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The Binding of Antimalarial Aminoquinolines to Nucleic Acids and Polynucleotides

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SUMMARY

The binding of antimalarial 8-aminoquinolines (including primaquine and pentaquine), some of their potential metabolites, and chloroquine to DNA, RNA, and various polydeoxyribo- and polyribonucleotides has been studied by equilibrium dialysis and direct spectrophotometry. The extent of binding of primaquine, pentaquine, and chloroquine to native calf thymus DNA, as measured by equilibrium dialysis, does not vary in the range of pH 6.0–8.0. A major portion of each 6-hydroxy derivative of the 8-aminoquinolines binds to DNA, although, in the two examples studied, the extent of binding is somewhat less than for the corresponding 6-methoxy parent compound. Removal of the protonated terminal nitrogen of the 8-diamino side chain decreases the binding of the 8-aminoquinolines to very low levels. The structure and composition of polynucleotides affect their binding of the 8-aminoquinolines and chloroquine. Data from equilibrium dialysis show that the extent of binding of 8-aminoquinolines is greatest to native and denatured DNA, followed, in order, by soluble RNA and purine-containing polyribonucleotides (poly rI, poly rA, poly rG), poly rU, and poly rC. The nucleotide sequence and composition also affect the polynucleotide-induced decrease in the absorbance of an aminoquinoline. Thus, double-stranded homopolymers like poly dAdT and poly dGdC cause only a slight decrease (less than 3%) in the absorbance of pentaquine, but the double-stranded alternating copolymer poly dAT causes a major decrease (more than 35%; similar to the decrease caused by native DNA) in the absorbance of the same aminoquinoline. Extensive binding of an aminoquinoline to a polynucleotide may occur without a marked concomitant decrease in the absorbance of the aminoquinoline. In equilibrium dialysis experiments, the presence of primaquine does not decrease the binding of chloroquine to native calf thymus DNA, even at a molar ratio of primaquine to chloroquine of 8:1. However, chloroquine decreases the binding of primaquine to native DNA. Although there is an apparent interaction between the ring systems of bound primaquine and the nucleic acid bases, primaquine does not appear to intercalate into the DNA structure to a sufficient extent to be detectable by an increase in the viscosity of native DNA.

INTRODUCTION

Antimalarial aminoquinolines, quinacrine, and quinine bind to DNA (1–6), and it has been suggested that the mechanism of the antimalarial activity may be related to in-

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hibition of the function of DNA in polymerase reactions as a result of binding of the drug. Some properties of the binding of 8-aminoquinolines to DNA have been reported previously (2).

The binding of the 8-aminoquinolines to DNA is (a) accompanied by a decrease in absorbance of the ligand, (b) decreased by an increase in ionic strength, and (c) decreased by Mg^{2+} to a greater extent than would be expected from the effects of ionic strength alone.

Additional studies by equilibrium dialysis and direct spectrophotometry have now been carried out on the binding of antimalarial 8-aminoquinolines (primaquine, pentaquine, plasmocid, and pamaquine), some of their potential metabolites, and the antimalarial 4-aminoquinoline chloroquine. The polymers studied include DNA from calf thymus and *Clostridium perfringens*, yeast soluble RNA, and various polydeoxyribo- and polyribonucleotides. A preliminary report of this investigation has been made (7).

MATERIALS AND METHODS

The experimental methodology was essentially the same as previously reported (2). The aminoquinolines studied were not detectably adsorbed to the dialysis tubing in the concentration ranges of free aminoquinolines used in these studies. The structures, pK_a values (equivalent to the pK_{a2}), and the molar absorptivities of primaquine, pentaquine, plasmocid, and pamaquine have been presented (2). The pK_a values and the molar absorptivities (measured at pH 6.0 in 0.01 M potassium phosphate within the range of 310–470 mμ) of other aminoquinolines are given as footnotes to the tables. In some experiments, binding was measured by the distribution of chloroquine-^{14}C or primaquine-^{3}H in equilibrium dialysis cells; both radioactive aminoquinolines were manufactured by New England Nuclear Corporation. Aminoquinolines not previously described and the radioactive primaquine were kindly supplied by the Army Malaria Research Program through the courtesy of Dr. David P. Jacobus of the Walter Reed Army Institute of Research.

The polynucleotides used for equilibrium dialysis were obtained from Biopolymers Laboratory, and polynucleotides used for direct spectrophotometry were obtained from Biopolymers Laboratory or from Miles Chemical Company; the DNA of calf thymus, from Sigma Chemical Company; the DNA of *C. perfringens*, from Worthington Biochemical Corporation; the DNA of *Micrococcus lysodeikticus*, from Miles Chemical Company; and the yeast soluble (transfer) RNA, from Schwarz BioResearch. The molar concentrations of DNA and other polynucleotides are expressed in terms of the concentrations of DNA phosphorus or polynucleotide phosphorus, which are equivalent to the concentration of the mononucleotide units of the polymers.

Viscosities were measured in a Cannon-Ubbelohde four-bulb shear dilution viscometer. From a plot of η_{sp} for each DNA concentration with respect to the shear stress calculated from data supplied with the viscometer and extrapolation to zero shear stress, the η_{sp} value at zero shear was determined for each DNA concentration.

In those experiments in which the binding was studied over a sufficiently wide range of polymer concentrations, the binding characteristics were evaluated (8) from the double-reciprocal plot of $1/r$ against $1/c$, where r = the molar concentration of aminoquinoline bound per molar concentration of polymer nucleotide and c = the molar concentration of the unbound aminoquinoline. From such a plot, the intercept on the vertical axis is equal to $1/n$, where n = the maximum number of aminoquinolines bound per polymer nucleotide. The association constant, K, was calculated from the relation slope = $1/nK$. Linear regression lines were determined with the aid of an Olivetti Underwood Programma 101 computer.

RESULTS AND DISCUSSION

Binding studies were routinely carried out in 0.01 M potassium phosphate (ionic strength 0.012) at pH 6.0, unless otherwise stated. At pH 6, the antimalarial 8-aminoquinolines (pK_2 3.0–3.2) occur as monovalent cations and the 4-aminoquinoline chloroquine (pK_2 7.8) exists predominantly as a doubly protonated, divalent cation (2).

However, within the pH range of 5.9–8.0, there is essentially no change in the extent of binding of the aminoquinolines measured by equilibrium dialysis with changes in pH if constant ionic strength is maintained (Fig. 1). Although the protonation of the 8-aminoquinolines does not change significantly in this pH range, the diprotonated form of chloroquine varies from more than 98% at the lower value to approximately 40% at the upper pH value. The binding results are not in contradiction to the report by Cohen and Yielding (3) that it is the divalent form of chloroquine which binds to DNA. There is ample time during the 20-hr equilibrium dialysis procedure for re-establishment of the equilibrium between free monovalent and free divalent chloroquine, as divalent chloroquine is, in effect, removed from solution by binding to DNA, and more than 90% of the total chloroquine in the dialysis cell is bound to DNA at equilibrium.

Binding of the primaquine and pentaquine to native and denatured DNA of *C. perfringens* is compared with the binding to calf thymus DNA in Fig. 2a and b. The adenine

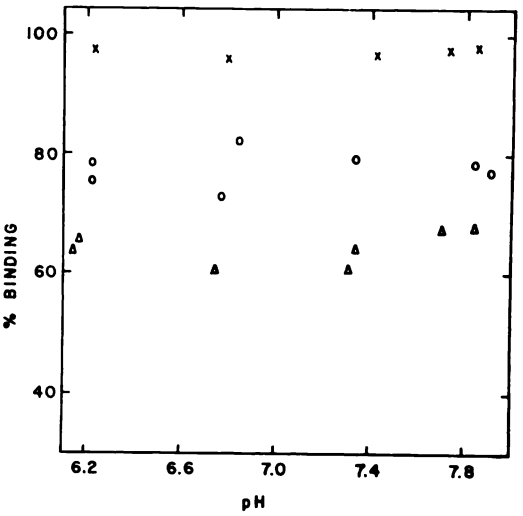

FIG. 1. *Percentage of aminoquinoline bound to native calf thymus DNA at various pH values and at a constant ionic strength of 0.025 (in potassium phosphate buffers of various molarities).*

The DNA-P to aminoquinoline molar ratios were 10–12:1; the DNA-P concentrations ranged from 4.3 to 5.0 mM. ×, chloroquine; ○ pentaquine; △, primaquine.

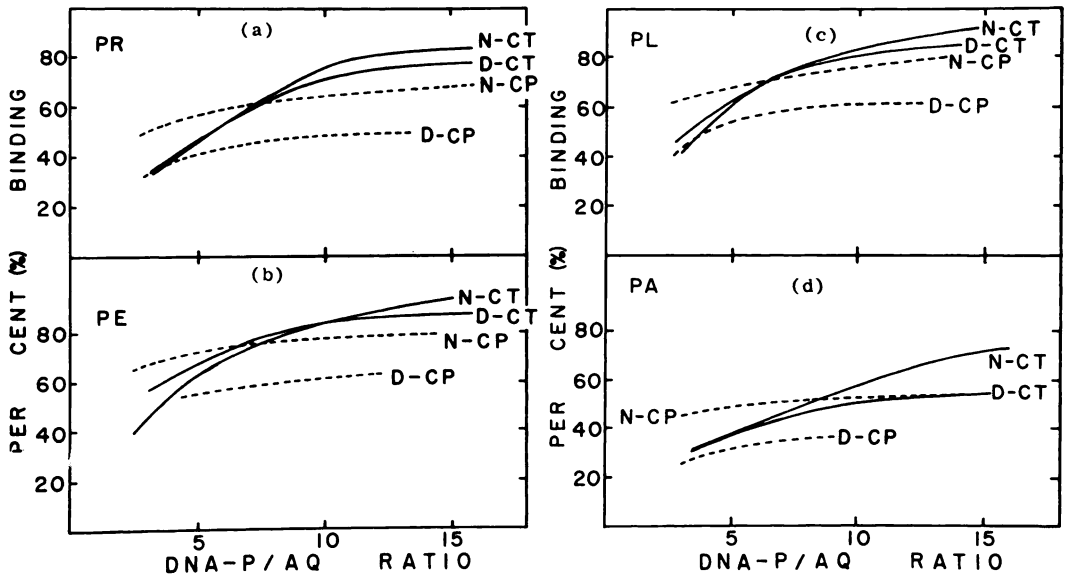

FIG. 2. *Percentage of aminoquinoline bound, as measured by equilibrium dialysis, at various DNA-P to aminoquinoline (AQ) ratios in 0.01 M potassium phosphate, pH 6*

The DNA-P concentrations ranged from 1.1 to 4.4 mM. DNA used: N-CT and D-CT, native and denatured calf thymus DNA; N-CP and D-CP, native and denatured DNA of *C. perfringens*. PR, primaquine; PE, pentaquine; PL, plasmocid; PA, pamaquine.

plus thymine content is considerably higher in the DNA of *C. perfringens* (73%) than in DNA of calf thymus (56%); the (A + T):(G + C) ratios are 2.7 and 1.3 for the DNA of *C. perfringens* and calf thymus, respectively.

At low DNA phosphorus to aminoquinoline ratios, the binding to native bacterial DNA exceeds the binding to native calf thymus DNA. However, at high DNA-P[2] to aminoquinoline ratios, binding to the native calf thymus DNA exceeds the binding to the native bacterial DNA. Similar results were obtained when two other antimalarial 8-aminoquinolines (plasmocid and pamaquine) were studied in the same system (Fig. 2c and d). These results are consistent with the existence of a larger number of strong binding sites and fewer weak binding sites within the calf thymus DNA compared to the bacterial DNA. Cohen and Yielding (3) have shown the presence of at least two (a strong and a weak) binding sites for the interaction between DNA and chloroquine. The occurrence of two such sites, as inferred from Scatchard plots and from the existence of two spectrally distinct bound forms of the antimalarial 8-aminoquinolines, has been found in this laboratory (2). Thus, at high DNA-P to aminoquinoline ratios, it appears that more 8-aminoquinoline is bound to the calf thymus DNA because of its larger number of strong binding sites. In contrast, at low DNA-P to aminoquinoline ratios (that is, at relatively high aminoquinoline to DNA-P ratios), there is a tendency to saturate all of the strong binding sites and an increasing proportion of the weaker binding sites. Native DNA of *M. lysodeikticus* [A + T equal to 29% of the base composition; the (A + T):(G + C) ratio of 0.4] was studied in equilibrium dialysis experiments similar to those shown in Fig. 2a for primaquine. The binding of primaquine to native calf thymus DNA is less than the binding to either of the native bacterial DNAs at low DNA-P to aminoquinoline molar ratios (5:1–7:1). The extent of binding of primaquine to native DNA of calf thymus and *M. lysodeikticus* is approximately equal at high DNA-P to aminoquinoline ratios (13:1–18:1), although the base compositions of the DNAs differ markedly. Thus, the binding data do not suggest that the number of strong (or weak) binding sites for primaquine (or pentaquine) in a DNA sample is directly proportional to the presence of a specific base or base pair within the DNA. However, it is not possible to exclude completely a possible effect of trace amounts of contaminating protein in the DNA samples on the observed levels of binding.

The common antimalarial 8-aminoquinolines have a 6-methoxy group which, at least in some species, undergoes extensive *O*-demethylation and subsequent oxidation at position 5 to yield the 5,6-dihydroxy derivatives (9, 10). It was of interest, therefore, to study the binding to DNA and other polynucleotides by these potential metabolites of several 6-methoxy-8-aminoquinolines. Data from equilibrium dialysis on the percentage of binding to native and denatured calf thymus DNA by pentaquine (the 6-methoxy compound) and the 5,6-dihydroxy and 5,6-dimethoxy derivatives are given in Table 1 (left-hand portion). All three compounds have the same side chain on the 8-amino nitrogen. A major portion of the potential 5,6-dihydroxy metabolite binds to native and denatured calf thymus DNA, although the extent of binding is only two-thirds of the binding by pentaquine. The 5,6-dimethoxy derivative binds to calf thymus DNA to a somewhat lesser extent than the 5,6-dihydroxy derivative. A second comparison of the binding of another 6-methoxy-8-aminoquinoline and its corresponding 6-hydroxy derivative is given in the right-hand portion of Table 1. In this case also, the binding of the 6-hydroxy derivative to DNA is similar to, but slightly less than, the binding of the 6-methoxy derivative.

The specific 6-hydroxy- and 5,6-dihy-

[2] The abbreviations used are: DNA-P, DNA phosphorus or DNA nucleotides; PN-P, polynucleotide phosphorus; ϵ = molar absorptivity of free, unbound aminoquinoline; the ribocopolymers (poly rAU, poly rAC, and poly rAG) are polynucleotide strands with randomly distributed nucleosides within the designated strand. The structures of the deoxyribopolymers are discussed in the text and Fig. 3.

TABLE 1

Binding of methoxy- and hydroxy-8-aminoquinolines to DNA

The percentage of binding was measured by equilibrium dialysis with calf thymus DNA in 0.01 M potassium phosphate, pH 6, at a DNA-P to aminoquinoline ratio of 10:1. The DNA-P concentrations ranged from 2.2 to 4.4 mM.

Polymer	8-NH—$(CH_2)_5$—NH—$CH(CH_3)_2$			8-NH—CH_2—CH_2—CH_2—NH_2	
	6—OCH_3[a]	5,6-di-OH[b]	5,6-di-OCH_3[c]	6-OCH_3[d]	6-OH[e]
	%	%	%	%	%
Native DNA	84	58	46	89	76
Denatured DNA	84	51	41	82	72

[a] Pentaquine.
[b] $pK_a < 1.0$; $\epsilon = 4810$ at 455 mμ.
[c] $pK_a = 3.2$; $\epsilon = 3520$ at 370 mμ.
[d] $pK_a = 3.1$; $\epsilon = 2480$ at 347 mμ.
[e] $pK_a = 3.2$; $\epsilon = 3970$ at 337 mμ.

droxyaminoquinolines discussed above are relatively stable under the conditions of equilibrium dialysis (pH 6 at room temperature for 20–24 hr). Comparison of the absorption spectrum immediately after preparation of the aminoquinoline solution and after 24 hr at room temperature indicated that less than 5% of each of the two hydroxylated aminoquinolines undergoes decomposition. The dihydroxypentaquine undergoes rapid aerobic decomposition in alkaline solution (11), and the 6-hydroxy-8-butylaminoquinoline (corresponding to the hydroxy derivative of the second compound of Table 2) undergoes very rapid decomposition (more than 60%) in 24 hr at pH 6. The rapid decomposition of the latter aminoquinoline prevented the study of its binding to DNA by equilibrium dialysis.

Other alterations in the structures of the 8-aminoquinolines affect the binding of these ligands to DNA as measured by equilibrium dialysis (Table 2). In some species, the terminal amino group of the side chain of chloroquine undergoes *N*-deethylation (9, 12), although this reaction appears to be relatively slower than the *O*-demethylation in pentaquine (9). The substitution of an 8-primary amino group for the 8-diamino side chain abolishes almost all of the binding to DNA. The absence of a terminal amino group on the substituted 8-amino side chain also decreases the percentage of binding to DNA to very low levels.

TABLE 2

Binding to DNA of aminoquinolines with various 8-amino substituents

The methodology was the same as in Table 1.

Aminoquinoline	Binding to native DNA
	%
6-OCH_3, 8-NH_2[a]	<5
6-OCH_3, 8-NH—CH_2—CH_2—CH_2—CH_3	<5
6-OCH_3, 8-NH—CH_2—CH_2—CH_2OH	<15
6-OCH_3, 8-NH—CH_2—CH(OH)—CH_2OH	15

[a] $pK_a = 3.6$; $\epsilon = 3070$ M^{-1} cm^{-1} at 357 mμ.

Polynucleotide structure and composition also markedly affect the binding of the 8-aminoquinolines and chloroquine to nucleic acids and polynucleotides. The 8-aminoquinolines and chloroquine bind to both polydeoxyribo- and polyribonucleotides. The binding of the 8-aminoquinolines to various polynucleotides (r = moles of aminoquinoline bound per mole of PN-P) as a function of the free aminoquinoline concentration (c) is plotted for primaquine, pentaquine, and dihydroxypentaquine[3] in Fig. 3. From such

[3] The compound referred to as "dihydroxypentaquine" is the 5,6-dihydroxyaminoquinoline, which has the same 8-diamino side chain as pentaquine; its systematic name is 5,6-dihydroxy-8-(5-isopropylaminopentylamino)quinoline.

data the relative order of binding of the 8-aminoquinolines to various polynucleotides was determined. The percentages indicated after the various polymers are the percentages of aminoquinoline bound to the respective polymers at a PN-P to aminoquinoline molar ratio of 10:1, where the total PN-P concentrations were 2.0–4.4 mM.

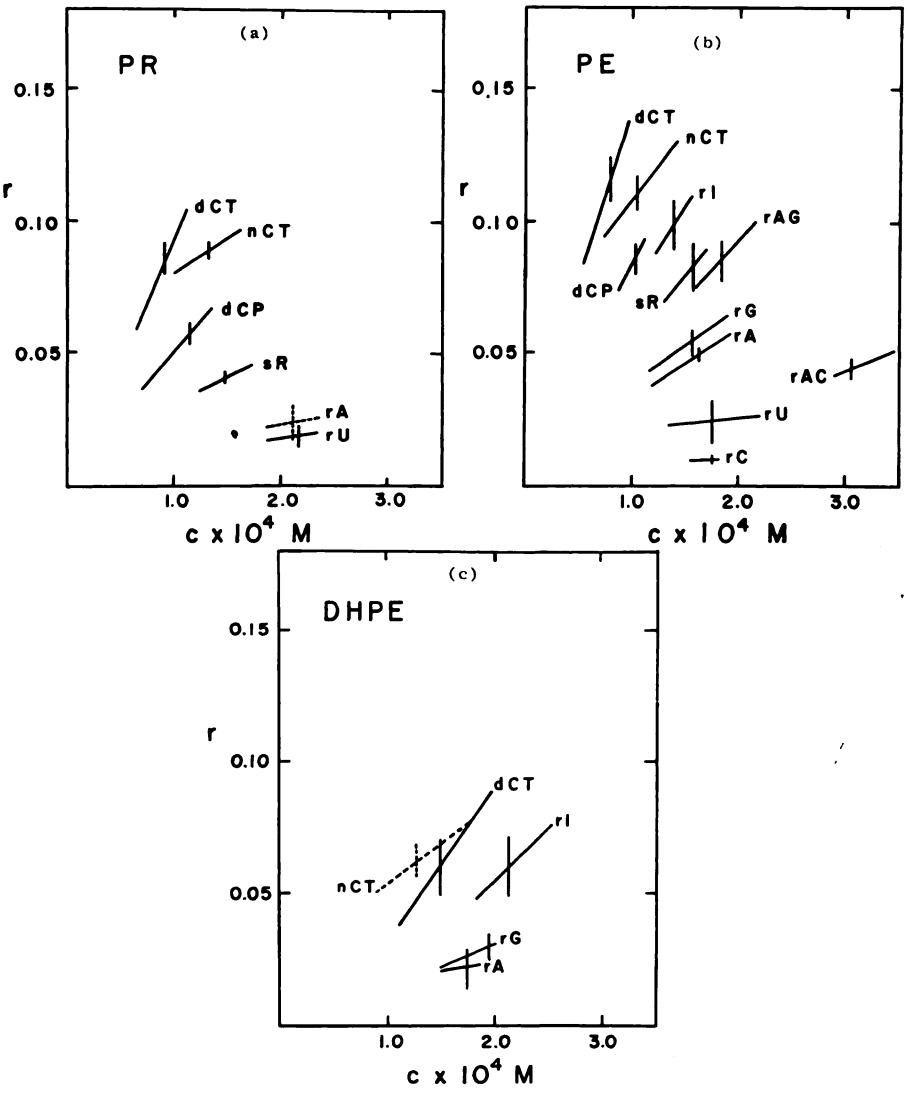

FIG. 3. *Binding of 8-aminoquinolines to various polynucleotides*

For each polymer, the inclined line is the calculated least-squares regression line (plot of r against c) of data from equilibrium dialysis measurements; r = moles of aminoquinoline bound per mole of DNA-P or PN-P; c = molar concentration of free aminoquinoline. In order to indicate the experimental variation among samples for each polymer, the mean of the differences between observed and calculated (from regression line) r is indicated as a vertical line plotted at the mean c of the experimental samples. An average of 11 (range, 6–17) determinations were made with each polymer and aminoquinoline combination. Polynucleotides: nCT and dCT, native and heat-denatured calf thymus DNA, respectively; nCP and dCP, native and denatured *C. perfringens* DNA, respectively; sR, yeast soluble RNA; rA, rC, rG, rI, and rU, polyribonucleotides of the indicated nucleoside. Aminoquinolines: PR, primaquine; PE, pentaquine; DHPE, dihydroxypentaquine.

For pentaquine, the relative order of binding to various polynucleotides was as follows: native and denatured calf thymus DNA (80–85%) > poly rAG, poly rI, yeast soluble RNA > poly rG ≧ poly rA (45%) ≫ poly rU (25%) > poly rC. For primaquine, the relative order of binding was as follows: native and denatured calf thymus DNA (75%) ≫ yeast sRNA > poly rA ≧ poly rU (20%). For dihydroxypentaquine, the relative order of binding was as follows: native and denatured calf thymus DNA (50–60%) > poly rI, poly rG > poly rA (25%).

The binding of the 8-aminoquinolines occurs to the greatest extent to native (double-stranded) and denatured (single-stranded) DNA, followed, in order, by yeast soluble RNA (single-stranded, with regions of intramolecular double-stranded structure), the purine-containing homopolymeric polyribonucleotides (poly rI, poly rA, poly rG), poly rU, and poly rC. Each of the ribohomopolymers is single-stranded at pH 6. In the case of pentaquine, the binding to the random ribocopolymer poly rAG (51% adenosine nucleotide) exceeds the binding to the ribohomopolymers poly rA and poly rG, as well as to soluble RNA.

The binding of chloroquine to these polynucleotides exceeds the binding of any of the 8-aminoquinolines, and it was not possible to demonstrate selectivity for polynucleotide binding under these experimental conditions (at very low ionic strength and at a molar ratio of PN-P to aminoquinoline of 10:1). In 0.01 M potassium phosphate, pH 6, chloroquine is bound extensively (more than 90%) to native and denatured calf thymus DNA, poly rA, poly rAU, poly rAG, and yeast sRNA at a PN-P to chloroquine molar ratio of 10:1 (total PN-P concentration of 3.7–5.0 mM). In these binding studies, performed at pH 6, chloroquine exists predominantly as a divalent cation and the 8-aminoquinolines exist as monoprotonated cations (2). Thus, at low ionic strengths, electrostatic interaction is probably a contributing factor to the greater binding of chloroquine to the anionic polynucleotides. An increase in the ionic strength (which markedly decreases electrostatic interaction) to 0.15 results in a decreased but equal binding—namely 25%—of chloroquine, pentaquine, and plasmocid to native calf thymus DNA at a molar ratio of DNA-P to aminoquinoline of 10:1 (2). Electrostatic interaction is not the only, or even the major, method of binding of the 8-aminoquinolines to polynucleotides measured by equilibrium dialysis. If electrostatic interaction were the only method of binding, one would expect approximately equal binding of a single cationic 8-aminoquinoline to each of the anionic polynucleotides (at a single concentration expressed as PN-P), and such is clearly not the case.

Binding parameters for the interaction of primaquine, pentaquine, and dihydroxypentaquine[3] to various nucleic acids are given in Table 3. Plots of r/c vs. r ("Scatchard plots") of the data from equilibrium dialysis failed to show an appreciable change in slope, and thus did not indicate the presence of more than one binding site within the limited concentration ranges studied. However, an earlier comparison of data from equilibrium dialysis and direct spectrophotometry showed that binding occurs by more than one method of interaction (2). The maximum number of aminoquinoline-binding sites per polynucleotide phosphorus is usually in the range of 0.12–0.20, as reported earlier (2). A 2-fold increase in the ionic strength, from 0.012 to 0.024 (in 0.01 and 0.02 M potassium phosphate, pH 6.0, respectively), markedly decreases the association constant of primaquine and pentaquine with native DNA without a significant change in the maximum number of binding sites per nucleotide. Binding parameters of plasmocid and pamaquine were n = 0.14 and K = 28,800, and n = 0.12 and K = 9500, respectively, for the interaction with native calf thymus DNA in 0.01 M potassium phosphate (pH 6). The association constants, which take into consideration the actual number of free binding sites on the polynucleotides, are greater than those constants reported previously (2), which considered only the unbound DNA nucleotides.

The binding of the aminoquinolines to DNA is accompanied by a decrease in ab-

TABLE 3

Binding parameters for interaction of aminoquinolines with polynucleotides

Binding was studied by equilibrium dialysis in 0.01 M potassium phosphate, pH 6.0, unless specified otherwise. The values of K are expressed in liters per mole.

Polymers	Primaquine	Pentaquine	Dihydroxypentaquine
Native calf thymus DNA	$n = 0.15$[a]	$n = 0.20$[a]	$n = 0.13$[a]
	$K = 10,000$	$K = 11,700$	$K = 7,200$
Denatured calf thymus DNA	$n > 0.11$[b]	$n > 0.14$[b]	$n > 0.09$[b]
Native *C. perfringens* DNA	$n > 0.12$[b]	$n > 0.16$[b]	
Denatured *C. perfringens* DNA	$n > 0.07$[b]	$n > 0.09$[b]	
Native calf thymus DNA[c]	$n = 0.14$[a]	$n = 0.22$[a]	
	$K = 5,300$	$K = 3,700$	
Denatured calf thymus DNA[c]	$n = 0.11$[a]	$n > 0.10$[b]	
	$K = 5,900$		
Poly rA	$n = 0.09$[a]		
	$K = 1,600$		
Yeast sRNA	$n = 0.12$[a]		
	$K = 3,400$		
Poly rG		$n = 0.17$[a]	$n = 0.15$[a]
		$K = 2,900$	$K = 1,200$
Poly rI		$n > 0.12$[b]	$n > 0.09$[b]

[a] Values of n (and K) determined from the double reciprocal plot of $1/r$ vs. $1/c$ (MATERIALS AND METHODS).

[b] From Fig. 3. These values of n are the minimum values, i.e., the highest values of r observed under the experimental conditions.

[c] In 0.02 M potassium phosphate, pH 6.0.

sorbance of the aminoquinoline, as well a as small bathochromic shift. Whereas measurements by equilibrium dialysis yield data on the total binding of aminoquinolines to polynucleotides, the polynucleotide-induced decrease in the absorbance of an aminoquinoline is generally accepted as indicative of an interaction between the ring system of the aminoquinoline and the ring systems of the polynucleotide bases (2, 13–15). Spectrophotometric evidence has been reported for an interaction between chloroquine and RNA (1), including soluble or transfer RNA (16) and purine-containing polyribonucleotides (14).

The decreases in the absorbance of pentaquine, primaquine, and chloroquine in the presence of the various polynucleotides have been examined (Table 4). Since the 8-aminoquinolines undergo only a small bathochromic shift (usually 2–5 mμ) in binding to DNA species and show essentially no change in binding to ribohomopolymers, the decrease in absorbance of the 8-aminoquinolines with various polymers is due to hypochromicity rather than to an appreciable shift in the wavelength of maximum absorption of the 8-aminoquinoline. The wavelength of the maximum absorption of chloroquine undergoes a somewhat greater shift, namely, 4–8 mμ, upon interaction with DNA species and most polyribonucleotides. Native and denatured DNA cause the largest decrease (usually 30% or greater) in the absorbance of the 8-aminoquinolines. Soluble RNA and the ribohomopolymers—poly rA, poly rI, poly rU, and poly rC—elicit little or almost no change (usually 6% or less) in the absorbance of pentaquine and primaquine.

The structures of three of the four polydeoxyribonucleotides studied are shown in Fig. 4. Poly dAdT is a duplex of one strand of repeating deoxyadenosine units hydrogen-bonded to a strand of repeating thymidine units; poly dGdC is a similar duplex of strands of homopolymers of deoxyguanosine and deoxycytidine. Poly dAT, however, is a double-stranded copolymer in which deoxyadenosine and thymidine units alternate in each strand, and two strands are

hydrogen-bonded together to form the double-stranded complex. Three polydeoxyribonucleotides, including the DNA-like double-stranded poly dAdT and poly dGdC, did not cause a significant decrease in the absorbance of pentaquine (Table 4). In contrast, however, the double-stranded alternating copolymer poly dAT caused a marked decrease (more than 35%) in the absorbance of pentaquine, an extent similar to the decrease caused by DNA.

TABLE 4

Percentage decrease in absorbance of aminoquinolines in the presence of polynucleotides

Spectrophotometry was carried out in 0.01 M potassium phosphate, pH 6.0, at the wavelength of maximum absorption within the range 343–352 mμ.

Polymer	Decrease in absorbance		
	Pentaquine	Primaquine	Chloroquine
	%	%	%
Native calf thymus DNA	43	39	57
Denatured calf thymus DNA	30	20	52
Yeast sRNA	8	4	31
Poly rA	7	<3	30
Poly rI	<3	<3	22
Poly rU	<3	<3	10
Poly rC	6	<3	8
Poly dA	<3		
Poly dGdC	<3	<3	35
Poly dAdT	<3	5	<5
Poly dAT	>35		>50
Poly rAU	10	<3	28
Poly rAC	<3	<3	18
Poly rAG	17	13	35

The largest changes in the absorbance of pentaquine appear to occur in the presence of those polynucleotides containing, within each strand, sequences of a purine adjacent to a pyrimidine (DNA, poly dAT, and poly rAU, but less with sRNA), and lesser changes in absorbance occur in those polynucleotides containing sequences of two different purines (poly rAG).

The polynucleotide-induced changes in the absorbance of chloroquine were considerably greater than the absorbance changes observed for pentaquine for each of the polymers tested. However, the relative extent of the total binding of an aminoquinoline to a series of polynucleotides, as measured by equilibrium dialysis, cannot be inferred from the extent of the polynucleotide-induced decreases in absorbance of that aminoquinoline. Soluble RNA, poly rA, poly rAG, and poly rU all bind chloroquine more than 90% and also decrease the absorbance of chloroquine by at least 30%, with the exception of poly rU, which causes only a 10% decrease in the absorbance of chloroquine. The lack of correlation between the extent of binding of chloroquine and the decrease in the absorbance of this aminoquinoline is in agreement with the report of Blodgett and Yielding (14). They reported that the decrease in absorbance of chloroquine in the presence of poly rA was only

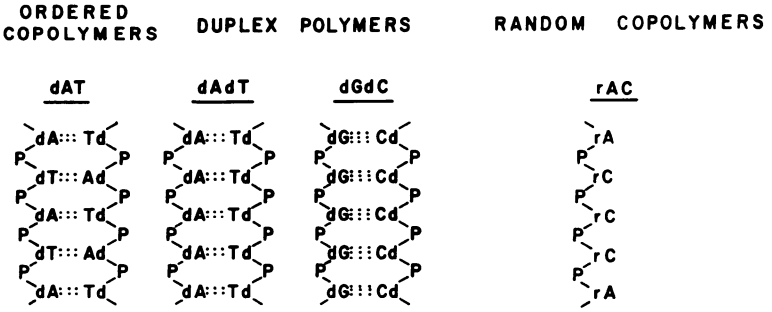

FIG. 4. *Structures of poly dAT, poly dAdT, poly dGdC, and a representative random copolymeric polyribonucleotide, poly rAC*

one-half the decrease caused by poly rG, even though the chloroquine was fully bound to the polynucleotide in each case. Poly dGdC and poly dAT cause large decreases, and poly dAdT causes essentially no change, in the absorbance of chloroquine (Table 4), in agreement with results published earlier for poly dGdC (3, 13), poly dAdT (13), and poly dAT (3). Although both polymers are double-stranded and have the same base composition, the interaction of chloroquine or pentaquine with the alternating copolymer (poly dAT) is accompanied by a large decrease in the absorbance of the aminoquinoline, whereas a possible interaction of either of these aminoquinolines with the homopolymer duplex (poly dAdT) cannot be detected spectrophotometrically. Wells (17) has reported the importance of the nucleotide sequence of guanine-containing polydeoxyribonucleotides in the binding of actinomycin D.

Samples of dextrorotatory and levorotatory chloroquine showed the same decreases in absorbance upon binding to native calf thymus DNA. The chloroquine was resolved (and kindly supplied by Dr. K. H. Dudley of this Center) to the extent of the rotations reported (18), although the absolute optical purities are still unknown.

The intercalation of a compound into DNA is accompanied by changes in the physical properties of the DNA, including an increase in viscosity (5, 19). Chloroquine has been shown to increase the viscosity of native DNA (5). Primaquine, however, does not cause an observable increase in the viscosity of native calf thymus DNA at a DNA-P to aminoquinoline molar ratio of 5:1 (Fig. 5). For comparison, the increase in viscosity of DNA in the presence of chloroquine is shown (Fig. 5). Primaquine also did not affect the chloroquine-induced increase in the viscosity of DNA; the addition of equimolar amounts of primaquine and chloroquine (each at a DNA-P to aminoquinoline molar ratio of 5:1) resulted in no change in the chloroquine-induced increase in the viscosity of DNA (as shown in the topmost line of Fig. 5). The absence of an increase in the viscosity of DNA in the presence of primaquine is consistent with a lack

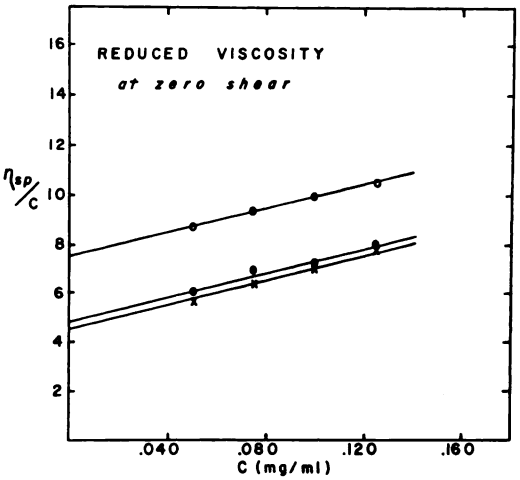

FIG. 5. *Effect of chloroquine and primaquine on viscosity of native calf thymus DNA.*

Reduced viscosities (η_{sp}/c) are expressed in milliliters per milligram of DNA; the intrinsic viscosity, the reduced viscosity extrapolated to zero DNA concentration (c), of the DNA was 4.8 ml/mg, equivalent to 48 dl/g, in 0.01 M potassium phosphate (pH 6) at 37°. ●, DNA with no aminoquinoline; ○, at a molar ratio of DNA-P to chloroquine of 5:1; ×, at a molar ratio of DNA-P to primaquine of 5:1.

of an appreciable intercalation of primaquine in the native DNA structure. The lack of a measurable increase in the viscosity of native DNA induced by primaquine does not completely rule out the binding of a minor portion of primaquine to DNA by intercalation. However, it appears from the spectrophotometric studies that there is an interaction between the ring systems of bound primaquine and the nucleic acid bases.

In equilibrium dialysis experiments in 0.01 M potassium phosphate (pH 6) at DNA-P to chloroquine ratios of 8:1 or 4:1, the addition of nonradioactive primaquine did not affect the binding of radioactive chloroquine to native calf thymus DNA, even at a molar ratio of primaquine to chloroquine of 8:1. In converse experiments, the addition of nonradioactive chloroquine decreased the binding of radioactive primaquine to DNA. The binding of primaquine was decreased by approximately one-third and two-thirds by the addition of chloroquine at molar ratios of chloroquine to primaquine of 0.5:1 and 1.3:1, respectively.

Binding of chloroquine, the antimalarial 8-aminoquinolines, and some of the potential metabolites of the 8-aminoquinolines occurs to a considerable extent to DNA, soluble RNA, and various polyribonucleotides. Despite their greater affinity for polynucleotides that contain purines, binding of the antimalarial aminoquinolines does not require the presence of specific purine nucleotides, a double-stranded polynucleotide, or a specific base pair in a double-stranded polymer.

The binding of the antimalarial aminoquinolines to polyribonucleotides suggests that a portion of the antimalarial activity and drug toxicity of these compounds may be effected by alterations in the function of various RNA fractions in protein synthesis. This proposal is in addition to the previously proposed mechanism (4, 20) that nucleic acid synthesis is inhibited by binding to DNA. The suggestion that antimalarial compounds may affect protein synthesis as well as nucleic acid polymerases as a result of interaction with nucleic acids has also been made for the antimalarial quinacrine (21).

It must be emphasized that the binding of antimalarial aminoquinolines to DNA, RNA, and related polynucleotides as measured by physical techniques points only to a *potential* for the biological mechanisms of action, specifically the antimalarial activity and the toxic effects of these drugs in animals. The 8-aminoquinolines and 4-aminoquinolines (including chloroquine) act at different stages in the life cycle of plasmodia. For the similar binding of 8-aminoquinolines and chloroquine to nucleic acids to be of biological significance, it must be assumed that other factors are involved in the selective activity of the 4-aminoquinolines and the 8-aminoquinolines against different stages in the life cycle of the parasite. Such factors might include (a) changes in the nature of the nucleic acids or their protein complexes that would alter the binding, or the functional results of such binding, on nucleic acid or protein synthesis during various stages in the parasite life cycle, and (b) differences between the 4- and 8-aminoquinolines with regard to distribution within the various host tissues and the permeability of the parasite at different life stages.

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