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BINDING OF SANGUINARINE TO DEOXYRIBONUCLEIC ACIDS OF DIFFERING BASE COMPOSITION

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Abstract—The binding of the alkaloid sanguinarine to natural DNAs of differing GC content has been studied by spectrophotometry and viscometry techniques. Binding parameters determined from spectrophotomeric measurements by Scatchard analysis, according to an excluded-site model, indicate a very high specificity of sanguinarine binding to GC rich DNA. In the strong binding region, the increase of contour length of DNA depends strongly on its base composition, being larger with GC rich DNA than with AT rich DNA. It is concluded that the alkaloid binds preferentially to the GC pairs in DNA template.

Sanguinarine, a benzophenanthridine alkaloid, has been reported to possess antitumour and antimicrobial activities [1–3]. It shows pH dependent changes in its absorption and fluorescence spectra [4]. Good evidence for an intercalation model has been obtained from the findings that sanguinarine binding increases the contour length of sonicated rod-like duplex DNA and induces an unwinding–rewinding process of covalently closed supercoiled DNA like ethidium bromide [5, 6]. It has also been observed that the alkaloid-DNA complexes stabilize DNA against thermal strand separation by a significant degree and GC rich DNA exhibits a much higher increase in transition temperature than AT rich DNA [6].

The present study has been undertaken in order to delineate nucleic acid structural features, necessary for binding, from the measurement of absorption spectrophotometry and viscometry in buffer of pH 7.1, where almost all the alkaloid molecules are in cationic form [4].

MATERIALS AND METHODS

Chemically pure sanguinarine sulphate was kindly supplied by Dr. S. B. Mahato of this institute. The alkaloid concentrations were determined spectrophotometrically using the molar extinction coefficient (ε) of 325 nm [4].

Clostridium perfringens DNA (type XII), calf thymus DNA (type I) and *Micrococcus lysodeikticus* DNA (type XI) were obtained from Sigma Chemical Co. (St Louis, MO) and were always tested for their nativeness and purity [7–9]. *Vibrio cholerae* DNA, *Escherichia coli* B DNA and NAG vibrio DNA used in this study were isolated, purified and characterized in our laboratory as described elsewhere [8–10]. DNA concentrations in terms of nucleotide phosphate were determined spectrophotometrically by using molar extinction coefficient (ε) at 260 nm for *Cl. perfringens* DNA ($6300\,M^{-1}\,cm^{-1}$), for calf thymus DNA ($6600\,M^{-1}\,cm^{-1}$), for *V. cholerae* DNA ($6400\,M^{-1}\,cm^{-1}$), for *E. coli* B DNA ($6500\,M^{-1}\,cm^{-1}$), for NAG vibrio DNA ($6300\,M^{-1}\,cm^{-1}$) and for *M. lysodeikticus* DNA ($6900\,M^{-1}\,cm^{-1}$). All experiments were conducted in 0.0015 M tri-sodium citrate + 0.015 M NaCl (0.1 SSC) buffer, pH 7.1. Glass distilled deionised water and analytical grade reagents were used throughout. The alkaloid obeyed Beer's law in the concentration range used in this study.

Absorption spectra of sanguinarine mixed with or without DNA were obtained at 22° using the Zeiss automatic recording spectrophotometer SPECORD UV-VIS (Carl Zeiss JENA DDR) against an appropriately prepared reference sample in 1 cm cuvette. Spectrophotometric titrations of the alkaloid-DNA mixtures were carried out at 22° generally following the method described earlier [5–7, 10, 11]. The changes in absorbance of sanguinarine at 325 nm due to varying amounts of DNA content were recorded in Beckman model 24 spectrophotometer (Beckman Instrument Inc. U.S.A.).

The viscometric measurements of DNA or DNA-alkaloid complex were performed with a type 75 Cannon Manning semimicro viscometer (Cannon Instrument Co. U.S.A.) at 22 ± 0.1° according to the method of Cohen and Eisenberg [12] as described previously [5, 6, 13, 14]. For the viscosity experiments samples of DNA were sonicated [5, 14] to fragments having an estimated molecular weight of approximately 3.5×10^5 [15] by using an MSE sonicator.

RESULTS AND DISCUSSION

The ultraviolet-visible absorption spectrum of sanguinarine was characterized by two maxima at 273 nm and 325 nm. The effect of progressively increasing concentrations of *M. lysodeikticus* DNA on the absorption spectrum of sanguinarine is illustrated in Fig. 1. The spectral changes involve essentially a progressive red shift and hypochromicity in the complex until saturation is reached, indicating

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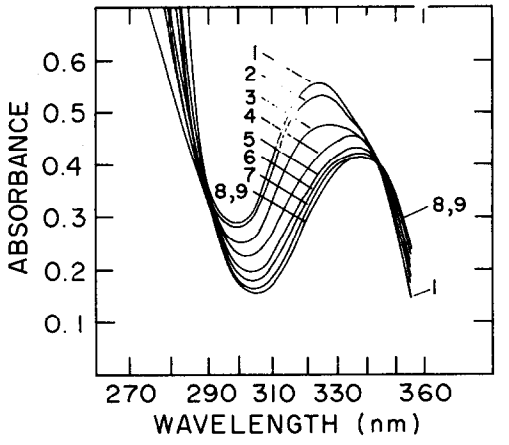

Fig. 1. Absorption spectra of 38 μM sanguinarine in 0.1 SSC buffer, pH 7.1 in the absence of DNA (curve 1) or *M. lysodeikticus* DNA added to yield a nucleotide/alkaloid ratio of 0.2 (curve 2), 0.6 (curve 3), 1.6 (curve 4), 2.8 (curve 5), 3.2 (curve 6), 4.2 (curve 7), 6.0 (curve 8), or 15.0 (curve 9).

possible intercalative interaction between chromophores and the DNA. Figure 2 shows the spectrophotometric titration data for the interaction of sanguinarine with the different DNAs of varying GC content. It can be seen from Fig. 2 that the hypochromicities of alkaloid-DNA complexes are significantly related to the GC content of the DNA.

The binding data were expressed in the form of Scatchard plot [16].

$$\frac{r}{C} = (n - r)\mathrm{K}. \qquad (1)$$

The variables of r (moles of alkaloid bound/mole of nucleotides) and C (the molar concentration of free

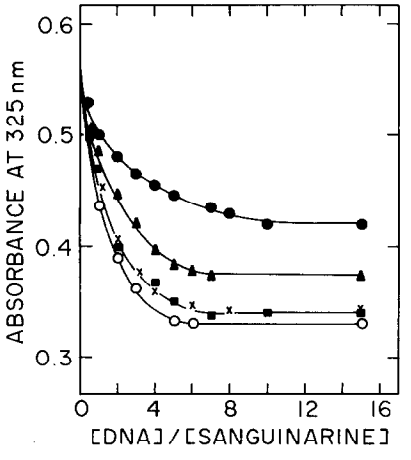

Fig. 2. Spectrophotometric titration data on the binding of sanguinarine (38 μM) to *Cl. perfringens* DNA (●——●), calf thymus DNA (▲——▲), *V. cholerae* DNA (×——×), *E. coli* DNA (■——■) and *M. lysodeikticus* DNA (○——○) in 0.1 SSC buffer pH 7.1. Each point represents the mean of three experiments.

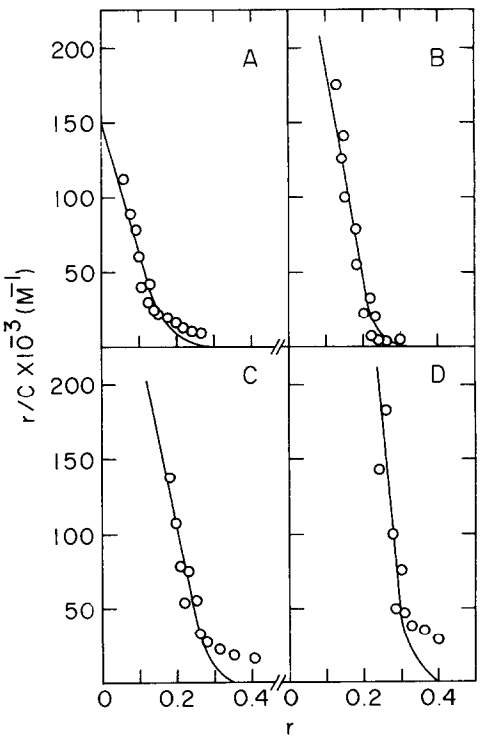

Fig. 3. Scatchard plots of the binding of sanguinarine to DNA of varying GC content in 0.1 SSC buffer pH 7.1. Experimental points are shown with the best fit theoretical line (solid line) obtained by using equation (2). The intercept in the ordinate and the abscissa by the solid curve represents the values of K(0) and $1/n_0$, respectively. (a) *Cl. perfringens* DNA with K(0) = 1.5×10^5 M^{-1} and $n_0 = 3.84$; (b), Calf thymus DNA with K(0) = 3.5×10^5 M^{-1} and $n_0 = 3.33$; (c) *E. coli* DNA with K(0) = 4.2×10^5 M^{-1} and $n_0 = 2.8$; (d) *M. lysodeikticus* DNA with K(0) = 8.0×10^5 M^{-1} and $n_0 = 2.5$.

alkaloid) were calculated according to the method of Peacocke and Skerret [11]. The binding isotherms of sanguinarine to the various DNAs are illustrated in Fig. 3. Experimental data were fitted to the theoretical curve which was drawn according to the excluded-site model developed by McGhee and von Hippel [17] as described previously [13, 18, 19].

$$\frac{r}{C} = \mathrm{K}(0)(1 - n_0 r)\left[\frac{1 - n_0 r}{1 - (n_0 - 1)r}\right]^{n_0 - 1}. \qquad (2)$$

The binding constant of equation (1) is the apparent binding constant, K, of the alkaloid to DNA molecule whereas the binding constant K(0) of equation (2) is the intrinsic binding constant for an isolated site, given by the intercept of the theoretical curve on the axis of r/C. In the corresponding treatment of the Scatchard plot this intercept would be interpreted as K/n_0. The symbol n (equation 1) is r_{max}, the maximum number of sites on the DNA which are available to the intercalator, and n_0 (equation 2) is the number of nucleotides occluded by the binding of a single alkaloid molecule. Two data treatments may be compared by examining r/C in the limit when r approaches to zero, sometimes referred to as the

Table 1. Binding parameters for sanguinarine with DNAs of varying GC content

DNA	GC mole %	K 10^{-6} (M^{-1})	σ 10^{-5} (M^{-1})	K(0) 10^{-5} (M^{-1})	$\sigma(0)$ 10^{-5} (M^{-1})	α^*
Cl. perfringens	30	1.55	2.24	1.50	1.50	
Calf thymus	42	2.24	4.60	3.50	3.50	2.33
V. cholerae	48	2.50	5.50	3.60	3.60	2.40
E. coli	50	2.60	5.98	4.20	4.20	2.80
NAG vibrio	62	2.75	7.97	5.60	5.60	3.73
M. lysodeikticus	72	3.70	11.84	8.00	8.00	5.33

* The ratio is based on the equation $\alpha = \sigma(0)_x/\sigma(0)_{Cl.\ perfringens}$.

affinity of binding [20] and designated σ (for the Scatchard analysis) or $\sigma(0)$ (for the excluded-site analysis). Equations (3) and (4) define these parameters.

$$\sigma = \lim_{r \to 0}\left(\frac{r}{C}\right) = Kn, \tag{3}$$

$$\sigma(0) = \lim_{r \to 0}\left(\frac{r}{C}\right) = K(0). \tag{4}$$

Since they correspond to the intercept on the axis of r/C, they should be in close agreement with one another. The binding parameters are summarized in Table 1. It is interesting to note that the degree of binding of sanduinarine varies with a tendency for DNAs richer in GC to bind the alkaloid more tightly.

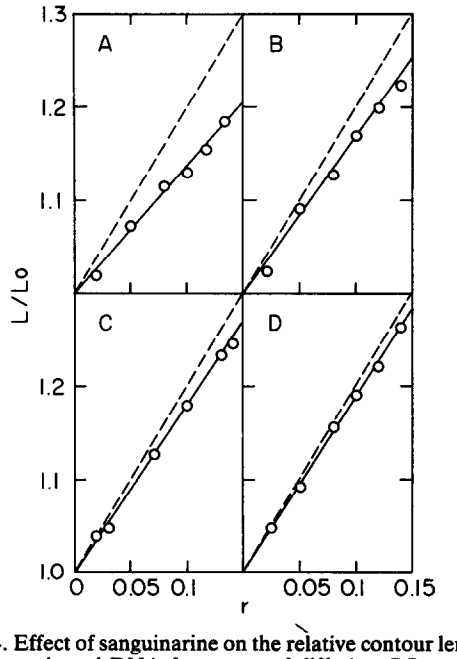

Fig. 4. Effect of sanguinarine on the relative contour length of the sonicated DNA fragments of differing GC content in 0.1 SSC buffer, pH 7.1. The theoretical line (----) represents a value of $\beta = 2$ predicated for an idealized intercalation process. Each point represents the mean of three experiments. (a), *Cl. perfringens* DNA; (b), Calf thymus DNA; (c), *E. coli* DNA; (d), *M. lysodeikticus* DNA.

The dependence of GC on binding of sanguinarine to DNA can be examined in terms of α defined by equation (5) for excluded-site analysis.

$$\alpha = \frac{\sigma(0)_x}{\sigma(0)_y} = \frac{K(0)_x}{K(0)_y}, \tag{5}$$

where suffix x and y are for two different species of DNA. An α value of greater than 2 (Table 1) would, of course, denote GC specificity in binding. Thus, sanguinarine exhibits a definite GC base pair specificity in binding to DNA. The majority of intercalative agents show GC specificity in DNA binding [20–22], and even those that do not (such as ethidium [23]), may exhibit a CG sequence preference in binding [24–27].

The viscometric titrations of sonicated duplex DNA of differing GC content were done by sanguinarine. The increase of sheared DNA helix length after intercalation of sanguinarine can be calculated from the experimental results of intrinsic viscosity according to the approximation [12].

$$\frac{L}{L_0} = \left[\frac{\eta}{\eta_0}\right]^{1/3} = 1 + \beta r, \tag{6}$$

where L and L_0 are the contour lengths of DNA in presence and absence of the alkaloid, respectively, η and η_0 are the corresponding values of intrinsic viscosity (approximated by the reduced viscosity) of the solution, and β is the slope when L/L_0 or $[\eta/\eta_0]^{1/3}$ is plotted against r. The β values obtained from the slope (Fig. 4) are found to be 1.90 ± 0.05 for *M. lysodeikticus* DNA, 1.80 ± 0.05 for *E. coli* DNA, 1.70 ± 0.05 for calf thymus DNA and 1.38 ± 0.05 for *Cl. perfringens* DNA. This technique is well established as a method for investigating the extension of DNA helix associated with intercalation [12, 28–32]. The slope β is a parameter related to the functional increase in the contour length of rod like DNA molecule, induced by intercalation agents. The data obtained with calf thymus DNA is in good agreement with those already published [5, 32]. The main result is that the increase of contour length of DNA on binding to sanguinarine is higher for GC rich than AT rich DNA (Fig. 4). These findings are comparable with the results previously reported for proflavine-DNA complexes [33], where the increase of contour length of duplex DNA depends strongly on its base composition.

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