FLUORESCENCE-DETERMINED PREFERENTIAL BINDING OF QUINACRINE TO DNA

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ABSTRACT Quinacrine complexed with native DNA (Calf thymus, *Micrococcus lysodeikticus, Escherichia coli, Bacillus subtilis,* and *Colstridium perfringens*) and synthetic polynucleotides {poly(dA)·poly(dT), poly[d(A-T)]·poly[d(A-T)], poly(dG)·poly(dC) and poly [d(G-C)]·poly[d(G-C)]} has been investigated in solution at 0.1 M NaCl, 0.05 M Tris HCl, 0.001 M EDTA, pH 7.5, at 20°C. Fluorescence excitation spectra of complexes with dye concentration D = 5–30 μM and DNA phosphate concentration P = 400 μM have been examined from 300 to 500 nm, while collecting the emission above 520 nm. The amounts of free and bound quinacrine in the dye-DNA complexes have been determined by means of equilibrium dialysis experiments. Different affinities have been found for the various DNAs and their values have been examined with a model that assumes that the binding constants associated with alternating purine and pyrimidine sequences are larger than those relative to nonalternating ones. Among the alternating nearest neighbor base sequences, the Pyr(3'-5')Pur sequences, i.e., C-G, T-G, C-A and T-A seem to bind quinacrine stronger than the remaining sequences. In particular the three sites, where a G·C base pair is involved, are found to display higher affinities. Good agreement is found with recent calculations on the energetics of intercalation sites in DNA. The analysis of the equilibrium shows also that the strength of the excitation spectrum of bound dye depends strongly upon the ratio of bound quinacrine to DNA. This effect can be attributed to dye-dye energy transfer along DNA.

INTRODUCTION

Several studies on the interaction of acridine derivatives with nucleic acids have been performed using spectroscopic techniques (1–17). Because acridine-nucleic acids complexes are model systems that have been investigated to understand the various aspects of DNA breathing processes and protein-DNA interactions, it is important to learn where and how dyes bind to DNA. Fluorescence (quantum yield, time decay, polarization, etc.) has been found useful for studying the nature of this interaction, which under specific conditions gives rise to the so-called intercalation of the dye between adjacent base pairs of DNA. From these studies it appears that the optical properties of a given intercalated dye and its equilibrium binding constants depend upon the nature of the host DNA, which suggests that heterogeneous sites might be involved in the binding (1–7). Heterogeneity of dye binding appears then a fundamental problem.

In this paper we have examined quinacrine in its interaction with native DNAs and synthetic polynucleotides for its relevance as a fluorescent probe in monitoring dye-DNA solutions (8–11) and chromosome structures (11–15). Previous studies of quinacrine-DNA complexes have shown that guanine, when close to the bound dye, quenches its fluorescence

(8–10) and that a high quantum yield appears to be due to intercalating triplets (or longer sequences) of A·T base pairs, whereas the affinity of bound dye has been found to change in various DNAs (4, 6, 10, 16, 17).

Here the affinities of quinacrine for native DNAs and synthetic polynucleotides have been measured by equilibrium dialysis and the fluorescence excitation intensities of the bound component have been correlated with the nearest neighbor base sequence frequencies of each DNA (18). It has been found that the alternating (Pyr-Pur and Pur-Pyr) neighboring base sequences have larger binding strengths for quinacrine and that, among them, the Pyr-Pur sequences dominate the binding. This agrees with recent theoretical predictions (19) and with experimental data on dye dinucleotide complexes (20–22).

EXPERIMENTAL

Instrumentation

The fluorescence of solutions was excited by monochromatic light focused onto the narrow side of quartz cuvettes ($2 \times 10\text{-mm}^2$ section). A Xenon high-pressure 450-W lamp was used as a source of excitation, chopped at 900 Hz, and dispersed by means of a double monochromator with 1.2–nm bandpass. A quartz beam splitter interposed on the exciting beam sent a fraction of the light to a rhodamine B cuvette acting as a photon counter and whose fluorescence was detected with a photomultiplier followed by a lock-in amplifier. The apparatus has been tested with a rhodamine B standard solution and found to give the expected yield within 5% in the 260–580 nm range. The quinacrine fluorescence at $\lambda > 520$ nm (Kodak Wratten Filters [Eastman Kodak Co., Rochester, N.Y.] No. 8 and 15) was detected by a 9558 Q EMI photomultiplier (EMI Gencom Inc., Plainview, N.Y.) followed by a lock-in amplifier. To measure correct excitation spectra, the two signals were divided with a ratiometer whose output was fed into a stripchart recorder (Leeds and Northrup, Philadelphia, Pa.), sent to a multichannel analyzer (Laben, Milan, Italy) and then a punched tape (one data point every 2 nm) in order to be processed by a computer (Univac 1100/80; Sperry Univac, Blue Bell, Pa.). During fluorescence measurements the samples were kept at constant temperature by circulating water at 20 ± 0.2°C.

Materials and Methods

Quinacrine dihydrochloride was a gift of Professor J.B. Le Pecq (Institut Gustave Roussy, Villejuif, France). Its concentration in 0.05 M Tris HCl, 0.1 M NaCl 10^{-3} M EDTA, pH 7.5, was determined assuming $\epsilon_{424} = 7270\ M^{-1}\ cm^{-1}$ according to Drummond et al. (23). *Micrococcus lysodeikticus, Escherichia coli,* calf thymus, and *Clostridium perfringens* DNA were obtained from Sigma Chemical Company (St. Louis, Mo.). *Bacillus Subtilis* DNA was purchased from Serva Feinbiochemica Gmbh & Co. (Heidelberg, Germany). All DNAs have been dialyzed against 0.05 M Tris HCl, 0.1 M NaCl, 10^{-3} M EDTA, pH 7.5 in dialyzer tubings mol wt cutoff 12,000 (Arthur H. Thomas Co., Philadelphia, Pa.) and used without further purification. The concentrations of the DNA solutions were determined spectrophotometrically at 260 nm using the molar extinction coefficients given by Felsenfeld and Hirschmann (24): *M. lysodeikticus,* $\epsilon = 6923\ M^{-1}\ cm^{-1}$; *E. coli,* $\epsilon = 6565\ M^{-1}\ cm^{-1}$; calf thymus, $\epsilon = 6412\ M^{-1}\ cm^{-1}$; *B. subtilis,* $\epsilon = 6412\ M^{-1}\ cm^{-1}$; *Cl. perfringens,* $\epsilon = 6225\ M^{-1}\ cm^{-1}$. Hyperchromicity of DNA solutions was checked by means of alkali denaturation according to Müller (5).

Synthetic polynucleotides poly(dA)·poly(dT), poly[d(A-T)]·poly[d(A-T)], poly(dG)·poly(dC) and poly[d(G-C)]·poly[d(G-C)] were obtained from Boehringer, Mannheim, dissolved in 0.05 Tris HCl, 0.1 M NaCl, 10^{-3} M EDTA (pH 7.5), and used without further purification. Concentrations were determined according to the following molar extinction coefficients (25, 26): poly[d(A-T)]·poly [d(A-T)], $\epsilon_{max} = 6.600\ M^{-1}\ cm^{-1}$; poly(dA)·poly(dT), $\epsilon_{max} = 6000\ M^{-1}\ cm^{-1}$; poly[d(G-C)]·poly [d(G-C)], $\epsilon_{max} = 8.400\ M^{-1}\ cm^{-1}$; poly(dG)·poly(dC), $\epsilon_{max} = 7.400\ M^{-1}\ cm^{-1}$.

The quinacrine DNA complexes were freshly prepared by adding microvolumes from a quinacrine

solution, 400 μM, to DNA solutions at constant concentration of phosphate P = 400 μM. Dye concentrations in the samples studied here ranged from D = 1 to 30 μM.

The fluorescence intensity for the excitation spectra can be written at wavelength λ as

$$I_{meas}(\lambda) = A[1 - T(\lambda)] \cdot \phi \tag{1}$$

where A is an arbitrary constant, T is the transmittance and ϕ is the fluorescence quantum yield. T can be expanded with respect to $\epsilon(\lambda)D\ell$, with ℓ being the cell length (1 cm), as

$$T = 10^{-\epsilon D\ell} = 1 - 2.3\, D\epsilon\ell + \frac{2.3^2}{2} D^2\epsilon^2\ell^2 + \cdots$$

and therefore Eq. 1 becomes

$$I_{meas}(\lambda) = 2.3\, A \cdot \phi \cdot D\epsilon\ell(1 - 1.15\, D\epsilon\ell + \cdots) \tag{2}$$

This shows that the fluorescence intensity vs. dye concentration is only linear at low values of D ($D\epsilon\ell \ll 1$). Therefore at all values of D the nonlinear contribution has been taken into account. All spectra shown in this paper have been processed according to the above equations in order to plot the product $\epsilon\phi$ which from now on will be referred as η, i.e., the excitation spectrum for unit dye concentration. In order to determine the contribution of free and bound dye to fluorescence of a dye-DNA complex, attention has been given only to the excitation spectra since they display differences larger than those found in emission spectra. In fact, the emission spectrum of bound quinacrine is blue shifted with respect to that of the free dye by only 4 nm (17); whereas for the excitation spectra a red shift of 26 nm is found, as shown in the following section. We note also that because of the slight shift in the emission of the bound and free dye, the ratio η_F/η_B is slightly altered by a constant factor ($\simeq$1.05) because of the presence of the cut-off filter.

The equilibrium parameters from every complex have been obtained using dialysis cells with 1-ml volumes of solutions on both sides of the membrane (Thomas tubing, mol wt cutoff 12,000). We found considerable losses of quinacrine mainly due to the membrane (of the order of 20% of the free dye concentration in the complex). To keep the equilibrium of bound and free dye approximately constant

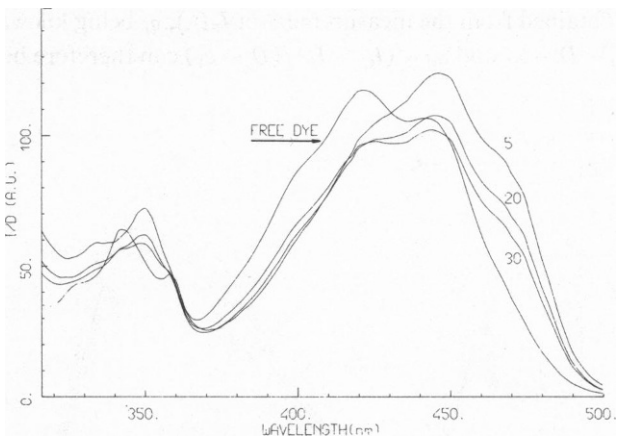

FIGURE 1 Fluorescence excitation spectra of quinacrine-calf thymus complexes with constant DNA concentration P = 400 μM and dye concentration D = 5, 20, and 30 μM. Intensities have been divided by D and processed according to Eq. 2. For comparison, the excitation spectrum of free quinacrine, D = 10 μM, is multiplied by 4.5 to obtain a correct intensity (peak value $I_F/D = \eta_F$ = 450 A.U.). A.U., arbitrary units.

during the dialysis, it is necessary to compensate for dye losses. Therefore, a preliminary run of the cell has been made with a free dye solution at a concentration close to that of the free dye expected to occur in the complex. The cells were then filled with DNA-dye solution and free dye solution in the two compartments, kept at constant temperature and in motion for ~18 h, to allow diffusion equilibrium of the dye through the membrane (after 12 h no significant diffusion was observed). The amounts of free and bound dye have been determined by measuring the fluorescence intensity of the samples taken from both sides of the dialysis cells as will be shown in the next section.

RESULTS

Fluorescence excitation spectra of quinacrine-calf thymus complexes with constant DNA concentration $P = 400\ \mu M$ and different amounts of dye are reported in Fig. 1. The excitation spectrum of the free dye is shown for comparison.

To evaluate the equilibrium parameters that determine the amount of free and bound dye, equilibrium dialysis experiments have been performed on quinacrine-DNA solutions and the fluorescence spectra have been measured. The spectra (corrected according to Eqs. 1 and 2) for the solutions of the two compartments of the cell are then the following:

$$\text{free dye compartment,} \quad I_F(\lambda) = c_F \eta_F(\lambda);$$

$$\text{complex compartment,} \quad I_C(\lambda) = c_F \eta_F(\lambda) + c_B \eta_B(\lambda) \tag{3}$$

$$= c_F \eta_F(\lambda) + (D - c_F)\eta_B(\lambda); \tag{4}$$

where $I_F(\lambda)$ and $I_C(\lambda)$ are the excitation spectra of the free dye and of the complex, respectively; c_F and c_B the concentrations of the free and bound dye in the complex, respectively; $D = c_F + c_B$ the total dye concentration; η_F and η_B the excitation spectra for unit concentration of free and bound dye, respectively. By subtracting Eq. 3 from 4 we get

$$I_C - I_F = (D - c_F)\eta_B \tag{5}$$

where c_F is directly obtained from the measurement of $I_F(\lambda)$, η_F being known from data on free dye fluorescence. $c_B = D - c_F$ and $\eta_B = (I_C - I_F)/(D - c_F)$ can therefore be determined, if the

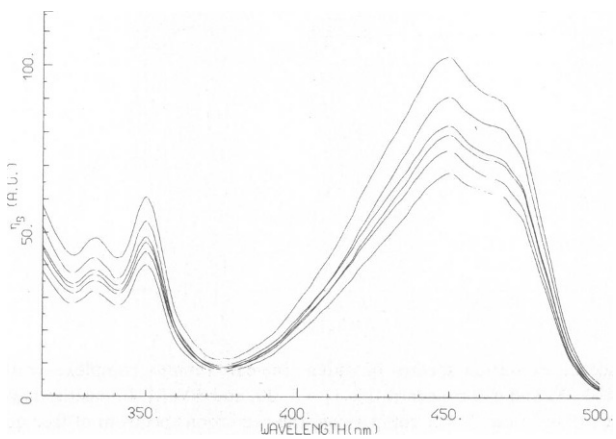

FIGURE 2 Fluorescence excitation spectra of bound quinacrine for unit concentration η_B, in Q-calf thymus complexes at $P = 400\ \mu M$ and $D = 5$, 10, 20, 25, and 30 μM in order of decreasing intensities.

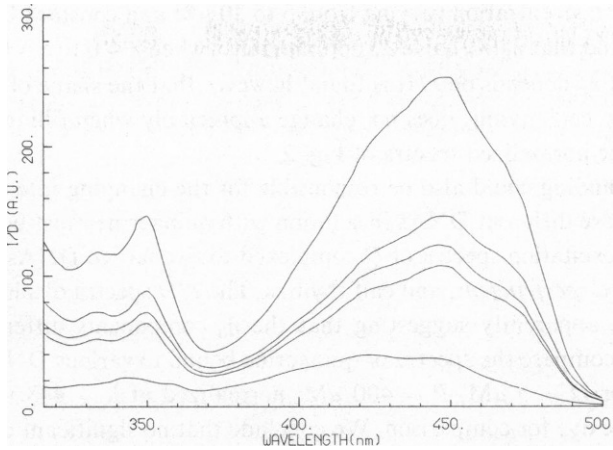

FIGURE 3 Fluorescence excitation spectra of quinacrine-DNA complexes at $P = 400\ \mu M$ and $D = 5\ \mu M$ for *M. lysodeikticus*, *E. coli*, calf thymus, *B. subtilis*, and *C. perfringens* DNAs in order of increasing intensities.

value of the total dye concentration D after dialysis is known. The accuracy of the evaluation of the equilibrium parameters depends upon the accuracy of D. Small changes of the values of D after dialysis due to inexact compensation of dye loss in the cell can be easily taken care of by simple interpolation. By plotting I_C vs. D before dialysis we find the correct values of total dye concentration in the dialyzed complexes.

It is therefore easy to separate the spectrum of the complex, Eq. 4, in two components: spectral intensity η_F of the free dye, and spectral intensity η_B of the bound dye. Following this procedure the correct equilibrium parameters can be obtained from dialysis experiments. The fluorescence response of the bound component η_B can also be obtained at all values of D.

In Fig. 2 we show the excitation spectrum of bound quinacrine in the complex with calf

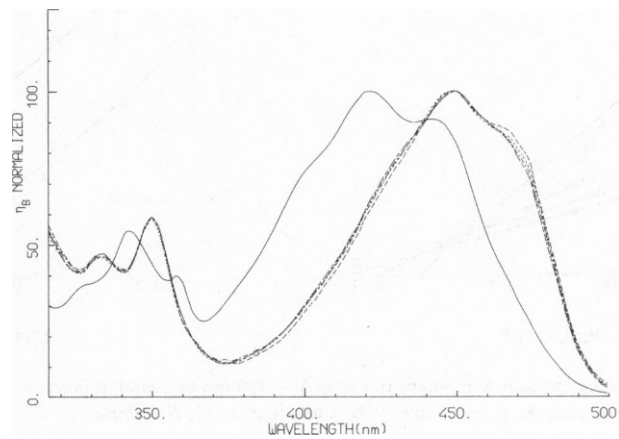

FIGURE 4 Fluorescence excitation spectra η_B of bound quinacrine in the five complexes with native DNAs of Fig. 3 normalized at $\lambda = 450$ nm for comparison. Free dye excitation spectrum is reported for comparison.

thymus at total dye concentration varying from 5 to 30 μM and constant DNA concentration $P = 400$ μM. We find that $\eta_B(\lambda)$ halves, approximately, when $r \sim 0$ to $r \sim 0.06$–0.08, showing that the strength of η_B depends on r. It is found however that the shape of the η_B spectrum of quinacrine bound to calf thymus does not change appreciably when filling DNA with dye as can be seen from the normalized spectra of Fig. 2.

Heterogeneous binding could also be responsible for the changing intensities of the bound component. Therefore different DNAs in solution with quinacrine must be examined. In Fig. 3 are reported the excitation spectra of Q complexed to five native DNAs: *M. lysodeikticus, E. coli, B. subtilis, C. perfringens,* and calf thymus. The I/D spectra display large differences in their intensities, apparently suggesting that the η_B components differ markedly for the various DNAs. To compare the spectra of quinacrine bound to various DNAs we show in Fig. 4 the η_B spectra for $D = 5$ μM, $P = 400$ μM, normalized at $\lambda = 448$ nm, along with the spectrum of the free dye for comparison. We conclude that no significant differences occur in the spectral shape of bound quinacrine when r increases for the same DNA or when spectra of different native DNAs are compared.

In Fig. 5 are reported the peak intensities of η_B (448 nm) vs. r for the five complexes. There are at least two reasons for the decrease of η_B with increasing r: (*a*) heterogeneous binding and/or (*b*) transfer of excitation among intercalated dyes. In (*a*) sites of higher quantum yield would be filled first (higher partial affinity) whereas sites with lower yield would be involved at larger values of r; in (*b*) energy transfer would weaken the fluorescence when r increases if quenching sites are available for intercalation. A further examination of the equilibrium constants is therefore required in order to understand the relevance of heterogeneity and transfer.

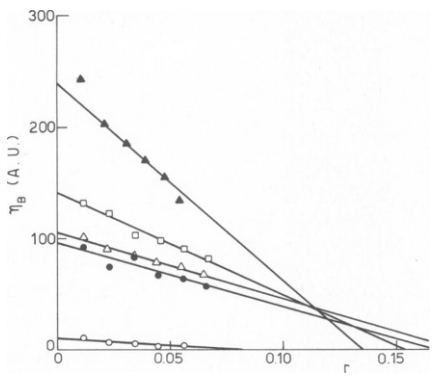

FIGURE 5

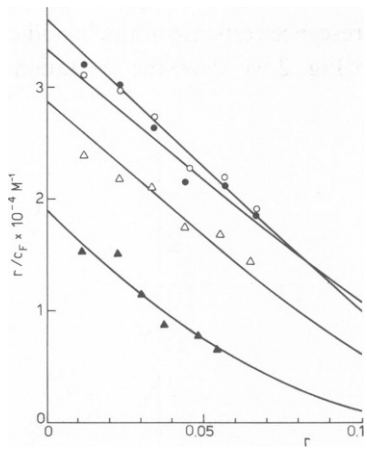

FIGURE 6

FIGURE 5 Fluorescence excitation intensities η_B at $\lambda = 450$ nm of bound quinacrine vs. $r = c_B/P$ for the five complexes of Fig. 2. ▲, *C. perfringens;* △, Calf thymus; □, *B. subtilis;* ○, *M. lysodeikticus;* ●, *E. coli.*

FIGURE 6 Scatchard plots of quinacrine-DNA complexes for *M. lysodeikticus,* ○; *E. coli,* ●; calf thymus, △; and *C. perfringens,* ▲. $D = 5, 10, 15; 20, 25$ μM. Experimental data have been fitted according to Eq. 9; calculated binding isotherms are shown as full lines.

Having determined the bound dye excitation spectrum we now evaluate the equilibrium concentration of bound and free dye in the complex with native DNAs. Using the values of c_F and c_B, estimated with the above procedure, we can plot the equilibrium data according to the Scatchard representation, i.e., r/c_F vs. r as shown in Fig. 6. The values of D have been limited to 30 μM so that $c_B/P = r$ does not exceed 0.1 in order to reduce cooperativity effects that interfere with external binding. From the intercepts on the r/c_F axis of a Scatchard plot we obtain the affinities of the dye for the various DNAs, which are reported in Table I, together with the extrapolated values at $r = 0$ for η_B^{max}, obtained from Fig. 5. The data relative to the complex of quinacrine with calf thymus reported in Fig. 6 agree very well with those recently obtained from absorption measurements by Wilson and Loop (27) when the effect of different ionic strength is taken into account ($I = 0.15$ in our measurements).

In order to obtain further information on the effect of DNA base composition on the equilibrium, the complexes of quinacrine with the synthetic polynucleotides poly(dA)·poly(dT), poly[d(A-T)]·poly[d(A-T)], poly(dG)·poly(dC), poly[d(G-C)]·poly[d(G-C)] were examined. The results obtained, as described above for the native DNAs, are reported in Table I. DNA alternating purine-pyrimidine copolymers appear to bind more strongly than the corresponding nonalternating isomers. The intensity of the excitation spectrum η_B of bound quinacrine in poly(dG)·poly(dC) and poly[d(G-C)]·poly[d(G-C)] can be considered essentially zero. This is in agreement with the quenching effect of guanine on the dye as reported by other authors (8–10, 16).

DISCUSSION

Our equilibrium data show that the various DNAs display different affinities for quinacrine. These differences strongly suggest heterogeneous binding and it is therefore interesting to try to understand the source of binding specificity. The problem has been studied by several authors (1–7) and recently the heterogeneous binding of quinacrine to DNA has been correlated with DNA base composition, under the assumption of a random distribution of base pairs (17).

We can try to express the heterogeneity of independent binding sites by writing the concentration of bound dye c_B as a sum of the partial concentrations c_{B_i}:

$$c_B = \sum_i c_{B_i} = \sum_i \frac{F_i n k_i P c_F}{1 + k_i c_F} \tag{6}$$

where P is the molar concentration of phosphates in DNA; k_i is the binding constant of site i; n is the number of binding sites per phosphate and is assumed to be the same for each site; F_i is the fraction of sites i in a given DNA, with $\Sigma_i F_i = 1$.

It is easily shown that if the constants k_i are of the same order of magnitude, Eq. 6 can be approximated by

$$\sum_i c_{B_i} \sim \frac{nKPc_F}{1 + Kc_F} \tag{7}$$

where K is the apparent equilibrium constant and the equilibrium isotherm in the Scatchard plot may appear as a straight line for values of $r \ll n$:

$$r/c_F \simeq K(n - r), \tag{8}$$

masking the heterogeneity of the binding constants. When $r \to 0$ we have $r/c_F \to nK$ and from Eq. 6:

$$nK = \sum_i F_i nk_i. \tag{9}$$

This is the expression in terms of the partial affinities nk_i of the ordinate intercepts of the Scatchard plot, i.e., the effective affinity nK of the dye for a given DNA.

Because, in DNA, there are 16 different intercalation sites arising from all combinations of base pair arrangements, we expect at most 16 different values of the affinities nk_i in the binding. The 16 sites can be labeled according to 16 nearest neighbor pair sequences which, because of base pairing such as [C-A] = [T-G], become 10 independent sequences. In order to estimate 10 values of nk_i from Eq. 7 at least 10 different DNAs would be required with their affinities. The data presented in this paper (Table I) refer to five native DNAs and four synthetic polynucleotides. Therefore at most nine partial affinities could be estimated from Eq. 9. However, caution must be used in interpreting the data on synthetic polynucleotides (6), since they display structures that might differ from those of native DNAs. As can be seen from Table I, the alternating polymers (... Pur-Pyr-Pur ...) are found to bind more efficiently than the nonalternating ones (Pur-Pur ... and Pyr-Pyr ...) suggesting that the former sequences may dominate the binding in native DNAs. In order to check this assumption in Fig. 7 we plot the values of the measured affinities for five native DNAs and four synthetic polynucleotides vs. the fraction of alternating nearest neighbor (n.n.) Pyr-Pur and Pur-Pyr sequences: γ = (Pyr-Pur + Pur-Pyr)/(total n.n. base sequences) which are reported in Table II together with the n.n. frequencies for the DNAs examined. From the graph one draws the following conclusions: the affinities increase with the fraction γ of alternating n.n. base sequences. At the two extrema, corresponding to the synthetic polymers, there appear saturation trends. Native DNAs appear narrowly grouped together, $0.44 < \gamma < 0.52$.

The steplike behavior of the affinities vs. γ might be due to conformational changes induced in the DNA helix by repeating purine-pyrimidine alternating sequences, which have been

TABLE I
AFFINITIES AND FLUORESCENCE INTENSITIES OF QUINACRINE BOUND TO DNA

	$\eta_B(r = 0)$*	nK_{expl}‡
M. lysodeikticus	11 ± 2	3.40 ± 0.30
E. coli	98 ± 3	3.55 ± 0.30
C. thymus	108 ± 4	2.50 ± 0.30
B. subtilis	145 ± 30	3.40 ± 0.30
C. perfringens	245 ± 10	1.80 ± 0.20
poly [d(A-T)] · poly [d(A-T)]	1,030 ± 40	3.5 ± 0.3
poly (dA) · poly (dT)	960 ± 40	0.95 ± 0.2
poly [d(G-C)] · poly [d(G-C)]	~10	3.1 ± 0.3
poly (dG) · poly (dC)	~10	0.9 ± 0.2

*Fluorescence intensities at $\lambda > 520$ nm excited at $\lambda = 450$ nm, arbitrary units. Intensity of free dye excited at $\lambda = 424$ nm $\eta_F^{max} = 450$.
‡Measured affinities in units $10^4 M^{-1}$.

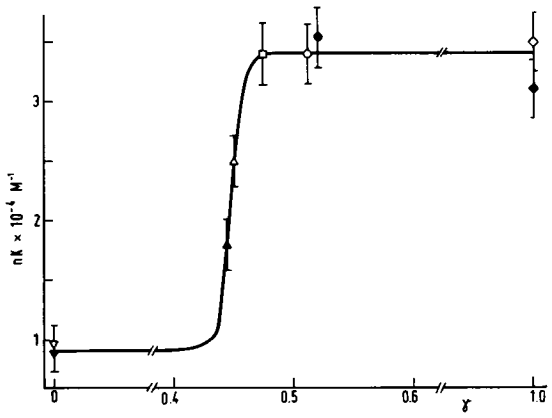

FIGURE 7 Affinities of native and synthetic DNAs plotted against the fraction of alternating n.n. base sequences γ, reported in Table II. For native DNAs see caption to Fig. 5. ◊, poly [d(A-T)]·poly[d(A-T)]; ♦, poly[d(G-C)]·poly[d(G-C)]; ▽, poly(dA)·poly(dT); ▼, poly(dG)·poly(dC).

TABLE II
NEAREST NEIGHBOR FREQUENCIES IN DNA (× 10^3)

	Source of DNA				
	*M. lysodeikticus**	*E. coli**	Calf thymus*	*B. subtilis**	*C. perfringens*‡
Pur-Pur					
A-G	49	55	72	58	59
A-A	19	71	89	92	129
G-G	112	56	50	46	26
G-A	65	55	64	67	49
Pyr-Pyr					
T-C	63	56	67	65	53
T-T	17	76	87	95	149
C-C	113	56	54	46	26
C-T	50	55	67	57	65
Pyr-Pur					
T-G	54	71	76	68	48
T-A	11	51	53	52	124
C-G	139	67	16	50	5
C-A	52	71	80	67	45
Pur-Pyr					
A-T	22	68	73	80	121
A-C	57	54	52	48	38
C-T	56	55	56	48	41
G-C	121	83	44	61	23
γ§	512	520	450	474	445

*Data from Josse et al. 1961. *J. Mol. Biol.* 236:864.
‡Data from Russell et al. 1973. *J. Mol. Biol.* 2:277.
§γ = [Pyr-Pur] + [Pur-Pyr]/total n.n. base sequences × 10^3.

observed by x-ray analysis on synthetic polynucleotides (28–30). The trend of the data in Fig. 7 is taken as a strong indication that the partial affinities of synthetic DNA cannot be directly transferred (Eq. 9) to the analysis of native DNAs. However a general trend can be inferred for the latter: alternating n.n. sequences bind more strongly than the nonalternating ones. If we assume therefore that the nonalternating n.n. pairs have negligible affinities for quinacrine in native DNAs, we are left with six partial affinities in Eq. 9 that refer to the alternating sequences. Moreover, data on dye dinucleotides complexes (20–22) and recent calculation on the energetics of DNA intercalation (19) indicate that Pyr(3'-5')Pur sequences have the largest affinities for intercalating drugs. In accordance with the above suggestions we use Eq. 9 under the simplifying assumption that only the four Pyr-Pur n.n. sites (TA, CG, CA and TG) have relevant affinities. Three partial affinities nk_i are sufficient however, since CA = TG. A fourth affinity is introduced in order to take into account the average contribution of all the remaining sequences (Pyr-Pyr, Pur-Pur, and Pur-Pyr). The frequencies F_i of the n.n. sequences which enter Eq. 9 have been reported in Table II for the five native DNAs examined here. In order to solve the system of linear Eq. 9 in the four unknowns nk_i, four experimentally determined affinities should be sufficient. However, experimental uncertainties will not allow an exact solution of the system. We therefore employed a multilinear regression, MINUIT Program (European Center for Nuclear Research, CERN, Geneva, Switzerland), applied to the experimental data from five native DNAs. The following values of the partial affinities are obtained from the computer fit: nk(T-A) $\simeq 2 \pm 0.4$, nk(C-G) $\simeq 13 \pm 2$, nk(C-A) $\simeq nk$(T-G) $\simeq 16 \pm 2$, and $nk_0 \sim 0.1$, in 10^4 M^{-1} units.

The fact that the value obtained for nk_0, the average partial affinity for the remaining sites, is much lower confirms that Pyr(3'-5')Pur sequences dominate the binding, in agreement with the assumptions. It appears also that when one or two G·C base pairs are involved in a binding site its affinity is found to be large.

According to the above results the binding isotherm r/c_F vs. r can be described by Eq. 6, now rewritten as

$$r/c_F = \sum_i nk_i F_i/(1 + k_i c_F). \tag{10}$$

In this expression the values of partial affinities $n_i k_i$ are taken from the previous fitting. F_i is, as before, the n.n. frequencies given in Table II, and n is a parameter which can be determined by a best fit of the data as shown in Fig. 6. It is found that n assumes values that are close to 0.5 (n = 0.50 *M. lysodeikticus;* 0.55 *E. coli;* 0.55 calf thymus; 0.60 *B. subtilis,* 0.45 *Cl. perfringens*) whereas the r-intercepts of the Scatchard plot of Fig. 6, when linearly extrapolated from the data points, n_{ap}, occur at $r \simeq 0.11$ to 0.15. These values are close to the values one can predict by considering that the apparent number of sites per phosphate can be written as $n_{ap} = n \cdot \Sigma_i F_i$, the sum being extended to the strongly binding pyr-pur sites. Since it has been found that $n \sim 0.5$ and sites T-A, C-G, C-A and T-G, occur with a frequency of about ¼, n_{ap} turns out of the order of ⅛ and therefore close to the linearly extrapolated experimental intercepts. The value of $n \sim 0.5$ suggests that n.n. could perhaps be neglected in the intercalation process at low values of r when DNA binding heterogeneity is taken into account.

A final comment is due for the fluorescence intensity η_B of the bound dye in the complex.

The above analysis has shown that pair sequences containing guanine, a quencher for quinacrine fluorescence (7, 11), display affinities which are greater than those of the other sequences. Therefore the fluorescence intensity of the bound dye would be expected to increase when r increases since the quenching sites are filled first and, with increasing values of r, fewer guanine rich sites remain available for intercalation. However, it has been shown in the previous section (see Fig. 5) that in native DNAs the fluorescence of bound quinacrine η_B decreases when r increases. Therefore the observed fall of fluorescence intensity in native DNAs is interpreted as a further evidence of dye-dye energy transfer a process which occurs to intercalated quinacrine (10, 11) and other molecules (33–36). We recall also that the fluorescence intensity of quinacrine intercalated in synthetic polynucleotides poly(dA)·poly(dT) and poly[d(A-T)]·poly[d(A-T)] has been found to be independent of r and this has been attributed to the absence of G·C quenching sites (10). A model for energy transfer from fluorescing sites to quenching sites along the DNA helix is under study.

CONCLUSIONS

From analysis of fluorescence excitation spectra of quinacrine-DNA complexes it has been found that heterogeneity occurs in the binding. Among the nearest neighbor base sequences of DNA, alternating purine and pyrimidine sequences (Pyr-Pur and Pur-Pyr) have been found to bind quinacrine with an average affinity that is larger than that of the other sequences (Pyr-Pyr and Pur-Pur). Moreover, in agreement with recent calculations on the energetics of intercalation (19), it appears that the Pyr(3′-5′)Pur sequences dominate the binding. From the experimental isotherms, examined assuming binding heterogeneity, it appears also that the excluded site model is not necessary, at least during the earlier stages of the intercalation process ($r \ll 0.1$). In fact, according to the model discussed above, many sites are prevented from binding quinacrine essentially because of their very low binding constants rather than by the regular stacking geometry suggested with the excluded site model. The values of r have been kept below 0.1 in the present study in order to lessen cooperativity effects which, according to Wilson and Lopp (27), seem to occur in the binding of quinacrine to calf thymus DNA. Moreover, external binding, which becomes competitive with the weaker intercalating sites at high values of r, should not affect the equilibrium in the present work because of the relatively high ionic strength $I = 0.15$ and the low D/P ratio. It might become difficult therefore to understand the equilibrium at large values of r since heterogeneous binding, external binding, anticooperativity and n.n. exclusion (27, 31) are effects which can all occur at the same time.

In conclusion, the different affinities of quinacrine for the five DNAs examined are attributed to heterogeneous binding, which, we suggest, is the dominant effect when a drug at low D/P ratios is intercalated in DNA (31, 5).

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