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Involvement of Nucleic Bases in the Quenching of the Fluorescence of Acridine by Methylviologen

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Hybrid molecules 5a–5c having a 9-acridinyl moiety connected to a viologen moiety through alkyl chains of three, six and nine carbons, respectively, were synthesized. The acridine and viologen rings of 5a were stacked in an aqueous solution. The other compounds, 5b and 5c, exist in a conformation separating the acridine and viologen units. Using absorption changes of the acridine chromophore of 5a-5c in the presence of calf thymus DNA, binding constants were determined to be 10^5–10^6 M^{-1} even at high salt concentrations (0.1–0.3 M). The binding affinity and the number of the associated counterions with the DNA complex of 5a were smaller than those of 5b and 5c. This is in agreement with the molecular model consideration in which the methylene chain of 5a was too short to allow interaction of the viologen with the phosphate anions of DNA. Electrochemical study of the viologen units of 5a–5c in the presence of DNA also supported the binding behavior obtained by the spectrophotometric method. Fluorometric study revealed that the quenching of the fluorescence of the acridine by the viologen is enhanced on the DNA matrix.

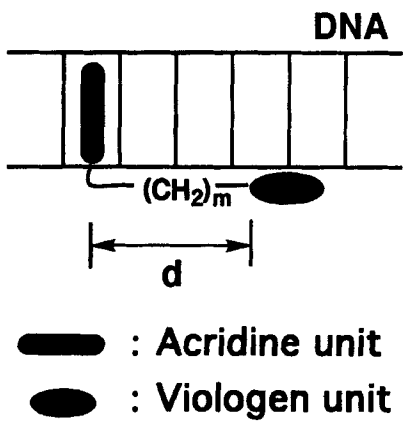
Keywords: Acridine intercalator, methyl viologen, DNA, fluorescence quenching

INTRODUCTION

Intercalators and groove binders are the compounds which bind in the cavities created by base pairs and the minor groove of DNA, respectively [1]. Recently, hybrid molecules having both intercalating and groove binding moieties within the same molecule were studied by Bailly and co-workers [2] from a viewpoint of the development of more effective anticancer agents. These hybrids demonstrated both high affinity and selectivity for the proper DNA sequence [2]. In these studies, netropsin or distamycin analogues were used in the part of groove binding moiety in the hybrid molecules [2]. Methylviologen is another well-known groove binder [3]. Although it does not show DNA sequence selectivity, because of the lack of the hydrogen bonding sites, its unique redox activity found interesting applications. For example, DNA was cleaved by a methylviologen anion radical generated by the photoinduced electron transfer from the guanine base and by a superoxide radical generated by reoxidation of one electron-reducted viologen [4, 5]. Another is the study of the photoinduced electron transfer to methylviologen acceptor from ethidium donor on the DNA matrix [3]. However, the methylviologen and ethidium were stochastically arranged on DNA in such studies, and the

distance between them could not be precisely regulated [3].

In this paper, we synthesized 9-acridinyl intercalators connected to a viologen moiety through alkyl chains of three, six and nine carbons (**5a–5c**) and studied their DNA-binding properties. It is known that acridine can undergo a photoinduced electron transfer to viologen intermolecularly [4]. When these two units are joined together, the distance between them will be important for the efficiency of the photoinduced electron transfer. CPK model building shows that alkyl chains of three, six and nine carbons of these compounds separate the acridine and the viologen by 4.8, 8.8 and 12.8 Å or cover 3, 4 and 5 base pairs, respectively, when bound to DNA [6] (Scheme 1). Thus, the interaction and subsequent reaction of the two units



SCHEME 1

may be carried out in a controlled fashion on the DNA matrix. This could lead to an increase in the efficiency and specificity of the reaction. With this goal in mind, we undertook the following basic experiments.

EXPERIMENTAL SECTION

Apparatus

Melting points are uncorrected. NMR spectra were recorded on a Jeol PMX60SI or Jeol GSX-400 spectrometer operating at 60 and 400 MHz, respectively, using tetramethylsilane as internal standard. Electronic absorption spectra were recorded on a Hitachi U-3210 UV-visible spectrophotometer equipped with a temperature controller Hitachi SPR-10 and a 1 cm pathlength quartz cell. Fluorescence emission spectra were recorded with a Hitachi F-4010 spectrofluorometer. Cyclic voltammograms and differential pulse voltammograms were obtained with a BAS-50W electrochemical analyzer. Glassy carbon working electrodes (ID 3.0 mm) were used together with Pt wire as the counter electrode. Ag/AgCl was used as the reference electrode.

Experimental Procedures

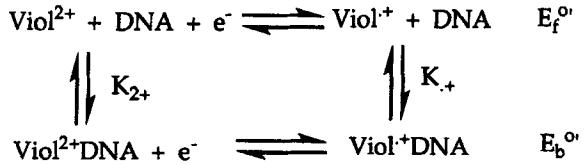
Experiments were conducted in MES buffer [10 mM 2-(N-morpholino)ethane sulfonic acid, 1 mM EDTA] adjusted to pH 6.25 with NaOH. Where necessary, NaCl was added to this buffer. Calf thymus DNA was obtained from Sigma Chemical Co. and, following sonication, was used in the experiments [7, 8]. Poly[d(A-T)]$_2$ was obtained also from Sigma.

The pKa of compounds **5a–5c** were determined at 25 °C as previously described [9]. The pH of a solution of 20 μM **5a**, **5b**, or **5c** in 5.7 mM citric aid, 5.7 mM KH_2PO_4, 5.7 mM boric acid, and 5.7 mM barbital was adjusted with 7 M sodium hydroxide. The volume of sodium hydroxide was kept less than 0.1 % of the total volume. Absorbance of the solutions was monitored at 424 nm as a function of pH.

Extinction coefficients and binding constants of **5a–5c** were determined as previously described [8, 10]. A concentrated solution of **5a–5c** was mixed with the buffer in a cuvette, and absorption spectra were recorded from 600 to 300 nm. Small volumes of a solution of concentrated DNA were then added to the **5a–5c** solutions and the absorption spectra rescanned.

Synthesis

Homogeneity of the compounds was established by TLC on silica gel with a fluorescence indicator (Kieselgel PF 254). Compounds **5a–5c** were synthesized according to the route of Scheme 2.



SCHEME 2

9-Chloro-2-methoxy-6-phenoxyacridine (1)

To a heated solution of 80 g of phenol at 100 °C were added 3.2 g (80 mmol) of sodium hydroxide. After the latter went into solution, a 15 g (54 mmol) portion of 6, 9-dichloro-2-methoxyacridine was added to the solution. The mixture was heated at 100 °C for 90 min. After being cooled, the solid formed was filtered, washed with water and acetone successively. Compound **1** was obtained as a yellow solid (31.3 g, 86%): mp 157–158 °C (lit[11] 158–159°C), ^{1}H-NMR (DMSO-d_6) δ 3.96 (3 H, s) and 6.82–8.64 (11 H, m) ppm.

Bromoalkylamines (2)

3-Bromopropylamine hydrobromide (**2a**) was purchased from Aldrich. 6-Bromohexylamine hydrobromide (**2b**) and 9-bromononylamine hydrobromide (**2c**) were synthesized by the Gabriel method [12]. An example is described below for **2b**.

Phthaloyl-6-Bromohexylimide

To a solution of 20 ml of DMF were added 14.6 g (60 mmol) of 1,6-dibromohexane and 7.4 g (40 mmol) of potassium phthalimide. The resulting solution was heated at 120 °C for 30 min. The precipitate formed was filtered off. After removing the DMF under reduced pressure, the excess of 1,6-dibromohexane was removed from the residue under vacuum (150 °C/1 mmHg). The residual oil was chromatographed on silica gel (Merck 60, chloroform eluent). The product was obtained as a white solid (7.3 g, 59 %), mp 56–57 °C, ^{1}H-NMR ($CDCl_3$) δ 1.16–2.32 (8 H, m), 3.40 (2 H, t, J=7.0 Hz), 3.66 (2 H, t, J=7.0 Hz), 7.67–7.89 (4 H, m) ppm. Phthaloyl-9-nonylimide was synthesized analogously as a white solid (46 %), mp 32–33 °C, ^{1}H-NMR ($CDCl_3$) δ 1.00–2.32 (14 H, m), 3.06 (2 H, t, J=7.0 Hz), 3.83 (2 H, t, J=7.0 Hz), 7.53–7.84 (4 H, m) ppm.

6-Bromohexylamine Hydrobromide (2b)

To a solution of 100 ml of acetic acid and 50 ml of 47 % HBr were added 7.0 g (22.6 mmol) of phthaloyl-6-bromohexylimide. The resulting solution was refluxed for 8 hr. The solution was poured into 200 ml of ice-water and filtered. The filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in a small amount of methanol and poured into ether (1 1). The solid obtained was dried under reduced pressure to yield 3.0 g (51%), mp 113–114 °C, ^{1}H-NMR (DMSO-d_6) δ 1.16–2.32 (8 H, m), 2.80–3.64 (4 H, m) and 7.64–8.32 (3 H, m) ppm. 9-Bromononylamine hydrobromide (**2c**) was synthesized analogously as a white solid (40 %), mp 114–115 °C, ^{1}H-NMR (DMSO-d_6) δ 1.00–2.32 (14 H, m), 2.64–3.32 (2 H, m), 3.50–4.00 (2 H, m) and 7.67–8.67 (3 H, m) ppm.

6-Chloro-2-Methoxy-9-[(3-Bromopropyl) Amino]Acridine (3a)

To a heated solution of 80 g of phenol at 120°C were added 31 g (93 mmol) of **1** and 20 g (93 mmol) of **2a**. The mixture was heated at 120°C for 2 hr. After being cooled, the mixture was poured into 1 1 of ether and the precipitate formed was filtered, washed with ether. Compound **3a** was obtained by recrystallization from methanol as a yellow solid (17.6 g, 50%), mp 209–210 °C (lit[13] 210–211 °C), ^{1}H-NMR (DMSO-d_6) δ 2.50–3.00 (2 H, m), 3.84–4.06 (2 H, m), 4.16 (3 H, s), 4.11–4.46 (2 H, m)

7.58–8.76 (6 H, m) and 9.66–10.16 (1 H, m) ppm. Compounds **3b** and **3c** were synthesized analogously: **3b**, yellow solid (55%), mp 89–90°C, ^{1}H-NMR (DMSO-d_6) δ 1.16–2.32 (8 H, m), 3.16–4.00 (4 H, m), 4.13 (3 H, s), and 7.15–8.20 (6 H, m) ppm; **3c**, yellow solid (64 %), mp 92–93°C, ^{1}H-NMR (DMSO-d_6) δ 1.00–2.32 (14 H, m), 3.50–3.83 (2 H, m), 4.00–4.45 (2 H, m), 4.16 (3 H, s) and 7.52–8.16 (6 H, m) ppm.

4-(4'-Pyridyl)-1-Methylpyridinium Iodide (4)

A solution of 2.0 g (12.8 mmol) of γ,γ'-bipyridyl and iodomethane was refluxed in 100 ml of ethyl acetate for 2 hr. The precipitate formed was collected by filtration and then recrystallized from ethanol. Compound **4** was obtained as a yellow solid (1.9 g, 49%), mp 253–254°C, ^{1}H-NMR (DMSO-d_6) δ 4.47 (3 H, s) and 8.04 (2 H, brd, J=7.0 Hz), 8.72 (2 H, brd, J=7.0 Hz), 8.90 (2 H, brd, J=7.0 Hz), 9.10 (2 H, brd, J=7.0 Hz) ppm.

9-Acridinylviologens (5)

To a solution of 50 ml of DMF were added 1.0 g (2.7 mmol) of **3a** and 0.80 g (2.7 mmol) of **4**. The mixture was heated at 90 °C for 6 hr. After being cooled, the solid formed was filtered. Compound **5a** was purified by recrystallization from methanol as a yellow solid (0.60 g, 28%), mp 253–254°C, ^{1}H-NMR (DMSO-d_6) δ 2.65–2.79 (2 H, m), 3.96 (3 H, s), 4.30–4.40 (2H, m), 4.46 (3 H, s), 4.85–4.91 (2 H, m), 7.53 (1 H, d, J=9.1 Hz), 7.65 (1 H, dd, J=9.5, 0.8 Hz), 7.83 (1 H, d, J=9.1 Hz), 7.89–7.99 (2 H, m), 8.55 (1 H, d, J=9.1 Hz), 8.74 (2 H, d, J=6.8 Hz), 8.75 (2 H, d, J=6.8 Hz), 9.31 (2 H, d, J=6.8 Hz), 9.41 (2 H, d, J=6.8 Hz) and 9.59 (1 H, brs) ppm. Anal. Calcd. for $C_{28}H_{28}N_4OBr_2ClI \cdot 1.5\ H_2O$: C, 42.80; H, 3.79; N, 7.13. Found: C, 42.87; H, 3.97; N, 7.06.

Compounds **5b** and **5c** were synthesized analogously: **5b**, yellow solid (24%), mp 263–264°C, ^{1}H-NMR (DMSO-d_6) δ 1.25–1.59 (4 H, m), 1.82–2.15 (4 H, m), 3.95 (3 H, s), 4.00–4.22 (2 H, m), 4.49 (3 H, s), 4.68–4.83 (2 H, m), 7.52 (1 H, d, J=8.9 Hz), 7.67 (1 H, d, J=8.9 Hz), 7.87 (1 H, d, J=8.9 Hz), 7.93 (1 H, s), 8.00 (2 H, brs), 8.50 (1 H, m), 8.82–8.88 (4 H, m), 9.36 (2 H, d, J=6.7 Hz), 9.50 (2 H, d, J=6.7 Hz) and 9.77 (1 H, brs) ppm. Anal. Calcd. for $C_{31}H_{34}N_4OBr_{2.5}ClI_{0.5}$: C, 47.90; H, 4.41; N, 7.21. Found: C, 47.86; H, 4.49; N, 7.26. **5c**, yellow soild (25%), mp 198–199 °C, ^{1}H-NMR (DMSO-d_6) d 1.25–1.59 (10 H, m), 1.89–2.01 (4 H, m), 3.95 (3 H, s), 4.12 (2 H, m), 4.51 (3 H, s), 4.75 (2 H, m), 7.54 (1 H, d, J=7.9 Hz), 7.71 (1 H, d, J=9.1 Hz), 7.86 (1 H, d, J=9.1 Hz), 7.88 (1 H, s), 7.95 (1 H, brs), 8.52 (1 H, d, J=7.9 Hz), 8.80 (2 H, d, J=6.4 Hz), 8.83 (2 H, d, J=6.4 Hz), 9.32 (2 H, d, J=6.4 Hz), 9.44 (2 H, d, J=6.4 Hz) and 9.64 (1 H, brs) ppm. Anal. Calcd. for $C_{34}H_{40}N_4OBr_{2.5}ClI_{0.5} \cdot 4\ H_2O$: C, 45.80; H, 4.99; N, 6.29. Found: C, 45.71; H, 4.76; N, 6.25.

RESULTS

pKa Determinations

9-Aminoacridine derivatives undergo acid dissociation with a dissociation constant (pK_a) of 8.0–10 [14]. Compounds **5a–5c** have a dicationic viologen unit separated from the acridine by 3, 6 or 9 carbon chains, respectively. We tested the electrostatic effect of these viologens on the pK_a of acridine. The pKa values of **5a–5c** were determined from a pH-dependence of the absorbance at 424 nm to be about 8.0 (Tab. I).

TABLE I Extinction coefficients and pK_a values for **5a–5c**

Compd	free		bound		%H[b]	pK_a[c]
	λ_f (nm)	$10^{-3}\varepsilon_f^a$ (M^{-1} εcm^{-1})	λ_b (nm)	$10^{-3}\varepsilon_f^a$ (M^{-1} cm^{-1})		
5a	424	7.8	430	3.4	38	8.0
5b	423	9.4	430	3.4	50	8.4
5c	423	9.4	430	3.4	51	8.4

[a] Experiments were conducted in MES buffer at 25°C. The extinction coefficients for the DNA-bound form were determined in the presence of a 50-times excess of calf thymus DNA.
[b] Hypochromicity as defined by %H $100 \times (\varepsilon_f - \varepsilon_b)/\varepsilon_f$.
[c] Determined by spectroscopic titrations.

This shows that the dicationic viologen unit did not affect the acid dissociation of the acridine moiety and that all compounds **5a**–**5c** behave as a tricationic molecule under the following experimental conditions of 10 mM MES and 1 mM EDTA at pH 6.25.

Spectrophotometric Titrations

Addition of sonicated calf thymus DNA to **5a**–**5c** brought about a hypochromic and red shift of the absorption bands of acridine, as shown in Figure 1 for **5b**. The extinction coefficients of the free and DNA-bound forms of **5a**–**5c** are summarized in Table I. Free **5a** has an absorption maximum at 424 nm, whereas absorption maxima of free **5b** and **5c** are at 423 nm. The extinction coefficient of free **5a** was 20% smaller than those of **5b** and **5c**, suggesting an intramolecular stacking between the acridine and viologen units of **5a**. By contrast, the extinction coefficients of the bound form of **5a**–**5c** were the same, suggesting that all of them bind to DNA in the same conformation. In other words, free **5a** undergoes a conformational transition upon binding to DNA.

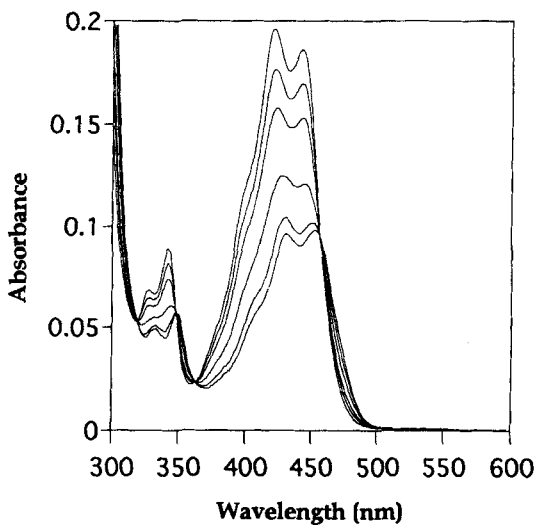


FIGURE 1 Spectral shifts of 20 μM **5b** on titration with sonicated calf thymus DNA. The DNA base concentrations were 0, 13.2, 26.4, 52.7, 79.1 and 105.4 μM from top to bottom. The experiments were carried out in 10 mM MES buffer and 1 mM EDTA (pH 6.25).

Binding Strength

Data on spectrophotometric titration of **5a**–**5c** with calf thymus DNA were analyzed by Scatchard plots (Fig. 2) with the extinction coefficients of the free and bound forms (Tab. I). The titration curves were fitted with the site exclusion model of McGhee and von Hi pel: [15]

$$\begin{aligned} \gamma/L = K(1-n\gamma)\{[(2\omega-1)(1-n\gamma) \\ +\gamma-R]/[2(\omega-1)(1-n\gamma)]\}^{n-1} \\ \times\{[1-(n+1)\gamma+R]/[2(1-n\gamma)]\}^2 \\ R=[(1-(n+1)\gamma)^2+4\omega\upsilon(1-n\gamma)]^{1/2} \end{aligned} \quad (1)$$

where γ is the moles of compound per DNA base pair, L is the free compound concentration, K is the intrinsic equilibrium binding constant, n is the number of base pair units per binding site, and ω is the cooperativity parameter. Table II lists the fitting values for **5a**–**5c**. The binding constants of **5a** with DNA are smaller than those of **5b** and **5c**, whereas those of **5b** and **5c** do not differ much at any salt concentrations.

The binding constants of **5a**–**5c** with DNA decreased with an increase in the salt concentra-

TABLE II Ionic strength dependence of the binding constants of **5a**–**5c** with sonicated calf thymus DNA[a]

Compd	[NaCl] (M)	$10^{-5}K(M^{-1})$	n	ω
5a	0.3	0.7	3	0.5
	0.2	0.8	3	0.5
	0.15	1.5	4	0.3
	0.1	1.7	4	0.4
5b	0.3	1.2	3	0.4
	0.2	2.5	3	0.4
	0.15	5.3	2	0.4
	0.1	11.4	3	0.3
5c	0.3	1.3	3	0.5
	0.2	4.0	3	0.4
	0.15	7.1	3	0.5
	0.1	13.2	2	0.3

[a]Experiments were conducted in 10 mM MES buffer (pH 6.25) in the presence of specified amounts of NaCl at 25 °C. Binding constants were determined by fitting experimental results with the McGhee-von Hippel eq. (1).

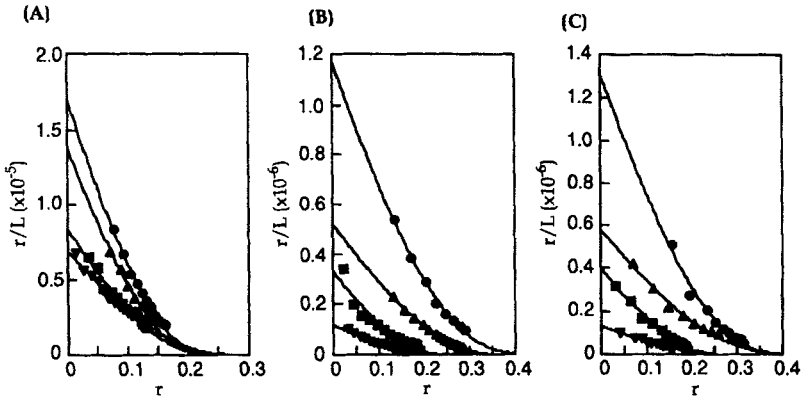


FIGURE 2 Scatchard plots for binding of **5a** (A), **5b** (B), and **5c** (C) to calf thymus DNA in 10 mM MES buffer, 1 mM EDTA (pH 6.25), and 100 mM (●), 150 mM (▲), 200 mM (■), or 300 mM (▼) NaCl.

tion and the plots of log$K$ vs $-$log[Na$^+$] were linear as expected (Fig. 3). For highly charged polymers like DNA, the dependence of the observed equilibrium constant (K_{obs}) on counterion concentration is given by eq. (2) [15] where m' depends on the number of ionic interactions found in the compound-DNA complexes, ψ is a characteristic of polymer which depends on the number of counterions thermodynamically associated with each charge unit on the polymer, and C depends on the charge in associated counterions which occurs as a result of a polymer conformational change which is coupled to the binding of the ligand.

$$\partial \log K_{obs}/\partial \log[Na^+] = -m'\psi - C \qquad (2)$$

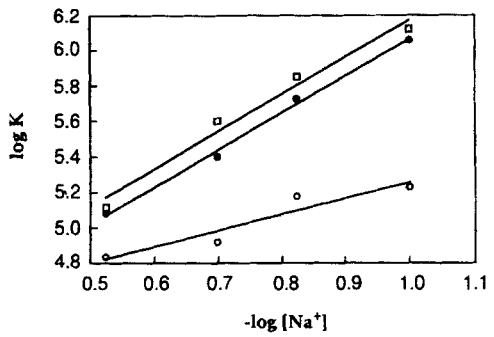


FIGURE 3 Dependence on the sodium ion concentration of log K, the binding constants for **5a** (○), **5b** (●), and **5c** (□) with calf thymus DNA. Slopes of the linear fits are 0.9±0.2 for **5a** and 2.1±0.1 for **5b** and **5c**.

The slope of the plots was 2.1 for **5b** and **5c** whereas that for **5a** was 0.9, implying that the former two bind to DNA as a divalent cation, while the latter as a monovalent cation.

Voltammetric Studies of the Interaction of 5a–5c with DNA

Compounds **5a**–**5c** have a viologen unit within the same molecule and should undergo a two-step redox reaction. Figure 4 shows differential pulse voltammograms of **5a**–**5c** in MES buffer (pH 6.25) with 0.1 M KCl at 25°C. Table III summarizes the redox potentials obtained from Figure 4 as well as the half-wave potentials $E_{1/2}$ of **5a**–**5c** obtained by cyclic voltammetry. The redox potential of **5a**–**5c** increased with an increase in the alkyl chain length.

TABLE III Voltammetric behavior of **5a**–**5c**[a]

Compd	−DPV peak[b] (mV)	half-height width[b] (mV)	$-E_{1/2}$[c] (mV)
5a	510	111	500
5b	490	111	499
5c	450	94	453
viologen	628	117	660

[a] Sweep rate: 50 mV/sec. Supporting electrolyte, 0.1 M KCl, 10 mM MES buffer, and 1mM EDTA (pH 6.25). [compound]=1 mM.
[b] These values were obtained from DPV measurements. Pulse amplitude: 50 mV, Pulse width: 50 msec, Pulse period: 200 msec, Quiet time: 2 sec.
[c] These values were obtained from cyclic voltammetric measurements.

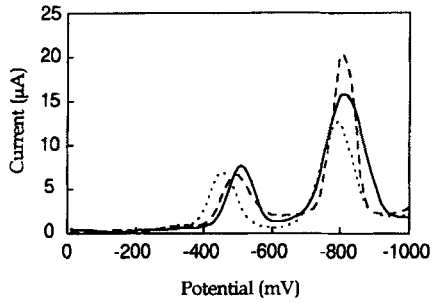


FIGURE 4 Differential pulse voltammograms for 0.10 mM **5a** (——), **5b** (– –), and **5c** (- - -) in 10 mM MES buffer and 1 mM EDTA (pH 6.25), and 10 mM KCl at 25 °C. Scan rate = 50 mV/s, Pulse amplitude = 50 mV.

Upon addition of calf thymus DNA, the peak potentials of **5a**–**5c** shifted to the more negative side, concomitant with a decrease in the peak current as the concentration of DNA was increased, as shown in Figure 5. Since the shift of the peak potential equals to the shift of the $E_{1/2}$, the net shift in $E_{1/2}$ ($\Delta E_{1/2}$) of **5a**–**5c** can be determined from Figure 5. $\Delta E_{1/2}$ decreased with an increase in the DNA concentration and then reached a plateau. These phenomena were analyzed by the following scheme [16, 17]; where $E_f^{o'}$ and $E_b^{o'}$ are the intrinsic redox potentials of the free and DNA-bound forms, respectively, and K_{2+} and $K_{.+}$ are the binding constants for the $Viol^{2+}$ and $Viol^{\cdot+}$ species to DNA, respectively, where Viol stands for the viologen unit of compounds **5a**–**5c**. K_{2+} is equivalent to K obtained spectroscopically. The shift in $E_{1/2}$ can be used to estimate the ratio of binding constants of $Viol^{2+}$ and $Viol^{\cdot+}$ species to DNA by eq. (3).

The relation between the intrinsic potentials and equilibrium constant is given by:

$$E_b^{o'} - E_f^{o'} = 0.059 \cdot \log(K_{.+}/K_{2+}) \qquad (3)$$

The shift in $E_{1/2}$ of **5b** and **5c** is −120 mV, whereas that of **5a** is −100 mV. The ratio of binding constants $K_{.}/K_{2+}$ was 0.0092 in **5b** and **5c**, in other words, the $Viol^{2+}$ species is bound ca 100 times more strongly than the Viol species. In the case of **5a**, the ratio of binding constants $K_{.+}/K_{2+}$ was half those of **5b** and **5c**. This means that the electrostatic interaction of **5a** with DNA is smaller than that of **5b** and **5c** and is in agreement with the spectroscopic data.

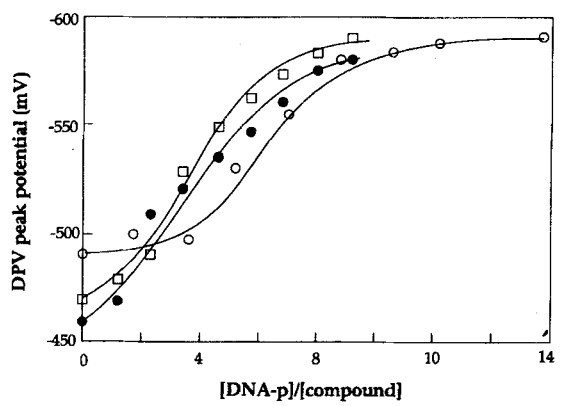


FIGURE 5 Changes in the $Viol^{2+/\cdot+}$ half-wave potential of 0.10 mM **5a** (O), **5b** (●), and **5c** (□) immersed in 10 mM MES buffer, 10 mM KCl and 1 mM EDTA (pH 6.25) at 25 °C containing various amounts of calf thymus DNA.

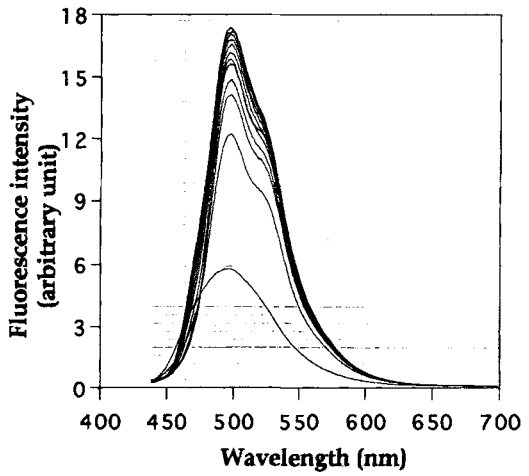


FIGURE 6 Fluorometric titration of 10 μM **5c** with poly $[d(A\text{-}T)]_2$ in 10 mM MES buffer, and 1 mM EDTA (pH 6.25) at 25 °C. Acridine was excited at 423 nm.

Fluorescence Studies of 5a–5c

The fluorescence spectrum of a 9-aminoacridinyl chromophore such as quinacrine is drastically changed, when intercalated into DNA [17]. Thus, the fluorescence intensity of quinacrine increased from 1.9 (arbitrary unit) up to 7.6 with an increase in poly[d(A-T)]$_2$ concentration in 10 mM MES and 1 mM EDTA buffer (pH 6.25) at 25 °C (Fig. 7). This result was essentially identical to that described previously[19]. By contrast, an equimolar amount of methylviologen did not affect the fluorescence of quinacrine at all, but in the presence of poly[d(A-T)]$_2$, the fluorescence intensity of a mixture of quinacrine and methylviologen did change. Thus, the intensity increased up to DNA/dye=4, then slightly decreased, and finally reached 5.3. This intensity was 30% smaller than that for quinacrine and DNA, demonstrating that methylviologen quenched the fluorescence of quinacrine when bound to DNA. It is presumed that quinacrine and methylviologen bind DNA at random and hence at high molar ratios of DNA to dye the separation is so large that their interaction is unlikely to occur. The fact that nonetheless the quenching occurred suggests that the nucleic bases mediate the fluorescence quenching by methylviologen. Alternatively, DNA may mediate association of quinacrine and methylviologen so that the latter can quench the fluorescence of the former directly.

The fluorescence intensities of compounds **5a–5c** were smaller considerably than that of quinacrine alone and the intensity decreased in the order of **5c**, **5b** and **5a** (y-intercept of Fig. 7). These data demonstrate that the intramolecular quenching by the methylviologen group is taking place and that its efficiency depends on the separation between the two chromophores. The effect of poly[d(A-T)]$_2$ on the fluorescence of these compounds was again biphasic; the intensity first increased, then decreased and finally leveled off, as shown in Figure 7. At high molar ratios of poly[d(A-T)]$_2$ to ligand (ca. DNA/dye = 20) where intermolecular quenching should be negligible, the fluorescence intensity of **5a–5c** increased with an increase in the alkyl chain length and all of these intensities were smaller than that of the mixture of quinacrine and methylviologen.

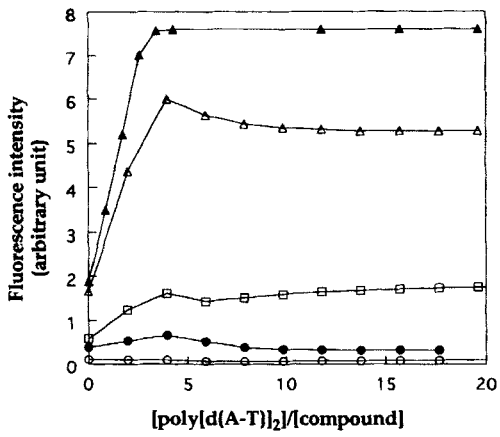


FIGURE 7 Changes in the fluorescence intensity of 10 μM **5a** (○), **5b** (●), and **5c** (□) as a function of the concentration of poly[d(A-T)]$_2$. Also shown are the changes in the fluorescence intensity of quinacrine in the absence (△) and presence (▲) of methylviologen. All experiments were conducted in 10 mM MES buffer, 10 mM KCl, and 1 mM EDTA (pH 6.25) at 25 °C.

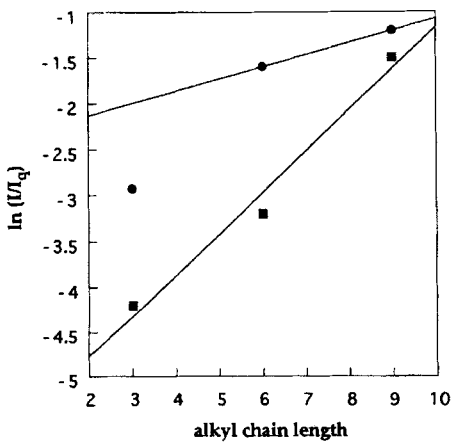


FIGURE 8 Correlation of $\ln(I/I_q)$ values of compounds **5a–5c** with the alkyl chain length in the absence (●) or presence (■) of poly[d(A-T)]$_2$. poly[d(A-T)]$_2$/[**5a–5c**]=20. I and I_q stand for the fluorescence intensity of **5a–5c** and quinacrine, respectively.

The fluorescence data of Figure 7 were transformed into $\ln(I/Iq)$, where Iq and I stand for the fluorescence intensity of quinacrine and **5a–5c**, respectively. By doing so, the quenching efficiency can be assessed more properly. The result of this analysis is presented in Figure 8 as a function of the alkyl chain length. The fluorescence intensity of the acridine chromophore increased with an increase in the alkyl chain length of **5a–5c** both in the absence and presence of DNA. The fluorescence intensity of **5a** in the absence of DNA was abnormally small because of the intramolecular stacking of the acridine and viologen units (see above). The data in the presence of poly[d(A-T)]$_2$ fell on a straight line, suggesting that the binding mode and quenching mechanism of **5a–5c** are identical. In other words, the intramolecular stacking was relieved upon binding of **5a** to DNA. It should be noted that the slope of the line in the presence of poly[d(A-T)]$_2$ was greater than that in its absence. This suggests that the DNA matrix is involved in the quenching of the fluorescence by viologen.

DISCUSSION

We synthesized 9-aminoacridinyl derivatives **5a–5c** carrying a viologen unit connected by the alkyl chain length of three, six and twelve carbons. Since the pKa of the acridine moiety of these compounds is 8.0–8.4, all of these compounds behave as a trication including the two charges of viologen at **pH** 6.25. The molar extinction coefficient of **5a** is smaller than those of **5b** and **5c**. The latters are in agreement with the molar extinction coefficients of the acridines **3a–3c** having no viologen moiety. In addition, the fluorescence intensity of **5a** was ca. 80% smaller than those of **5b** and **5c**. Taken together it is concluded that the acridine and viologen moieties of **5a** stacked within the same molecule, whereas **5b** and **5c** have an extended conformation in aqueous solution. When bound to DNA, the molar extinction coefficients of **5a–5c** became identical with each other, suggesting that the DNA bound acridine moieties of **5a–5c** exist in the same environment on DNA. However, the binding affinity of **5a** with DNA is smaller than those of **5b** and **5c** and the effective charge contributed to the formation of the DNA complex, estimated from the salt-dependence of binding affinity, was 0.9 for **5a** and 2.1 for **5b** and **5c** (Fig. 3). This behavior of **5a** may arise from the disadvantage at the DNA binding: **5a** has the structure with the acridine and viologen moieties stacking. Because of the short linker of three methylenes, the viologen cations of **5a** cannot be placed in an ideal position to interact with the DNA phosphate anions, when the acridine moiety is intercalated between DNA base pairs. This notion is further reinforced by the electrochemical data that only one of the two positive charges of viologen of **5a** contributes to the formation of its DNA complex.

Space-filling model building suggests that **5a–5c**, when fully extended cover an area of DNA 4.8, 8.8 and 12.8 Å in length which correspond to 3, 4 and 5 base pairs, respectively [6]. Although it remains to be proven experimentally whether the linker chain is extended as in solution or slack on the DNA, it seems certain that the binding mode of **5a–5c** is roughly identical and the separation between the two units is proportional to the methylene chain length (Fig. 8). In other words, the linker chain of **5a–5c** should lie over the DNA groove as shown in Scheme 1.

With this conformation of the DNA-bound form of **5a–5c**, intramolecular quenching was more pronounced than for their free counterparts (Fig. 8). This phenomenon may be explained in part by a conformational "freezing" of **5a–5c**; they have an extended conformation in solution, but when bound to DNA, the conformational freedom is reduced drastically. This facilitates the interaction of the acridine and viologen units, resulting in the effective quenching of the fluorescence. Fluorescence quenching is brought about either through energy transfer

or electron transfer. With viologen as the quencher, the latter mechanism seems to be more plausible [4]. If the quenching is indeed effected through electron transfer, the aromatic rings of the DNA matrix may enhance it, as Formherz [3], Harriman [20–22], Barton [23], and associates reported that nucleic bases mediated electron transfer. Whatever the validity of this notion, DNA offers a suitable matrix for DNA-interacting ligands to assume a specific conformation, some of which are favorable for the subsequent events such as electron transfer and chemical reactions.

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