The Benzoxazolinate of C-1027 Confers Intercalative DNA Binding

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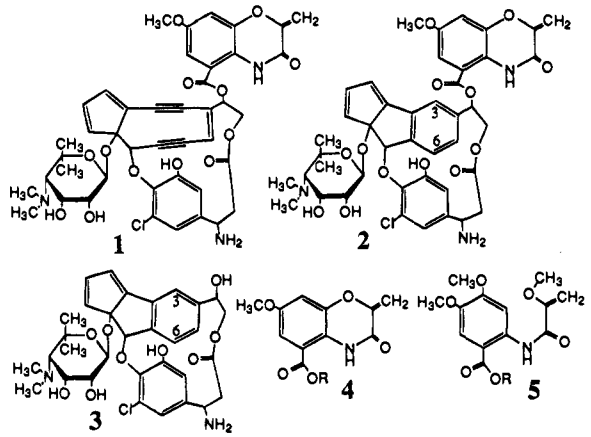
C-1027 (**1**) is a member of the enediyne family of antitumor antibiotics.[1] These compounds produce DNA damage following rearrangement to form a diradical species which abstracts hydrogen atoms from deoxyribose.[1,2] The 9-member enediyne ring system of C-1027 chromophore bears a greater structural resemblance to the chromophores of neocarzinostatin (NCS)[1c] and kedarcidin[3] than to the 10-member systems of calicheamicin (CAL) and esperamicin A1 (ESP A1). However, the benzoxazolinate moiety (**4**) of C-1027 is a bicyclic analogue of the *N*-(2-methoxyacrylyl)anthranilate group (**5**) of ESP A1. Recent studies have revealed that the anthranilate of ESP A1 intercalates in DNA.[4] In this Communication, we present hydrodynamic and spectroscopic evidence consistent with DNA intercalation by the benzoxazolinate of C-1027.



The present studies were performed with the stable aromatized derivatives **2** and **3**,[5] since C-1027 chromophore is unstable and undergoes cycloaromatization in the absence of reducing agents.[2,5] Previous studies with CAL,[6] NCS,[7] and ESP A1[8] suggest that **2** will bind to DNA in a manner similar to that of the parent compound **1**.

Prior to undertaking the intercalation studies, binding constants for **2** and **3** with λ DNA (48 502 bp) were estimated by Scatchard plot analysis (Figure 1).[9] The affinity of **2** for DNA, $K_a \approx 5.1 \times 10^5\ M^{-1}$, is in accord with the binding constant for the aromatized form of CAL.[10] However, removal of the

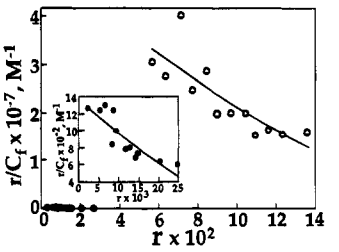


Figure 1. Scatchard plots for the binding of **2** (○)and **3** (●) to λ DNA.[9] r is the molar ratio of drug molecules bound per bp of λ DNA; C_f is the concentration of drug free in solution. The curves represent the best fit of the data to the site exclusion model of McGhee and von Hippel.[18]

benzoxazolinate moiety to produce **3** reduced the DNA affinity ~400-fold ($K_a \approx 1.2 \times 10^3\ M^{-1}$). This observation stands in contrast to the 10-fold reduction in the binding affinity of ESP A1 for DNA resulting from the removal of the deoxyfucose anthranilate.[4a] These two studies must be compared with caution, however, since **2** and **3** are soluble in aqueous solution[9] while ESP A1 solubility requires the presence of organic solvent.[4a]

To test the hypothesis that the benzoxazolinate of C-1027 intercalates in DNA, compounds **2** and **3** were examined for their ability to unwind negatively-supercoiled DNA, to increase the viscosity of a DNA solution, and to undergo DNA-induced quenching of their absorbance of UV light.

Hypochromism in the absorbance spectrum of an intercalated chromophore is necessary, but not sufficient, to prove intercalation.[11a] With compound **2** ($\lambda_{max} = 345$ nm), ~75% of the absorbance at 345 nm can be attributed to the benzoxazolinate ($\epsilon_{345} \approx 10\,000\ cm^{-1}\ M^{-1}$ in ethyl acetate; $\lambda_{max} = 345$ nm), while the aromatized core, amino sugar, and β-tyrosine moiety of **3** account for the remaining ~25% ($\epsilon_{345} \approx 3000\ cm^{-1}\ M^{-1}$ in ethyl acetate; $\lambda_{max} = 328$ nm). As shown in Figure 2, the absorbance of **2** at 345 nm decreases as the proportion of DNA-bound drug increases, with a maximal reduction of ~30%.[11b] This hypochromicity is in good agreement with other known intercalators.[11a]

Another critical feature of intercalation is unwinding of the

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(9) Isotherms for the binding of **2** and **3** to phage λ DNA were established by separating DNA-bound from unbound drug by ultracentrifugation as described elsewhere, except that organic solvent was not used in the present study.[4a] Both **2** and **3** were judged to be soluble in aqueous solution, since centrifugation at 217000*g* for 20 min did not change the 345 nm absorbance of aqueous solutions of **2** and **3**.

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(11) (a) Satyanarayana, S.; Dabrowiak, J. C.; Chaires, J. B. *Biochemistry* **1993**, *32*, 2573-2584. (b) The effect of DNA binding on the 345 nm absorbance of **2** was assessed with calf thymus DNA fragments as described elsewhere, except no organic solvent was present.[4a] Due to its relatively low affinity for DNA, technical limitations did not permit a study of DNA-induced spectral changes with **3**.

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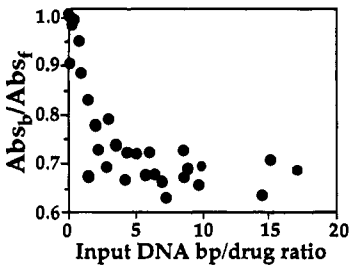

Figure 2. DNA-induced quenching of the 345 nm absorbance of **2**. The ratio of the absorbance of **2** at 345 nm in the presence (Abs_b) and absence of DNA (Abs_f) is plotted relative to the input molar ratio of DNA (bp) to drug.[11b] The data points were derived from three experiments.

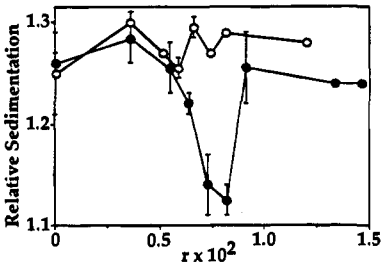

Figure 3. Unwinding of negatively-supercoiled DNA by **2**. The sedimentation of negatively-supercoiled plasmid DNA relative to nicked closed-circular DNA was determined in the presence of **2** (●, 1–6 μM) and **3** (○, 3.6–14.5 μM).[13] The molar ratios of bound drug to DNA bp, r, were calculated from the binding isotherms assuming an initial DNA concentration of 385 μM. The data points are derived from two experiments; error bars represent variation about the mean for duplicate values.

DNA helix upon binding.[12] The effect of **2** and **3** on the helical state of DNA was assessed by the differential sedimentation of negatively-supercoiled plasmid DNA relative to nicked, open-circular DNA in the presence of the drugs.[13] As shown in Figure 3, increasing concentrations of **2** altered plasmid sedimentation in a manner consistent with progressive relaxation of negative superhelical tension followed by induction of positive superhelices. These effects are analogous to the helical unwinding caused by intercalation of the naphthoate of NCS[14] and the anthranilate of ESP A1.[4a] Furthermore, similar levels of bound **3** did not change the sedimentation of the supercoiled DNA (Figure 3).

The third test for intercalation is lengthening of the DNA helix, as judged here by a drug-dependent increase in the viscosity of a DNA solution.[12b] As shown in Figure 4, **2** is capable of increasing the viscosity of the DNA solution, while **3** is not.[15]

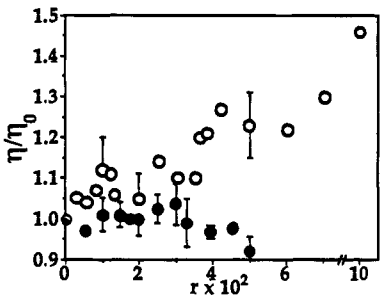

Figure 4. Compound **2** increases the viscosity of a DNA solution. Relative viscosities (η/η_0) were determined for solutions of DNA containing compounds **2** (○) or **3** (●).[15] r is the number of moles of bound drug per mole DNA bp. Data points represent an average of 3–5 flow times; error bars represent the standard deviation or error about the mean for 2–3 experiments.

Any one of the three criteria is insufficient to establish classical intercalation. However, it is unlikely that another DNA binding mode can account for all three observations. To our knowledge, there is no precedent for all three effects occurring with groove-binding small molecules and cross-linking agents.[16] In the case of C-1027, it is likely that the benzoxazolinate intercalates in DNA, given the structural similarity to the anthranilate of ESP A1 and the requirement of the benzoxazolinate for lengthening and unwinding of the DNA helix. As with ESP A1,[4c] intercalation by C-1027 is also supported by the observation of linker-selective nucleosomal DNA damage produced by C-1027 in nuclei,[17] presumably due to the constrained dynamics of DNA wrapped around the histone core.

In summary, we have presented evidence that the benzoxazolinate of C-1027 intercalates in DNA. These observations strengthen the hypothesis that the anthranilate and benzoxazolinate groups represent DNA binding elements of conserved structure and function.

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(15) Viscosity studies were performed with calf thymus DNA (~400 bp average) in an Ostwald capillary viscometer (25 °C), as described previously.[4a] Flow times (t) of solvent (10 mM HEPES/1 mM EDTA, pH 7) without (t_0) and with (t; 500 μg/mL) DNA were used to calculate the specific viscosity, $\eta = (t - t_0)/t_0$, of the DNA solution without (η_0) or with (η) drug.

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