

Interaction of sanguinarine with double stranded RNA structures

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Interaction of sanguinarine with A-form RNA structures of poly(rI).poly(rC) and poly(rA).poly(rU) has been studied by spectrophotometric, spectrofluorimetric, UV melting profiles, circular dichroism and viscometric analysis. The binding of sanguinarine to A-form duplex RNA structures is characterised by the typical bathochromic and hypochromic effects in the absorption spectrum, increasing steady state fluorescence intensity, an increase in fluorescence quantum yield of sanguinarine, an increase in fluorescence polarization anisotropy, an increase of thermal transition temperature, an increase in the contour length of sonicated rod-like RNA structure and perturbation in circular dichroic spectrum. Scatchard analysis indicates that sanguinarine binds to each polymer in a non-cooperative manner. Comparative binding parameters determined from absorbance titration by Scatchard analysis, employing the excluded site model, indicate a higher binding affinity of sanguinarine to poly(rI).poly(rC) structure than to poly(rA).poly(rU) structure. On the basis of these observations, it is concluded that the alkaloid binds to both the RNA structures by a mechanism of intercalation.

Interaction of naturally occurring and synthetic biologically active organic compounds with nucleic acid structures are an active area of research. One important class of these compounds binds to DNA by a mechanism of intercalation. Such compounds are important tools in molecular biology, and some are used for the treatment of cancer in man^{1,2}. Although there have been a few detailed studies on RNA binding mode of organic compounds³⁻¹¹, very little is known about how alkaloid interacts with various structures of RNA. Because of the serious human diseases are caused by RNA viruses, such as HIV and Hemorrhagic fever viruses such as dengue, west nile and ebola¹², attempts have been made to design drugs against these viruses¹²⁻¹⁴.

Sanguinarine (Fig. 1), a naturally occurring benzophenanthridine plant alkaloid has been the focus of recent attention from the standpoint of its diverse biological, DNA binding and photophysical properties¹⁵⁻³². It exhibits excellent antimicrobial, antiplaque, antitubulin and antitumour activities¹⁶⁻²⁰. At the same time, the alkaloid is phototoxic and produces hydrogen peroxide and singlet oxygen^{21,22}. We have demonstrated that sanguinarine iminium form binds to B-form duplex DNA by the mechanism of intercalation with a high preference to GC base pairs²⁴⁻³², while the sanguinarine alkonalamine form does not bind to DNA³¹. The mode, mechanism and

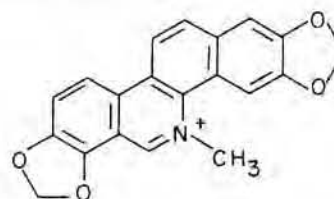


Fig. 1—Chemical structure of sanguinarine

specificity of binding of sanguinarine to DNA structures is now clearly established, but its interactions with RNA structures have been still unexplored. In this paper we have elucidated various spectroscopic and hydrodynamic aspects of sanguinarine-RNA interactions.

Materials and Methods

Sanguinarine chloride was purchased from Aldrich Chemical Co., Milwaukee, WI, USA, and was used after checking its purity by thin layer chromatography and melting point determination and characterizing by mass and NMR spectrum. The alkaloid solution was freshly prepared by dissolving appropriate amounts in acidic buffer and the concentration was obtained spectrophotometrically using a molar extinction coefficient (ϵ) of $30,700 \text{ M}^{-1}\text{cm}^{-1}$ at 327 nm in 0.1 N HCl³³. The alkaloid obeyed Beer's law in the concentration range used.

The RNA polymers poly(rI).poly(rC) and poly(rA).poly(rU) were purchased from Sigma Chemical Co., St. Louis, MO, USA. Each duplex

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polynucleotide was sonicated in a Labsonic 2000 sonicator (B. Braun Swiss) by using a needle probe of 4 mm diameter. After sonication, the polymer was extensively dialyzed under sterile conditions. The average length of sonicated polynucleotide was found to be 280 ± 40 base pairs determined from viscometric measurements as described by Maiti *et al.*²⁴ Their nativeness and purity were tested by using UV spectral characteristics, circular dichroic and T_m analysis²⁵. Their concentrations in terms of nucleotide phosphate were estimated spectrophotometrically^{34,35} using known molar extinction coefficients (ϵ) $5300 \text{ M}^{-1}\text{cm}^{-1}$ at 264 nm for poly(rI).poly(rC) and $7140 \text{ M}^{-1}\text{cm}^{-1}$ at 260 nm for poly(rA).poly(rU). Binding experiments were performed in citrate-phosphate-EDTA (CPE) buffer (4.254 mM citric acid monohydrate, 9.75 mM Na_2HPO_4 anhydrous purified, 0.25 mM EDTA, pH 5.2 ± 0.01). Deionized triple distilled water and analytical grade reagents were used throughout spectrophotometric study.

Absorption spectral studies were performed on a Shimadzu UV-260 spectrophotometer (Shimadzu Corporation, Japan) in matched quartz cells of 1 cm path length as described earlier^{32,36,37}. The decrease in absorbance at 327 nm of sanguinarine on binding to RNA polymers was used to calculate the equilibrium concentrations of free and bound alkaloid. Binding data were cast into the form of Scatchard plot³⁸ of r/C_f versus r , where r is the number of alkaloid molecules bound per mole of nucleotide and C_f is the molar concentration of the free alkaloid. The data were fitted to a theoretical curve, which is drawn according to the excluded site model³⁹ developed by McGhee and von Hippel⁴⁰.

$$\frac{r}{C_f} = K'(1 - nr) \left[\frac{(1 - nr)}{1 - (n-1)r} \right]^{(n-1)} \quad \dots (1)$$

where K' is the binding constant to an isolated RNA binding site and n is the exclusion parameters in nucleotides. To analyze the binding data the programme SCATPLOT, Version 1.2⁴¹ was used which works on an algorithm as described in Nandy *et al.*⁴² to determine the best fit parameters for a nonlinear noncooperative ligand binding system.

Thermal melting study

Melting profiles were also recorded on the Shimadzu UV-260 unit equipped with a temperature programmer (KPC-5) and controller (SPR-5) in

stoppered quartz cells of 1 cm path length, monitoring the absorbance change at each maximum of RNA polymers. The thermal transition temperature, T_m was determined from the first derivative plots of the transition profiles.

Spectrofluorimetric study

Fluorescence measurements were recorded at 22°C on a Hitachi-F-4010 spectrofluorimeter (Hitachi Ltd., Tokyo, Japan) equipped with a thermoelectric temperature controller model EYELA UNICOOL UC-55 (Tokyo Rika Kikai Co. Ltd., Tokyo, Japan). Measurements were made in fluorescence free quartz cells of 1 cm path length as described Nandi *et al.*³⁶. Uncorrected fluorescence spectra are reported. Quantum yields were calculated as described²³.

Fluorescence polarisation anisotropy measurements were carried out as described by Larsson *et al.*⁴³ using:

$$A = (I_{VV} - I_{VH}G)/(I_{VV} + 2 I_{VH}G) \quad \dots (2)$$

where G is the ratio I_{HV}/I_{HH} used for instrumental correction. I_{VV} , I_{VH} , I_{HV} and I_{HH} represent the fluorescence signal for excitation and emission with the polarizer set at $(0^\circ, 0^\circ)$, $(0^\circ, 90^\circ)$, $(90^\circ, 0^\circ)$ and $(90^\circ, 90^\circ)$ respectively.

Spectropolarimetric study

Circular dichroism (CD) spectra were recorded on a JASCO J-720 CD spectropolarimeter attached with a temperature controller and thermal programmer model PTC-343 interfaced with a COMPAQ PC-486 in a rectangular quartz cuvette of 1 cm path length as reported earlier^{36,37}. Spectral measurements were carried out at 20°C and the reported CD spectra were averages of four scans. The ellipticities were expressed in terms of per nucleotide phosphate and the molar ellipticity (θ) was expressed in $\text{deg.cm}^2.\text{dmol}^{-1}$.

Results

Absorption spectral analysis of the sanguinarine-RNA complex

The absorption spectrum of sanguinarine iminium form (Fig. 2) shows three peaks at 327, 340 and 475 nm respectively, with a kink at 351 nm in 20 mM CPE buffer at 20°C . The effect of increasing the concentration of A-form RNA duplexes on the absorption spectrum of sanguinarine shows hypochromism and bathochromism in these bands

until saturation was reached in each case. The observed spectral changes are illustrated in Fig. 2A and B respectively. Three clear isosbestic points are observed at 303, 365 and 498 nm respectively and are indicative of equilibrium between bound and free alkaloid molecules.

Evaluation of binding parameters

The Scatchard plots derived from equilibrium concentrations of free and bound alkaloid in all A-form RNAs were concave upwards and represent more than one type of binding mode. Since we found no sign of sigmoidal behaviour for the occurrence of cooperativity in our system, we adopted the neighbour exclusion model^{39,40} for noncooperative binding phenomena to fit our experimental data. We have also found that such a model adequately fits the data within the regions of Scatchard plot corresponding to the range 30% (lower) to 95% (upper) of each polynucleotide bound alkaloid. Representative

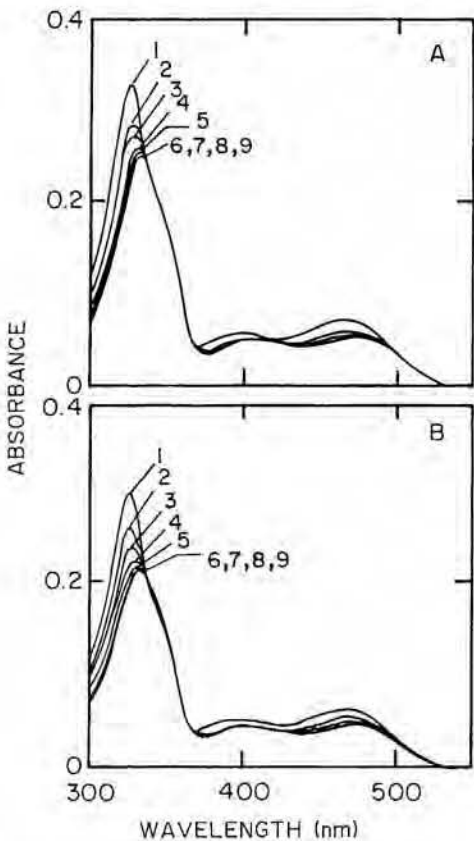


Fig. 2—Representative absorption spectrum of sanguinarine (9.36 μ M, curve 1) treated with (A) 9.86, 19.7, 29.6, 39.4, 49.3, 59.2, 78.9 and 98.6 μ M of poly(rI).poly(rC) (curves 2-9) and (B) 9.86, 19.7, 29.6, 39.4, 59.0, 78.5, 98.6 and 118.0 μ M of poly(rA).poly(rU) (curves 2-9) respectively in 20 mM CPE buffer pH 5.2 at 20°C

binding isotherms for A-form RNAs are illustrated in Fig. 3. The quantitative binding parameters presented on Table 1 indicate that the value of K' for poly(rI).poly(rC) is higher than that of poly(rA).poly(rU) structure.

Fluorescence spectral analysis of the sanguinarine-RNA complex

The characteristic steady-state emission spectrum of sanguinarine iminium form in the region 500 to 650 nm has an emission maximum at 577 nm when excited at 475 nm. A progressive enhancement of the steady state fluorescence of sanguinarine with increasing concentrations of double stranded A-form RNAs was observed (Fig. 4A and B). The relative quantum yield data (ϕ/ϕ_0) calculated for each sanguinarine-RNA complex at a fixed P/D ratio are presented in Table 2. The data indicate that enhancement in quantum yield value upon interaction with RNA structures.

Fluorescence anisotropy or polarization measurements reveal the time-average rotational motion of fluorescent molecules. When the benzophenanthrene chromophore of sanguinarine intercalates into the RNA helix, its rotational motion

Table 1—Binding parameters for the interaction of sanguinarine with RNA structures

[Data represent average of five determinations each]

RNA	$K' \times 10^{-5} (M^{-1})$	n
Poly(rI).Poly(rC)	9.42 ± 0.5	3.25 ± 0.12
Poly(rA).Poly(rU)	5.16 ± 0.4	3.12 ± 0.11

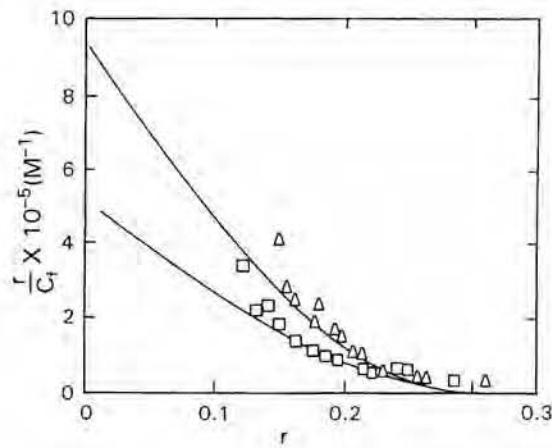


Fig. 3—Representative Scatchard plots of sanguinarine binding to poly(rI).poly(rC) (Δ — Δ) and poly(rA).poly(rU) ($\square$ — $\square$) in 20 mM CPE buffer pH 5.2 at 20°C. [K' and n values are presented in Table 1]

is restricted and hence fluorescence from bound chromophore is polarized. The fluorescence from sanguinarine iminium form in solution is weakly polarized (the value is 0.0332), owing to the rapid tumbling motion of the chromophore in the aqueous medium. The values of anisotropy (A) were evaluated from equation (2) at a particular P/D ratio and are listed in Table 2. The data show a large increase of polarization which indicate restricted orientation of planar aromatic ring of the alkaloid and larger anisotropy suggests better orientation to the binding site. This is possible if the alkaloid is intercalated in the nucleic acid helix.

Circular dichroic spectral analysis of sanguinarine-RNA complex

RNA polymers used showed their respective characteristic dichroic features under the experimental conditions and matched with existing reports⁴⁴⁻⁴⁶. The effects of increasing concentrations of sanguinarine on the CD spectrum RNA structure are shown in Fig. 5A and Fig. 5B respectively. Increase in the positive ellipticity of both the RNA structures upon interaction with sanguinarine was observed. Poly(rI).poly(rC) structure exhibited only one isodichroic point at 210 nm (Fig. 5A), while poly(rA).poly(rU) structure showed three isodichroic point at 238, 250 and 302 nm respectively (Fig. 5B). Extrinsic CD band at 340 nm appeared in both the double-stranded RNA structures.

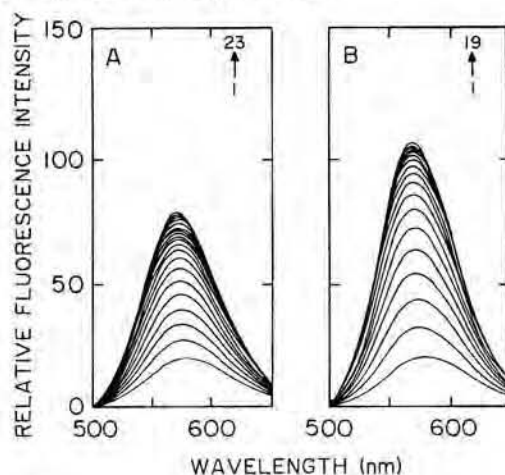


Fig. 4—Representative steady state fluorescence emission spectrum of sanguinarine (9.26 μM , curve 1) treated with (A), 9.2 to 204 μM of poly(rI).poly(rC) (curves 2-23) and (B) 9.2 to 187 μM of poly(rA).poly(rU) (curves 2-19), respectively in 20 mM CPE buffer pH 5.2 at 20°C [Arrow indicates the addition of increasing concentration of RNA. The excitation was fixed at 475 nm. The excitation and emission band passes were 1.5 and 20 nm respectively]

Thermal stabilization of sanguinarine-RNA complex

Further evidence to the strong interaction of the alkaloid to double stranded RNA is obtained from the measurement of the thermal denaturation temperature of the RNA in presence of the alkaloid. The thermal melting profiles (data not shown) show enhancement of thermal melting stability of the alkaloid. The T_m value was determined from the first derivative of each

Table 2—Binding data for the sanguinarine complexation with the RNAs obtained from relative quantum yield values, fluorescence polarization anisotropy values and thermal melting analysis

[Data presented from 5 determination in each case. ^aRelative quantum yield and fluorescence polarization anisotropy monitored at P/D = 8.0. The molecular weight of the sonicated RNA is in the range 2 – 2.5 $\times 10^5$ dalton. Average anisotropy for the free alkaloid is (3.32 $\pm$ 0.02) $\times 10^{-3}$; ^b ΔT_m is the [T_m of bound – T_m of native RNA]. T_m of poly(rI).poly(rC) and poly(rA).poly(rU) and 37.5 and 41°C respectively in 20 mM CPE buffer, pH 5.2. Melting studies in presence of sanguinarine were performed for a D/P ratio of 0.2 in both the cases]

RNA	ϕ/ϕ_a^a	A^a	ΔT_m^b
Poly(rI).Poly(rC)	3.6 $\pm$ 0.3	0.17 $\pm$ 0.02	13.5 $\pm$ 0.5
Poly(rA).Poly(rU)	4.5 $\pm$ 0.3	0.15 $\pm$ 0.02	16.5 $\pm$ 0.5

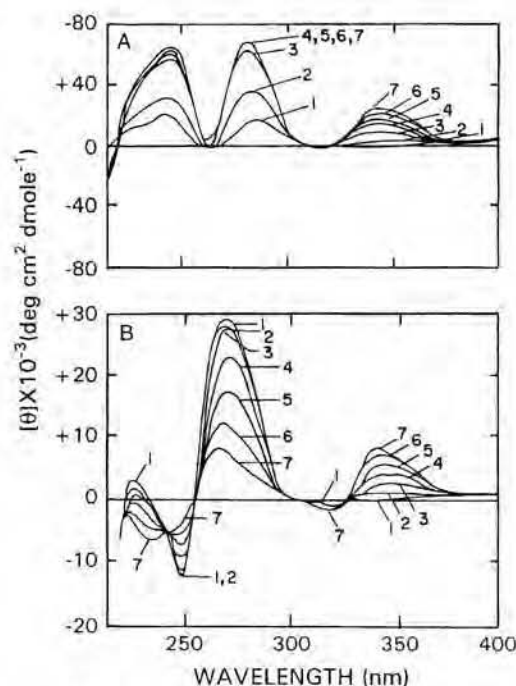


Fig. 5—Representative CD spectrum resulting from the interaction of (A) poly(rI).poly(rC) (38.5 μM , curve 1) treated with 1.14, 2.2, 3.4, 4.46, 5.8 and 7.25 μM of sanguinarine (curves 2-7); and (B) poly(rA).poly(rU) (42.7 μM , curve 1) treated with 1.78, 3.85, 9.0, 14.1, 19.3 and 24.5 μM of sanguinarine (curves 2-7) in 20 mM CPE buffer pH 5.2 at 20°C [The expressed molar ellipticity is based on the RNA concentration]

melting profile (data not shown). Melting profiles indicate a cooperative melting transition with percentage hyperchromicity changes within the normal range of A-form RNA at pH 5.2. At saturation ($D/P \sim 0.2$) the T_m 's of the A-form poly(rI).poly(rC) and of poly(rA).poly(rU) were increased by 13.5° and 16.5°C respectively (Table 2). It was observed that melting profile of each polymer was identical for a heating rate of either 1.0 or 0.5°C indicating that enough time was allowed for thermal equilibration.

Hydrodynamic properties of sanguinarine-RNA complex

The effect of sanguinarine on the viscosity of linear RNA duplexes is reflected by the L/L_0 versus r plots (Fig. 6) which show rise in L/L_0 values with increasing r . The β values evaluated from the slopes of plots of Fig. 6 are presented in Table 3. Extent of length enhancement of RNA duplexes upon binding of sanguinarine was estimated with respect to a standard value ($\beta = 2$ corresponds to a length enhancement of 0.34 nm) and are presented in Table 3. The percent length enhancement is greater in the complex formation with poly(rI).poly(rC) structure compared to poly(rA).poly(rU) structure.

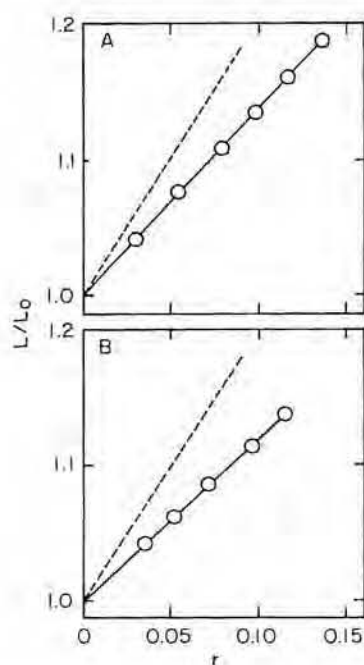


Fig. 6—Plot of increase in helix contour length (L/L_0) versus r for the complexation of (A), poly(rI).poly(rC); and (B), poly(rA).poly(rU) with sanguinarine in 20 mM CPE buffer pH 5.2 at 20°C . [The theoretical line (---) represents a slope (β) of 2 predicted for perfect monofunctional intercalator]

Discussion

The spectroscopic aspects of sanguinarine-RNA complexation show the following quantitative behaviours – the hypochromism and bathochromism of alkaloid absorption spectra, increase in fluorescence quantum yield and polarization anisotropy, perturbation to CD spectra in analogy with earlier reports⁸⁻¹⁰. The quantitative data of binding are higher than those reported for ethidium-RNA interaction. Several drugs like ethidium, propidium, DAPI (4',6-Diamidino-2-phenylindole) have been shown to intercalate with RNA duplexes^{10,11} and models for RNA binding models relative to DNA have been proposed. The major and minor grooves of A-form RNA duplexes, however, differ very significantly from those of B-form DNA^{4,8}. The grooves in RNA have distinctive steric and chemical characteristics, different relative molecular electrostatic potentials^{4,44-46}. The minor groove in the A-form family is shallow and wide, while the major groove is narrow and deep relative to the corresponding DNA grooves. Additionally the unique structural folds present in RNA but not in DNA offer the possibility of much higher recognition specificity by small molecules than that of DNA⁸.

It is known that intercalation of many ligands between neighbouring base pairs changes the secondary structure of nucleic acid and causes an increase in thermal stability of the duplexes^{1,2}. There are various factors that contribute to the stabilizing ability of the ligand on the duplex helical structures. There are: (i), molecular shape of the complex; (ii), van der Waals interaction between ligand with base pairs; (iii), the formation of H-bonded structure by the ligand with base pairs or to the groove of the helix.

The thermal denaturation studies showed that sanguinarine enhanced T_m of poly(rI).poly(rC) and poly(rA).poly(rU). Further, the cooperativity of the

Table 3—Viscometric properties of the binding of sanguinarine with RNA structures

[Data presented from 5 determination in each case. ^aLength enhancement per intercalation site, $L = 0.34$ nm corresponds to the standard value of β is 2 (Lerman 1961); ^bPercent helix length enhancement at $r_{\max}$ of each polymer (L_p %). Relative length enhancement $\Delta L/L_0$ is given by $\Delta L/L_0 = \beta r$ and hence, L_p is calculated from $(\Delta L_{\max}/\Delta L_0) \times 100 = (\beta r_{\max}) \times 100$]

RNA	β	$L(\text{nm})^a$	$L_p(\%)^b$
Poly(rI).Poly(rC)	1.37 ± 0.02	0.23 ± 0.005	19.2
Poly(rA).Poly(rU)	1.20 ± 0.015	0.20 ± 0.002	14.0

melting transition of RNA was unaffected in presence of the alkaloid but a larger hypochromicity of the RNA-sanguinarine complexes as compared to RNA alone was observed, which indicated a contribution of the liberated sanguinarine to the overall absorbance at 260 nm. The value of ΔT_m (T_m of complex- T_m of native RNA) was dependent on the D/P ratio. Table 2 illustrated the values of ΔT_m at D/P of 0.2 for each complex. The results indicated that the alkaloid stabilized poly(rA).poly(rC) more as compared to poly(rI).poly(rU) polymer. The extent of stabilization is well in the range of intercalators binding to poly(rA).poly(rU) studied by Tanious *et al.*¹⁰ and Wilson *et al.*¹¹. Thermal denaturation study adds support to the spectroscopic data indicating intercalative binding mode.

The viscometric technique is well established as a method for investigating the extension of the nucleic acid helix associated with intercalation^{1,2}. The slope β of the L/L_0 versus r plot is a parameter related to the fractional increase in the contour length of the rod-like RNA molecule induced by intercalative agents. The intercalation model given by Lerman⁴⁷ requires that the helix should be extended by an amount equal to the thickness of an alkaloid molecule (0.34 nm) for each intercalation event. So it is reasonable to assume that each intercalated alkaloid molecule makes a contribution of a 0.34 nm to the RNA contour length, equivalent to 0.28 nm for the distance between two base pairs of RNA^{10,45,46}. Generation of an intercalation site in the A-form of RNA structures is quite straight forward and the geometry of the site is similar to DNA intercalation sites¹⁰.

Present data show that the β values and calculated values for helix extension per bound alkaloid (Table 3) for each complex lie well within the range of values (*i.e.*, 0.15 to 0.37 nm) reported for other intercalating ligands¹¹, indicating that sanguinarine binds to these polymers by a mechanism of intercalation. However, recently we have demonstrated that sanguinarine binds to poly(rG).poly(rC) structure in a nonintercalative mechanism under identical experimental conditions⁴⁸.

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References

1. Waring M J (1981) *Annu Rev Biochem* 50, 159-192

2. Wilson W D (1999) in *Comprehensive Natural Products Chemistry* (Kool E T ed), pp 427-476, Elsevier Science Ltd, Oxford
3. Hermann T & Westhot E (1999) *J Med Chem* 42, 1250-1261
4. Niedle S (1999) *Oxford Handbook of Nucleic Acid Structure*, Oxford University Press, Oxford
5. Wong C H, Hendrix M, Manning D D, Rosenbohm C & Greenberg W A (1998) *J Am Chem Soc* 120, 8319-8327
6. Bresloff J L & Crothers D M (1981) *Biochemistry* 20, 3547-3553
7. Kapuscinski J, Darzynkiewicz Z & Melamed M R (1983) *Biochem Pharmacol* 32, 3679-3694
8. Tinoco I J & Bustamauto C (1999) *J Mol Biol* 293, 271-281
9. Babayan Y, Manzini G, Xodo L E & Quadrifoglio F (1987) *Nucl Acids Res* 15, 5803-5812
10. Tanious F A, Veal J M, Buczak H, Ratmeyer L S & Wilson W D (1992) *Biochemistry* 31, 3103-3112
11. Wilson W D, Ratmeyer L, Zhao M, Strekowski L & Boykin D (1993) *Biochemistry* 32, 4098-4110
12. Oldstone M B A (1998) *Viruses, Plaques and History*, Oxford University Press, Oxford
13. Li K, Davis T M, Baily C, Kumar A, Boykin D W & Wilson W D (2001) *Biochemistry* 40, 1150-1158
14. Wilson W D & Li K (2000) *Curr Med Chem* 7, 73-98
15. Preininger V (1975) *The Alkaloids* 15, 207-2621
16. Cordell A G & Farnsworth N R (1977) *Lloydia* 40, 1-44
17. Nandi R, Maiti M, Chaudhuri K, Mahato S B & Bairagi A K (1983) *Experientia* 39, 524-525
18. Kornman K S (1986) in *Producing Dental Plaque Control Workshop* (H Loe & D V Kleinman, eds), pp.121-142, U.K
19. Wolff J & Knipling L (1993) *Biochemistry* 32, 13334-13339
20. Babich H., Zuckerbraun H L, Barber I B, Babich S B & Borenfreund E (1996) *Pharmacol Toxicol* 78, 397-403
21. Turveson R W, Larsson R A, Marley K A, Wang G & Berenbaum, M R (1989) *Photochem Photobiol* 50, 733-738
22. Maiti M & Chatterjee A (1995) *Curr Sci*, 68, 734-736
23. Maiti M, Nandi R & Chaudhuri K (1983) *Photochem Photobiol* 38, 245-249
24. Maiti M, Nandi R & Chaudhuri K (1982) *FEBS Lett* 142, 280-284
25. Maiti M, Nandi R & Chaudhuri K (1984) *Indian J Biochem Biophys* 21, 158-165
26. Nandi R, Chaudhuri K & Maiti M (1985) *Photochem Photobiol* 42, 497-503.
27. Nandi R & Maiti M (1985) *Biochem Pharmacol* 34, 321-324
28. Maiti M & Nandi R (1987) *J Biomol Struct Dyn* 5, 159-175
29. Smekal E, Kobova N & Kleinwachter V (1984) *Stud Biophys* 101, 125-132
30. Faddejeva M D, Belyaeva T N, Rosanev Y M, Sedova M & Sokovskaya E L (1984) *Stud Biophys* 104, 267-269
31. Sen A & Maiti M (1994) *Biochem Pharmacol* 48, 2097-2102
32. Sen A, Ray A & Maiti M (1996) *Biophys Chem* 59, 155-170
33. Jones R R, Harkrader R J & Southard G L (1986) *J Nat Prod* 49, 1109-1111
34. Riley M, Maling B & Chamberlin M J (1966) *J Mol Biol* 20, 359-389
35. Hung S H, Yu Q, Gray D M & Ratliff R L (1994) *Nucl Acids Res* 22, 4326-4334
36. Nandi R, Chakraborty S & Maiti M (1991) *Biochemistry* 30, 3715-3720
37. Ray A & Maiti M (1996) *Biochemistry* 35, 7394-7402
38. Scatchard G (1949) *Ann N Y Acad Sci* 51, 660-672
39. Crothers D M (1968) *Biopolymers* 6, 575-584

- 40 McGhee J D & von Hippel P H (1974) *J Mol Biol* 86, 469-489
- 41 Ray A, Maiti M & Nandy A (1996) *Comput Biol Med* 26, 497-503
- 42 Nandy A, Kumar G S & Maiti M (1993) *Indian J Biochem Biophys* 30, 204-208
- 43 Larsson A, Carlsson C, Jonsson M & Albinson B (1994) *J Am Chem Soc* 116, 8459-8465
- 44 Pullman A & Pullman B (1991) *Q Rev Biophys* 14, 289-315
- 45 Saenger W (1984) *Principles of Nucleic Acid Structure*, Springer-Verlag, New York
- 46 Blackburn M (1990) in *Nucleic Acids in Chemistry & Biology* (Blackburn M & Gait M, eds) Chapt 2, pp.17-70, Oxford IRL Press Ltd., Oxford
- 47 Lerman L S (1961) *J Mol Biol* 3, 18-30
- 48 Das S, Banerjee A, Sen A & Maiti M (2000) *Curr Sci* 79, 82-87