Volume 13 Number 18 1985 Nucleic Acids Research

Is the bithiazole moiety of bleomycin a classical intercalator?

Jean-Pierre Hénichart, Jean-Luc Bernier, Nicole Helbecque[1] and Raymond Houssin[2]

INSERM U 16, Place de Verdun, [1]Département de Biochimie, Faculté de Médecine, Place de Verdun, and [2]Laboratoire de Chimie de Synthèse des Médicaments, Faculté de Pharmacie, rue Laguesse, 59045 Lille, France

Received 8 July 1985; Revised and Accepted 6 September 1985

ABSTRACT

Bleomycin is a widespread anticancerous drug, the biological activity of which having been extensively studied. Its metal ion-chelating portion has been shown to cleave DNA whereas the role of the bithiazole moiety is still questionable. In order to elucidate this problem some 2',4-disubstituted bithiazoles structurally related to the "tripeptide S" moiety of bleomycin were synthesized and their interaction with DNA was studied using ΔTm, fluorescence, EPR and viscometry techniques. The results of ΔTm and fluorescence quenching determinations were in favour of a binding of the bithiazole part by an intercalation process. Nevertheless, the use of the spin-label probes indicated only a partial intercalation of the ring between the base-pairs. Moreover, viscometry data which clearly exhibited a slight decrease of DNA length in the presence of bithiazole derivative led to the proposal of a binding model involving a partial insertion of a thiazole ring which wedges in between the bases at a bending point of DNA.

INTRODUCTION

Bleomycin (Blm) is an antitumour antibiotic drug employed for the treatment of lymphomas, squamous cell carcinomas and testicular carcinomas (1). In fact, it is the generic name of closely related natural glycopeptides which differ only in the substituent on their heterocyclic unit (2,3). The biological activity of bleomycin involves two well defined parts of the molecule. A metal ion-chelating portion is able to form with iron (II) and molecular oxygen an active complex which generates free radicals responsible for the cleavage of DNA. On the other hand, a bithiazole containing moiety contributes to the binding of bleomycin to DNA.

DNA-binding and DNA-cleaving are believed to be the two essential steps of the cytotoxic process (4). The metal-binding properties of bleomycin and the role of the iron(II)-bleomycin-oxygen complex in the initiation of the chain of reactions leading to single and double-strand DNA scissions is well documented (5-10) but the exact involvement of the bithiazole group in bleomycin action on DNA has yet to be established. Whether the bithiazole intercalates between DNA bases or simply binds in the minor groove is

questionable. The coplanarity of the bithiazole rings (11) and their parallelism to the aromatic rings of DNA bases (12) are in favour of an intercalation process such as already suggested by Murakami (13) on the basis of model studies.

Binding of bleomycin to DNA has been studied by a variety of techniques including nuclear magnetic resonance (NMR), quenching of bithiazole fluorescence (14), equilibrum dialysis (12,15) and circular dichroism (16).

None of them demonstrate unambiguously the location of bithiazole rings in the DNA helix.

In addition, according to NMR studies of the poly (dA-dT)-bleomycin complex, the non exchangeable bithiazole protons do not exhibit the large upfield shifts seen for protons of classical intercalators (17-19).

Furthermore, if the work of Povirk et al(12) indicates that binding of bleomycin relaxes supercoiled DNA, suggesting that part of the drug intercalates between base pairs, bleomycin is also reported, on the basis of viscosity study (14), to cause an only modest increase in the length of DNA indicating that binding of bleomycin is not intercalative in nature.

This prompted us to reinvestigate the problem and in an effort to obtain more detailed information about the binding of bleomycin, the interaction with calf thymus DNA of some synthetic 2,4-disubstituted bithiazoles structurally related to the "tripeptide S" moiety of bleomycin was undertaken by using electron paramagnetic resonance (EPR) spectroscopy, variation of thermal denaturation of DNA (ΔTm), fluorescence spectroscopy and viscometry techniques.

The bithiazoles are substituted by aminoalkyl chains on 2' and/or 4-positions since this seems to be an important feature involved in the DNA-bleomycin binding process. Moreover the role of the cationic terminus in the formation of electrostatic bindings with the backbone phosphates has been emphasized and was taken in account in the elaboration of our experimental models. In the case where these molecules are used as reporter probes in EPR and fluorescence spectroscopy studies, a nitroxide group or a fluorescent chromophore is linked to the end of different length spacers (Fig. 1).

MATERIALS AND METHODS

Materials

Bleomycin was used as obtained from Roger Bellon laboratories and was a mixture of 60% bleomycin A_2, 30% B_2 and 10% of other bleomycins.

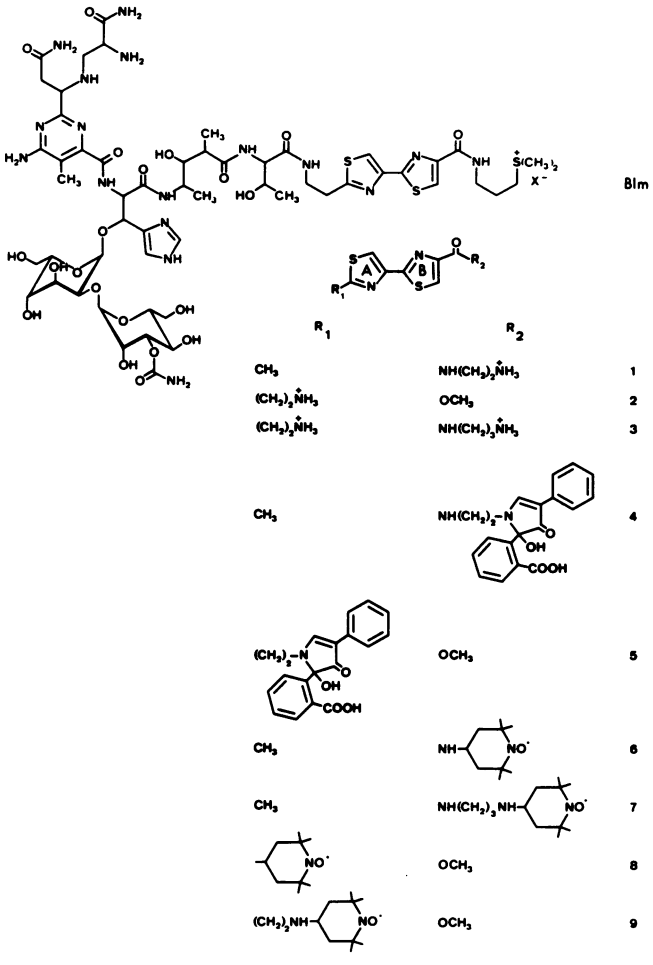

Figure 1 : Structures of Bleomycin A_2 (Blm) and of synthetic bithiazole derivatives (1-9).

Synthetic probes

The synthesis of the bithiazole derivatives used for thermic denaturation and fluorescence studies was previously described (20) and is close to the principle of the preparation of the spin-labeled bithiazole derivatives used in the EPR study (21). The spin-labeled probes bear their nitroxide group either in the vicinity of the bithiazole rings, with the piperidinooxy moiety linked to the heterocycle itself, or at the cationic termini (Fig. 1). Fluorophors used in the fluorescence polarization study

were obtained following the general procedure of Weigele (22) and used without isolation.

DNA solutions

Calf thymus DNA (type I, highly polymerized ; Sigma Chemical Co.) was used throughout the experiments . U.v. and fluorescence measurements were conducted in phosphate buffer, pH 6.9, whereas viscometry experiments were done in 0.01 SHE buffer, (9.4 mM NaCl/2 mM HEPES (N-(2-hydroxyethyl)piperazine-N'-2-ethanesulfonic acid)/10 µM EDTA (ethylenediaminetetraacetic acid) buffer, pH 7.0) as described by Wakelin and Waring (23).

For EPR determinations, DNA (200 mg) was dissolved in 1 liter of 0.1 SSC buffer (0.15 M NaCl/0.015M sodium citrate, pH 7.0).

The concentration expressed in terms of the nucleotide as a unit of DNA was determined spectrophotometrically at 260 nm by using a molar absorption coefficient of 6600 $M.cm^{-1}$ (24).

Methods

Absorption coefficients and "melting" curves were measured by using a Uvikon Kontron 810/820 spectrophotometer coupled to a Uvikon Recorder 21 and a Uvikon Thermoprinter 48. Samples were placed in a thermostatically controlled cell-holder (10 mm path length). The cuvette was heated by circulating water from a Haake unit set. The temperature inside the cuvette was monitored by using a thermocouple in contact with the solution. The absorbance at 260 nm was measured over the range 20-95°C with a heating rate of 1°C/min. The "melting" temperature Tm was taken to be the mid-point of the hyperchromic transition.

Fluorescence spectra were recorded at 20°C on a Jobin-Yvon J-Y-3 spectrophotofluorometer equipped with an X-Y recorder. All measurements were made in a 1 cm light path cuvette in a 0.05 M sodium borate buffer pH 9.0.

Fluorescence polarization studies have been achieved using a Spectrofluopolarimeter SLM 4048. The degree of linear polarization of the fluorophors bithiazole-fluorescamine free or bound to DNA was measured at 20°C. The freshly prepared samples were excited with linearly polarized light at 365 nm. The ratio of the intensities of the emitted vertically and horizontally polarized light was measured after passing a 480 nm cutoff filter. The fluorescence anisotropy factor was calculated using the following equation :

$$r = \frac{I_{//}/I_{\perp} - 1}{I_{//}/I_{\perp} + 2}$$

Helical lengthening measurements were made by using a Ubbelohde semi-micro dilution viscometer. Temperature was maintained at 20 ± 0.01°C in a thermostatically controlled water bath. Flow times were electronically measured to an accuracy of 0.1 s (Schott ABS/G type detector). Calf thymus DNA was sonicated as described by Wakelin and Waring (23). Solutions were filtered through 0.45 µm Millipore filters before measurements. Unwinding studies using closed circular DNA (a generous gift of Dr P. Lemay) were performed essentially as described by Saucier et al (25) and Revet et al (26) on the same apparatus described above. The viscometer contained 2.0 ml of a 150 µM solution of DNA. Drugs were added in increments of 5-10µl from a stock solution (c = 150 µM). Flow times were measured with an accuracy of 0.1 s. Ethidium bromide was used as reference inducing an unwinding angle of 26°, according to Wang (27).

EPR measurements were recorded on a Varian E-109 X-band spectrometer with an E-238 cavity operating in the TM_{110} mode. A 100 KHz high-frequency modulation was used with a 20 mW microwave power. The sample solutions were dispensed into a flat quartz cell. The preparation of a bithiazole-DNA complex in solution was performed by stirring a solution of a spin-labeled bithiazole derivative with a DNA solution in 0.1 SSC buffer at room temperature. The mixture could then be studied directly.

RESULTS

Melting temperature studies

The effects of Blm and analogues 1, 2 and 3 on the Tm of the helix-coil transition are shown in Table 1. First, it can be noted that Blm causes a slight increase in the thermal denaturation temperature of calf thymus DNA, indicating a weak but significant stabilization of the helix.

Previous data on the reaction of bleomycin derivatives with DNA have been reported. Whereas phleomycin increased the Tm of E. Coli DNA (28),

Table 1

Compounds	Blm	1	2	3
ΔTm	+4°	+4.5°	+5°	+7°

Effect of Blm, 1, 2 and 3 (0.5×10^{-4} M) on the Tm of the helix-coil transition of calf thymus DNA (10^{-4} M) in phosphate buffer (pH 6.9). The concentration expressed in terms of the nucleotide as a unit of DNA was determined spectrophotometrically at 260 nm by using a molar absorption coefficient of 6600 $M.\ cm^{-1}$.

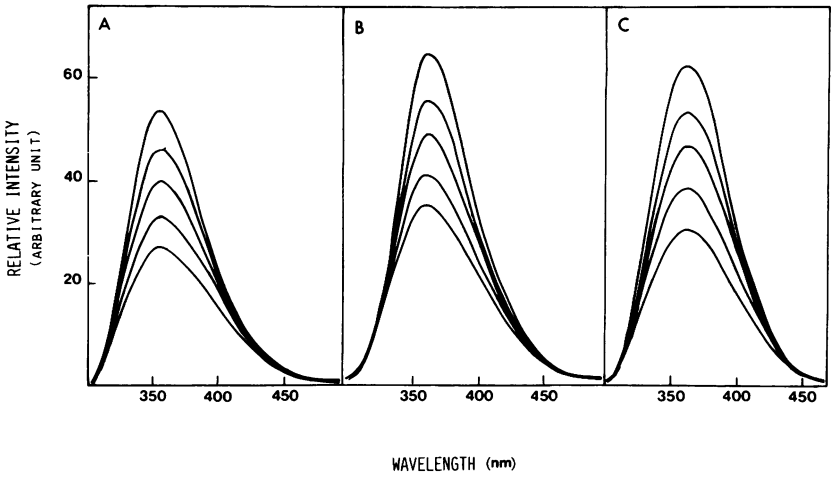

Figure 2 : Fluorescence quenching of Blm (A), 1 (B) and 2 (C) by calf thymus DNA ; with 2.5 x 10^{-5}M Blm, 1 and 2 in 0.05 M sodium borate buffer pH 9.0 ; concentrations of DNA are : 0.05 x 10^{-4}M, 1 x 10^{-4}M, 1.5 x 10^{-4}M, 2 x 10^{-4}M.

bleomycin A_2 in the copper-containing form did not significantly affect the Tm of DNA (29).

The ΔTm values observed for bithiazole derivatives 1 and 2 are comparable to those of Blm, indicating comparable DNA binding strengths.

In addition, it is found that 3 shows a much larger ΔTm and then causes a greater stabilization of the helix than respectively Blm, 1 or 2, suggesting that the presence of an additional positive charge further enhances the stability of the drug-DNA complex.

Fluorescence spectra

For Blm at pH 9.0, excitation and emission maxima lie at 300 and 355 nm respectively. At the same pH, addition of DNA to Blm quenches fluorescence at 355 nm (Fig. 2). Furthermore, there was no shift in maximum emission wavelength.

The fluorescence spectra of 1 and 2 in the presence of calf thymus DNA at different concentrations and pH 9.0 are shown in Fig. 2. The excitation and emission maxima are 297 and 363 nm respectively. By adding increasing concentrations of DNA, the fluorescence of 1 and 2 was quenched analogously to Blm with 50% maximum extinction in both cases.

Polarization fluorescence studies

The fluorescence anisotropy factors obtained for the bithiazole-

Table 2

DNA μM	0	25	50	75	100	125	150	175	200
$r_{\underline{4}}$	0.036	0.036	0.036	0.036	0.038	0.037	0.038	0.037	0.038
$r_{\underline{5}}$	0.021	0.022	0.020	0.021	0.024	0.024	0.023	0.022	0.020

Effect of increasing concentrations of calf thymus DNA on fluorescence anisotropy of adducts 4 and 5 (2.5×10^{-5} M) in phosphate buffer (pH 6.9) at 20°C.

fluorescamine adducts, 4 and 5 issued from 1 and 2, at 20°C, pH 9.0 and in the presence of increasing concentration of DNA are listed in Table 2.

Although direct intensity measurements were expected to vary as a result of a binding of the bithiazole to DNA, the polarization values remained constant over a wide range of concentrations.

These results indicate that on the adducts 4 and 5 the fluorophor is positioned so that the interaction of 1 and 2 with DNA is impeded.

Viscometry

This is a technique of choice to appreciate helical lengthening and DNA unwinding and therefore to study the intercalative properties of drugs in DNA. A drug is commonly admitted as an intercalator if (i) it produces a helix extension corresponding to 3.4 Å per drug chromophore ; (ii) the chromophore is aligned parallel to these pairs ; or (iii) DNA unwinding is produced (30).

Experiments designed to measure the helix extension produced by binding of bithiazole 1 and 2 were performed essentially by the method of Cohen and Eisenberg (31,32). The data were transformed directly from flow times to values for the relative contour length using the expression

$$\frac{L}{L_o} = \left[\frac{t_c - t_o}{t_D - t_o}\right]^{1/3}$$

where L is the contour length in the presence of drug, L_o is the contour length of free DNA, t_c is the flow time for the complex, t_D is the flow time for pure DNA, and t_o is the flow time for buffer at a given total volume. This expression derives directly from the theory of Cohen and Eisenberg (31,32) with the added assumption that the intrinsic viscosity approximates to the reduced viscosity for the complexes. Results obtained with 1 and 2

showed a small length decrease on adding each of these drugs to sonicated DNA at pH 7.0. In order to see if no electrostatic interactions between the positively charged bithiazole moieties and DNA occur, similar measurements were done at pH 9.25 leading to the same observation as precedently. Such results are to be compared with previous studies about binding of dipeptide amides to DNA (33) where the observed shortening of the DNA helix was interpreted as reflecting partial intercalation.

Unwinding of base pairs at and around the binding site is a necessary consequence of intercalation. The degree of angular unwinding, measured by the reversal of negative supercoiling in a covalently closed circular double-stranded DNA, is conventionally calculated relative to the 26° value for ethidium. The determination of unwinding angles using natural closed-circular DNA means that the values are averaged over all the possible sites. As intercalating drugs are added to superhelical DNA, the double helix is unwound, and initially the natural right-handed superhelical turns are removed until the DNA has no remaining superhelical structure. At this point it is hydrodynamically equivalent to nicked circular DNA. If more intercalating ligand is added, the double helix continues to unwind with the formation of a left-handed superhelical structure. The maximum in a viscometric titration corresponds to closed circular DNA with no remaining superhelical turns. Unwinding angles values of 16° and 17° $\pm$ 1° were found for 1 and 2 respectively. These values are smaller than that of most intercalators. However they are higher than those measured for bleomycin (about 12° at pH 5.5) and tripeptide S (12° at pH 8.0) (12) and can be thought to reflect partial intercalation of the bithiazole moiety in both cases.

EPR Spin-label studies

The usefulness of the spin-label technique to obtain information about small molecule-macromolecule interactions has been demonstrated in various cases (34). When a stable paramagnetic nitroxide is chemically attached to a molecule, its EPR spectrum is sensitive to the polarity of the environnement and to the type of motion. The spin-label method has been shown to be a very efficient tool for providing evidence of physical binding of drugs to DNA. Particularly, the intercalation of planar drugs in DNA has been studied (35) and determination of affinity parameters has been established (36-38) for 9-amino acridine derivatives. The introduction of a bulky moiety such as 2,2,6,6-tetramethyl-1-piperidinyloxy or "TEMPO" into the 9-amino group did not abolish the ability of the drug to intercalate into DNA. In dilute

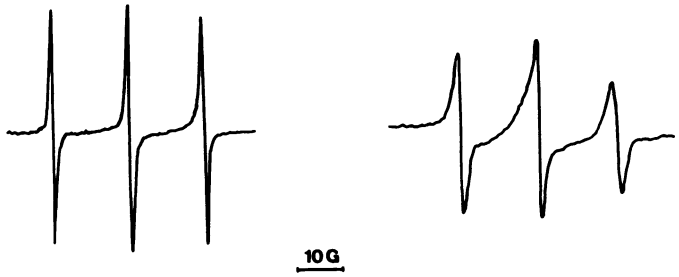

Figure 3 : EPR spectra of 6, 7, 9 (left) and 8 (right) 0.5 X 10^{-4} M in the presence of calf thymus DNA (200mg/l) in 0.1 SSC buffer (0.15 M NaCl, 0.015 M sodium citrate : SSC), pH 7.0, at 20°C.

aqueous solution, the EPR spectrum of 9-amino-TEMPO-acridine consists of three sharp lines which result from the anisotropic hyperfine interaction between the unpaired electron and the nuclear spin of the nitrogen atom. In the presence of calf thymus DNA, the EPR spectrum of the spin-label becomes broad and asymetric, characteristic of a high degree of immobilization of the nitroxide. To establish whether the bithiazole ring of bleomycin could bind to DNA in a manner similar to that of 9-amino acridine, the TEMPO substituent was chemically introduced on the ring. Moreover, varying the distance through aliphatic chains between the nitroxide and the bithiazole ring allowed the depth of the site to be outlined. The EPR spectra of spin-label 6, 7, 8, and 9 in aqueous solution and in the absence of DNA were very similar. They exhibited the classical three sharp lines with a splitting of 17 G between adjacent lines. In the presence of DNA, the EPR spectra of 6, 7 and 9 did not show any modification. Only the spectrum of 8 was different and exhibited a shortening of the third band as well as a broadening of the three bands (Fig. 3). These results demonstrate that when 8 binds to DNA, the motion of the nitroxide is restricted but is not as fully immobilized as the TEMPO moiety of the intercalated amino acridine.

DISCUSSION

Data obtained from various physico-chemical studies have been cited as evidence for an interaction of the bithiazole moiety of bleomycin to DNA, but the exact nature of this binding is poorly understood.

It has been claimed, on the basis of NMR studies, that the bithiazole ring could intercalate in the nucleic base pairs. Clearly the bithiazole aromatic CH resonances preferentially broadened in the presence of DNA

(14) or poly (dA-dT) (17). Furthermore, in the presence of poly (dA-dT), the aromatic resonances showed a moderate high field shift (17,19). This NMR assay alone does not provide sufficient evidence to conclude that a complete or a partial intercalation could occur.

The high-field shift of C_5H and $C_{5'}H$ protons resonances could be consistent with the placement of those hydrogens in positions where they experience the ring-current effects emanating from the base pairs. Nevertheless, the very small magnitude of the chemical shift perturbations (0.3 ppm) could be the reflect of a simple binding in the minor groove for example, or the result of a charge and polarizability effects (39).

The identification of the bithiazole ring as an intercalator must result from a pool of evidences given by various techniques such as ΔTm, fluorescence and viscometry.

In particular, the studies of variations of hydrodynamic properties of DNA in the presence of a drug are believed to be essential in determinating the reality of the intercalation.

The interaction of the bithiazole part of bleomycin with DNA has been here monitored by the quenching of fluorescence of the bithiazole derivative 1 and 2 in the presence of DNA. It is noticeable that the degree of quenching was similar to that exhibited by Blm itself and these results confirmed earlier works (14). Quenching is a useful tool to estimate binding constants, but it has been assumed that this method is a sensitive measure of non-intercalative as well as intercalative binding.

The thermal denaturation results reported here clearly demonstrate the role of bithiazole ring in the stabilization of the DNA helix. This effect is slight but significant and the ΔTm values obtained with probes 1 and 2 are of the same order as those of Blm. Yet it is fundamental to point out the importance of the side chain in the binding process. The presence of positive charges appears to be essential and this assumption is attested by the higher ΔTm value obtained with 3 which bears two protonated aminoalkyl chains.

These results are corroborated by the fluorescence polarization study. The fluorescent probes 4 and 5 do not bind to DNA. The presence of a bulky substituent at the end of the 2' or 4-side chains causes some loss of binding. Additionally the bulk of the fluorescamine moiety sterically could restrict association of 4 and 5 with DNA but more surely does not allow the formation of electrostatic bonds with the phosphate backbone. In order to delineate the influence of the side chains on the mode of binding to DNA,

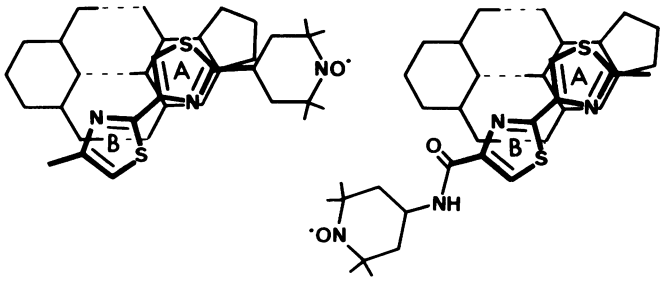

Figure 4 : Proposed representation of the partial stacking of 6 (right) and 8 (left) with the base pairs (C-G) of DNA.

spin-labeled bithiazole derivatives and EPR technique were used. It has been found that the motion of the piperidinyloxy moiety was slightly restricted when it was directly linked to the thiazole ring A at the 4-position since characteristic spectral changes might be observed (Fig. 3). Indeed when the nitroxide was freely rotating (6, 7, 9), the anisotropy was averaged to zero and three sharp lines of equal intensities and about 14 Gauss separation were observed. When the rotational motion of the nitroxide was reduced, high and low field lines reduced in height and broadened (8, Fig. 3).

Taking into account the above results, it could be suggested that a "partial" intercalation of the bithiazole ring occurs in the binding to DNA, in such a way that only the ring A is involved in a stacking interaction with the bases.

The proposed mode of insertion is shown in Fig. 4. The thiazole B is located outside the neighbouring base and excluded from the central region rich in hydrogen-bondings.

Such a position and stacking of the thiazole ring A are in agreement with the NMR data of Chien et al (14) and Chen et al (17).

The problem remains to know whether the insertion of one of the bithiazole could be considered as a classical intercalation. The viscometry data are essential on this point. The unwinding angle of 17° for DNA in the presence of 1 and 2 is the result of the thiazole insertion. However, DNA intercalation of a planar aromatic nucleus of usual thickness (3.4 Å) would be expected to provide a relatively constant degree of unwinding. A maximum attainable value of 26° is provided by ethidium (27) and some related phenanthridines (40). Lower unwinding angles such as reported for acridines, 17°-20° (41,42), adriamycin, 12° (43) or daunorubicin, 10° (25) can be attributed to reflect either strong influences changing the interaction

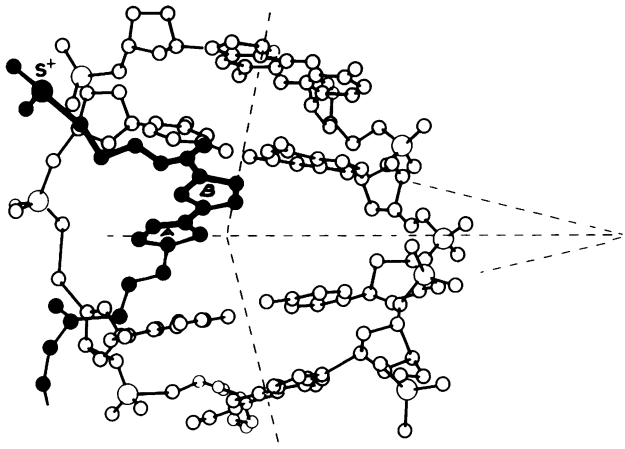

Figure 5 : Proposed model for the insertion of the bithiazole part of Blm into a DNA kink.

between the intercalated ligand and the two adjacent base pairs or the existence of more than one type of DNA binding site available.

On the other hand helix extension studies would be of valuable interest to get an insight in the mode of insertion of thiazole into DNA. In an extensive series of experimental and theoretical studies, Cohen and Eisenberg (31,32) have characterized the hydrodynamic properties of rod-like segments of double-helical DNA and its complexes with ligands. They found that the average increase in length produced by proflavine binding to DNA was of 2.72 Å per bound proflavine molecule. Saucier et al (25) also using sonicated calf thymus DNA and other similar experimental techniques analyzed the DNA viscosity enhancement of several planar aromatic molecules. They found that daunorubicin and 9-methoxyellipticine gave the expected increase in viscosity corresponding to the theoretical helix extension of 3.4 Å per bound drug molecule.

Unexpectedly, a small but significant decrease of the helix length was observed upon binding of 1 and 2. On the basis of the preceeding results, insertion in DNA of a ring of the bithiazole cannot be excluded but the DNA lenghtening determinations are not in favour of an intercalative interaction.

In fact, Gabbay and co-workers, with reporter molecules and peptides containing small aromatic ring systems which could insert a part of their molecule into the bases of DNA (44-47), suggested that several derivatives

could produce a very different effect on helix length and viscosity. One possibility proposed is that these compounds cause a bending of the double helix favouring a partial intercalation and resulting in a slight decrease in length on complex formation. This concept has received support from the X-ray analysis of intercalating drugs with dinucleotides (48).

Thus, bending of the helix at the point of insertion of the bithiazole between base-pairs would adequately agree with the observed data (Fig. 5).

In conclusion, considering the small aromatic surface of the bithiazole of bleomycin and the possible steric hindrances of its side chains, and bearing in mind the above results, we propose a binding model where only one of the thiazole ring is inserted between base pairs resulting in a slight bending at the point of complexation.

In such a complex (Fig. 5), the bithiazole wedges in between the base pairs which occurs at bends in a kinked DNA.

This position is in agreement with the formation of an ionic interaction between the sulfonium side chain and the nucleic acid backbone. Furthermore, this linkage precludes the bithiazole from full overlap, leading to "partial intercalation" such as shown by EPR and unwinding studies. May be the introduction of longer side chains at the 2' or 4-position of the bithiazole moiety would help in understanding the mechanism of interaction of this planar chromophore with DNA.

ACKNOWLEDGEMENTS

We express our gratitude to Dr Jean-Pierre Catteau for recording the EPR measurements and Michèle Lohez for her excellent technical assistance. This work was supported by grants from the "Institut National de la Santé et de la Recherche Médicale" and the "Fédération des Centres de Lutte contre le Cancer".

REFERENCES

1 Carter, S.K. (1978) in Bleomycin : Current Status and New Developments (Carter, S.K., Crooke, S.T., and Umezawa, H., Eds.) pp 9-14, Academic Press, New-York.
2 Crooke, S.T. (1978) in Bleomycin : Current Status and New Developments (Carter, S.K., Crooke, S.T., and Umezawa, H., Eds.) pp 1-12, Academic Press, New-York.
3 Umezawa, H. (1979) in Bleomycin : Chemical, Biochemical, and Biological Aspects (Hecht, S.M., Ed) pp 24-36, Springer-Verlag, New-York.
4 Hutchinson, F., and Povirk, L.F. (1979) in Bleomycin : Chemical, Biochemical, and Biological Aspects (Hecht, S.M., Ed) pp 255-266, Springer-Verlag, New-York.

5 Sausville, E.A., Stein, R.W., Peisach, J., and Horwitz, S.B. (1978) Biochemistry 17, 2746-2754.
6 Dabrowiak, J.C., Greenaway, F.T., and Grulich, R. (1978) Biochemistry 17, 4090-4096.
7 Oppenheimer, N.J., Rodriguez, L.O., and Hecht, S.M. (1979) Proc. Natl. Acad. Sci. USA 76, 5616-5620.
8 Sugiura, Y. (1980) J. Am. Chem. Soc. 102, 5208-5215.
9 Hénichart, J.P., Houssin, R., Bernier, J.L., and Catteau, J.P. (1982) J. Chem. Soc. Chem. Commun. 1295-1297.
10 Hénichart, J.P., Bernier, J.L., Houssin, R., Lohez, M., Kenani, A., and Catteau, J.P. (1985) Biochem. Biophys. Res. Commun. 126, 1036-1041.
11 Koyama, G., Nakamura, H., Muraoka, Y., Takita, T., Maeda, K., and Umezawa, H. (1968) Tetrahedron Lett. 4635-4638.
12 Povirk, L.F., Hogan, M., and Dattagupta, N. (1979) Biochemistry 18, 96-101.
13 Murakami, H., Mori, H., and Taira, S. (1976) J. Theor. Biol. 59, 1-23.
14 Chien, M., Grollman, A.P., and Horwitz, S.B. (1977) Biochemistry 16, 3641-3647.
15 Roy, S.N., Ott, G.A., Brewer, C.F. and Horwitz, S.B. (1981) Cancer Res. 41, 4471-4477.
16 Krueger, W.C., Pschigoda, M., and Reusser, F. (1973) J. Antibiot. 26 424-428.
17 Chen, D.M., Sakai, T.T., Glickson, J.D., and Patel, D.J. (1980) Biochem. Biophys. Res. Commun. 92, 197-205.
18 Glickson, J.D., Pillai, R.P., and Sakai, T.T. (1981) Proc. Natl. Acad. Sci. USA 78, 2967-2971.
19 Booth, T.E., Sakai, T.T., and Glickson, J.D. (1983) Biochemistry 22, 4211-4217.
20 Houssin, R., Bernier, J.L., and Hénichart, J.P. (1984) J. Heterocyclic Chem. 21, 681-683.
21 Houssin, R., Bernier, J.L., and Hénichart, J.P. (1984) J. Heterocyclic Chem. 21, 465-469.
22 Weigele, M., De Bernardo, S., Tengi, J., and Leimgruber, W. (1972) J. Am. Chem. Soc. 94, 5927-5928.
23 Wakelin, L.P.G., and Waring, M.J. (1976) Biochem. J. 157, 721-740.
24 Malher, H.R., Kline, B., and Mehrotra, B.D. (1964) J. Mol. Biol. 9, 801-811.
25 Saucier, J.M., Festy, B., and Le Pecq, J.B. (1971) Biochimie 53, 973-980.
26 Revet, B., Schmir, M., and Vinograd, J. (1971) Nature New. Biol. 229, 10-13.
27 Wang, J.C. (1974) J. Mol. Biol. 89, 783-801.
28 Falaschi, A., and Kornberg, A. (1964) Federation Proc. 23, 940.
29 Nagai, K., Yamaki, H., Suzuki, H., Tanaka, N., and Umezawa, H. (1969) Biochim. Biophys. Acta 179, 165-171.
30 Neidle, S., and Abraham, Z. (1984) C.R.C. Crit. Rev. Biochem. 17, 73-121.
31 Cohen, G., and Eisenberg, H. (1966) Biopolymers 4, 429-440.
32 Cohen, G., and Eisenberg, H. (1969) Biopolymers 8, 45-55.
33 Sheardy, R.D., and Gabbay, E.J. (1983) Biochemistry 22, 2061-2067.
34 Piette, L.H., and Hsia, J.C. (1979) in Spin Labeling. Theory and Applications (Berliner, L.J., Ed.) pp 247-290, Academic Press, New-York.
35 Hong, S.J., and Piette, L.H. (1976) Cancer Res. 36, 1159-1171.
36 Bernier, J.L., Hénichart, J.P., and Catteau, J.P. (1981) Anal. Biochem. 117, 12-17.

37 Bernier, J.L., Hénichart, J.P., and Catteau, J.P. (1981) Biochem. J. 199, 479-484.
38 Hénichart, J.P., Bernier, J.L., and Catteau, J.P. (1982) Hoppe Seyler's Z. Physiol. Chem. 363, 835-841.
39 Krugh, T.R., and Nuss, M.E. (1979) in Biological Applications of Magnetic Resonance, Shulman, R.G., Ed., pp 113-175, Academic Press, New-York.
40 Wakelin, L.P.G., and Waring, M.J. (1974) Mol. Pharmacol. 10, 544-561.
41 Jones, R.L., Lanier, A.C., Keel, R.A., and Wilson, W.D. (1980) Nucleic Acids Res. 8, 1613-1624.
42 Jones, R.L., and Wilson, W.D. (1981) Adv. Pharmacol. Chemother. 18, 177-221.
43 Waring, M.J. (1971) Prog. Mol. Subcell. Biol. 2, 216.
44 Kapicak, L., and Gabbay, E. (1975) J. Am. Chem. Soc. 97, 403-408.
45 Adawadkar, P., Wilson, W.D., Brey, W., and Gabbay, E.J. (1975) J. Am. Chem. Soc. 97, 1959-1961.
46 Gabbay, E.J., Adawadkar, P.D., Kapicak, L., Pearce, S.W., and Wilson, W.D. (1976) Biochemistry 15, 152-157.
47 Gabbay, E.J., Adawadkar, P.D., Wilson, W.D. (1976) Biochemistry 15, 146-151.
48 Sobell, H.M., Reddy, B.S., Bhandray, K.K., Jain, S.C., Sakore, T.D., and Seshadri, T.P. (1978) Cold Spring Harbor Symp. Quant. Biol. 42, 87.