9) along with the restrictions on M of $M(0) = 0$ and $M'(0) = 0$. Within these limitations, Eq. 13 is a rather general relationship between the disintegration and dissolution of a tablet. Any function M that fits the tablet dissolution data and meets these restrictions may be substituted into Eq. 13 to give an explicit expression for the disintegration.

Another ramification of Eq. 13 is that it would be possible to determine the disintegration profile in real time if k is known by using the output signal from the spectrophotometer or potentiometer that is monitoring the tablet dissolution as input for an analog computer programmed for Eq. 13. The dissolution and disintegration profiles could thereby be determined simultaneously.

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ACKNOWLEDGMENTS

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Binding of 3-Aminoacridinium and 7-Aminoquinolinium Monocations to Double-Stranded DNA: Evidence for Nonlinear Binding Isotherm in Intercalative Region

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Abstract □ The complexations of the singly charged cations of 3-aminoacridine and 7-aminoquinoline by double-stranded, calf thymus DNA were studied by electronic absorption spectrophotometry. At high ratios of total DNA phosphate concentration to total probe concentration (the region associated with intercalative binding), four DNA phosphate units are associated with each bound monocation. However, a binding isotherm based on four DNA phosphate groups in a single binding site does not yield a reproducible equilibrium constant for the binding process. Rather, the sequestration of the monocations by two binding sites, each containing two DNA phosphate units, yields a satisfactory equilibrium expression. This result suggests that the intercalative mode of binding by DNA is not a simple one-step process, which is in agreement with previous kinetic studies. In the region of low DNA phosphate concentration to monocation concentration (the external binding region), the binding is described adequately by a linear isotherm.

Keyphrases □ 3-Aminoacridinium monocations—binding to double-stranded DNA, equilibrium expressions developed □ 7-Aminoquinolinium monocations—binding to double-stranded DNA, equilibrium expressions developed □ Binding—3-aminoacridinium and 7-aminoquinolinium monocations to double-stranded DNA, equilibrium expressions developed □ DNA, double stranded—binding to 3-aminoacridinium and 7-aminoquinolinium monocations, equilibrium expressions developed

Numerous investigators have examined the binding of aminoacridines and aminoquinolines to double-stranded and denatured deoxyribonucleic acid (DNA) (1–8). The aminoacridines have received particular attention because of their well-known antibacterial activity, which has been attributed to their binding by bacterial DNA (9–11). The ability of aminoacridinium compounds to induce frameshift mutations by causing a deletion or insertion of a single nucleotide in the complementary chain of a replicating chromosome is believed to be due to the strong or intercalative binding of these compounds to nucleic acid (12–14).

Aminoquinolines, particularly the 4-amino and 8-amino analogs, have been employed extensively as antimalarial agents (15). Their antimalarial activity has been said to be due to their interaction with the DNA of infectious plasmodia found in malaria-infested mammals (16).

BACKGROUND

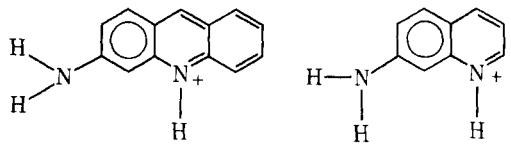
The various interactions of aminoacridines and aminoquinolines with nucleic acids can be classified into two groups: intercalation and external binding (6, 11). Intercalation or strong binding of the aminoacridine or aminoquinoline involves insertion of these compounds, to varying degrees, into the DNA molecule and results in some disruption of the latter (1–3). The intercalative binding of drugs or probes to double-stranded DNA is strongest with compounds possessing three fused aromatic rings, linearly annulated, as in the aminoacridines (6–17). A decrease in the size of the aromatic portion of the intercalating probe results in weaker intercalative binding, as demonstrated by DNA–aminoquinoline complexes (18).

Previous studies (19–23) utilizing temperature-jump relaxation kinetics indicated that the intercalation of the singly charged cations of proflavine and acridine orange may occur in two kinetically discrete steps at high DNA phosphorus to probe ratios. However, this behavior has not been established for all aminoacridine cations. External binding to the double-stranded DNA polyanion occurs after all intercalative binding sites are saturated. The interaction apparently occurs mainly between the cationic probe molecule and the negatively charged DNA phosphate groups and, as such, is primarily an electrostatic interaction (6).

Albert (9) demonstrated that it is the cation of aminoacridine that is necessary for bacteriostasis. The inference is that the cationic form is a necessary condition for intercalation of aminoacridines and aminoquinolines. Therefore, electrostatic forces are believed to provide the initial driving force behind the formation of the DNA–probe complex, prior to intercalation of the probe into the DNA molecule, as well as a substantial portion of the forces that maintain the integrity of the complex (6, 9, 18). However, electrostatic forces are said to be less important in intercalative binding than in the binding of cationic drugs or probes to the exterior of the DNA molecule (5, 6).

Data obtained from investigations of the complexation of small cations to DNA are usually treated according to a model developed by Scatchard (24) to describe the binding of small molecules to proteins. This model was later modified and adapted by other investigators to describe binding to nucleic acids (5, 6, 11, 25).

As a consequence of the pharmacological and physicochemical significance of the complexation of aminoquinolines and aminoacridines to double-stranded and denatured DNA, as well as of an interest in im-



I II

proving the methods employed in developing experimental data obtained from these complexation studies, it seemed desirable to investigate the comparative binding affinities of these compounds. The 3-aminoacridinium (I) and 7-aminoquinolinium (II) monocations were chosen as model compounds since they are not only benzologs of each other but are directly related to the much studied compounds proflavine and acridine orange but are less susceptible to problems associated with molecular aggregation (6, 11, 25–28).

EXPERIMENTAL

Instrumentation—Electronic absorption spectra were taken in 1-cm silica cells on a spectrophotometer[1]. The pH measurements were made on a pH meter[2] with a combination silver–silver chloride glass electrode[1]. Calculations were made on a programmable calculator[3] utilizing an extensive cassette program library. Solutions were mixed on a rotating mixer[4] and an ultrasonic mixer[5]. Solutions of DNA or probe were delivered with micropipets[6].

Materials and Solutions—Aqueous buffered solutions at a specified pH were prepared from analytical grade monobasic and dibasic potassium phosphates[7] in which the ionic strength was maintained at 0.005 *M*. Calf thymus DNA[8] as the A grade sodium salt, containing 8.04% phosphorus and 12.21% nitrogen, was used without further purification. A sufficient amount of the DNA sodium salt was added to 10 ml of buffer solution in a volumetric flask to make a stock DNA solution (1.10×10^{-2} mole of DNA phosphorus/liter).

Solutions of DNA were prepared at the desired pH for each titration and were mixed for 6 hr on a rotating mixer at room temperature. After mixing, the flask containing the stock solution was placed in an ultrasonic mixer for 10 min. These solutions were then divided into 0.5-ml aliquots and stored at 0° for not more than 2 weeks prior to use.

3-Aminoacridine, prepared by a literature method (29), and 7-aminoquinoline[9] were determined to be at least 98% pure according to literature melting-point and molar absorptivity values (29–32).

Methods—Ethanolic solutions ($\sim 1.00 \times 10^{-3}$ *M*) of the compounds to be studied were delivered from a 20-μl micropipet into a 1-cm silica cell containing 2.00 ml of the aqueous buffer solution at the desired pH. This 2 ml of solution was titrated, in the cell, with small increments of the DNA solution at the desired pH. Several micropipets that delivered 5–100 μl reproducibly were employed during each titration. The inherent viscosity of the DNA solution did not appear to be a factor in its reproducible delivery.

The total concentration of DNA phosphorus at each point in the titration was calculated from the dilution of the added DNA stock solution by the 2 ml of test solution. The total concentration of I or II at each point in the titration was calculated from the initial value, taking account of dilution by the volume of added DNA solution. All experiments were conducted at ambient temperature.

Calculations—The concentrations of free base, [B], free monocation, [BH^+], and bound monocation, [BHS_q], in solution were calculated from the simultaneous solution of the equations representing the protolytic equilibrium:

$$K_a = \frac{[H^+][B]}{[BH^+]} \qquad \text{(Eq. 1)}$$

the conservation of mass for the probe I or II:

$$C_t = [B] + [BH^+] + [BHS_q] \qquad \text{(Eq. 2)}$$

and the photometric absorbance at any point in the titration of I or II with DNA:

$$A = \epsilon_B[B]l + \epsilon_{BH^+}[BH^+]l + \epsilon_{BHS_q}[BHS_q]l \qquad \text{(Eq. 3)}$$

Explicitly:

$$[BHS_q] = \frac{\left(\epsilon_B \frac{K_a}{[H^+]} + \epsilon_{BH^+}\right)C_t l - A(1 + K_a/[H^+])}{\left\{\epsilon_B \frac{K_a}{[H^+]} + \epsilon_{BH^+} - \epsilon_{BHS_q}(1 + K_a/[H^+])\right\}l} \qquad \text{(Eq. 4)}$$

$$[BH^+] = \frac{A - \epsilon_{BHS_q}C_t l}{\left\{\epsilon_B \frac{K_a}{[H^+]} + \epsilon_{BH^+} - \epsilon_{BHS_q}(1 + K_a/[H^+])\right\}l} \qquad \text{(Eq. 5)}$$

$$[B] = \frac{(A - \epsilon_{BHS_q}C_t l)K_a/[H^+]}{\left\{\epsilon_B \frac{K_a}{[H^+]} + \epsilon_{BH^+} - \epsilon_{BHS_q}\left(1 + \frac{K_a}{[H^+]}\right)\right\}l} \qquad \text{(Eq. 6)}$$

It was also useful to calculate the ratio directly:

$$\frac{[BHS_q]}{[BH^+]} = \frac{\left(\epsilon_B \frac{K_a}{[H^+]} + \epsilon_{BH^+}\right)C_t l - A(1 + K_a/[H^+])}{A - \epsilon_{BHS_q}C_t l} \qquad \text{(Eq. 7)}$$

K_a = dissociation constant for the drug or probe
$[H^+]$ = hydronium-ion concentration
ϵ_{BHS_q} = molar absorptivity of the bound monocation at the specified analytical wavelength
ϵ_{BH^+} = molar absorptivity of the free monocation at the specified analytical wavelength
ϵ_B = molar absorptivity of the free base at the specified analytical wavelength
l = absorption cell path (1 cm)
A = absorbance at any point during the titration at the specified analytical wavelength
C_t = total concentration of drug or probe

The molar absorptivities of I and II were calculated at each absorption maximum as well as at each distinct vibrational band and agreed with published values (31, 32). The molar absorptivities of the bound monocations of I and II were calculated from the absorbances of solutions of the probes in which pH = pKa − 2 and to which a sufficient excess of DNA was added so that further addition of DNA produced no perceptible changes in the absorption. Since C_t is equal to [BHS_q] when I or II is completely sequestered by the DNA, ϵ_{BHS_q} for I and II may be readily calculated as $A/C_t l$.

The analytical wavelengths at which all calculations were made were 363 and 453 nm for I and 393 nm for II.

The pKa values of 8.04 and 6.65 of I and II, respectively, were taken from the literature (9, 31).

RESULTS AND DISCUSSION

Figures 1 and 2 are representative of the absorption spectra obtained when aqueous solutions of I and II were titrated with double-stranded DNA. Titrations were conducted at five different pH values from 5.90 to 7.90 for I and at three different pH values from 4.90 to 6.40 for II. Figures 1 and 2 contain the electronic absorption spectra of the free drug or probe, three intermediate spectra, and a final spectrum of the DNA–probe complex in which the probe has been sequestered completely by the DNA. Each titration consisted of 13–18 spectral measurements. However, some intermediate spectra were omitted from these figures for clarity.

Calculation of the solution concentrations of the free base, [B], the free monocation, [BH^+], and the bound monocation, [BHS_q], derived from I and II represents an attempt to account for all spectroscopically distinct species derived from the probes throughout the titration with DNA. The advantage of this approach is that the pH of a solution titrated with DNA need not be maintained such that the drug or probe is fully protonated. The ability to vary the pH of the test solution offers the alternative of measuring either BHS_q or B in cases where the spectra of BHS_q and BH^+ overlap to a great extent or when the affinity of BH^+ for DNA is so great that little free BH^+ is in solution.

Moreover, the reproducibility of binding constants at different values of solution pH is a good test of the absence of processes competing with the binding equilibria of interest over a given pH range. Therefore, a wider

[1] Beckman Instruments, Fullerton, Calif.
[2] Orion Research, Cambridge, Mass.
[3] Wang Laboratories, Tewksburg, Mass.
[4] Scientific Industries, Springfield, Mass.
[5] Precision Cells, Hicksville, N.Y.
[6] Unimetrics Universal Corp., Anaheim, Calif.
[7] Mallinckrodt Chemical Corp., Anaheim, Calif.
[8] Calbiochem, LaJolla, Calif.
[9] Courtesy of Dr. D. Jackman, Texas Tech University, Lubbock, Tex.

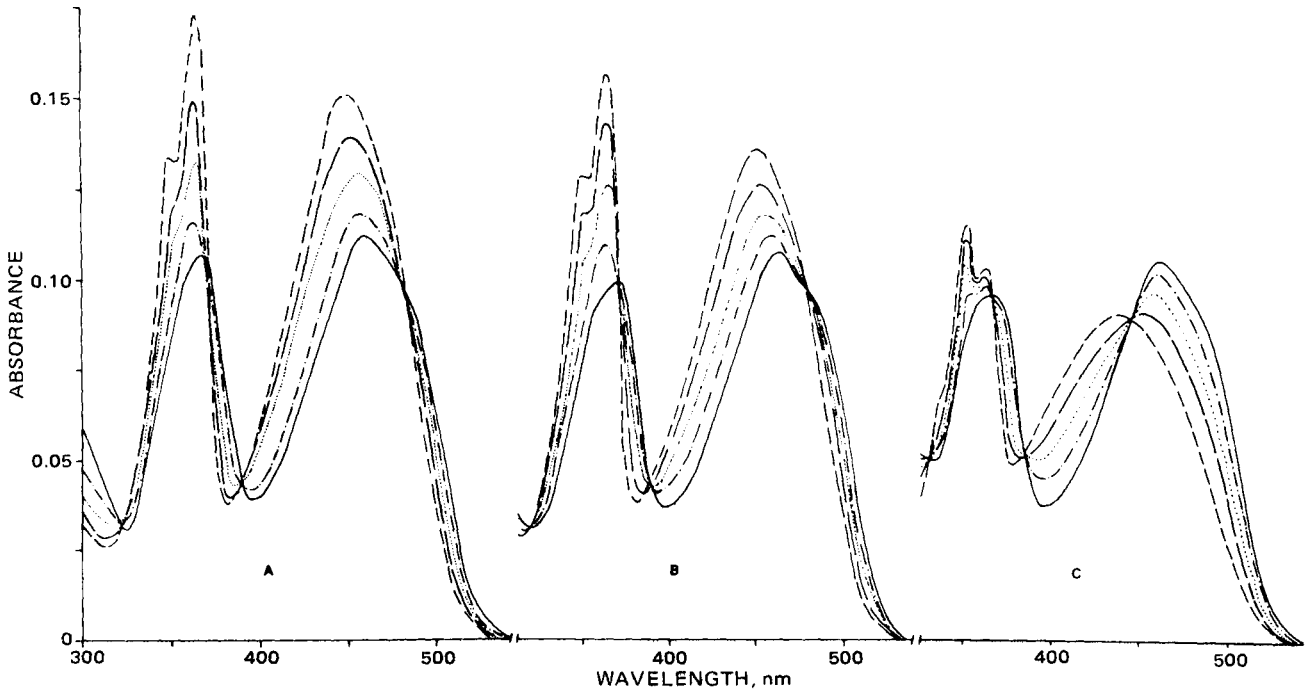


Figure 1—*Variation of the electronic absorption spectra accompanying the titrations of 1.00 × 10^{-5} M I with double-stranded DNA at pH 5.90 (A), 6.90 (B), and 7.90 (C). The* P_t *is the total DNA phosphate concentration at any point during the titration. Key:* $P_t = 0$, –; $P_t = 1.38 \times 10^{-5}$ *mole* P/l, —; $P_t = 4.13 \times 10^{-5}$ *mole* P/l, . . . ; $P_t = 8.19 \times 10^{-5}$ *mole* P/l, –·–; *and* $P_t = 14.9 \times 10^{-5}$ *mole* P/l, *solid line.*

variety of compounds may be utilized to study the complexation behavior of DNA. In studies with double-stranded DNA, this approach is limited to the pH range in which the integrity of the double helix is maintained [*i.e.*, double-stranded DNA becomes denatured at pH values below 4.00 and greater than 11.00 (33)]. Furthermore, at a high pH (pH > pKa), the competition of alkali metal ions with BH^+ for the DNA binding sites may complicate the interpretation of the titration curves.

Stoichiometry of Binding—The number of DNA phosphate groups associated with each bound monocation was estimated by the mole ratio method (34). Plots of absorbance against P_t/C_t (total DNA phosphate concentration/total probe concentration) for I with double-stranded DNA were obtained in acidic solution (pH 5.90). The stoichiometry of binding is apparently four or five phosphates for each bound BH^+. The stoichiometry is in agreement with that obtained by other investigators for different amino-substituted acridines such as proflavine, acridine orange, 9-aminoacridine, and ethidium bromide (5, 6, 35, 36).

The high degree of curvature of the mole ratio plots for II with double-stranded DNA prevented estimation of the binding stoichiometry and suggested that II is more weakly bound by double-stranded DNA than is I. This observation is supported by the weaker association constants calculated for II relative to those calculated for I (Table I).

Methods for Determining Binding Constants—Most available information concerning the binding of the aminoacridines and aminoquinolines to DNA has been gathered using the method first proposed by Scatchard (24) and then modified by Peacocke and Skerrett (5, 6) to explain spectrophotometric data acquired directly or subsequent to separation of the free and bound probes by equilibrium dialysis. In this approach, the average number of moles of probe bound per mole of DNA phosphate, $[BHS_q]/P_t$, is divided by the free protonated probe concentration, $[BH^+]$, and plotted against $[BH^+]$. This plot yields a hyperbolic curve resolvable into two linear components, one of steep slope at high values of $[BHS_q]/P_t[BH^+]$ (low values of $[BH^+]$) and one of shallow slope at low values of $[BHS_q]/P_t[BH^+]$ (high values of $[BH^+]$). The line of steep slope is associated with the intercalative (strong) binding, and the line of shallow slope is associated with external (weak) binding of the probe.

The slope of each line segment is taken to be the negative of the equilibrium constant and the intercept on the vertical axis, the product of the equilibrium constant, and the number of DNA phosphates associated with each mode of binding. It is implicit in this treatment that the number of DNA phosphate groups associated with each mode of binding is equivalent to a single binding site (*i.e.*, regardless of the number of phosphates in each type of binding site, $q = 1$ in BHS_q). Upon careful examination of the data obtained for the strong binding of I and II using this treatment, definite curvature was exhibited in the plots of $[BHS_q]/P_t[BH^+]$ against $[BH^+]$ at low values of $[BH^+]$. This result suggested that the linearity in binding site concentration, implicit in the method of Peacocke and Skerrett (5), might not adequately describe the chemistry of binding in the intercalative region. Therefore, the binding of I and II by DNA was examined by employing an empirical mass action approach.

For the systems under study, the binding equilibria may be expressed as:

$$BH^+ + qS \rightleftarrows BHS_q$$

Scheme I

where BH^+ is the free I or II monocation, q is the number of DNA binding sites that complex one BH^+, and S is the free DNA binding site, which may contain $1, 2, 3, \ldots, m$ DNA phosphates. The binding sites are defined in terms of DNA phosphate since the formal concentration of the DNA and the stoichiometry are expressed in these terms. Although binding sites on the exterior of the DNA may differ physically and chemically from intercalative (internal) binding sites, each is defined, for the present, in terms of the number of DNA phosphates engaged in binding since DNA phosphate is their common component.

The association constant, K, may be determined from:

$$K = \frac{[BHS_q]}{[BH^+][S]^q} \qquad \text{(Eq. 8)}$$

Table I—Binding Constants for the Association of I and II at Different pH Values with Double-Stranded DNA[a]

I			II	
pH	$K_1 \times 10^{-9}$	$K_2 \times 10^{-5}$	pH	$K_1 \times 10^{-6}$
5.90	6.8 ± 0.5	0.97 ± 0.04	4.90	4.2 ± 0.1
6.40	8.0 ± 0.5	1.3 ± 0.3	5.40	6.5 ± 0.7
6.90	8.3 ± 0.6	1.0 ± 0.06	6.40	6.2 ± 0.6
7.40	8.9 ± 0.7	1.0 ± 0.1		
7.90	7.0 ± 0.7	—		

[a] The constants in the intercalative (strong-binding) region of the titration of the probe with DNA (K_1) were calculated from the expression:

$$K_1 = \frac{[BHS_q]}{[BH^+](P_t/2 - [BHS_q])^2}$$

The constants in the external (weak-binding) region of the titration were calculated from the expression:

$$K_2 = \frac{[BHS_q]}{[BH^+](P_t/3 - [BHS_q])}$$

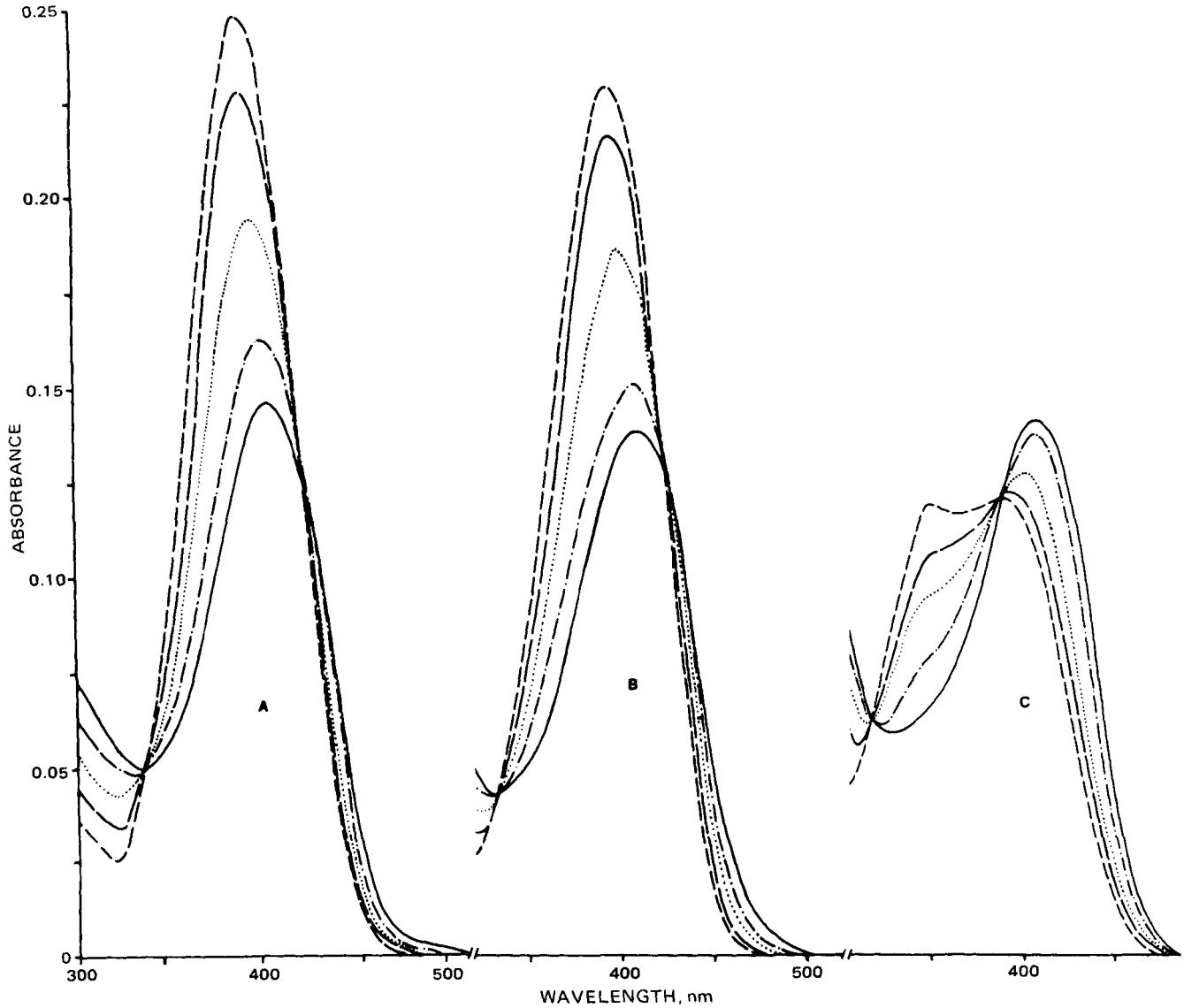


Figure 2—*Variation of the electronic absorption spectra accompanying the titrations of 1.00 × 10^{-5} M II with double-stranded DNA at pH 4.90 (A), 5.90 (B), and 6.90 (C). The* P_t *is the total DNA phosphate concentration at any point during the titration. Key:* P_t *= 0, –; P_t = 52.4 × 10^{-5} mole* P/l, —; P_t = *100 × 10^{-5} mole* P/l, . . . ; P_t = *183 × 10^{-5} mole* P/l, – –; *and* P_t = *285 × 10^{-5} mole* P/l, *solid line.*

The equilibrium concentration of free binding sites, in a given class [S], is evaluated by:

$$S_t = [S] + S_c \qquad \text{(Eq. 9)}$$

where S_t is the total concentration of DNA binding sites in moles per liter and S_c is the equilibrium concentration of complexed DNA binding sites in moles per liter. If q is the number of occupied sites, S_c, in each complex of a given class, then:

$$S_c/q = [\mathrm{BHS}_q] \qquad \text{(Eq. 10)}$$

and since P_t is the total concentration of DNA phosphate in moles per liter:

$$S_t = P_t/m \qquad \text{(Eq. 11)}$$

where m is the number of DNA phosphates in a binding site. Then [S] becomes:

$$[S] = (P_t/m - q[\mathrm{BHS}_q]) \qquad \text{(Eq. 12)}$$

Substituting Eq. 12 into Eq. 8 yields:

$$K = \frac{[\mathrm{BHS}_q]}{[\mathrm{BH}^+](P_t/m - q[\mathrm{BHS}_q])^q} \qquad \text{(Eq. 13)}$$

which, upon rearrangement, becomes:

$$[\mathrm{BHS}_q]/[\mathrm{BH}^+] = K(P_t/m - q[\mathrm{BHS}_q])^q \qquad \text{(Eq. 14)}$$

Values for [BH^+] and [BHS_q] were calculated at each point in the titration using methods described under *Experimental.*

The appropriate values for m and q were empirically determined by solving for K in Eq. 13 using probable combinations of the two constants based on the approximate stoichiometry obtained from the mole-ratio plots (the stoichiometric number of DNA phosphates bound must equal $m \times q$). Combinations of q = 1 and m = 1–6, q = 2 and m = 1–4, q = 3 and m = 1–3, and q = 4 and m = 1, 2 were attempted to fit the data of the titrations to Eq. 13. The combination of q = 2 and m = 2 gave the best fit of K to the data points in the intercalative region. In the region generally associated with external binding, the combination of q = 1 and m = 3 gave the most reproducible value of K. The results of these calculations are shown in Table I.

Equation 14 can be expressed in logarithmic form such that:

$$\log [\mathrm{BHS}_q]/[\mathrm{BH}^+] = \log K + q \log (P_t/m - q[\mathrm{BHS}_q]) \qquad \text{(Eq. 15)}$$

This equation allows a rapid graphical estimate of the suitability of estimated values of m and q over an extended titration range. Log [BHP]/[BH^+] against log ($P_t/m - q[\mathrm{BHS}_q]$) was plotted for I and II, with double-stranded DNA, using the empirically chosen value of 2 for both

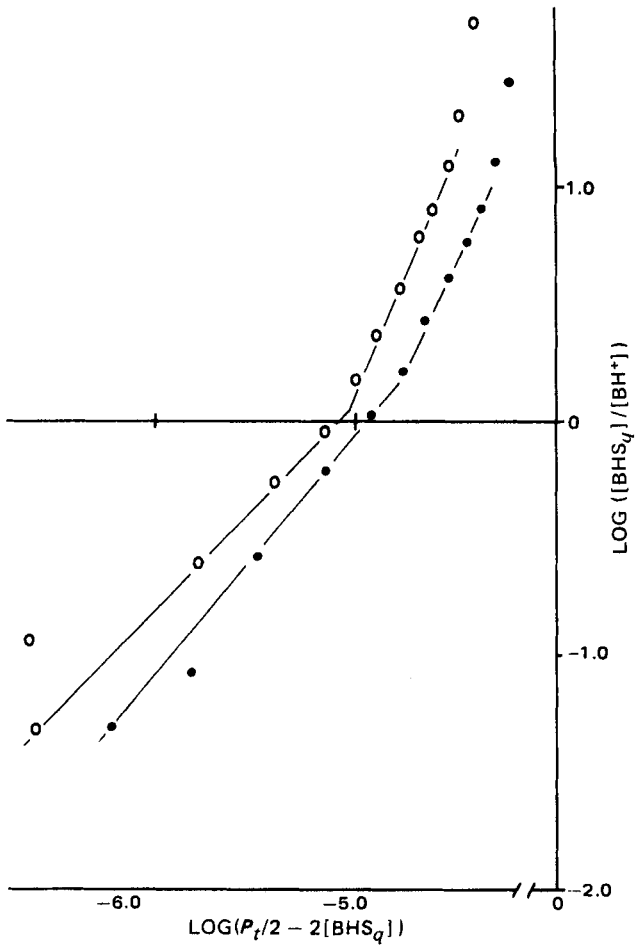


Figure 3—*Logarithmic plots of the ratio of bound monocation [BHS$_q$] to free monocation [BH$^+$] against (P_t/2 − 2[BHS$_q$]) for I titrated with double-stranded DNA at pH 6.40 (○) and 7.40 (●).*

m in the P_t/m term and q in the q[BHS$_q$] term. The slope of the plot should be 2 and the intercept should be log K where $m = q = 2$ correctly represents the binding isotherm.

Representative results for I with double-stranded DNA are found in Fig. 3. This figure exhibits two distinct regions of linearity. The linear region of least slope corresponds to the external binding region of each titration, although this line segment has no rigorous physical significance when m and q are chosen as 2. The linear region of greatest slope corresponds to the intercalative region of each titration. The presence of two regions of linearity strongly suggests that there are at least two separate modes of binding. One occurs at the beginning of the titration at concentrations of I such that only external binding sites on the DNA molecule are available. The other occurs near the latter part of the titration at total concentrations of I such that intercalative binding could predominate (*i.e.*, $P_t/([BH^+] + [BHS_q]) > 10$).

The slopes of these two linear regions, $q = 2.0 \pm 0.3$ and $q = 1.00 \pm 0.04$, indicate that the nature of the binding site differs in each mode of binding. The poor precision in the former slope may indicate overlap of the two modes of binding when the free binding site concentration is high. If $q = 2$ is substituted in Scheme I:

$$BH^+ + 2S \rightleftarrows BHS_2$$

Scheme II

However, the binding stoichiometry of I to double-stranded DNA was determined to be four or five DNA phosphates per BH$^+$. This result can be accounted for by considering that one BH$^+$ may be complexed by two binding sites, each containing two phosphate groups, in separate steps. Scheme II will then be equivalent to:

$$BH^+ + 2[PO_4]_2 \rightleftarrows BH([PO_4]_2)_2$$

Scheme III

where $[PO_4]_2$ is conceived as representing one binding site. Kinetic studies demonstrated that the binding of proflavine and acridine orange to

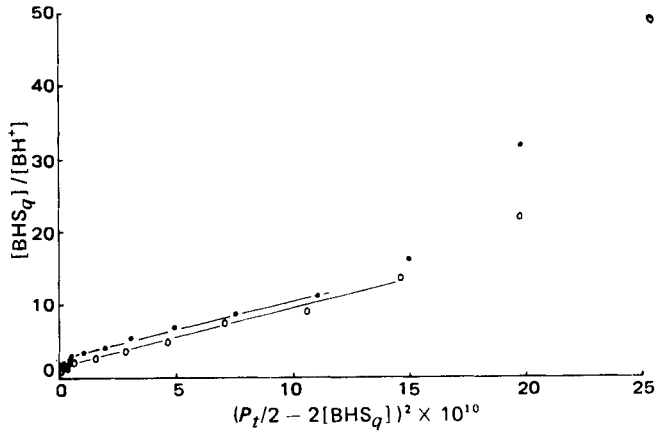


Figure 4—*Linear plots of the ratio of bound monocation [BHS$_q$] to free monocation [BH$^+$] against (P_t/2 − 2[BHP])^2 for I titrated with double-stranded DNA at pH 6.40 (○) and 7.40 (●).*

double-stranded DNA occurs in two discrete steps at concentrations of probe and DNA such that only intercalative binding can occur (19–23).

The mathematical model of the binding equilibrium in the intercalative region, represented in Scheme III, does not establish a detailed mechanism for the interaction. Moreover, the physical significance of the occurrence of two DNA phosphates in each intercalative binding site is not clear from the present study. The diphosphate units are not independently diffusing entities and, on the DNA polyanion, may actually represent two singly charged phosphate groups, two adjacent bases on one DNA strand, or one DNA base pair on complimentary strands.

Furthermore, it is even possible that the two diphosphate units represented in Scheme III may not represent identical physical entities but may actually be a combination of two different binding sites of the types just mentioned. For example, the intercalative process could entail a diffusion-controlled encounter of a monocation with the phosphate backbone of DNA, with a strong electrostatic interaction occurring between the cation and two neighboring phosphate groups. As suggested by the experiments of Li and Crothers (19), the externally bound cation could then move, in a second discrete step, into the interior of the double helix. This would require that two more DNA phosphates (and their associated bases) would be removed from the pool of "free" phosphate groups and thereby account for the four phosphates associated with each intercalated monocation. However, at equilibrium, the interaction with the DNA base pairs may actually be more important than the interaction with the phosphate groups.

The complexation of II by double-stranded DNA was comparable to that observed for I. Empirically determined values for m and q were the same as those for I under the same conditions ($m = q = 2$). However, values for the slopes of the logarithmic plots were not as consistent as those calculated for I. The correlation coefficients, although still indicating that the slopes drawn correlate to the equation for a straight line, were not quite as good, nor were the errors as small, as those obtained for I. Moreover, it was not possible to observe a well-defined region of weaker external binding for II. Possibly this is because external binding is so weak that it does not occur to a significant degree with the concentrations of reagents and buffer species employed in the present experiments.

The adherence of the binding reaction to an isotherm linear in the concentration of the binding site appears to be valid in the beginning of the titration of the monocations with DNA where saturation of the intercalative binding sites of double-stranded DNA by the monocations exists. The available DNA binding sites in this region of the titration contain three DNA phosphates.

General Power Curve, $y = Kx^b$—A nonlinear regression analysis was conducted at each pH to test the two regions of linearity observed in the logarithmic plots obtained from titrations of I and II with double-stranded DNA. The data points constituting the region of greatest slope in these plots followed the equation of the power curve and:

$$[BHS_q]/[BH^+] = K(P_t/2 - 2[BHS_q])^b \qquad \text{(Eq. 16)}$$

The power $b \simeq q \cong 2.0 \pm 0.2$ while the average correlation coefficient was 0.996 ± 0.003. This result supports the conclusion that the power, q, cannot be neglected when writing equilibrium expressions to describe the strong complexation (intercalation) of I and II and perhaps other aminoacridines and aminoquinolines to double-stranded DNA. The data

points comprising the region of least slope in these logarithmic plots also followed Eq. 16, with $b \cong a \cong 1 \pm 0.1$. The average correlation coefficient was 0.997 ± 0.002.

Linear Plots—To confirm the significance of the exponent q in the equilibrium expression describing the intercalative binding region, elements of Eq. 14 were plotted against each other. Linear plots of $[BHS_q]/[BH^+]$ against $(P_t/2 - 2[BHS_q])$ were drawn in which the power, q, was set equal to 2 for each pH at which the titrations were conducted. Figure 4, which is representative, shows that the linear plots exhibit a definite region of linearity, corresponding to the intercalative region of the titration.

A linear regression analysis of the linear portions in these plots revealed an average correlation coefficient of 0.997 ± 0.002 for I titrated with double-stranded DNA; the value for a similar titration of II was 0.991 ± 0.006. The results indicate that Eq. 14 fits well the experimental data obtained from the titrations of I with double-stranded DNA, when q is set equal to 2. The experimental fit of the data obtained from similar titrations with II, however, is more approximate.

CONCLUSIONS

The complexation of I and II by double-stranded DNA at high P_t/C_t (total DNA phosphate concentration/total probe concentration) values may be described by a nonlinear binding isotherm in which one monocation is sequestered in at least two successive steps by two separate entities on the DNA polymer, each containing two phosphate groups. This process may be described by Scheme III.

However, the complexation of the same compounds by double-stranded DNA, having only external binding sites vacant, evidently follows a linear binding isotherm in which one monocation is complexed by one binding site containing three phosphate groups. The latter binding process may be described by:

$$BH^+ + [PO_4]_3 \rightleftarrows BH([PO_4]_3)$$

Scheme IV

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