The Binding of Primaquine, Pentaquine, Pamaquine, and Plasmocid to Deoxyribonucleic Acid

LEONA P. WHICHARD, CARL R. MORRIS, JUDY M. SMITH, AND DAVID J. HOLBROOK, JR.[1]

Center for Research in Pharmacology and Toxicology,[2] School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27514

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SUMMARY

The binding of four 8-aminoquinoline antimalarials (primaquine, pentaquine, pamaquine, and plasmocid) to native and denatured calf thymus DNA has been studied by equilibrium dialysis and direct spectrophotometry. 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, (c) decreased by addition of Mg^{2+} to a greater extent than would be expected from ionic strength effects alone, and (d) decreased under some conditions by the presence of 4 M urea. In 0.01 M potassium phosphate (pH 6), the total binding of the 8-aminoquinolines to various DNA preparations at DNA nucleotide to aminoquinoline ratios of 6 or greater occurs in the following order: native DNA = denatured DNA > native DNA in 4 M urea > denatured DNA in 4 M urea. At low ionic strengths and pH 6, the binding of the singly protonated 8-aminoquinolines is less than, but comparable to, the binding of chloroquine, a divalent cation at the same pH. At a DNA nucleotide to aminoquinoline ratio of 10 and an ionic strength of 0.012 (pH 6), the percentages of the aminoquinolines bound to native DNA decrease in the following order: chloroquine > pentaquine > plasmocid > primaquine > pamaquine. At an ionic strength of 0.15 or greater, the binding of pentaquine and plasmocid equals or exceeds the binding of chloroquine to native DNA. Evidence is presented for the occurrence of at least two spectrally distinct bound forms for each 8-aminoquinoline.

INTRODUCTION

The binding of the antimalarial 4-aminoquinolines (chloroquine and a number of related analogues), quinacrine, and quinine to DNA has been demonstrated by physical techniques, including difference spectra, equilibrium dialysis, and changes in the melting temperature of DNA (1–3). It has been suggested that the mechanism of the antimalarial action of these compounds is related to their binding to DNA (1) and may be due to inhibition of the function of DNA in the polymerase reactions (3, 4). It has been proposed, however, that the 8-aminoquinolines have a different mechanism of antimalarial action; namely, they are converted initially in the host to the 6-hydroxy and 5,6-dihydroxy derivatives, which interfere in oxidation-reduction reactions (5, 6).

Studies of the binding of the 8-aminoquinoline antimalarials to nucleic acids have not been reported previously. This report concerns the binding of the 8-aminoquinolines primaquine,[3] pentaquine,

[1] To whom inquiries should be directed.

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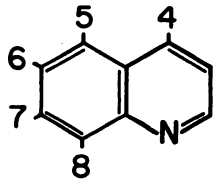
[3] Systematic names: primaquine, 8-(4-amino-1-methylbutylamino)-6-methoxyquinoline; pentaquine, 8-(5-isopropylaminopentylamino)-6-methoxyquinoline; pamaquine, 8-(4-diethylamino-1-methylbutylamino)-6-methoxyquinoline; plasmocid, 8-(3-diethylaminopropylamino)-6-methoxyquinoline; chloroquine, 7-chloro-4-(4-diethylamino-1-methylbutylamino)quinoline.

TABLE 1
Structures of the antimalarial aminoquinolines



Primaquine	6 —OCH_3	8 —$NHCH(CH_3)CH_2CH_2CH_2NH_2$
Pentaquine	6 —OCH_3	8 —$NHCH_2CH_2CH_2CH_2CH_2NHCH(CH_3)_2$
Plasmocid	6 —OCH_3	8 —$NHCH_2CH_2CH_2N(CH_2CH_3)_2$
Pamaquine	6 —OCH_3	8 —$NHCH(CH_3)CH_2CH_2CH_2N(CH_2CH_3)_2$
Chloroquine	7 —Cl	4 —$NHCH(CH_3)CH_2CH_2CH_2N(CH_2CH_3)_2$

pamaquine, and plasmocid to DNA as measured by equilibrium dialysis and direct spectrophotometry. The binding of chloroquine to DNA under the conditions of this study has been included as a standard of reference, since such binding has been extensively studied (1–3). The structures of the antimalarial aminoquinolines are shown in Table 1. The extent of binding of the 8-aminoquinolines, which occur predominantly as monoprotonated cations at pH 6, is comparable to the binding of chloroquine, a divalent cation at the same pH. A preliminary report of this investigation has appeared (7).

MATERIALS AND METHODS

Materials. Highly polymerized calf thymus DNA was obtained from Worthington Biochemical Corporation and Sigma Chemical Company. Heat-denatured DNA was prepared by treatment for 20 min at 90° in 0.01 M potassium phosphate buffer (pH 6.0), followed by rapid cooling in an ice-salt bath. The heat-denaturation of the DNA samples caused a 26–30% increase in absorbance at 260 mμ as measured at room temperature. The molar concentrations of DNA are expressed in terms of concentrations of DNA phosphorus (DNA-P), which are equivalent to those of DNA nucleotide.[4]

[4] The abbreviations used are: DNA-P, concentration of DNA phosphorus or DNA nucleotide; ϵ_f = molar absorptivity (molar extinction coefficient) of free, unbound aminoquinoline; ϵ_b = molar absorptivity of aminoquinoline bound to DNA.

Pamaquine hydroiodide and pentaquine hydrochloride 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. Chloroquine diphosphate and primaquine diphosphate were obtained from Sigma Chemical Company, and plasmocid dihydroiodide, from Aldrich Chemical Company.

Spectrophotometry. Ultraviolet spectra were measured at room temperature (25° ± 1°) on a Cary model 15 spectrophotometer over a wavelength range from 310 to 380 mμ, a region in which DNA itself gives minimal interference. The higher absorption of the aminoquinolines in the range from 240 to 280 mμ could not be used conveniently in the binding studies, since it is the wavelength range of maximum absorption of DNA. In the concentration ranges used in these studies, the aminoquinolines obeyed Beer's law. The percentage of ligand bound, evaluated by direct spectrophotometry, was expressed as 100α (where α is the fraction of the total ligand bound), and α was calculated by the methods of Cohen and Yielding (ref. 2, Eq. 2).

Equilibrium dialysis. Aminoquinoline and DNA solutions were allowed to equilibrate at room temperature (25° ± 1°) in from Sigma Chemical Company, and dialysis cells such as those distributed by Chemical Rubber Company and TechniLab Instruments. The cells contained either 1 ml or 5 ml of solution on each side of the dialysis membrane (Fisher Scientific dial-

ysis tubing). The cells were rotated slowly (approximately 40 rpm) on an inclined rotating platform; dialysis was continued routinely for 20 hr, although equilibration occurred in 6–7 hr. The aminoquinolines studied were not adsorbed to the dialysis tubing in the concentration ranges of free aminoquinolines used in these studies. The aminoquinoline concentrations were determined from the ultraviolet spectra of (a) the total aminoquinoline placed in the dialysis cells and (b) the external, or DNA-free, side of the cell after equilibration. In some of the experiments with plasmocid, pentaquine, and primaquine, one of a pair of equilibrium dialysis cells was prepared with the DNA and aminoquinoline initially on opposite sides of the dialysis membrane and dialyzed to equilibrium against each other. In the other cell, both the DNA and aminoquinoline were initially placed on the same side of the membrane and dialyzed against buffer. The extent of binding measured in the paired cells was equal for each of the compounds studied. Corrections were made for the inequality of free aminoquinoline concentration on the two sides of the membrane due to the Donnan effect. The correction for the Donnan effect was small: the maximum internal (DNA side) to external (DNA-free side) ratio of free aminoquinoline was 1.2 (in 0.01 M potassium phosphate solution), and the largest correction required was 5–6% of the aminoquinoline bound.[5] The pH values of the internal and external solutions after equilibration were within 0.04 pH unit or less of each other, which also indicates the small Donnan effect occurring under these experimental conditions.

Ionization constants. The ionization constants for the second protonation of the 8-aminoquinolines were determined spectrophotometrically (9). Measurements were made in formate buffers of ionic strength 0.015–0.030, and the ionization constants were expressed as pK_a after correction to zero ionic strength (ref. 10, Eqs. 16 and 17).

[5] The correction for Donnan effect was made on the basis of 1 negative charge per DNA phosphate. It is probable, however, that part of the charges on the DNA are neutralized by bound potassium and sodium ions (8), and the actual Donnan effect may be even less than the small correction used in these experiments.

RESULTS AND DISCUSSION

In order to compare the binding of the 8-aminoquinolines and of chloroquine, it was desirable to select a single pH so that observed differences would not be due to changes in the nature of the DNA caused by changes in pH. For the second protonation of the 8-aminoquinolines (Table 2), the pK_a values were found to be 3.0–3.2, which is consistent with the pK (after calculation to zero ionic strength) of 3.1–3.2 (at 30°) reported for pamaquine by Irvin and Irvin (11). In contrast, for the second protonation of chloroquine, the pK is 7.8 at 30° (9). A pH of 6.0–6.1 was selected for the binding studies, since the aminoquinolines are then approximately 2 pH units from the nearest pK value, and thus a single type of charged aminoquinoline predominates in the solution at pH 6.0; chloroquine occurs predominantly as a divalent cation, and the 8-aminoquinolines occur as singly protonated cations.

TABLE 2
Molar absorptivities and pK values of 8-aminoquinolines

The molar absorptivities (ϵ_f) of the free 8-aminoquinolines were measured at pH 6.0 at the wavelength of maximal absorption between 310 and 380 mμ.

Aminoquinoline	pK_a (25°)	Wavelength (λ_{max}) of free aminoquinoline	ϵ_f
		mμ	$M^{-1}\ cm^{-1}$
Primaquine	3.2_2	351	3280
Pentaquine	3.2_2	348	2760
Pamaquine	3.0_4	352	3310
Plasmocid	3.0_4	347	3250
Chloroquine	7.8[a]	343	18950

[a] From Parker and Irvin (9); at 30°.

The molar absorptivities (molar extinction coefficients) of the free aminoquinolines are given in Table 2. At pH 6 in the 320–400 mμ range, the molar ab-

sorptivities of the 8-aminoquinolines are approximately one-sixth of the molar absorptivity of chloroquine, a 4-aminoquinoline. This difference in the molar absorptivities is not due to the degree of protonation, since the molar absorptivities of the divalent 8-aminoquinolines (at pH 1) are also one-fifth or less of the molar absorptivity of divalent chloroquine. The molar absorptivities of the free aminoquinolines do not change measurably with ionic strength at pH values that are 1.8 or more units removed from the pK values.

Upon binding to DNA, the 8-aminoquinolines undergo decreases in absorbance in the wavelength range of 310–380 mμ. Figure 1 shows the spectra of free plasmocid and of plasmocid in the presence of native DNA at DNA-P to plasmocid ratios of 5.6 and 11.2. Comparable changes in spectra are obtained for the other 8-aminoquinolines in the presence of native DNA. The spectrum of each 8-aminoquinoline undergoes a bathochromic shift, i.e., a shift in the wavelength of maximum absorbance to longer wavelengths, on binding to either native or denatured calf thymus DNA. In 0.01 M potassium phosphate (pH 6), the bathochromic shift is usually 3–6 mμ, but it is not possible to establish a definite relationship between the extent of binding and the extent of the bathochromic shift.

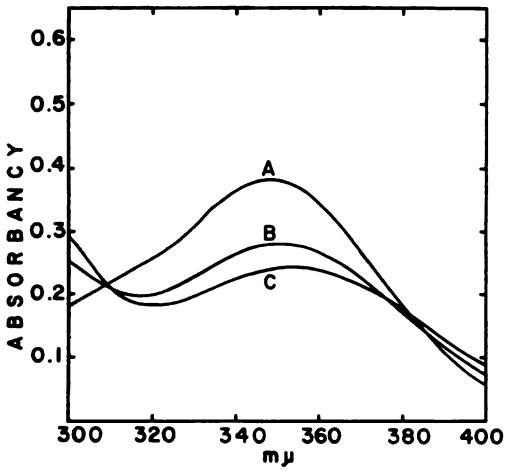


FIG. 1. *Spectra of plasmocid in the presence of various amounts of native DNA in 0.01 M potassium phosphate (pH 6.0–6.1)*

Curve *A*, free plasmocid; curve *B*, at a DNA-P to plasmocid molar ratio of 5.6; curve *C*, at a DNA-P to plasmocid molar ratio of 11.2.

As is customary in binding measurements obtained by direct spectrophotometry, the apparent molar absorptivity of the bound aminoquinoline was determined by measurement of the absorbance of the free aminoquinoline solution and that of the aminoquinoline solution after addition of an excess of polymer, i.e., until no change in absorbance occurred (after correction for dilution) upon further addition of aliquots of the DNA solution. The evaluation of the apparent molar absorptivity of the bound aminoquinoline (expressed as a percentage of the ϵ_f) in this manner depends on the assumption that the concentration of the free aminoquinoline approaches zero when the absorbance does not change upon further addition of the polymer. If there are two or more methods of binding, and each bound form has a different molar absorptivity, a further assumption becomes necessary: that the proportion of the various bound forms remains constant as greater amounts of polymer are added. If the percentages of binding as evaluated by equilibrium dialysis and direct spectrophotometry show good agreement, the following statements are applicable. (a) If there is only one bound form of aminoquinoline, all binding is accompanied by a concomitant change in the absorption spectrum of the ligand at DNA-P to aminoquinoline ratios of 5–20 (the range where comparisons can be made between the data from equilibrium dialysis and direct spectrophotometry). If there are two or more methods of binding, the proportion of the bound forms remains essentially constant in the range of concentrations of DNA and aminoquinolines under study. (b) The binding is essentially complete, and the concentration of the free aminoquinoline approaches zero, at high DNA-P to aminoquinoline ratios (molar ratios of 18–20). (c) Appropriate corrections have been applied for the Donnan effect to the data from the equilibrium dialysis measurements.

Figure 2 shows the percentage of amino-

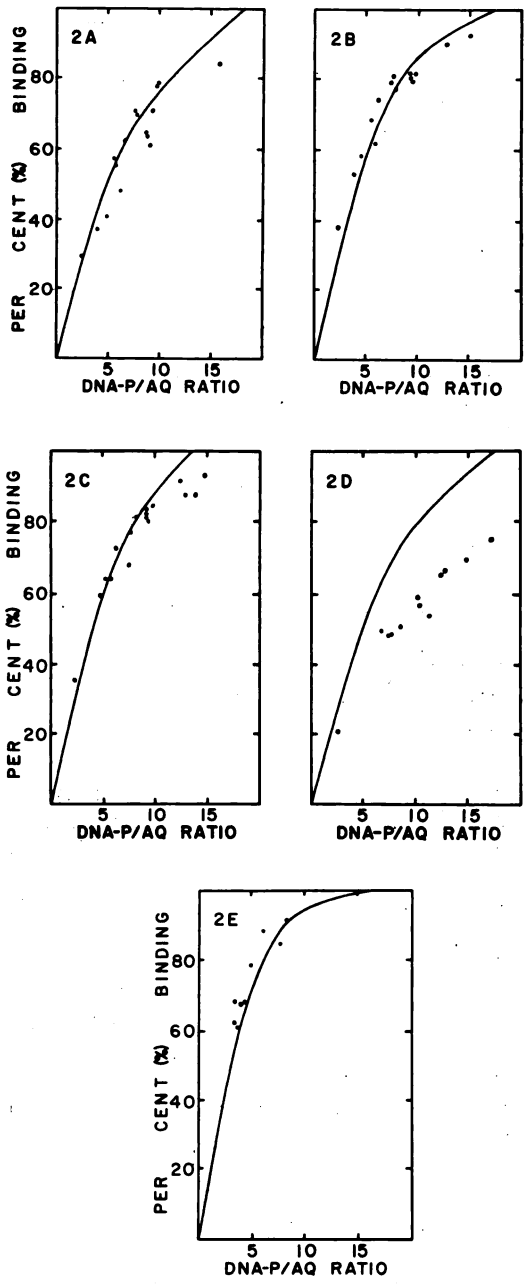


FIG. 2. *Percentage of aminoquinoline bound to native DNA at various DNA-P to aminoquinoline (AQ) ratios in 0.01 M potassium phosphate (pH 6)*

The lines represent the extent of binding measured by direct spectrophotometry, and the points indicate binding measured in individual cells in equilibrium dialysis experiments. A, Primaquine; B, pentaquine; C, plasmocid; D, pamaquine; E, chloroquine.

quinoline bound to native DNA at various molar ratios of DNA nucleotide to aminoquinoline in 0.01 M potassium phosphate solution (pH 6). The percentage of binding as determined by direct spectrophotometry is shown by the line in each graph. The experimental points represent the results obtained from individual cells in equilibrium dialysis experiments. The results show good agreement between the extents of binding measured by the two methods for primaquine, pentaquine, plasmocid, and chloroquine in Fig. 2A, B, C, and E, respectively. Thus, the three assumptions involved in the binding experiments (preceding paragraph) are valid within the limits of measurement. However, the apparent binding of pamaquine as determined by direct spectrophotometry is significantly greater than the binding measured by equilibrium dialysis (Fig. 2D). The discrepancy can be explained by the finding in the equilibrium dialysis experiments that only about 80% of the pamaquine is bound at DNA-P to pamaquine ratios of 18–20, the ratios at which the absorbance of all of the 8-aminoquinolines no longer decreases on the further addition of DNA. Thus, the actual ϵ_b for pamaquine is less than the value estimated by direct spectrophotometry, and the percentage of binding is actually less than that indicated by direct spectrophotometry. In the case of the other 8-aminoquinolines (pentaquine, plasmocid, and primaquine) and chloroquine, the actual extent of binding is greater than 90% at DNA-P to aminoquinoline ratios of 18–20, and the extents of binding measured by the two methods are experimentally indistinguishable.

The cause for the lower binding of pamaquine to DNA, compared to the other 8-aminoquinolines, is unknown. Since both plasmocid and pamaquine have a tertiary amino group at the terminal nitrogen of the side chain and the pK values of the quinoline ring nitrogen are comparable, the difference in the extent of binding of these compounds does not reside with these two possible determinants.

Figure 3 shows the effect of increasing

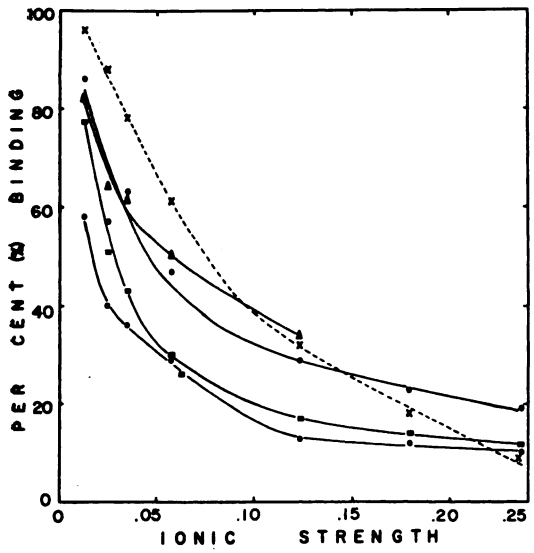


FIG. 3. *Percentage of aminoquinoline bound to native DNA, as measured by equilibrium dialysis, at DNA-P to aminoquinoline ratio of 10 at various ionic strengths*

×, Chloroquine; ○, pentaquine; △, plasmocid; □, primaquine; ●, pamaquine.

ionic strength on the binding of the aminoquinolines to native DNA, as measured by equilibrium dialysis, at a DNA nucleotide to aminoquinoline molar ratio of 10. The extent of binding of the aminoquinolines to DNA decreases with increasing ionic strength, as reported by others for the binding of chloroquine and of quinacrine to DNA (1, 2, 12). At an ionic strength of 0.012 (in 0.01 M potassium phosphate, pH 6), 58–85% of the four 8-aminoquinolines is bound to DNA at a DNA-P to aminoquinoline ratio of 10. This is equivalent to approximately 6–8.5 aminoquinoline molecules bound per 100 DNA nucleotides. At lower ionic strengths, chloroquine exhibits greater binding to DNA than any of the 8-aminoquinolines studied. At an ionic strength of 0.15 or higher, however, the extent of binding of plasmocid and pentaquine equals or exceeds the binding of chloroquine to DNA. The binding of the 8-aminoquinolines to DNA is not affected by the substitution of a solution of NaCl–dilute potassium phosphate for a solution of equal ionic strength containing potassium phosphate alone.

The addition of Mg^{2+} decreases the binding of the 8-aminoquinolines to DNA to a greater extent than would be expected from ionic strength effects alone. In 0.01 M potassium phosphate–1 mM $MgCl_2$, the binding of pentaquine and plasmocid is 40–60% of the binding expected at an equal ionic strength contributed by potassium phosphate alone.

The binding of the aminoquinolines to native and heat-denatured DNA was studied by equilibrium dialysis to determine whether the denaturation of the DNA affected the total binding of the aminoquinolines. Although a range of values was obtained, the data show that each 8-aminoquinoline binds to an approximately equal extent to native and denatured DNA in 0.02 M potassium phosphate (pH 6) at DNA-P to aminoquinoline ratios of 10 (Table 3) or higher. In addition, the extent

TABLE 3

Percentage of aminoquinoline bound to native and denatured DNA

The DNA-P to aminoquinoline molar ratio was 10 ± 2. The solvent was 0.02 M potassium phosphate (pH 6).

Aminoquinoline	Binding to native DNA	Binding to denatured DNA
	%	%
Primaquine	48 (41–53; 5)[a]	48 (41–55; 4)
Pentaquine	57 (52–62; 4)	57 (52–62; 4)
Plasmocid	64 (64–64; 2)	61 (60–62; 2)
Pamaquine	40 (38–43; 4)	40 (31–45; 4)
Chloroquine	87 (85–90; 2)	84 (83–84; 2)

[a] The range and number of determinations are given in parentheses.

of the total binding of chloroquine in 0.02 M potassium phosphate is not appreciably altered by denaturation of the DNA (Table 3). (It should be emphasized that the equilibrium dialysis measurements represent total binding and, if two or more methods of binding are involved, the proportions of bound forms may differ with native and denatured DNA.)

At a lower ionic strength (in 0.01 M potassium phosphate), the binding of each 8-aminoquinoline to denatured DNA is approximately equal to the binding of the

corresponding aminoquinoline to native DNA, except that the binding of pamaquine to denatured DNA may be somewhat less than to native DNA at DNA-P to aminoquinoline ratios above 10 (Fig. 4A–D).

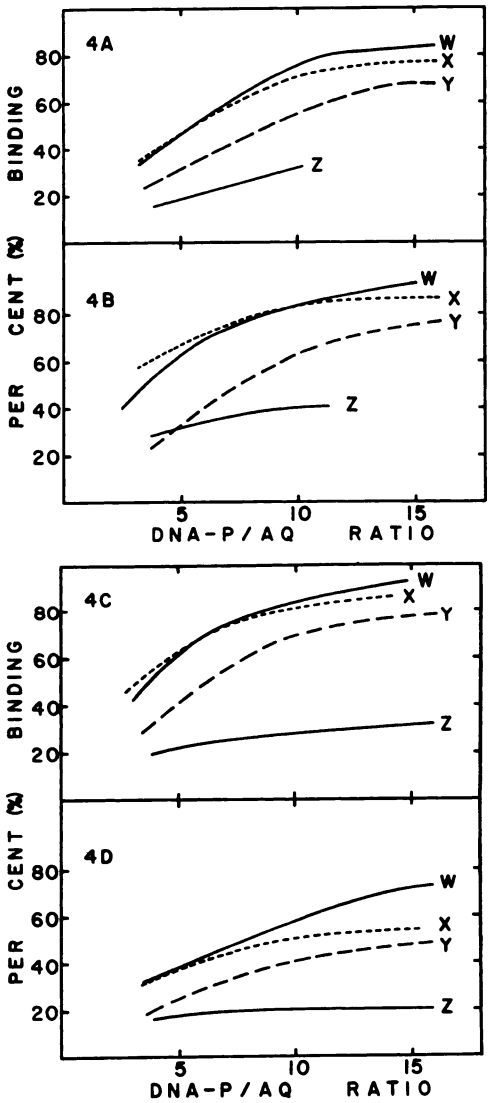


FIG. 4. *Percentage of aminoquinoline bound, as measured by equilibrium dialysis, at various DNA-P to aminoquinoline (AQ) ratios in 0.01 M potassium phosphate (pH 6)*

W, Native DNA; *X*, heat-denatured DNA; *Y*, native DNA in 4 M urea; *Z*, denatured DNA in 4 M urea added after denaturation. A, Primaquine; B, pentaquine; C, plasmocid; D, pamaquine.

At DNA-P to aminoquinoline ratios above 8, the presence of 4 M urea decreases the total binding of the 8-aminoquinolines to native DNA by less than one-third, but the relative effect of the urea on binding to native DNA becomes greater as the DNA-P to aminoquinoline ratio becomes smaller. It is apparent that the binding process affected by urea is the predominant method of binding to native DNA at high aminoquinoline to DNA-P ratios, i.e., at low DNA-P to aminoquinoline ratios.

The binding of the 8-aminoquinolines to denatured DNA in the presence of 4 M urea is markedly less than the binding to native DNA in 4 M urea or to denatured DNA in the absence of urea. The effect of urea on binding to denatured DNA increases as the DNA-P to aminoquinoline ratio increases (Fig. 4A–D).

The manner in which urea affects the binding of the 8-aminoquinolines to native and denatured DNA has not been established. One proposal consistent with the observations reported here is that there are at least two methods of binding. Heat denaturation may decrease the proportion bound by one method but increase the potential to form hydrogen bonds between aminoquinoline and DNA by increasing the number of available sites through disruption of the interstrand hydrogen bonds of the native DNA. Urea may then prevent, by competition, the hydrogen bonding of the 8-aminoquinolines to the denatured DNA. If urea affects hydrogen bonding to DNA, hydrogen bonding represents a relatively small proportion of the total binding of the 8-aminoquinolines to native DNA at the higher DNA-P to aminoquinoline ratios. O'Brien *et al.* (3) found that urea decreases the binding of quinine to DNA, and suggested that quinine is bound to DNA by hydrogen bonding and that these bonds are disrupted in the presence of 6 M urea. It has not been established whether the effect of 4 M urea on the binding of 8-aminoquinolines to denatured DNA in these studies is due solely or in part to the disruption of hydrogen bonds. It is necessary to emphasize that urea may affect the binding of ligands to

TABLE 4

Percentage decrease in absorbance of aminoquinolines upon binding to calf thymus DNA

The medium was potassium phosphate buffer, pH 6.0–6.2, of the molar concentration indicated, with the addition of urea where specified. DNA was added until no decrease in absorbance occurred on further addition of DNA; the molar ratio of DNA-P to aminoquinoline was about 18–20.

DNA	Potassium phosphate, pH 6.0–6.2	Decrease in absorbance				
		Primaquine	Pentaquine	Plasmocid	Pamaquine	Chloroquine
	M	%	%	%	%	%
Native	0.001	54	53	52	49	60
Native	0.01	39	43	42	31	57
Native	0.01 + 4 M urea	25	32	28	18	
Denatured	0.01	20	30	28	16	

polymers by means other than the disruption of hydrogen bonds, namely, by effects on solvent water (13). In addition, it has been found that 8 M urea or 4 M guanidinium chloride decreased the hypochromicity due to base stacking of denatured DNA and other single-stranded polynucleotides, but altered only slightly the hypochromicity due to base stacking and to the double-helical structure of native DNA (14). Thus, the decrease in binding of the 8-aminoquinolines to DNA, especially denatured DNA, observed in the presence of urea, may be due to effects on solvent water (13) or base stacking of the DNA (14), rather than to the disruption of hydrogen bonds by urea.

As mentioned previously, a hypochromic change in the spectra of the aminoquinolines occurs on binding to DNA. The magnitude of the change is dependent on the composition of the medium as well as on the nature of the DNA. Table 4 shows the percentage decrease in the absorbance of the aminoquinolines on binding to DNA at DNA-P to aminoquinoline ratios of 18–20, the range in which no further decrease in absorbance occurs upon addition of DNA. For each 8-aminoquinoline, the hypochromicity on binding to DNA varies with the ionic strength (compare 0.01 M and 0.001 M potassium phosphate) and according to whether native or denatured polymer is used. Regardless of the variation in hypochromicity exhibited on binding, more than 90% of the pentaquine, plasmocid, and primaquine is bound to native DNA in either 0.001 M or 0.01 M potassium phosphate. Thus, when certain variations in the medium are occurring, the extent of change in the ligand spectrum is not always a valid indication of the extent of binding. In addition, although essentially complete binding to native DNA occurs in either 0.01 M or 0.001 M potassium phosphate, the decrease in absorption is not the same in both media and thus there are two or more spectrally distinct forms of each bound aminoquinoline. The occurrence of more than one spectrally distinct bound ligand has been recognized in the binding of both proflavine and acridine orange to DNA (15).

In a plot of r/c vs. c, where r is the number of moles of aminoquinoline bound per mole of DNA nucleotide and c is the concentration of free aminoquinoline, the intercept on the horizontal axis is equal to the maximum number of moles of aminoquinolines bound per mole of DNA-nucleotide (1, 2). This type of plot of measurements obtained by direct spectrophotometry indicates that for the 8-aminoquinolines a maximum of 0.12–0.2 mole of aminoquinoline is bound per DNA nucleotide by the method(s) of binding predominant at DNA-P to aminoquinoline ratios between 5 and 18 in 0.01 M potassium phosphate (pH 6).

The apparent association constants, K, were calculated from the equation $K = [\text{DNA-aminoquinoline complex}] / [\text{free aminoquinoline}][\text{unbound DNA-P}]$. The association constants for the binding of

plasmocid, pentaquine, and pamaquine to native DNA were 1.7, 1.6, and 0.6 mM^{-1}, respectively, measured in 0.01 M potassium phosphate (pH 6). The association constants were minimum values, since the unbound DNA-P concentration was calculated on the basis that each of the bound aminoquinolines was bound to only one DNA nucleotide, although intercalation or stacking would involve one or two base pairs or two adjacent bases in a single polynucleotide strand. The resultant concentration of unbound DNA-P would actually be less than the estimated value and lead to a larger association constant. The constants for the 8-aminoquinolines are comparable to but are less than the association constants reported for the binding of chloroquine to DNA (1, 2) under similar conditions and less (by a factor of 10–100) than the association constants for the binding of proflavine and benzpyrene to DNA.

The mechanism of the binding of the 8-aminoquinolines to DNA is unknown. Some of the properties of this process, however, are consistent with various modes of binding. The binding of the 8-aminoquinolines to DNA is greatly decreased by an increase in the ionic strength of the medium. Such a phenomenon has been attributed to the involvement of binding of ligand to polymer by electrostatic attraction (1–3). It is of interest that the binding of the univalent 8-aminoquinolines is not appreciably less than that of divalent chloroquine at low ionic strength, and may exceed the binding of chloroquine at higher ionic strengths. This finding was somewhat unexpected, since Cohen and Yielding (2) reported that only the divalent chloroquine binds to DNA, whether the divalent or the monovalent chloroquine predominates at the pH at which binding studies are performed. The gain or loss of a proton from the terminal nitrogen is not accompanied by a change in the spectrum of an 8-aminoquinoline (11), and thus it is not anticipated that an interaction with only the protonated terminal nitrogen would be accompanied by a change in the 8-aminoquinoline spectrum. The occurrence of a spectral change of the 8-aminoquinolines on binding indicates that an ionic interaction involving the protonated terminal nitrogen is not the sole region of the 8-aminoquinolines involved in the binding to DNA.

Since there is such a relatively large decrease in the absorptivity of the 8-aminoquinolines upon binding to native and denatured DNA, some interaction presumably occurs between the quinoline ring and the rings of the nucleotides. A bathochromic shift of a ligand upon binding to DNA, such as that observed for the 8-aminoquinolines, has been established as a manifestation of a "face-to-face" arrangement, as in co-stacking or intercalation in the case of the interaction of benzpyrene to DNA and polynucleotides (16). O'Brien *et al.* (17) have presented evidence that the interaction of chloroquine with native DNA involves intercalation of the ligand between base pairs of DNA. A possible role of intercalation in the binding process is not excluded by the present finding that 8-aminoquinolines were bound equally well to native and to denatured DNA at DNA-P to aminoquinoline ratios of 6–8. Pritchard, Blake, and Peacocke (18) and Lloyd, Prutton, and Peacocke (19), in order to explain the equal binding of proflavine to native and denatured DNA, proposed a modified intercalation model for the interaction of aminoacridines and DNA, in which the aminoacridines are intercalated between two adjacent bases in the same polynucleotide chain. Thus, according to this proposal, neither the native nor the double-stranded form of DNA would be a requisite for binding to occur by intercalation.

Evidence is presented that there are at least two spectrally distinct bound forms of each 8-aminoquinoline. It is proposed that the binding of the 8-aminoquinolines to DNA involves electrostatic interaction, an interaction between the quinoline ring and the rings of the nucleotide bases, and possibly hydrogen bonding. In cognizance of the observed binding of the 8-aminoquinolines to DNA, it is proposed that a portion of the antimalarial activity of these

compounds is related to their binding to DNA and the inhibition of DNA function.

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