremely flexible and mobile structure of poly(dA-dT) allows well-defined proton NMR spectra to be obtained, in contrast with DNA, which exhibits only extremely broadened resonances due to its rigid rodlike structure. Proton resonances of the aromatic bithiazole group of Bleo-A_2 exhibited a temperature-dependent upfield shift upon complexation to poly(dA-dT), which suggested the presence of two modes of binding (Chen et al., 1980) and/or a preference of the drug for a partially opened helical structure (Sakai et al., 1981). Those studies also indicated that the drug is binding to the minor groove of the nucleic acid.

The present study examines in detail these chemical shift displacements, as well as the influence of temperature, pH, and ionic strength on the binding process, in an effort to further clarify the molecular geometry of the drug–nucleic acid complex.

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[1] Abbreviations: Bleo, bleomycin; Bleo-A_2, bleomycin A_2; pH_m, pH meter reading, uncorrected for isotope effects; BIT, bithiazole; THR, threonine; VAL, γ-amino-β-hydroxy-α-methylvaleric acid; HIS, β-hydroxyhistidine; PYR, pyrimidine; PRO, propionamide, ALA, β-aminoalanine; G, gulose; M, mannose; NMR, nuclear magnetic resonance.

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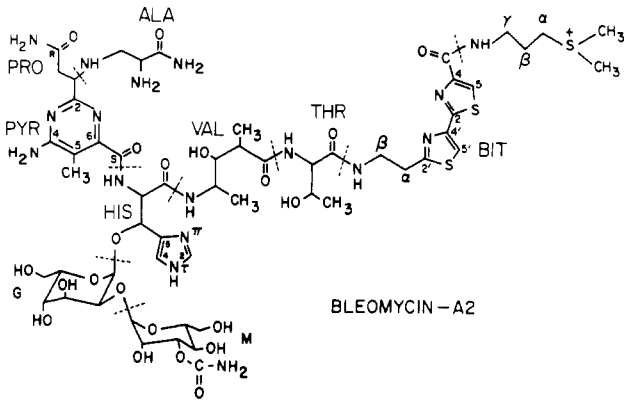

FIGURE 1: Structure of bleomycin A_2 indicating abbreviations of constituent residues.

Experimental Procedures

Materials

Poly(dA-dT) (s^w_{20} = 9.0) was obtained from P-L Biochemicals (Milwaukee, WI) and purified as described previously (Patel, 1979; Chen et al., 1980). Concentrations were determined by using a molar absorptivity at 262 nm of 6.60 × 10^3 cm^{-1} M^{-1} per nucleotide (Inman & Baldwin, 1962). The poly(dA-dT) employed in these experiments was deuterated in the adenine-8 position by heating the poly(dA-dT) in D_2O at 70–75 °C for 24–36 h. No differences were detectable in the NMR spectra or in the temperature dependence of the proton resonances of the exchanged or unexchanged preparations of the polynucleotide.

Bleo-A_2 was purified from commercial blenoxane (Bristol Laboratories, Syracuse, NY) by ion-exchange chromatography on a column of carboxymethyl-Sephadex C-25 (Pharmacia Fine Chemicals, Inc., Piscataway, NJ) by employing a linear gradient of ammonium formate (Fujii et al., 1973). After repeated lyophilization to remove excess formate, the purified Bleo-A_2 was converted to the chloride salt on a column of Dowex 1-X8 (chloride form). Concentrations of Bleo-A_2 were determined by using a molar absorptivity of 1.5 × 10^4 cm^{-1} M^{-1} at 292 nm (Chen et al., 1980).

Methods

Proton Fourier-transform NMR spectra were obtained at 400 MHz on a Bruker WH-400 NMR spectrometer. Each spectrum was the average of 128 or 256 transients (16K data points, 4-kHz sweep width, 1.0-Hz digital filter, 15-μs pulse width, 3.0-s relaxation delay). Samples were contained in 5 mm o.d. NMR tubes (Wilmad Glass Company, Buena, NJ). The temperature was determined to ±1 °C from the separation of ethylene glycol resonances (Van Geet, 1968). All chemical shifts were calculated with respect to the proton resonance of internal $(CH_3)_3SiCD_2CD_2CO_2Na$ (Stohler Isotope Chemicals, Waltham, MA).

The ionic strength studies were carried out in D_2O (99.8 atom %, Aldrich Chemicals, Milwaukee, WI) containing 10 mM sodium phosphate, pH_m 6.8, and sodium chloride concentrations of 0, 40, 90, and 140 mM and 1 M. The pH studies were performed in D_2O containing 90 mM sodium chloride and 10 mM phosphate at pH_m 4.5, 6.8, and 8.0.

Results and Discussion

General Characteristics. The binding of Bleo-A_2 to poly(dA-dT) has been studied by comparing the temperature dependence of the chemical shift of each resolvable proton resonance in the proton NMR spectra of solutions containing the drug and the nucleic acid with the corresponding resonances in spectra of the free drug and the free nucleic acid. Studies were generally carried out on samples with a nucleic acid phosphate to drug ratio (P/D) of 8.5 (see below). The 400-MHz proton NMR spectrum of the Bleo-A_2–poly(dA-dT) complex at 30 °C, in 10 mM sodium phosphate buffer (pH_m 6.8) containing 90 mM NaCl, is shown in Figure 2. Also shown in this figure are the spectra of the complex at 50 and 70 °C, as well as the spectrum of the free drug at 30 °C. Assignments of the resonances for Bleo-A_2 (Chen et al., 1977), poly(dA-dT) (Patel, 1978), and the Bleo-A_2–poly(dA-dT) binary complex (Chen et al., 1980) have been reported previously. The binding appears to be at least moderately fast on the chemical shift time scale since only one resonance is observed in the spectrum of the binary complex for each of the Bleo-A_2 or poly(dA-dT) protons. The broadness of some of the resonances of the complex results in some uncertainty in the measured chemical shifts. In these instances, repeated measurements of the chemical shifts were made on expanded spectra, and the errors were estimated from the reproducibility of these repeated determinations. The error estimates are given with each shift reported in the text.

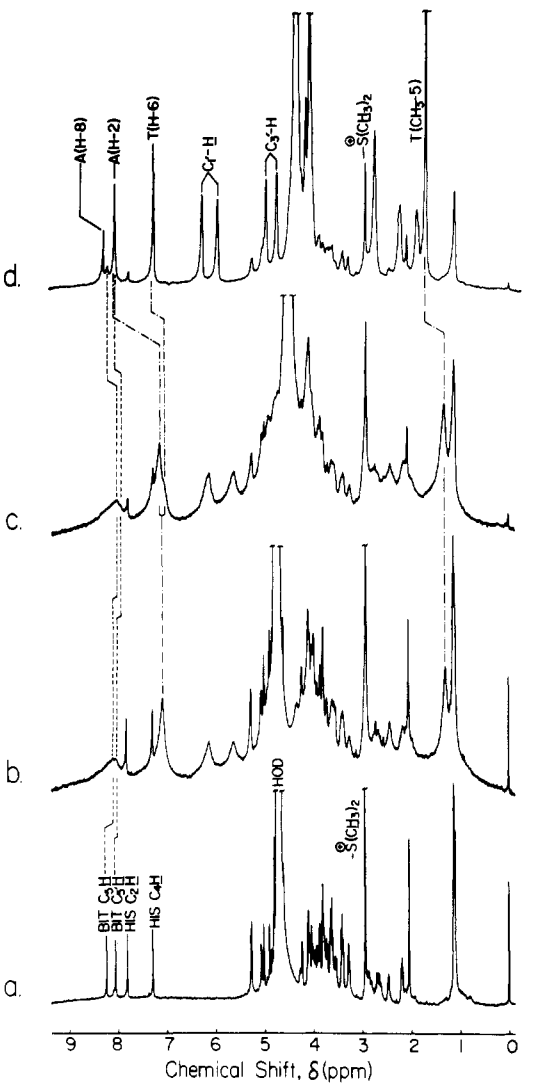

FIGURE 2: Proton NMR spectra at 400 MHz of (a) Bleo-A_2 (1.18 mM) at 25 °C and of Bleo-A_2 (1.18 mM) plus 10 mM poly(dA-dT) at (b) 25, (c) 55, and (d) 75 °C. The buffer is 10 mM sodium phosphate (NaP_i) (pH_m 6.8) and 90 mM NaCl.

The reversibility of all thermally induced perturbations in the spectra of Bleo-A_2, poly(dA-dT), and the binary complex was tested by monitoring the chemical shifts during both

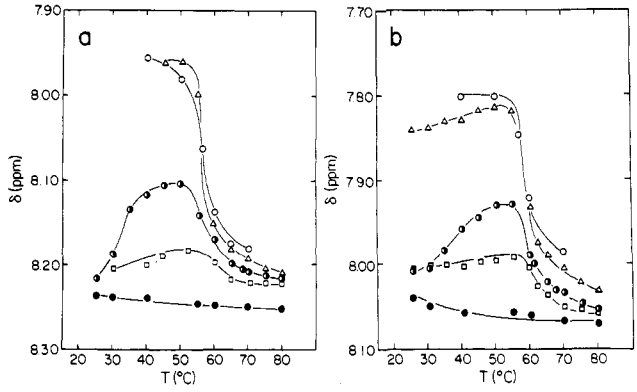

FIGURE 3: Temperature dependence of chemical shifts of (a) BIT C_5H and (b) BIT $C_{5'}H$ resonances of Bleo-A_2 (1.18 mM) in D_2O containing 90 mM NaCl (●) and in the presence of 10 mM poly(dA-dT) in D_2O containing 0 (○), 40 (△), 90 (◑), and 140 mM NaCl (□). All samples contained 10 mM NaP_i (pH_m 6.8).

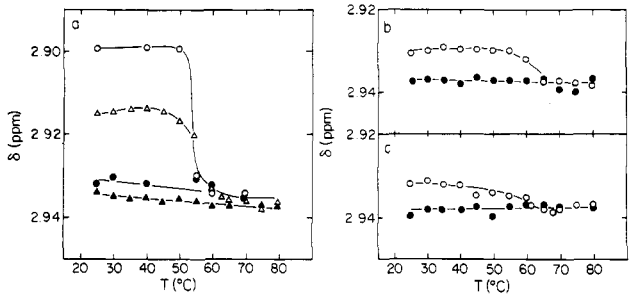

FIGURE 4: Temperature dependence of chemical shift of $S(CH_3)_2$ resonance of Bleo-A_2 (1.18 mM) in the absence (closed symbols) and presence (open symbols) of 10 mM poly(dA-dT) in (a) 0 (○, ●) and 40 mM NaCl (△, ▲), (b) 90 mM NaCl, and (c) 140 mM NaCl. All samples contained 10 mM NaP_i (pH_m 6.8).

heating and cooling cycles. No hysteresis effects were observed in any of the experiments. Temperature profiles for the BIT C_5H and $C_{5'}H$, the $S(CH_3)_2$, the CH_2S, and the BIT $C^{\beta}H_2$ resonances in the presence and in the absence of poly(dA-dT) are shown in Figures 3–6. As reported previously (Chen et al., 1980), the involvement of the bithiazole moiety in complex formation is indicated by high-field shifts and preferential line broadening of the BIT C_5H and $C_{5'}H$ resonances. The maximum chemical shift perturbations of these resonances occur at approximately 55 °C, just below the thermal denaturation temperature of poly(dA-dT) (T_m = 60 ± 1 °C), and are of the order of 0.15–0.30 ppm (±0.025 ppm), depending on the salt concentration (see below). Interestingly, both the C_5H and the $C_{5'}H$ resonances show virtually identical complexation shifts under each condition studied. Lesser perturbations are observed at both lower and higher temperatures. The high-field shift of these resonances is consistent with the placement of those hydrogens in positions where they experience the ring-current effects emanating from the nucleic acid base pairs. Lin & Grollman (1981) have observed shifts of similar magnitude in studies of a bleomycin fragment binding to deoxydinucleotides. In this case also, the shifts were attributed to partial intercalation of the bithiazole system in the base-pair region. The observation that the two thiazole aromatic hydrogens experience the same shift was noted previously by this laboratory (Chen et al., 1980) and more recently by Lin & Grollman (1981) in their bleomycin fragment–deoxydinucleotide system. It is suggestive of a mode of binding in which the two thiazole hyrogens are pointed in the same direction. Lin & Grollman (1981) have used extensive modeling studies to proposal a structure for the bleomycin–nucleotide complex in which the ligand binds from the major groove of

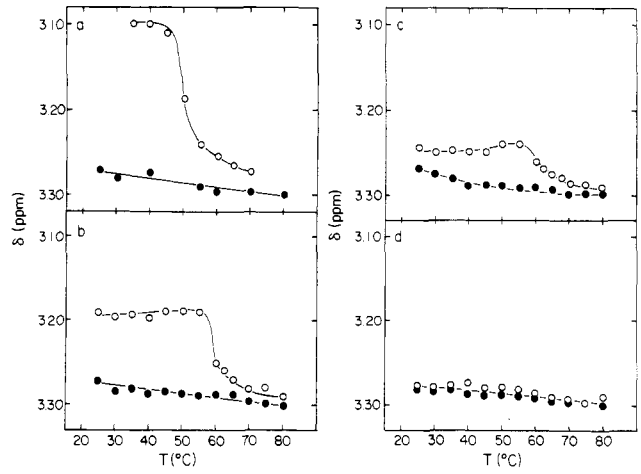

FIGURE 5: Temperature dependence of chemical shift of BIT $C^{\beta}H_2$ resonance of Bleo-A_2 (1.18 mM) in the presence (○) and in the absence (●) of 10 mM poly(dA-dT). The buffer is D_2O containing 10 mM NaP_i (pH_m 6.8) and (a) 0, (b) 40, (c) 90, and (d) 140 mM NaCl.

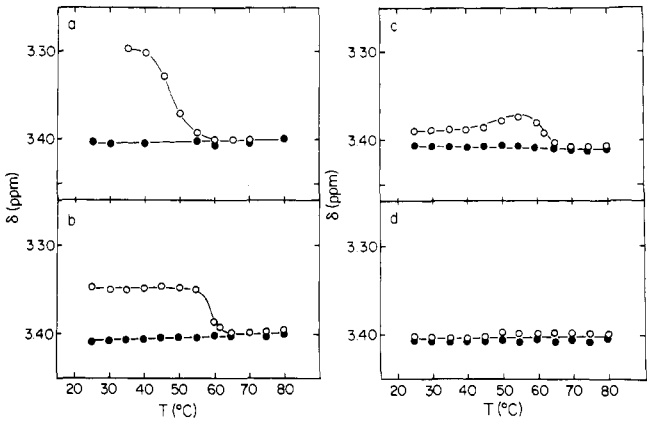

FIGURE 6: Temperature dependence of chemical shift of CH_2S resonance of Bleo-A_2 (1.18 mM) in the presence (○) and absence (●) of 10 mM poly(dA-dT). The conditions and panels correspond to those in Figure 5.

the nucleic acid with the bithiazole system partially intercalated in such a way that the two aromatic hydrogens point toward the minor groove. Possible models for the bleomycin–poly(dA-dT) complex are discussed below.

The decrease in the high-field shifts of the aromatic resonances at higher temperatures parallels the thermal denaturation of the nucleic acid, suggesting a preferential association of the drug with the double-stranded form of the nucleic acid. Other resonances of the antibiotic that exhibit perturbations in the presence of the nucleic acid include BIT $C^{\alpha}H_2$, CH_2-CH_2S, $CH_2CH_2CH_2S$, THR $C^{\alpha}H$, and VAL $C^{\alpha}H$. No other resolved drug resonances exhibit significant perturbations with the polynucleotide. These observations clearly show that the binding of Bleo-A_2 to poly(dA-dT) is localized to the cationic terminal dipeptide. While the BIT C_5H and $C_{5'}H$ exhibit anomalously smaller high-field shifts at lower temperatures (Figure 3), the other resonances originating from the cationic terminus do exhibit maximal perturbations of approximately 0.1 ppm at room temperature, and these perturbations persist with increasing temperature until the melting temperature of the polynucleotide is reached. Additionally, the BIT C_5H and $C_{5'}H$ resonances show preferential line broadening below the melting temperature of the polynucleotide. This broadening is especially pronounced at ambient temperature (see Figure 2b), indicating that the drug is bound to the nucleic acid under these conditions even though the bithiazole moiety is not experiencing its maximal chemical shift perturbations.

Previous studies on the binding of the acetyl derivative of the terminal dipeptide and other Bleo-A_2 analogues to poly(dA-dT) (Sakai et al., 1981, 1982) show the persistence of the high-field shifts of the BIT C_5H and $C_{5'}H$ resonances at temperatures at which the corresponding resonances of Bleo-A_2 have exhibited smaller high-field shifts. This suggests that in Bleo-A_2 the anomalous reduction in high-field shifts of these resonances may result from steric interactions between the nucleic acid and residues closer to the N terminus of the drug than is the BIT residue. Thus, it seems possible that the bithiazole system is forced from between the base pairs of poly(dA-dT) at low temperatures, perhaps by the bulky end of the Bleo-A_2 molecule, while the dipeptide and other analogues that lack bulky substituents are less sensitive to the compactness or flexibility of the nucleic acid helix.

The possibility has also been considered that the anomalous temperature dependence of the BIT aromatic resonances results from an equilibrium between intercalation (favored at temperatures just below the helix–coil transition) and external binding in a groove of the nucleic acid (favored at lower temperatures). A number of observations argue against this explanation: First, the persistence of most of the spectral perturbations (except of the BIT C_5H and $C_{5'}H$ resonances) at low temperatures suggests a highly localized conformational change rather than the more extreme change suggested by this model. Second, the various analogues of the bithiazole-containing end of Bleo-A_2 all exhibit maximal shifts of their BIT C_5H and $C_{5'}H$ resonances of about 0.3 ppm (i.e., very similar to the corresponding resonances of Bleo-A_2) (Sakai et al., 1982). This is true both for derivatives that exhibit the anomalous reductions in the high-field shift at low temperature and for those that do not. If the latter shift were the result of an increase in the mole fraction of externally bound species, then a larger maximal high-field shift would have been expected for derivatives not exhibiting this reduction in shift (since these derivatives would presumably have a higher mole fraction of the intercalated complex).

Several poly(dA-dT) resonances show chemical shift perturbations upon binding Bleo-A_2. The temperature dependence of the chemical shift of the A H-2 resonance under different ionic strength conditions is shown in Figure 7. This resonance exhibits a low-field shift in the presence of Bleo-A_2, indicating that the binding of the drug molecule causes the adenine ring to experience smaller ring-current effects than those encountered in the absence of the drug. This effect is generally attributed to intercalation by the drug, a process that serves to force apart the base pairs or to unwind the nucleic acid helix. The degree to which the nucleic acid conformation is altered by Bleo-A_2 cannot be determined from the magnitude of the chemical shift perturbation of the A H-2 resonance since several different factors may influence the chemical shift observed for this resonance. For example, the expected low-field shift of the A H-2 resonance resulting from helical unwinding may be partially offset by a high-field shift due to ring currents emanating from the bithiazole system. Conversely, the ring-current contributions from the intercalating system may not be sufficient to have an effect on the chemical shift of the A H-2 resonance. The low-field shifts (~0.1 ppm) of the A H-2 resonance induced by Bleo-A_2 persist as long as poly(dA-dT) is in the helical form and are lost as the nucleic acid undergoes the helix–coil transition, again indicating that the drug binds only to the double-stranded polynucleotide.

Other poly(dA-dT) resonances such as those of the deoxyribose H-3′, H-2′, and H-2″ also exhibit perturbations as a result of the binding of Bleo-A_2. Generally these shifts are

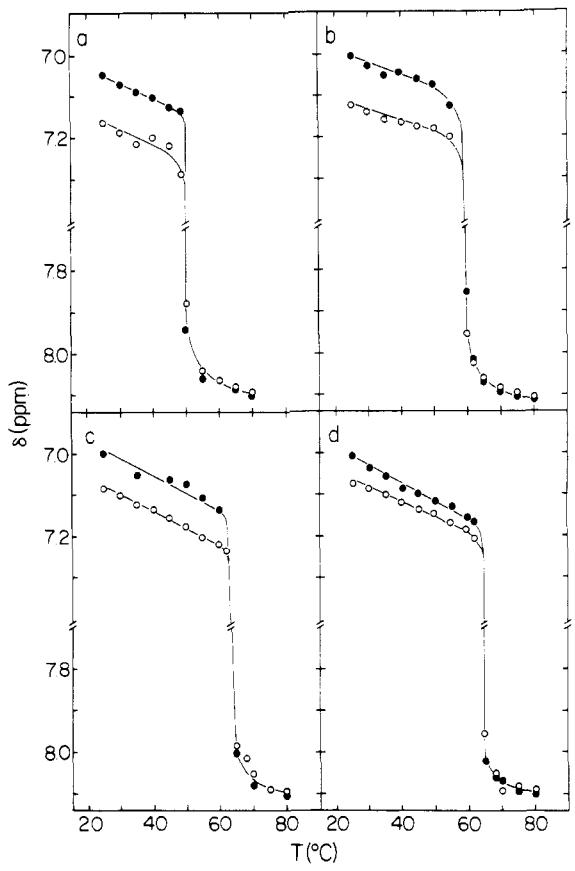

FIGURE 7: Temperature dependence of chemical shift of adenine H-2 resonance of poly(dA-dT) (10 mM) in the presence (○) and absence (●) of 1.18 mM Bleo-A_2. The conditions of each panel correspond to those in Figure 5.

small (≤0.08 ppm) and to high field and are observed only when the nucleic acid is in the helical form. Ambiguity about the assignment of these and other resonances specifically to A or T residues makes its difficult to obtain more information about likely sites of interaction on the polynucleotide or to draw definite conclusions regarding any possible changes in the pucker of the deoxyribose rings.

As indicated above, Lin & Grollman (1981) have proposed a model of the interaction between a bleomycin fragment and self-complementary deoxydinucleotides in which binding occurs from the major groove side. The data presented here show only small changes in the T H-6 resonance while the T CH_3-5 resonance exhibits no perturbations in the presence of the antibiotic. These data suggest minor groove binding since the thymine methyl groove extends into the major groove; however, they do not exclude major groove binding as a possibility.

P/D Titrations. The chemical shifts of the BIT C_5H and BIT $C_{5'}H$ resonances were monitored at 50 °C as the P/D was varied at fixed concentrations of poly(dA-dT). Titration curves for the C_5H resonance obtained at poly(dA-dT) concentrations of 10 and 20 mM and also in buffers containing 40 and 90 mM NaCl are shown in Figure 8. The largest high-field shift of 0.31 ppm (±0.025 ppm) is obtained in the low-salt buffer. A limiting complexation shift of 0.34 ppm is obtained when the chemical shifts of this hydrogen in both the free and complexed forms are extrapolated to infinite dilution. The $C_{5'}H$ resonance showed a similar titration curve (data not shown) and a similar maximum high-field shift. The magnitude of the chemical shift perturbation at any given P/D value is dependent on the concentration of the nucleic acid, with higher polynucleotide concentrations giving rise to the larger maximum displacement. This is an indirect ionic

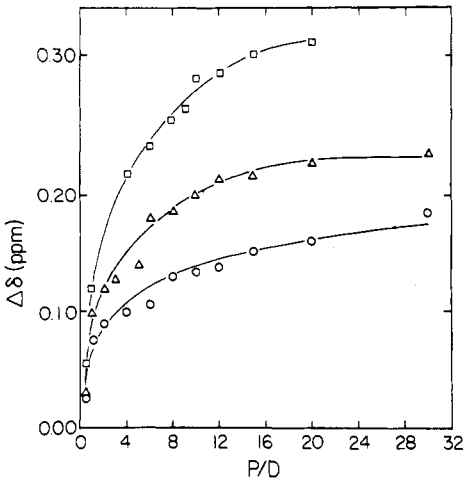

FIGURE 8: Dependence of chemical shift of BIT C_5H resonance of Bleo-A_2 on molar ratio of poly(dA-dT) phosphates to Bleo-A_2 (P/D) at fixed poly(dA-dT) concentrations of 10 (○, □) and 20 mM (△). The values of $\Delta\delta$ were measured at 50 °C. The buffer is 10 mM NaP_i (pH_m 6.8) and 90 (○, △) and 40 mM NaCl (□).

strength effect resulting from binding of sodium ions to the polynucleotide. Exhaustive dialysis of poly(dA-dT) during the purification process results in replacement of some of the sodium counterions by protons; solution in the saline buffer results in reabsorption of these metal ions and a decrease in the ionic strength of the buffer. For this reason, all other experiments involving poly(dA-dT) were conducted at a fixed poly(dA-dT) concentration of 10 mM in nucleotide residues and at a verified pH. The high Bleo-A_2 concentrations used in the measurments at low P/D contributed significantly to the bulk ionic strength of the medium, making it difficult to obtain useful binding data in this region of the titration curve. However, it is apparent from the titration curves that the drug is largely bound for P/D ≥6–8 since there is only a small response of the chemical shifts of either the drug or the nucleic acid residues to any further increases in the P/D. To ensure essentially total association of the drug with the nucleic acid, all experiments were run at a P/D value of 8.5.

Ionic Strength Effects. The binding of Bleo-A_2 to poly(dA-dT) was monitored at sodium chloride concentrations of 0, 40, 90, and 140 mM and 1 M with fixed concentrations of polynucleotide (10 mM) and phosphate buffer (10 mM, pH_m 6.8). The high-field shifts for both bithiazole resonances increase in magnitude with decreasing NaCl concentration to a maximum of approximately 0.3 ppm in the absence of sodium chloride (Figure 3). In 140 mM salt, the maximum shift is only about 0.1 ppm, while no chemical shift perturbations are observed in 1 M NaCl. The increased high-field shift of the bithiazole resonances at low ionic strength reflects the increased influence of ring currents emanating from the bases of the nucleic acid. As the ionic strength of the medium is lowered, the polynucleotide experiences increasing electrostatic repulsion between phosphate groups, leading to some unwinding of the helix or formation of local fluctuations in structure as has been described by Gabbay (1977) and by Sobell et al. (1977). This increases the distance between base pairs and facilitates a greater extent of insertion of the bithiazole moiety. The general loss of the high-field shifts at higher ionic strengths indicates that the binding process probably involves ionic interactions—electrostatic attraction between the cationic sulfonium groups and the anionic phosphate groups in the nucleic acid backbone. This interaction is reflected in the displacement and broadening of the $S(CH_3)_2$ resonance, which exhibits its largest change in chemical shift (approximately 0.04 ppm) at the lower salt concentration and minimal perturbations at the higher salt concentration. At low ionic strength, resonances are displaced to high field, probably due to increased electronic shielding of the $S(CH_3)_2$ hydrogens. Other resonances that exhibit ionic strength dependent chemical shift perturbations are CH_2S, CH_2CH_2S, $CH_2CH_2CH_2S$, BIT $C^{\alpha}H_2$, BIT $C^{\beta}H_2$, THR $C^{\alpha}H$, and VAL $C^{\alpha}H$, all of which are located at or near the cationic terminus of Bleo-A_2. The THR $C^{\alpha}H$ is the only drug resonance that exhibits a downfield shift (~0.04 ppm) upon complexation with the nucleic acid, suggesting that this residue is not located between the base pairs.

It is likely that the partial intercalative binding exhibited by the bithiazole rings and the ionic binding of the sulfonium group are interrelated since both interactions are lost as the poly(dA-dT) is melted. Since ionic interactions are expected to be more rapid than the conformational changes associated with intercalation, ionic binding probably precedes or coincides with intercalation. Binding of the dimethylsulfonium group to the polynucleotide backbone may position the bithiazole system near the base pairs so that intercalation can occur. Whether maintenance of the ionic interaction is necessary for the stabilization of the complex is not known; however, high concentrations of sodium chloride (1 M) completely prevent the upfield shifts normally associated with Bleo-A_2 complexation (not shown), indicating that Bleo-A_2 does not intercalate in the same sense that ethidium bromide intercalates in nucleic acids. The intercalative binding of the latter is enhanced by high salt.

Several poly(dA-dT) resonances also show ionic strength dependent chemical shift perturbations. The largest such effect is exhibited by the A H-2 resonance (Figure 7). The magnitude of the downfield shift of the A H-2 resonance upon complexation increases as the ionic strength decreases, indicating greater unwinding or separation of the bases concomitant with more extensive insertion of the bithiazole moiety of Bleo-A_2. Other affected poly(dA-dT) resonances include H-1′, H-2′, H-2″, and H-3′. These resonances show Bleo-A_2-induced perturbations only in the 0 and 50 mM NaCl samples, indicating that the drug is unable to substantially distort the geometry of the nucleic acid in the presence of the higher salt concentrations.

pH Effects. In addition to the studies at pH_m 6.8, the binding of Bleo-A_2 to poly(dA-dT) was monitored at pH_m values of 4.5 and 8.0. In this pH range, the imidazole group [$pK_a(D_2O)$ 5.29] and the primary amino group of the β-aminoalanyl moiety [$pK_a(D_2O)$ 8.23] are titrated (Mooberry et al., 1980). Over this pH range, Bleo-A_2 is not degraded, and poly(dA-dT) maintains its double-helical conformation.

The temperature profiles for the BIT C_5H resonance at each pH are presented in Figure 9. The chemical shift perturbations observed at pH_m 4.5 and 6.8 are roughly comparable; however, the perturbations exhibited by this resonance at pH_m 8.0 are considerably smaller. Furthermore, the resonances of hydrogens adjacent to the sulfonium group (CH_2S and CH_2CH_2S) exhibited diminished perturbations at pH_m 8.0 relative to displacements observed at pH_m 6.8; greater shifts for these resonances occurred at pH_m 4.5, suggesting a diminution of the ionic interaction between the cationic terminus of the drug and the nucleic acid phosphates at pH_m 8.0.

The temperature profiles of the resonances of the ALA CH_2, PYR CH_3, and HIS imidazole C_2H and C_4H hydrogens, which are sensitive to titrations of the nearby ionizable groups, show that these resonances are not perturbed by the binding to poly(dA-dT) at any pH examined. Similarly, no significant

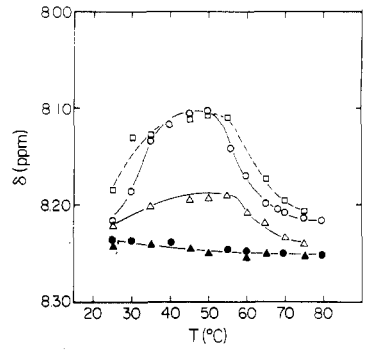

FIGURE 9: Temperature dependence of chemical shift of BIT C_5H resonance of Bleo-A_2 (1.18 mM) in D_2O containing 10 mM NaP_i and 90 mM NaCl at pH_m 6.8 (●) and 8.0 (▲) and in the presence of 10 mM poly(dA-dT) at pH_m 4.5 (□), 6.8 (○), and 8.0 (△).

shifts were observed for other groups proximal to the N terminus of the drug upon nucleic acid binding. There is no evidence for an ionic interaction between poly(dA-dT) and the protonated primary amino group of ALA. Unfortunately, the resonance of the ALA methine hydrogen, which is adjacent to the primary amino group, overlapped with the $S(CH_3)_2$ resonance in spectra of the drug–nucleic acid complex and could not be monitored. Thus, the reduction of the high-field shifts for the BIT C_5H and $C_{5'}H$ resonances at pH_m 8.0 is apparently due to either a decreased extent of ionic binding between the drug and polynucleotide or to a conformational change in the bound drug molecule.

Conclusions

The interaction of Bleo-A_2 with poly(dA-dT) appears to involve two different processes: the partial intercalation of the planar bithiazole system between the base pairs of the polynucleotide and an electrostatic interaction between the dimethylsulfonium cationic terminus of the drug and the anionic phosphate backbone of the nucleic acid. Intrinsic viscosity experiments (Chien et al., 1977), as well as the small magnitude of the chemical shift perturbation observed for the bithiazole protons (Chen et al., 1980), argue against a full or classical intercalation of the bithiazole moiety of Bleo-A_2. However, the significant broadening and chemical shift variation of the bithiazole and dimethylsulfonium resonances in the complex clearly indicate the binding of these moieties to the helical form of the nucleic acid.

The positioning of the bithiazole system for partial intercalation presumably involves an initial electrostatic interaction between the positively charged sulfonium group and the negative phosphate groups of poly(dA-dT). This interaction restricts the placement of the bithiazole moiety relative to the bases by requiring that the dimethylsulfonium group remain closely associated with the nucleic acid phosphate backbone. The steric bulk of the side chains attached to either end of the bithiazole ring system further limits the possible orientations of the drug relative to the nucleic acid. Despite these constraints, the placement of the bithiazole system cannot be uniquely determined.

Possible orientations of the bithiazole moiety relative to the base pairs of poly(dA-dT) have been examined by fitting the observed bithiazole chemical shift perturbations to the theoretical isoshielding contours described by Giessner-Prettre & Pullman (1976a,b). These contours, which show the spatial dependence of induced high-field shifts due to ring-current and atomic-anisotropy terms, allow the prediction of chemical shift displacements of protons located between nucleic acid base pairs. The predicted displacements for several different base-pair overlap geometries have been examined, including (i) an intercalation site as described by Berman et al. (1978), (ii) an "alternating B" geometry proposed for poly(dA-dT) by Klug et al. (1979), (iii) β-kinked sites postulated by Sobell et al. (1977), and (iv) the intercalation model of Lin & Grollman (1981) for the binding of a bleomycin fragment. In no case does a full intercalative mechanism give good agreement between experimental and predicted displacements; however, reasonable agreement has been found if the bithiazole moiety is only partially inserted between the bases. Unfortunately, it is evident that there is no unique geometry that can be determined for the complex in the region of the bithiazole group. For example, numerous orientations can be found that place the two aromatic hydrogens either on the same side (syn) or on opposite sides (anti) of the 2,4′ bond joining the two rings and that give rise to theoretical shifts similar to those observed experimentally. The problem is compounded by the small magnitude of the observed complexation shifts that are of the order of the uncertainty in ring-current calculations.

In addition to nearest-neighbor effects, next nearest neighbor effects may contribute to the observed shifts. Furthermore, the positioning of the bithiazole ring at axial distances other than 3.4 Å from the plane of the base pair or in a nonparallel orientation with respect to the base pair may also give the observed shielding effects. These considerations indicate that it is premature to ascribe a specific geometry to the Bleo-A_2–poly(dA-dT) complex.

The temperature dependence of binding shows that the maximum interaction between Bleo-A_2 and poly(dA-dT) occurs at temperatures at which "kinks" have been postulated to form in the polynucleotide (Sobell et al., 1977; Gabbay, 1977). It is tempting to ascribe the observed binding characteristics of Bleo-A_2 to an interaction with such a nucleic acid form. Stabilization of the helical state at lower temperatures decreases the probability of forming such kinked sites and makes intercalation of the bithiazole group less likely. The binding of Bleo-A_2 to such a nucleic acid structure could explain the lack of significant increase in the intrinsic viscosity of DNA upon binding of Bleo-A_2 (Chien et al., 1977) since the molecular rod length of β-kinked DNA is very close to that of native B DNA (3.5 Å vs. 3.4 Å axial length per base pair) (Sobell et al., 1977).

The absence of any significant effects upon titration of the hydroxyhistidine imidazole group or the primary amino group of the aminoalanine amide moiety indicates that these groups are probably not involved in the binding process. The data presented here fully support the idea that the association of Bleo-A_2 with poly(dA-dT) is restricted to the C terminal region of the antibiotic. Further experiments are in progress to help define more precisely the nature of the Bleo-A_2–poly(dA-dT) complex.

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Poly(8-methyladenylic acid): A Single-Stranded Regular Structure with Alternating Syn–Anti Conformations†

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ABSTRACT: Poly(8-methyladenylic acid) has been prepared by chemical synthesis of 8-methyladenosine 5′-diphosphate and enzymatic polymerization with polynucleotide phosphorylase. The polymer exhibits a large hypochromism and cooperative melting in neutral solution. The transition temperature is independent of salt concentration at moderate ionic strength and decreases slightly at high salt. The adenine ring vibration at 1626 cm^{-1} is independent of temperature. A high-resolution nuclear magnetic resonance spectrum is observed near the bottom of the melting range. The chemical shift of the H2 proton exhibits a large upfield shift in the ordered form, and the temperature profile of H2 is cooperative and congruent with the UV melting curve. The CH_3 proton signal, in striking contrast to H2, is independent of temperature. These results support a regular, single-stranded helix in the ordered form, in contrast to both poly(adenylic acid) and poly(8-bromoadenylic acid). We suggest that the contrasting temperature dependence of the H2 and CH_3 proton signals can be accounted for by regularly alternating syn and anti conformations of the 8-methyladenylic acid residues.

One of the most extensively investigated aspects of polynucleotide conformation in recent years has been that of syn and anti isomers, which result from torsion about the glycosidic bond (Donohue & Trueblood, 1960; Sundaralingam, 1969; Lakschminarayanan & Sasisekharan, 1969; Haschemeyer & Rich, 1967). In the naturally occurring purine nucleotides, the rotational energy barrier is relatively low. Both syn and anti conformations are encountered in crystals, though with the latter clearly predominating. One fruitful approach to the study of the less common syn conformation has been the synthesis of purine monomers substituted at the 8-position with groups sufficiently bulky to restrict the anti range of conformation [see, for example, Ikehara et al. (1969), Michelson et al. (1970), Tavale & Sobell (1970), and Howard et al. (1974)]. This method has been extended to polymers, providing the first examples of syn polynucleotides. Thus, for example, in the homopolymer poly(8-bromoadenylic acid) [poly(8brA)] the Br substituent causes all residues to adopt a syn conformation and to have properties radically different from the parent poly(adenylic acid) [poly(A)] [cf. Howard et al. (1974, 1975) and Govil et al. (1981)]. Poly(8brA) forms a hydrogen-bonded double helix of high stability rather than the nonregular, partially stacked single-stranded structure possessed by poly(A) [cf. Leng & Felsenfeld (1966), Applequist & Damle (1966), Brahms et al. (1966), Holcomb & Tinoco (1965), and Eisenberg & Felsenfeld (1967)]. In this report, we are concerned with an electronically less perturbing 8-substituent of about the same size as Br, namely, the methyl group. A series of dinucleoside monophosphates investigated by Ikehara and co-workers (Uesugi et al., 1978; Ikehara et al., 1978) indicated that the 8-methyladenylic acid (8-meA) residues could adopt a syn conformation in some of the dimers and an anti structure in others, as well as a syn–anti structure in the same molecule. It is likely that the dimers in solution are present as mixtures of more than one conformation. We find in the present study that poly(8meA) forms a regular, single-stranded helix, the

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