The interaction of plant alkaloids with DNA. II. Berberinium chloride

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ABSTRACT

The interaction of berberinium chloride with DNA has been investigated using spectrophotometry, viscometric titrations with sonicated and closed circular superhelical DNA, and flow polarized fluorescence. The binding results for berberinium were found to fit the neighbor exclusion model. The two viscometric titrations and flow polarized fluorescence results also indicated that berberinium binds to DNA by intercalation. Titration of sonicated DNA with berberinium produced viscosity increases which were less than those obtained with quinacrine and the titration of superhelical DNA indicated a significantly smaller unwinding angle for intercalation of berberinium than for quinacrine. These results can be interpreted in terms of a model in which (i) berberinium is partially intercalated into the double helix, or (ii) the alkaloid is more completely intercalated into the double helix, but causes bending of the helix due to the slight nonplanarity of the berberinium ring system, or (iii) a combination of (i) and (ii).

INTRODUCTION

Berberinium salts are members of the protoberberine class of isoquinoline alkaloids found widely distributed in various plant species [1]. An important compound in this category, berberinium chloride (Figure 1), displays a broad spectrum of antibacterial and antiprotozoal activity [2-5]. Berberinium chloride has been shown to convert certain yeast strains into respiration-deficient mitochondrial mutants [6] and the alkaloid is also effective in eliminating bacterial plasmids [7-9]. On the basis of this and other evidence, Hahn and Ciak [10] postulated that berberinium chloride exerts biological activity by binding to extra-chromosomal DNA, blocking transcription and/or replication.

As part of a continuing investigation on the mechanism and specificity of protoberberine alkaloid-DNA complex formation, we have initiated a study of the interaction between berberinium chloride and DNA. Earlier we demonstrated [11] that coralyne (Figure 1), a closely related alkaloid with anti-leukemic properties, binds strongly to DNA by a mechanism which includes

Figure 1.

intercalation of the unsaturated naphthoisoquinoline chromophore between DNA base pairs. Such an interaction *in vivo* might be responsible for coralyne antileukemic activity. Hahn and coworkers [12,13] have shown that berberinium chloride binds to calf thymus DNA and that complex formation is accompanied by an increase in the intrinsic viscosity of DNA. This suggests that the berberinium cation also binds to DNA by intercalation according to the classical Lerman model [14,15]. Viscosity enhancements are, however, also observed on binding certain non-intercalating compounds such as netropsin [16], 2,5-*bis*-(4-guanylphenyl)furan [17], and 2-hydroxystilbamidine [18] to DNA. Berberinium possesses a partial unsaturation in ring C (Figure 1) and, in contrast to the planar coralyne molecule, should have severe steric constraints imposed on any intercalation mechanism for this compound. To determine how these steric factors influence berberinium-DNA interactions, we have analyzed the binding using viscometric titrations of sonicated calf thymus DNA and closed circular superhelical plasmid DNA, and have utilized flow polarized fluorescence with high molecular weight chicken erythrocyte DNA. The amount of berberinium bound to DNA in these experiments was quantitated using the neighbor exclusion model [19-22] rather than the homogeneous, independent site model [23,24] which was applied previously [12] with berberinium but is now known to be incorrect.

MATERIALS AND METHODS

Chemicals

Berberinium chloride, obtained from Calbiochem (Lot #103032), was twice recrystallized from a 40% isopropanol/60% ethanol mixture. The melting point, ultraviolet-visible absorption spectrum, and elemental analysis (Atlantic Microlab Inc., Atlanta, Georgia) agreed with reported values and were consistent with the proposed structure [25,26]. After drying samples of berberinium chloride to constant weight in a vacuum at room temperature, stock solutions were prepared in standard buffer ($3.75 \times 10^{-3} \text{M}$ NaH_2PO_4 , $5 \times 10^{-4} \text{M}$ EDTA adjusted to pH 7.0 with NaOH, ionic strength 0.0095). Concentrated stock solutions of berberinium chloride (1.5 to $2.0 \times 10^{-3} \text{M}$) were found to be stable for a period of at least 2 months when stored at 4°C in the dark. Elemental analysis of quinacrine (Sigma Chemical Co.) indicated that the preparation was pure and it was used without further purification. Mefloquine hydrochloride, kindly provided by Dr. E. A. Steck of the Walter Reed Army Institute of Research, was twice recrystallized from acetonitrile and the purified product gave physical constants in agreement with reported values [27]. All other chemicals were of the highest purity commercially available. Water was redistilled from acid permanganate in an all glass still.

DNA

Calf thymus DNA (Worthington Lot #35M614) was dissolved in 2X standard buffer, made 2.0M in NaCl and sonicated as described [28]. Colicinogenic factor E_1 (Col E_1) closed circular superhelical DNA was prepared by a modification of the method described by Nomura and associates [28,29]. DNA concentrations are expressed as nucleotide equivalents per liter and were determined using an extinction coefficient of $6600 \text{ M}^{-1}\text{cm}^{-1}$ at 260 nm.

For the preparation of chicken erythrocyte nuclei, White Leghorn layer hens weighing 1-1.5 kg were exsanguinated by severing the jugular vein and blood was collected in 2-4 volumes of 0.1M EDTA, 0.05M tris (pH 7.8). Erythrocytes, separated from plasma proteins by centrifugation at $300 \times g$ for 20 min, were resuspended and washed with 5 volumes of sucrose-tris (0.25M sucrose and 0.05M tris, pH 7.8), resedimented and rewashed with 3-5 volumes of sucrose-tris made $3 \times 10^{-3} \text{M}$ in CaCl_2 (STC buffer). No hemolysis was noted during the washing procedure. The erythrocytes were then resuspended and lysed by stirring at room temperature for 1 hr in 2 volumes of STC buffer containing 0.5% saponin. The crude lysate was centrifuged at $600 \times g$ for 45 min to remove cellular debris and the supernate was collected and spun at $10^4 \times g$ for 30 min to pellet the nuclei. The gelatinous nuclear pellet

was collected, triturated in 3 volumes of sucrose-tris with 0.01M EDTA, and dispersed by mixing for 60 sec in a Waring blender. The resulting suspension was again sedimented at $10^4 \times g$ for 30 min. This process was repeated until most of the reddish hue had been removed from the pelleted nuclei (usually 3-5 washings).

The procedure for chicken erythrocyte DNA isolation is a modification of that described by Blin and Stafford [30]. Purified nuclei were resuspended with the aid of a Dounce homogenizer in one volume of 0.05M tris (pH 8.0) and an equal volume of 0.1M EDTA, 1.0% sarkosyl pH 8.0 was slowly added with gentle shaking. The resulting viscous mixture was rotated at 200 rpm and 50°C in a New Brunswick gyratory incubator/shaker (model #G-26). After 1 hr of shaking Proteinase K (Merck) was added (from a 10 mg/ml stock solution in 0.05M tris pH 8.0) to a final concentration of 100 µg/ml and rotation was continued for an additional 5 hr. The solution was then transferred to a 500 ml separatory funnel, an equal volume of phenol solution (500 ml phenol, 70 ml *meta*-cresol, and 0.1% w/v 8-hydroxyquinoline equilibrated with 0.05M tris and adjusted to pH 8.0) added and the phases mixed by placing the long axis of the separatory funnel parallel to the bench (supported by a ring stand) and rotating the funnel at 20 rpm by means of a short section of vacuum tubing placed over the funnel stem and connected to a G. K. Heller 1/40 hp motor. The separatory funnel allows the phenol layer to be easily removed without having to pour or pipette the viscous aqueous layer. Rotation of the mixture was continued for 1 hr before replacing the phenol layer. The extraction procedure was repeated four times after which, the DNA solution was transferred to a dialysis bag and dialyzed against 1000 volumes of a buffer comprised of 0.05M tris, 0.01M EDTA, and 0.01M NaCl pH 8.0. After dialysis, heat treated ribonuclease A (Worthington) was added to a final concentration of 100 µg/ml (from a 10 mg/ml stock solution in 0.05M tris pH 8.0) and the resulting solution incubated for four hours at 37°C in the New Brunswick shaker at 100 rpm. The solution was again transferred to a separatory funnel and three phenol extractions performed. After extraction, the DNA solution was dialyzed exhaustively against standard buffer. Purified chicken erythrocyte DNA had an A_{260}/A_{280} ratio between 1.8 and 1.9, an A_{260}/A_{230} ratio between 2.3 and 2.4, and a total hyperchromicity at 260 nm of 29%. This preparation contained less than 1% residual RNA analyzed by the method of Savitsky and Strand [31]. The viscosity average molecular weight, determined in a rotating cylinder viscometer, was calculated to be 26 megadaltons using the equation of Crothers and Zimm [32].

Spectrophotometry

Absorption spectra were recorded in 1 cm lightpath quartz cuvettes equipped with teflon stoppers using a Cary 17D spectrophotometer thermostatted with a Haake (model #F 4391) circulating water bath. Spectrophotometric titrations were performed by two methods: (i) small aliquots of concentrated DNA stock solution ($1-2 \times 10^{-2} \text{M}$) were added with a calibrated microliter syringe (Hamilton) to a dilute berberinium chloride solution in the sample cuvette and the absorption spectrum of the resulting mixture recorded, and (ii) small aliquots of a concentrated berberinium chloride stock solution in standard buffer were added to a dilute DNA solution in the sample cuvette, the resulting solution mixed by repeated inversion of the cuvette, and the absorbance at 345 nm recorded.

The extinction coefficient of free berberinium ($27300 \text{ M}^{-1}\text{cm}^{-1}$) at 345 nm was measured directly and the extinction coefficient, at the same wavelength, of berberinium bound to DNA ($16500 \text{ M}^{-1}\text{cm}^{-1}$) was calculated using the following relationship [33]:

$$\frac{1}{\epsilon_{app} - \epsilon_f} = \frac{1}{c_D} \cdot \frac{1}{(\epsilon_b - \epsilon_f) \cdot B_{app} K_{app}} + \frac{1}{\epsilon_b - \epsilon_f}$$

where ϵ_f and ϵ_b are the extinction coefficients of free and bound berberinium respectively, ϵ_{app} is the apparent extinction coefficient of berberinium at DNA concentration c_D , and B_{app} is the apparent number of binding sites per nucleotide with binding constant K_{app} . A plot of $1/(\epsilon_{app} - \epsilon_f)$ vs $1/c_D$ yields a straight line with intercept $1/(\epsilon_b - \epsilon_f)$ at high molar ratios of DNA to bound berberinium cations (no prior knowledge of B_{app} , K_{app} , or ϵ_b is required).

Viscometric Titrations

Flow times of DNA-drug complexes were recorded at 25.0°C using an Ubbelohde semi-micro dilution viscometer (Cannon Instruments #75-L711). Sonicated or closed circular DNA concentrations ranged between 2.0 and $3.0 \times 10^{-4} \text{M}$. Successive aliquots of concentrated drug stock solution were added to a 1.0 to 5.0 ml DNA solution in the viscometer, and the resulting solution mixed for 5 min by gentle airflow.

Flow Polarized Fluorescence

Fluorescence polarization spectra were recorded on a Perkin-Elmer MPF-44A spectrophotofluorometer fitted with Polacoat UV 105 polarizing filters adjacent to the sample cell in both the excitation and emission light paths. The polarizing effects of the emission monochromator were corrected for by the method of Azumi and McGlynn [34] so that the polarization (P) was cal-

culated from the formula:

$$P = \frac{I_{vv} - G \cdot I_{vh}}{I_{vv} + G \cdot I_{vh}} \quad \text{where} \quad G = \frac{I_{hv}}{I_{hh}}$$

I_{ij} is the fluorescence intensity obtained for orientation of the excitation (i) and emission (j) filters to pass light with the electric vector polarized in the vertical (v) or horizontal (h) plane. Using this method the fluorescence polarization spectrum of quinacrine bound to DNA was found to be essentially the same as that obtained by Lerman [15]. Static polarization spectra were recorded in a regular 1 cm quartz cuvette. For measurement of flow oriented samples, the solution was passed through a 2 mm i.d. quartz capillary oriented vertically in the light path. A Sage motor-driven syringe pump was utilized to produce a flow rate of 7.21 ml/min (producing an average shear rate of 102 sec^{-1}). The fractional change in fluorescence intensity due to orientation of the DNA helix axis parallel to the flow was determined by comparing the fluorescence intensity of the same sample solution with the pump on and off.

RESULTS

Binding Equilibria

DNA induces progressive hypochromic shifts in the visible absorption spectrum of berberinium as the ratio of nucleotides to drug molecule is increased (Figure 2). In addition, the berberinium absorption maxima shift to lower wavenumbers upon binding to DNA and approach a constant value as the ratio of DNA nucleotides to berberinium cation becomes greater than 10:1.

These binding results may be analyzed according to the nearest neighbor exclusion model described by Crothers [19], Bauer and Vinograd [20], McGhee and von Hippel [21], and Zasedatelev *et al* [22] utilizing the equation:

$$K = \frac{2r(1-2r)}{c(1-4r)^2} \quad (1)$$

where r is the ratio of bound berberinium cation per DNA nucleotide equivalent, c is the free cation concentration, and K is the intrinsic binding constant to an isolated potential binding site. Figure 3 illustrates the results obtained in four independent spectrophotometric titrations of berberinium chloride with sonicated calf thymus DNA. The solid line was calculated using equation (1) with a K value of 35400 which was determined by a least squares procedure. It is readily apparent that the binding of

Figure 2. Effect of sonicated calf thymus DNA on the visible absorption spectrum of berberinium chloride ($3.21 \times 10^{-5} \text{M}$). The berberinium spectrum before addition of DNA is the one having the greatest absorbance at 29000 cm^{-1} (345 nm). Successive decreases in absorbance occur as the DNA concentration is increased (DNA concentrations are, respectively: 0.182 , 0.451 , 0.804 , 1.24 , 1.75 , 2.40 , 3.19 , 4.17 , 5.24 , 6.38 , and $9.39 \times 10^{-4} \text{M}$). Spectra were recorded at 25°C on an Acta V spectrophotometer.

Figure 3. Binding analysis of berberinium chloride and sonicated calf thymus DNA in standard buffer at 27.1°C. The ratio of bound berberinium per DNA nucleotide divided by the free berberinium concentration (r/c) is plotted as a function of r . The solid line was calculated by a least squares procedure using equation (1). (○) and (●) are results from titration method (i) with initial berberinium concentrations of 2.86 and $2.00 \times 10^{-5}M$ respectively; (□), (■), (▲), and (△) are results obtained from titration method (ii) with initial DNA concentrations of 1.11 , 0.775 , 0.376 , and $0.153 \times 10^{-4}M$ respectively. Because of the low DNA concentrations involved, data points for the latter two titrations lie in the 10-30% bound berberinium range and are subject to increased scatter [50,51].

berberinium chloride to DNA at this temperature and ionic strength can be described in terms of an excluded site binding model [19-22] and that saturation of binding occurs at a r value of 0.25 or one berberinium cation per four DNA nucleotides. The finding that both titration methods (i) and (ii), described in the Methods section, give identical results indicates that the extinction coefficients and thermodynamic parameters determined for this system are accurate.

Viscometric Titration: Sonicated DNA

Effects of berberinium chloride, quinacrine, and the antimalarial quinolinemethanol, mefloquine, on sonicated calf thymus DNA viscosity are displayed in Figure 4. The DNA viscosity increase induced by binding berberinium is similar to the intercalating antimalarial quinacrine, but reduced in

Figure 4. Viscometric analysis illustrating the interaction of (○) quinacrine, (●) berberinium, and (□) mefloquine with sonicated calf thymus DNA. The quantity η/η_0 represents a ratio of drug-DNA complex reduced specific viscosity to that of DNA alone and is plotted as a function of the ratio of drug added per DNA nucleotide equivalent. (Δ) are the berberinium viscosity results corrected for the amount of berberinium bound using the association constant 35400 and equation (1). Calf thymus DNA was at a concentration of $2.0 \times 10^{-4}M$ in all cases.

magnitude. In contrast, mefloquine, which has been demonstrated not to form an intercalated complex with DNA [28,35], produces slight decreases in DNA viscosity. The rise in DNA viscosity reaches a maximum as the ratio of quinacrine molecules per nucleotide approaches the value of 0.25 corresponding to saturation of intercalation binding sites. For the more weakly binding berberinium, the viscometric titration does not reach a plateau until the molar ratio is above 0.4.

In figure 4 the results for berberinium are also plotted as a function of r calculated from equation (1). At these low ionic strengths, quinacrine is essentially fully bound over this r range. Using the K value determined from Figure 3, the berberinium curve is shifted to lower r values but remains

significantly lower in reduced viscosity than the quinacrine curve at all r values.

Viscometric Titration: Superhelical DNA

Convincing evidence for binding and intercalation of berberinium and quinacrine to plasmid closed circular superhelical DNA is presented in Figure 5. Both drugs induce unwinding of the DNA and produce viscosity increases at low molar ratios of drug to DNA nucleotide equivalents. As the ratio is increased, a point is reached where all superhelical turns are removed and a maximum is obtained in the viscometric titration. Addition of more drug generates left-handed superhelical turns in the closed circular DNA and causes viscosity decreases. Non-intercalating cations which bind to DNA (such as putrescine illustrated in Figure 5) have little effect on circular DNA superhelical density and consequently produce little change in viscosity. Because of the dependence of binding free energy on the superhelical density in Col E₁ DNA [20], we cannot, from the available data, correct the Col E₁ titration curve as was done for sonicated DNA in Figure 4. It can be seen from Figure 4, however, that at molar ratios of berberinium to DNA of less than 0.07, very little correction is required. Since the berberinium has completely removed superhelical turns at a molar ratio of 0.05 (peak position in Figure 5) this

Figure 5. The effect of (○) quinacrine, (●) berberinium, and (□) putrescine on the viscosity of Col E₁ closed circular superhelical DNA. The reduced specific viscosity ratio, described in Figure 4, is plotted as a function of drug added per Col E₁ DNA nucleotide equivalent on a logarithmic scale. Col E₁ DNA was at a concentration of 2.59×10^{-4} M in all cases.

should closely correspond to the moles of berberinium bound per DNA nucleotide at this low ratio. Quinacrine has completely removed superhelical turns at a molar ratio of 0.028 which at this low salt concentration also corresponds to bound quinacrine. These results indicate that almost twice as many berberinium molecules must be bound to produce the same unwinding as quinacrine.

Flow Polarized Fluorescence

The fluorescence polarization of berberinium (10^{-5}M) complexed to DNA ($3.1 \times 10^{-4}\text{M}$) remains positive throughout the 300 nm to 500 nm wavelength range, gradually decreasing from 0.40 at 480 nm to 0.17 at 300 nm. This indicates that the electronic transition vectors for berberinium in this range are much more nearly parallel than those for quinacrine in the same range and are, therefore, not suitable for determining the orientation of berberinium with respect to the DNA helix axis. The change in berberinium polarized fluorescence intensity when the berberinium-DNA complex is flow oriented indicates that the transition moments in the 350 nm to 450 nm regions are approximately perpendicular to the DNA helix axis as would be expected for an intercalated molecule (Table I).

Table I. Fractional change in polarized fluorescence intensity of berberinium bound to DNA when oriented by flow.

Polarization of Emission	V	V	H	H
Wavelength of Excitation (nm)	445	350	445	350
Exciting Light Vertically Polarized	-.066	-.051	- ^a	-.008
Exciting Light Horizontally Polarized	- ^a	-.016	+.062	+.053

^aThe change in intensity was obscured by signal noise.

The concentration of DNA was $3.1 \times 10^{-4}\text{M}$ and the berberinium concentration was 10^{-5}M . All measurements were conducted in standard buffer at 25°C.

DISCUSSION

Although there is a structural similarity, through the berberine nucleus, between coralyne chloride and berberinium chloride (Figure 1), the coralyne ring system is completely aromatic and planar while the berberinium ring system has partial saturation in ring C. This saturation causes a distortion in the plane of the berberinium aromatic ring system. Rings A and B are planar and should be capable of intercalation by the classical model [14]. Ring C is not totally planar and analysis of CPK space-filling models indicates that, as a result, Ring D is slightly twisted out of the Ring A-B plane. If the

van der Waals thickness of the coralyne ring system is 3.4 Å then the Ring A-B plane in berberinium has the same thickness, but the total berberinium molecule has a thickness of approximately 4.5 Å (CPK models[36]) due to the effects discussed above. This structural difference between coralyne and berberinium causes three dramatic differences in their properties: (i) coralyne dimerizes extensively in aqueous solution [37] with a dimerization constant greater than 10^4 on a molar scale while no berberinium dimerization can be detected at concentrations up to 10^{-4} M; (ii) at low ionic strengths, coralyne stacks (by self-association) along the polyanionic deoxyribose-phosphate backbone of DNA while such stacking is negligible for berberinium (as evidenced by the presence of isosbestic points in the berberinium spectrophotometric titration (Figure 2) which are absent in the coralyne titration [11]); and (iii) at moderate ionic strengths and low molar ratios of drug to DNA nucleotides, coralyne binds much more strongly to DNA than does berberinium. The binding mode for coralyne under these later conditions has been identified as intercalation [11].

Because of the lack of planarity in the berberinium ring system and the importance of determining how this affects the potential to bind to DNA, we have analyzed berberinium interaction with DNA by viscometric titrations with sonicated calf thymus DNA and closed circular superhelical plasmid DNA, and by flow polarized fluorescence. These are extremely sensitive methods for detecting structural perturbations in the DNA double helix induced by the binding of small molecules.

With sonicated DNA, berberinium induces an increase in DNA viscosity as would be expected for intercalation (Figure 4). Even after correcting for unbound drug, however, the berberinium induced viscosity increases are significantly less than for quinacrine. Analysis of the quinacrine viscosity results using the equation of Cohen and Eisenberg [38] gives a linear plot with the slope predicted for an intercalating molecule. The berberinium plot, using the same equation, is curved and at any r value has a slope less than that predicted by the classical intercalation model. This suggests that berberinium intercalates between DNA base pairs but causes a structural distortion of the double helix that is slightly different than classical intercalation.

The viscometric titration with closed circular superhelical DNA (Figure 5) also suggests that berberinium binds to DNA by intercalation. The unwinding angle for the Col E₁ plasmid DNA double helix is less for berberinium than for the planar molecules, quinacrine (Figure 5), coralyne [39], and similar

planar aromatic compounds [20,28,40]. The smaller apparent unwinding angle for berberinium also suggests that this compound induces a different structural change in the DNA double helix on intercalation than classical intercalating molecules. This could also partially explain why berberinium is less efficient than quinacrine in the frequency of elimination of bacterial plasmids *in vivo* [7,8].

The flow polarized fluorescence results are in agreement with an intercalation binding mode for berberinium but because the electronic transition vectors above 300 nm are nearly parallel, this technique cannot prove intercalation or probe the structural changes in DNA induced by berberinium binding.

Two non-classical intercalation models for berberinium binding to DNA can explain the above results: (i) only the planar Ring A-B system intercalates and because the space between base pairs is not filled, a slight bending of the helix occurs, and (ii) the entire berberinium molecule is intercalated but the slight non-planarity of the drug also causes bending of the DNA helix. The first model is similar to the one extensively developed by Gabbay and coworkers [41-43] for the binding of aromatic amino acid containing peptides and reporter molecules to DNA. The second model has precedence in the X-ray work on ethidium-dinucleoside monophosphate crystals [44]. Sobell [45] has extended these findings to a proposed model for the ethidium-DNA intercalation complex which involves slight bending of the DNA helix. It should be obvious that various combinations of these two models are possible.

A model in which the greater part of rings A, B, and D (Figure 1) of berberinium are intercalated, the double helix is slightly bent, and the berberinium molecule is slightly distorted bringing rings A, B, and D into nearly the same plane best fits the experimental data. For example, the quinine derivative, chloroquine which has been extensively investigated [46], does not cause significant bending of DNA [28]. The fluorescence quantum yield for berberinium increases on binding of the drug to DNA [47]. This can be explained if berberinium assumes a more planar rigid conformation in the intercalation complex. CPK models indicate this conformational change is possible with only slight bond angle distortion in ring C. This distortion of both the DNA double helix and of the berberinium molecule on intercalation should decrease the equilibrium constant for this reaction relative to classical intercalating molecules. In fact, the berberinium binding constant is at least two orders of magnitude less than that for quinacrine [48] in the same buffer [49].

The above results could be explained by a two-site binding model involving both intercalation and external stacking of the drug molecules [24]. This could account for both the smaller unwinding angle of superhelical DNA and decreased helix extension of sonicated DNA for berberinium since externally bound molecules make negligible contributions to these two effects [16-18]. Coralyne [11,37], proflavine [24], and related *planar* molecules [52], for example, bind to DNA by both mechanisms but still display linear DNA viscosity enhancements and superhelical DNA unwinding angles which are much closer to quinacrine than to berberinium. Also, this two site model is unlikely since (i) berberinium is *not planar* and does not dimerize (stack) readily; (ii) isosbestic points are obtained in the spectrophotometric titration of berberinium with DNA, but not with coralyne [11], proflavine [53], and related molecules; and (iii) treating the DNA molecule as a collection of independent discrete binding sites is inconsistent with a considerable body of experimental evidence [19-22, 45, 51, 54, 55].

In summary, the data presented here suggests that a large portion of the berberinium ring system intercalates into DNA, resulting in a distortion of the berberinium molecule to a more planar conformation. In addition, the DNA helix is slightly bent on intercalation of berberinium which causes a total length increase of less than 3.4 Å per bound berberinium cation, and an unwinding angle on intercalation which is significantly less than for quinacrine, ethidium and similar molecules.

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