MOLECULAR PHARMACOLOGY, 10, 686–695

Optical Studies on the Interaction of DL-Quinacrine with Double- and Single-Stranded Calf Thymus Deoxyribonucleic Acid

ANNE K. KREY AND FRED E. HAHN

Department of Molecular Biology, Walter Reed Army Institute of Research, Washington, D. C. 20012

(Received December 19, 1973)

SUMMARY

KREY, ANNE K., AND HAHN, FRED E.: Optical studies on the interaction of DL-quinacrine with double- and single-stranded calf thymus deoxyribonucleic acid. *Mol. Pharmacol.* **10**, 686–695 (1974).

The binding of the drug quinacrine to its bioreceptor, DNA, was studied by optical methods. Binding of quinacrine to duplex calf thymus DNA produced bathochromic shifts in the absorption spectrum of the drug, indicating the binding of single drug molecules rather than of dimers or aggregates. Results of a spectrophotometric titration of quinacrine with DNA were converted into a nonlinear adsorption isotherm (Scatchard plot) whose curvature suggests that the drug binds to more than one class of binding sites by more than one process. Strong binding with an apparent association constant of 1.2×10^6 M^{-1} and a stoichiometry of 1 drug molecule/$\sim$4 nucleotides is ascribed to the known intercalation of the drug into DNA. A weaker process, with an apparent association constant of 4.6×10^4 M^{-1} and a stoichiometry of 1 drug molecule/3 nucleotides, may represent a peripheral electrostatic attraction to phosphates of DNA. Fluorometric titration of quinacrine with DNA at low inorganic ion concentrations showed progressive quenching of fluorescence until a drug to nucleotide ratio (r) of 0.22 was attained. When titration with DNA was continued beyond this point, fluorescence increased and ultimately attained a plateau similar to that to which the fluorescence of the drug decreased in titrations at higher salt concentrations. Poly dA·dT strongly enhanced the fluorescence of quinacrine, while poly dG·dC quenched it. Quinacrine displaced 97.2% of DNA-bound methyl green at rates higher than those caused by aminoacridines which did not possess cationic aliphatic side chains. Depending upon the concentration of free quinacrine, the displacement of methyl green was either first- or second-order with time. Duplex and single-stranded DNAs induced Cotton effects in the optical rotatory dispersion spectrum of the drug, suggesting that the 9-imino group in the molecule is involved in intercalation binding. Quinacrine stabilized native DNA to thermal denaturation and formed a complex with denatured DNA which thermally dissociated in a cooperative manner.

INTRODUCTION

The antimalarial drug quinacrine (Atebrin) (1) owes its action as a blood schizontocide to its ability to inhibit nucleic acid biosyntheses in intraerythrocytic plasmodia (2, 3). It also inhibits DNA biosynthesis in *Escherichia coli* (4) and the DNA and RNA polymerase reactions *in vitro* (5). Quinacrine is a weak frameshift mutagen (6) but a potent antimutagen in bacteria (7), and

eliminates episomic genetic markers from *E. coli* (8). The drug has attracted much attention as a vital fluorescent stain of chromosomes (9) with specificity for A–T-rich regions of DNA (10, 11). These chemotherapeutic, antimicrobial, biochemical, genetic, and tinctorial effects result from the formation of complexes of quinacrine with DNA.

Quinacrine cosediments with DNA and, at higher concentrations, precipitates it in the form of yellow strands (12); this provides simple and direct evidence for the formation of DNA-drug complexes. The absorption spectrum of quinacrine is altered by DNA (5, 12, 13), but no exact spectrophotometric titrations have been reported which have lent themselves to the derivation of stoichiometries and apparent association constants. Binding of quinacrine increases the viscosity and reduces the sedimentation rate of DNA. The hydrolysis of the polymer with deoxyribonuclease is inhibited by the drug and it displaces methyl green from DNA (12). In a classical study Lerman (14) used quinacrine to demonstrate intercalation binding of acridines to DNA by measuring the linear dichroism and the polarized fluorescence of the DNA-drug complex. Duplex and single-stranded DNAs induce different ultraviolet Cotton effects in the ORD spectrum of DL-quinacrine (15).

The present work was undertaken as a definitive study of DNA-quinacrine complexes by optical methods.

MATERIALS AND METHODS

DL-Quinacrine was purchased from Winthrop Laboratories as the dihydrochloride dihydrate. Calf thymus DNA was obtained from Worthington Biochemical Corporation; poly dA·dT, from General Biochemicals; poly dG·dC, from Miles Laboratories; and the DNA–methyl green complex, from Sigma Chemical Company. DNA was rendered single-stranded as described previously (15) or denatured by boiling for 10 min, followed by rapid cooling in an ice bath. All experiments, except for those shown in Fig. 7, were carried out in 5 mM Tris-HCl buffer, pH 7.5. Optical paths were 10 mm.

Absorption spectra were recorded in a Cary model 14 spectrophotometer; ORD spectra, in a JASCO ORD/UV-5 spectropolarimeter; thermal denaturation profiles ("melting curves"), in a Gilford model 2000 spectrophotometer programmed for automatic recording of temperature and absorbance; and fluorescence emission spectra, in an Aminco-Bowman spectrophotofluorometer equipped with a high-pressure xenon arc lamp, an ellipsoidal condensing mirror and a 1P21 photomultiplier detector tube. Methyl green displacement was measured in the Cary model 14 instrument, and the reaction mixtures were kept in the dark except for illumination at 642 nm during periodic spectrophotometric measurements. The adsorption isotherm (Fig. 2) was derived by established methods and principles (16, 17). For the derivation of binding parameters, tangents to this isotherm were fitted arithmetically to the region of eight points with highest values of drug to nucleotide ratio (r) as well as to that composed of the five points with lowest values of r.

RESULTS

Effects of DNA on absorption spectrum of quinacrine. Diagrams depicting DNA-induced changes in the absorption spectrum of quinacrine (5, 12, 13, 15) at individual drug to nucleotide ratios have shown that DNA decreases the intensities of the quinacrine absorption bands and shifts their maxima to longer wavelengths. Figure 1 depicts a family of such spectra of 89 μM quinacrine alone or in the presence of native calf thymus DNA at drug to nucleotide ratios of 1.56, 0.42, and 0.06. With increasing DNA concentrations, the intensities of the quinacrine absorption bands decreased and showed progressive bathochromic shifts. All spectra passed through discrete isosbestic points (at 322 and 455 nm), indicating the presence of only two spectrophotometrically distinct molecular species (16), free quinacrine and DNA-bound quinacrine.

The bathochromic shifts of the absorption bands of the drug suggest that single quinacrine molecules are bound to DNA rather than aggregates (18). When solutions of quinacrine alone were progressively diluted from 0.1 mM, none of these dilutions exhibited bathochromic shifts, indicating that

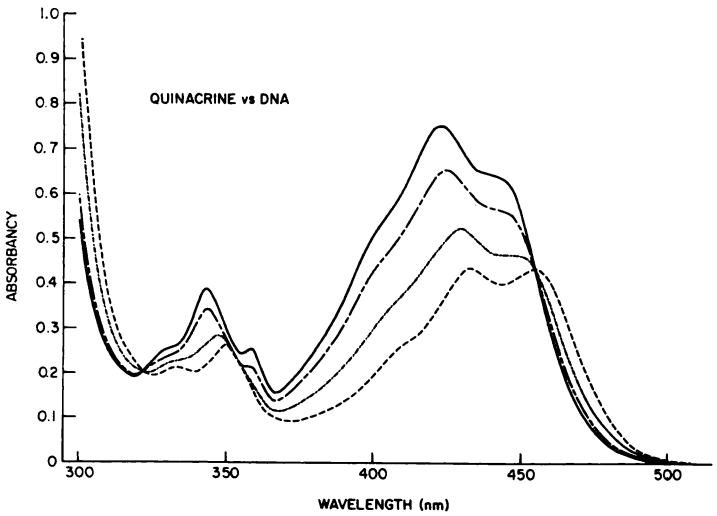

FIG. 1. *Effects of native calf thymus DNA on absorption spectrum of quinacrine* ——, quinacrine, 89 μM, plus DNA phosphorus (– — –, 57 μM; ·–·–, 210 μM; -----, 1.6 mM).

over the concentration range used in the present studies, quinacrine forms no aggregates, unlike metachromatic dyes, which have a tendency to self-aggregation and also bind to DNA as aggregates (18). The DNA-induced changes in absorption spectrum of quinacrine are not base-specific: they are similar for DNA, poly dG·dC, and poly dA·dT (5, 11).

These spectrographic observations indicate that the DNA-quinacrine interaction satisfies all necessary requirements for a spectrophotometric titration of the drug with DNA (16). We carried out such a titration.

Adsorption isotherm for binding of quinacrine to DNA. Results of a spectrophotometric titration of quinacrine (89 μM) with graded concentrations of calf thymus DNA were converted to the adsorption isotherm shown in Fig. 2. Assuming an independent binding site model, in which the partial population of binding sites by ligand molecules does not change the binding properties of still vacant sites, the curvature of the adsorption isotherm indicates the existence of more than one class of binding sites in DNA to which quinacrine becomes bound by more than one process (16). It is conventional to consider such a nonlinear adsorption isotherm the result of only two binding processes involving two corresponding classes of binding sites (16). Accordingly the curve (Fig. 2) was extrapolated to the axes of the coordinate system for the regions of the highest and lowest values of r (drug molecules bound per constituent nucleotide); the two corresponding stoichiometries were obtained from the intercepts of the two tangents with the abscissa, and the apparent association constants were derived from the intercepts with the ordinate (16).

By the stronger of the two processes, 1 molecule of quinacrine was bound per 4 nucleotides, i.e., per two base pairs, with an apparent association constant of 1.2×10^6 M^{-1}. Since quinacrine is known to intercalate into DNA (14) and maximal intercalation has been postulated to occur adjacent to every 2.2nd base pair (19), the strong binding of quinacrine to double-stranded calf thymus DNA can be attributed to intercalation. From less exacting spectrophotometric measurements, the stoichiometry of *total* binding of quinacrine to DNA had been estimated to amount to 1 drug molecule/4 nucleotides (12).

By the weaker of the two processes, 1 molecule of quinacrine was additionally bound per 3 constituent nucleotides of DNA, with an apparent association constant of 4.6×10^4 M^{-1}. Electrostatic forces have been implicated in the weaker binding process of aminoacridines to DNA in general

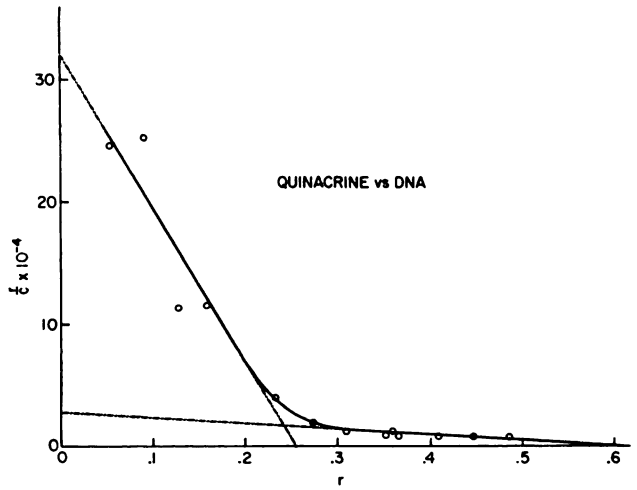

FIG. 2. *Adsorption isotherm for binding of quinacrine to DNA*

r is the number of drug molecules bound per nucleotide, and *c* is the concentration of unbound quinacrine. The quantities *r* and *c* were derived as described previously (16) from optical densities, obtained with increasing concentrations of DNA, for the maxima of the most intense visible absorption band of quinacrine. The optical density for the completely bound dye was obtained by extrapolation to zero of a double-reciprocal plot of optical densities with respect to polymer concentrations.

(16), and also may be responsible for the weak binding of quinacrine.

The maximal equilibrium binding of quinacrine by both processes hence has a stoichiometry of 7 quinacrine molecules/12 nucleotides, or $r = 0.58$, as shown in Fig. 2.

Displacement of methyl green from DNA by quinacrine. Methyl green, a triphenylmethane dye, forms a stable complex with DNA with a stoichiometry of 1 dye molecule/13 nucleotides (20). When methyl green becomes dissociated from DNA, the liberated dye undergoes a spontaneous molecular rearrangement with attending loss of color. This makes it possible to determine the displacement of the dye from DNA by measuring spectrophotometrically the decrease in absorbance at 642 nm (Table 1).

Displacement of methyl green from DNA by quinacrine had been observed at an arbitrary end point of 18 hr (12). We have developed methyl green displacement into a quantitative method (21) which determines both the kinetics of the displacement reaction and the true end point beyond which no further displacement occurs. Quinacrine, at a molar concentration 2.5 times in excess of that of DNA-bound methyl green, displaced 97.2% of the dye; this required incubation at room temperature of the DNA–methyl green–quinacrine mixture for several days. The kinetic course of the reaction was first-order with time during the initial 2 hr, i.e., as long as free quinacrine was present in excess. Thereafter the reaction changed to

TABLE 1

Displacement of methyl green from its complex with calf thymus DNA by quinacrine and related compounds

Compounds were present at 50 μM, and methyl green at 18.8 μM, bound to DNA at 245 μM mononucleotide.

Compound	First-order rate constant	Second order rate constant	End point (methyl green displaced)
	hr^{-1}	$\frac{M^{-1}}{hr^{-1}}$	%
Quinacrine	0.52	13290	97.2
9-Methylamino-3-chloro-7-methoxyacridine	0.11	2805	85.5
1-Diethylamino-4-aminopentane	0.01	221	23.3
Proflavine	0.23	5610	92.5
Acridine orange	0.03	738	38.5

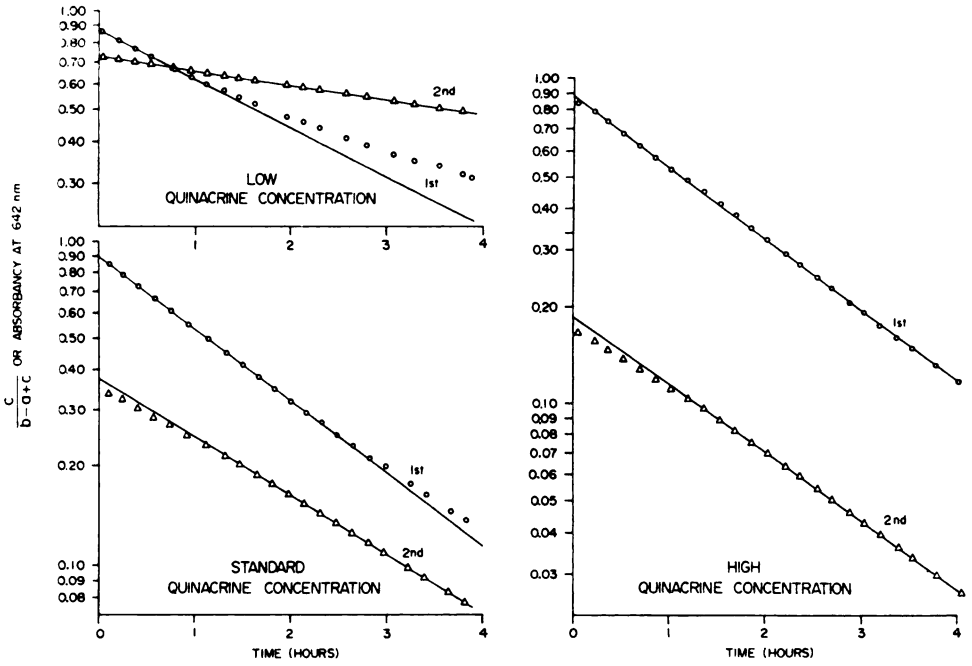

FIG. 3. *First-order (1st) and second-order (2nd) kinetics for displacement of methyl green from its complex with DNA by quinacrine*

a = initial concentration of DNA-bound methyl green (18.8 μM; b = initial concentrations of quinacrine (50 μM, standard concentration of quinacrine; 100 μM, high; 25 μM, low); c = concentration of the amount of methyl green which remained bound to DNA. On the ordinate absorbance is plotted for first-order kinetics, and $c/(b - a + c)$, for second-order kinetics.

second-order kinetics, in which the rate depended upon the remaining concentrations of both free quinacrine and DNA-bound methyl green. At a 5-fold molar excess of quinacrine the displacement reaction was first-order with time during its measured course of 4 hr, while at 1.25-fold molar excess second-order kinetics was observed from the beginning of the reaction. These different courses of the displacement reaction are shown in Fig. 3.

Over the 4-hr experimental period the absorbance of a DNA–methyl green solution did not change, while free methyl green decayed in a first-order reaction whose rate was greater than that of the decline of the absorbance of the DNA complex during displacement of methyl green by quinacrine. Hence the decay of free methyl green was not a rate-limiting process in the measurement of dye displacement from DNA.

The transition from first- to second-order kinetics at moderate molar excess (2.5-fold) of free quinacrine over bound methyl green may be explained by the following considerations. The stoichiometry of binding of methyl green to DNA is 0.077 dye molecule/nucleotide (20), while that for quinacrine, reported here, is 0.25 molecule/nucleotide for the strong binding process. It follows that more quinacrine binds to DNA than the number of methyl green molecules displaced by the drug. This stoichiometric excess of binding of quinacrine to DNA reduced the concentration of free drug to the extent that after 2 hr it became one of the two rate-limiting factors, the second one being the remaining concentration of bound methyl green.

Spectrophotofluorometric titration of quinacrine with DNA. Fluorometric titrations of quinacrine with various DNAs at inorganic salt concentrations of approximately 0.1 M showed a progressive, nonlinear decline in fluorescence intensity at 494 nm with increasing DNA concentrations (10, 11). The

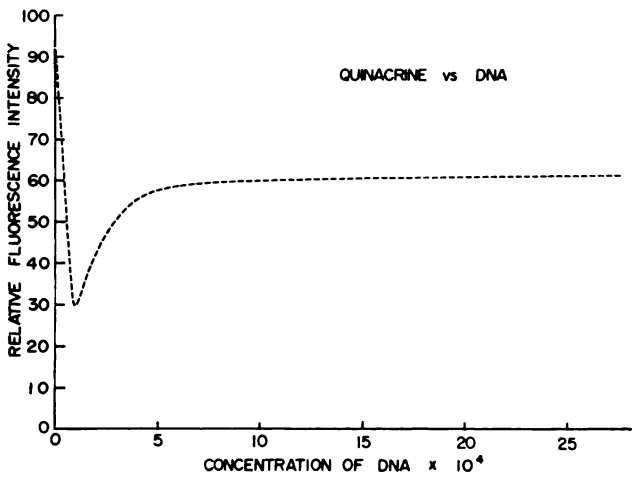

FIG. 4. *Fluorometric titration of quinacrine, 20 μM, with DNA*

Fluorescence was excited at 322 nm, the position of the short wavelength isosbestic point shown in Fig. 1, and emission intensities were measured at the maximum of each emission spectrum.

fluorescence of aminoacridines, in general, is quenched by DNA (16); for quinacrine, the extent of quenching is a function of the G–C content of the DNA species used (10). Experiments with synthetic polydeoxyribonucleotides have shown that poly dA·dT (10) and poly d(A–T) (11) enhance the fluorescence of the drug while poly d(G–C) quenches it (11). It has been estimated that at least four adjacent A–T pairs are needed to enhance the fluorescence of intercalated quinacrine (11).

We have reproduced fluorometric titrations of quinacrine with DNA at high salt concentrations (0.2 M) with results (not shown) similar to those of other workers (10, 11). However, when such titrations were carried out in 5 mM Tris-HCl, a different titration curve was obtained (Fig. 4). At lower concentrations of added DNA the fluorescence intensity of quinacrine steeply declined, until a drug to mononucleotide ratio of $r = 0.22$ was attained; upon further addition of DNA the fluorescence of quinacrine increased and eventually approached a plateau similar to that attained from higher fluorescence intensities in titrations at 20–40 times higher inorganic salt concentrations.

Under our low ionic conditions the duplex polymer poly dA·dT enhanced the fluorescence of quinacrine 5-fold (Fig. 5); the same polymer also increases the fluorescence of the drug in 0.1 M sodium phosphate (10). We have shown for the first time that poly dG·dC quenched the fluorescence of quinacrine (Fig. 5); similar results at high salt concentrations have been reported for poly d(G–C) (11). Our results and those of others (10, 11) converge on the conclusion that the presence of guanine in synthetic duplex polydeoxynucleotides, as well as in DNA itself, is responsible for the observed quenching. Based upon the postulate (11) that the fluorescence-enhancing site in DNA is an A–T quadruplet, it is readily apparent that the frequency of this sequence will be less than 1% of all possible quadruplets (disregarding differences between A–T and T–A pairs), that for any natural DNA the number of quenching sites will hence be in gross excess over the number of enhancing sites, and that all DNAs will produce net quenching of quinacrine fluorescence in a homogeneous liquid phase.

DNA-induced Cotton effects in ORD spectrum of quinacrine. The preparation of DL-quinacrine used in our work did not exhibit anomalous optical rotation which might have been associated with the asymmetrical carbon atom of the isobutylamino side chain. While the possibility was not experimentally excluded that for racemic quinacrine, associated Cotton effects of opposite signs might have cancelled each other out, our previous

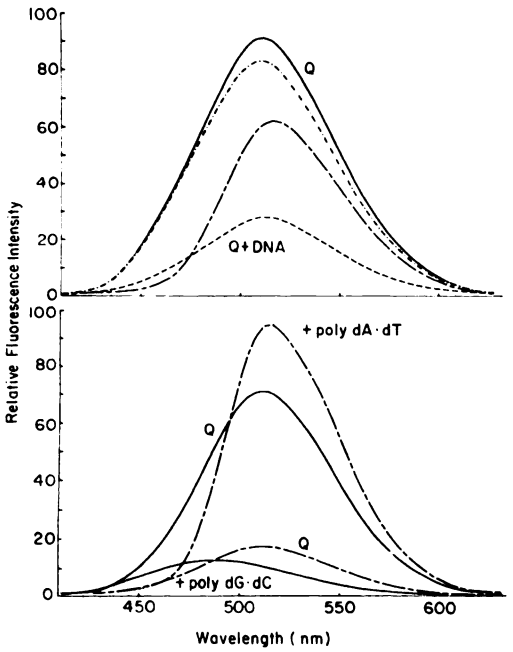

FIG. 5. *Effects of DNA (top spectra), of poly dA·dT (bottom spectra, broken lines) and of poly dG·dC (bottom spectra, solid lines) on fluorescence emission spectrum of quinacrine (Q)*

Concentrations: 20 μM quinacrine; 22 μM (·–·–), 100 μM (-----), and 2.7 mM (– – –) DNA phosphorus; and 120 μM synthetic polymers. Relative excitation slit width for the experiments with DNA and poly dG·dC was 1.35; for poly dA·dT, 0.4; the emission slit was set at 1.0.

studies with D-chloroquine (15) showed that this enantiomer, which is analogous to D-quinacrine, does not exhibit an associated Cotton effect. We assume, therefore, that the analogously positioned asymmetrical carbon atom does not cause asymmetrical perturbations in the electron distribution of the quinoline and acridine chromophores.

DNA induces anomalies in the ORD spectra of several aminoacridines (22–26); such induced Cotton effects in the ultraviolet ORD spectrum of quinacrine have been illustrated by us in a preliminary communication (15). Figure 6 depicts a complete recording of Cotton effects induced in the ORD spectrum of quinacrine by double-stranded and single-stranded DNAs.

Double-helical DNA at $r = 0.11$, at which nearly all quinacrine is bound by intercalation (Fig. 2), induced multiple Cotton effects in the ORD spectrum of the drug by rendering optically active two principal electronic transitions (425–450 nm) and, most strongly, that at 333 nm, which is responsible for a minor peak in the absorption spectrum of the drug (Fig. 1). In contrast, single-stranded DNA induced a single positive Cotton effect of smaller molecular amplitude by rendering optically active the transition at 345 nm (Figs. 1 and 6).

Influence of quinacrine on thermal denaturation profile ("melting curve") of DNA. Aminoacridines share the group property of stabilizing double-helical DNA to thermal denaturation (16). As expected, therefore, quinacrine shifted the thermal denaturation profile of calf thymus DNA to higher temperature. At 2 μM the drug caused a change in melting temperature (ΔT_m) of +8° in approximately 70 μM DNA (concentration with respect to mononucleotides). We have not expanded this result at $r = 0.029$ to measurements at other drug to nucleotide ratios; at this value of r nearly all the quinacrine present will have been bound to DNA by intercalation at room temperature (Fig. 2).

We explored the behavior of a complex of quinacrine with heat-denatured DNA in a temperature gradient (Fig. 7). Denatured DNA alone typically showed a slight, non-cooperative increase in absorbance at 260 nm when heated from room temperature to 95°, while the complex of this DNA with quinacrine exhibited above 45° a large increase in absorbance, which occurred over a narrower temperature range. The absorbance at 260 nm at 95° could be accounted for by the arithmetic sum of contributions from the absorbance increase of denatured DNA alone plus the hyperchromic contribution of quinacrine itself upon its liberation from the complex. We infer that denatured DNA forms a complex with quinacrine, which is dissociated by heat in a cooperative manner.

DISCUSSION

The binding of the drug quinacrine to its bioreceptor, DNA, was studied by optical methods. Although a partial adsorption isotherm for the binding of quinacrine to calf thymus DNA had been presented earlier for

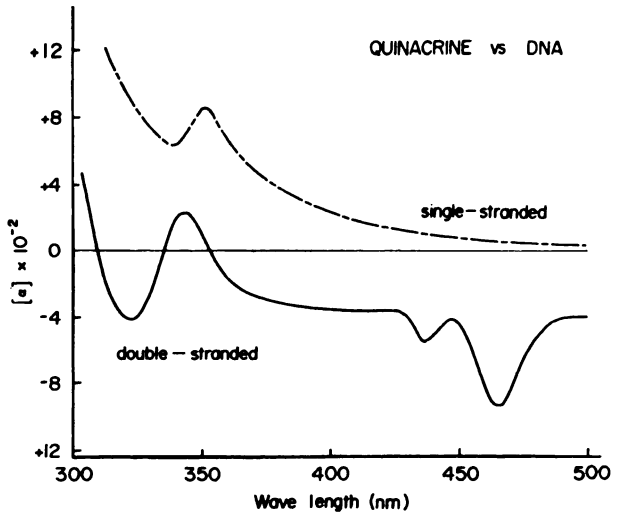

FIG. 6. *DNA-induced Cotton effects of quinacrine*

Concentrations: 890 μM double-stranded or single-stranded DNA phosphorus and 100 μM quinacrine. The induced effects are represented as ORD difference spectra obtained by arithmetic subtraction of the contribution of DNA from the recordings for the quinacrine-DNA complex. Optical activities are expressed as specific rotations.

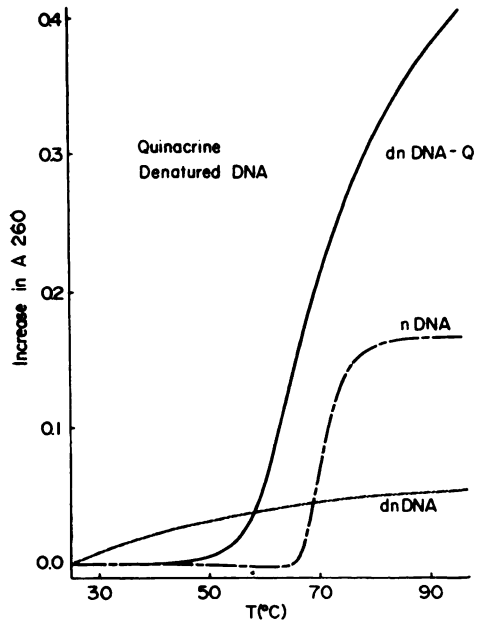

FIG. 7. *Melting profiles in 20 mM KCl of thermally denatured (dn) DNA in the absence and presence of quinacrine, and melting profile of native (n) DNA*

Concentrations: 63 μM DNA phosphorus and 31.5 μM quinacrine.

comparison with such curves for other aminoacridines (27), we report for the first time numerical values of stoichiometries and association constants for intercalative and peripheral binding of the drug to DNA. The reasons why intercalation of quinacrine, like that of proflavine (18) or chloroquine (28), is limited to 1 drug molecule/~4 nucleotides remain unknown. Cairns (19) has suggested that this intercalation stoichiometry results from conditions under which only every 2.2nd intercalation slot in DNA is available for physical occupancy. The determinants of this exclusion principle await study.

Changes in the absorption spectrum of quinacrine upon binding to DNA, which we used as an optical indicator in titration experiments, are not base-specific (5, 11). In this respect the drug differs from chloroquine (5) or quinine (29), for which the intensity of major absorption bands is preferentially decreased by polynucleotides which contain guanine.

Aminoacridines do, however, exhibit base specificities in their binding to DNA by other indications. The ΔT_m values caused by proflavine in the melting curves of DNAs of

different base compositions are a function of the A–T content of these polymers (30), and, as for quinacrine, the fluorescence of DNA-bound 3,6-diamino-9-methylacridinium chloride (31) or proflavine (32) is quenched as a function of the G–C content of DNAs.

For quinacrine we, like others (10), found that its fluorescence was strongly enhanced by binding to poly dA·dT; it was quenched by binding to poly dG·dC. Our fluorometric titration curve of quinacrine with DNA (Fig. 4) at a low inorganic ion concentration differs from such curves (10, 11) at 20–40 times greater ionic strengths, in that fluorescence decreased steeply with the amount of DNA added until $r = 0.22$ was attained, while supplying more DNA produced increases in fluorescence with eventual attainment of a plateau.

One possible explanation of this observation is that at initial high values of r quinacrine occupied indiscriminately all available binding sites of DNA with attendant fluorescence quenching, but that with progressively decreasing values of r the drug redistributed itself to bind preferentially to the fluorescence-enhancing A–T sites of DNA inasmuch as more of these sites became available. Preferential population of fluorescent A–T sites to which the aminoacridine binds 3–4 times more strongly than to quenching sites of DNA has been postulated for proflavine (32) on the basis of converse titrations of constant DNA concentrations with increasing concentration of dye. The low ionic concentration in our titration experiment would have favored, at high values of r, the occupancy by quinacrine of peripheral binding sites by electrostatic attraction, while the high salt concentrations in fluorometric titrations by others (10, 11) might have interfered with such occupancy. Titrations of quinacrine at high ionic strength, at which the existence of only one mode of binding was indicated for ethidium bromide (33), could not be carried out because of insolubility of our aminoacridine under the ionic experimental conditions.

A definitive interpretation of DNA-induced optical activities in the ORD spectrum of quinacrine would have to be based upon a correspondingly definitive interpretation of the absorption spectrum of the drug, which, to our knowledge, has not been made. The absorption bands in the region of 380–450 nm are those of monoprotonated 9-iminoacridines. The induction of optical activities in these transitions by duplex DNA indicates that the 9-imino moiety of the drug molecule is involved in the interaction of quinacrine with double-stranded DNA. Such involvement apparently does not exist for the interaction of quinacrine with single-stranded DNA, which does not induce Cotton effects in the wavelength region around 450 nm.

Acridine itself shares a series of electronic transitions around 350 nm with its carbocyclic analogue, anthracene (34). It is difficult to assign subtle differences between the spectra of anthracene and acridine to the influence of the ring nitrogen. For quinacrine, it has been assumed that the drug molecule exists as a resonance hybrid in proton equilibrium between the charges of the ring nitrogen and of the imino group in position 9 (35). For these various reasons, neither the Cotton effect induced by duplex DNA at 333 nm nor that induced by single-stranded DNA at 345 nm can be assigned with any degree of confidence to an interaction of a protonated ring nitrogen with DNA phosphates; we are left with the general conclusion that the interactions of the acridine ring structure of the drug with double- and single-stranded DNAs are different.

The ability of quinacrine to displace methyl green from its complex with DNA invites comparison with similar abilities of structurally related compounds, listed in Table 1 (from ref. 21). For 9-methylamino-3-chloro-7-methoxyacridine, an analogue of quinacrine in which the side chain is replaced by $—CH_3$, the reaction rates and the end point were significantly less than those for the drug itself. The side chain, in which the acridine ring system is replaced by hydrogen (1-diethylamino-4-aminopentane), showed low activity and displaced only 23.3% of methyl green from DNA. Proflavine and especially its tetramethyl derivative, acridine orange, which do not carry aliphatic and cationic side chains, were significantly less active than quinacrine. We conclude that the affinity of quinacrine for

DNA is a function of both the acridine ring system and the cationic side chain, and that both molecular moieties are involved in the binding of the drug to double-helical DNA. For the quinacrine analogue chloroquine it has been postulated that the side chain protrudes beyond the contour lines of the double helix and binds electrostatically to both complementary strands across the minor groove (36). The same side chain in quinacrine may act in an analogous manner.

REFERENCES

1. Mietzsch, F. & Mauss, H. (1932) German Patent 553,072; *Chem. Abstr.*, **26**, 4683.
2. Schellenberg, K. A. & Coatney, G. R. (1961) *Biochem. Pharmacol.*, **6**, 143–152.
3. Van Dyke, K. & Szustkiewicz, C. (1969) *Mil. Med.*, **134**, 1000–1006.
4. Ciak, J. & Hahn, F. E. (1967) *Science*, **156**, 655–656.
5. O'Brien, R. L., Olenick, J. G. & Hahn, F. E. (1966) *Proc. Natl. Acad. Sci. U. S. A.*, **55**, 1511–1517.
6. Ames, B. N. & Whitfield, H. J. (1966) *Cold Spring Harb. Symp. Quant. Biol.*, **31**, 221–225.
7. Johnson, H. G. & Bach, M. K. (1966) *Proc. Natl. Acad. Sci. U. S. A.* **55**, 1453–1456.
8. Hahn, F. E. & Ciak, J. (1971) *Ann. N. Y. Acad. Sci.*, **182**, 295–304.
9. Vosa, C. C. (1970) *Chromosoma*, **30**, 366–372.
10. Weisblum, B. & de Haseth, P. L. (1972) *Proc. Natl. Acad. Sci. U. S. A.*, **69**, 629–632.
11. Pachmann, U. & Rigler, R. (1972) *Exp. Cell Res.*, **72**, 602–608.
12. Kurnick, N. B. & Radcliffe, I. A. (1962) *J. Lab. Clin. Med.*, **60**, 669–688.
13. Irvin, J. L. & Irvin, E. M. (1954) *J. Biol. Chem.*, **206**, 39–49.
14. Lerman, L. S. (1963) *Proc. Natl. Acad. Sci. U. S. A.*, **49**, 94–102.
15. Hahn, F. E. & Krey, A. K. (1969) *Antimicrob. Agents Chemother.*, 15–20.
16. Blake, A. & Peacocke, A. R. (1968) *Biopolymers*, **6**, 1225–1253.
17. Peacocke, A. R. & Skerrett, J. N. H. (1956) *Trans. Faraday Soc.*, **52**, 261–279.
18. Michaelis, L. (1947) *Cold Spring Harb. Symp. Quant. Biol.*, **12**, 131–142.
19. Cairns, J. (1962) *Cold Spring Harb. Symp. Quant. Biol.*, **27**, 311–318.
20. Kurnick, N. B. & Foster, M. (1950) *J. Gen. Physiol.*, **34**, 147–159.
21. Krey, A. K. & Hahn, F. E. (1971) *Proc. 1st Eur. Biophys. Congr.*, **1**, 223–227.
22. Neville, D. M. & Bradley, D. F. (1961) *Biochim. Biophys. Acta*, **50**, 397–399.
23. Blake, A. & Peacocke, A. R. (1966) *Biopolymers*, **4**, 1091–1104.
24. Yamaoka, K. & Resnik, R. A. (1966) *J. Phys. Chem.*, **70**, 4051–4066.
25. Gardner, B. J. & Mason, S. F. (1967) *Biopolymers*, **5**, 79–94.
26. Yamaoka, K. (1968) *Biochim. Biophys. Acta*, **169**, 553–556.
27. Drummond, D. S., Simpson-Gildemeister, V. F. W. & Peacocke, A. R. (1965) *Biopolymers*, **3**, 135–153.
28. O'Brien, R. L., Allison, J. L. & Hahn, F. E. (1966) *Biochim. Biophys. Acta*, **129**, 622–624.
29. Estensen, R. D., Krey, A. K. & Hahn, F. E. (1969) *Mol. Pharmacol.*, **5**, 532–541.
30. Kleinwächter, V., Balcarová, A. & Boháček, J. (1969) *Biochim. Biophys. Acta*, **174**, 188–201.
31. Tubbs, R. K., Ditmars, W. E. & Van Winkle, Q. (1964) *J. Mol. Biol.*, **9**, 545–557.
32. Thomas, J. C., Weill, G. & Daune, M. (1969) *Biopolymers*, **8**, 647–659.
33. Lepecq, J.-B. & Paoletti, C. (1967) *J. Mol. Biol.*, **27**, 87–106.
34. Radulescu, D. & Ostrogovich, G. (1931) *Ber.*, **64**, 2233–2240.
35. Irvin, J. L. & Irvin, E. M. (1950) *J. Amer. Chem. Soc.*, **72**, 2743–2749.
36. O'Brien, R. L. & Hahn, F. E. (1966) *Antimicrob. Agents Chemother.*, 315–320.