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BINDING OF ANTIMALARIAL AMINOQUINOLINES TO CHROMATIN, RECONSTITUTED DEOXYRIBONUCLEOHISTONE AND RIBOSOMES FROM MAMMALIAN TISSUES*

MILDRED E. WASHINGTON, LIDIA A. WHITE and DAVID J. HOLBROOK, JR.†

Center for Research in Pharmacology and Toxicology, School of Medicine, University of North Carolina, Chapel Hill, N.C. 27514, U.S.A.

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Abstract—A study was conducted at low ionic strengths of the binding of three antimalarial aminoquinolines (primaquine, pentaquine and chloroquine) to DNA, to various DNA–protein complexes (chromatin isolated from calf thymus and reconstituted deoxyribonucleohistone preparations), and to ribosomes isolated from rat liver. The order of binding to any of the nucleoproteins was chloroquine > pentaquine > primaquine. The greatest level of binding of an aminoquinoline occurs to free DNA and to deoxyribonucleohistone containing 0·5 mg of histone/mg of DNA, an intermediate level of binding to deoxyribonucleohistone containing 1·0 or 1·2 mg of histone/mg of DNA, and the lowest level of binding to isolated chromatin. No binding of the aminoquinolines to free calf thymus histones was detected. The lower level of binding to nucleoproteins is attributable to a masking of some of the potential binding sites on the DNA by the protein (as measured by a decrease in the maximum number of binding sites per DNA–nucleotide); there is also a small decrease in the strength of binding (as measured by the association constants) to DNA and to DNA–protein complexes by an aminoquinoline.

VARIOUS antimalarial compounds such as chloroquine, quinacrine and quinine interact with DNA and, in some cases, with RNA and other polyribonucleotides.[1–10] The antimalarial 8-aminoquinolines, including primaquine and pentaquine, also interact with DNA, RNA and various polynucleotides.[8–10] Such interactions measured by physical techniques point to a potential for various mechanisms of biological action such as the inhibition of replication, transcription or translation.

The interaction of the aminoquinolines and other compounds with various nucleic acids may be appreciably modified by the presence of those proteins commonly associated with the nucleic acid in its intracellular environment, namely, the histones and other proteins associated with DNA within chromatin and the proteins attached to the RNA within ribosomes. This study was undertaken to measure the binding of the antimalarial aminoquinolines to the nucleoproteins and, in the case of the DNA–protein complexes, to obtain information on the possibly modifying effect of the proteins on the binding characteristics.

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† To whom reprint requests and inquiries should be addressed.

MATERIALS AND METHODS

The interaction of the aminoquinolines with the polymers was studied by equilibrium dialysis at 5° essentially as described previously.[9] Unless otherwise indicated, the medium consisted of 0·01 M potassium phosphate buffer (pH 7·0), with or without the addition of NaCl to a final concentration of 0·5 mM; ionic strength, 0·018. The calf thymus DNA was purchased from Sigma Chemical Company, the unfractionated calf thymus histone from Worthington Biochemical Corp., and the aminoquinolines have been previously described.[8,9] The calf thymus chromatin was prepared from frozen calf thymus by the method of Zubay and Doty.[11] The wavelengths of maximum and minimum absorptivity of six chromatin preparations were 257–258 and 237 nm, respectively; the ratios of A_{280}/A_{260} and A_{240}/A_{260} were 0·59 ± 0·01 and 0·67 ± 0·01 (mean ± standard error) respectively. The reconstituted deoxyribonucleohistones (RDNH) were prepared by (a) dissolution of native calf thymus DNA and unfractionated calf thymus histone in 1 M NaCl, (b) subsequent dialysis of the mixture against a series of stepwise decreasing concentrations of NaCl (namely 0·75, 0·50, 0·25 M) and (c) a final dialysis against either 1 mM NaCl or 0·01 M potassium phosphate (pH 7·0). The input ratio (w/w) of histone to DNA in the mixture is indicated immediately after the abbreviation. For example, RDNH-0·5 is the RDNH produced from the mixture of 0·5 mg of histone/1·0 mg of DNA. The binding of the aminoquinolines to three types of RDNH (with histone to DNA ratios of 0·5, 1·0 and 1·2) was studied. Where used, the molar concentrations of the polymers are expressed in terms of the nucleic acid–phosphorus (DNA–P or RNA–P). Ribosomes were isolated from rat liver by treatment of the postmitochondrial supernatant with 0·3% sodium deoxycholate and centrifugation for 2 hr at 105,000 *g*-max.

Three or four different preparations of each polymer (chromatin, each RDNH and ribosomes) were used for the binding studies. The binding data obtained with different preparations of the same polymer were comparable, and composite data are presented in the accompanying figures. The binding studies by equilibrium dialysis were conducted at 5° for approximately 20 hr. Dialysis of chromatin or ribosomes against buffer indicated that very little (<0·5 per cent) material absorbing at 260 nm diffused across the dialysis membrane under these conditions, and it is concluded that there was no interfering endogenous nuclease activity in the preparations of chromatin or ribosomes. No correction was made for the Donnan effect since (a) the correction is extremely small at ionic strengths above 0·01[2,9,12,13] (the current studies were done at ionic strength 0·018), and (b) the Donnan effect would be minimal in nucleoproteins where cationic proteins (e.g. histones) mask at least a portion of the anionic phosphates of the nucleic acid.

The data on the level of binding of an aminoquinoline to a nucleic acid or nucleoprotein are expressed as r, the moles of aminoquinoline bound either per mole of DNA–P or per milligram of polymer at various c, the concentration of free aminoquinoline. Measurements are utilized where the concentration of free aminoquinoline ranged from 0·7 to 2·3 × 10^{-4} M, unless otherwise indicated. Within this concentration range of free aminoquinoline, the values of r appear to be linear with c. The r values presented in the tables and figures are calculated from plots of the respective linear regression lines of r against c. The values of n, the maximum number of moles of aminoquinoline bound per unit of polymer, are obtained from the double-reciprocal plot of $1/r$ against $1/c$[7,8] where the intercept on the vertical axis is equal to $1/n$. The

association constants (K), expressed as M^{-1}, are calculated from $K = r/(n - r)c$. Since values of r/c were within a narrow range, the use of a Scatchard plot was not feasible.

RESULTS

The level of binding of primaquine to DNA, chromatin and RDNH is shown in Fig. 1a. The level of binding decreases as follows: native DNA = RDNH-0·5 > RDNH-1·0 = RDNH-1·2 > chromatin. Quantitatively, at a free primaquine concentration of $1{\cdot}5 \times 10^{-4}$ M, primaquine binding to native DNA and to chromatin amounts to 6 and 3 molecules, respectively, per 100 DNA–nucleotides; the corresponding levels for the RDNH are 7, 5 and 5 for RDNH-0·5, RDNH-1·0 and RDNH-1·2 respectively. As will be shown below, the interaction of primaquine with free calf thymus histone is negligible under these conditions.

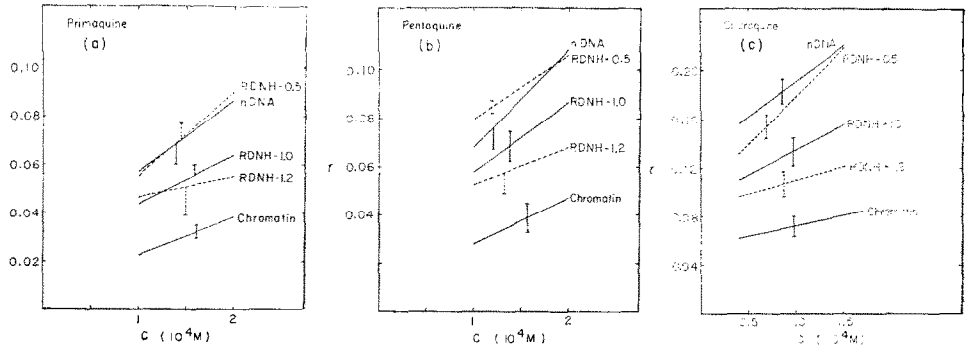

FIG. 1. Binding of aminoquinolines to DNA and DNA–protein complexes. The values of r, the vertical co-ordinate, are expressed as the millimoles of aminoquinoline bound per millimole of DNA-P in the respective polymer. Each line is drawn from the calculated least-squares regression line of data from equilibrium dialysis measurements. The median difference between observed and calculated (from linear regression line) r is indicated as a vertical line plotted at the mean c of the experimental samples. The samples of polymer–aminoquinoline combination had, after equilibration, a free aminoquinoline concentration within the range $0{\cdot}7$–$2{\cdot}3 \times 10^{-4}$ M in the case of primaquine and pentaquine, or within $0{\cdot}4$–$1{\cdot}5 \times 10^{-4}$ M in the case of chloroquine. For each aminoquinoline–polymer combination, there are 16–24 samples with free DNA, 13–16 samples with each RDNH and 28–34 samples with chromatin.

The order of binding of pentaquine to DNA and various DNA–protein complexes (Fig. 1b) is essentially the same as the binding of primaquine, although the level of binding of pentaquine is somewhat greater. The level of binding of pentaquine decreases as follows: native DNA = RDNH-0·5 > RDNH-1·0 > RDNH-1·2 > chromatin. Quantitatively, at a free pentaquine concentration of $1{\cdot}5 \times 10^{-4}$ M, pentaquine binding to native DNA and to chromatin amounts to 9 and 4 molecules, respectively, per 100 DNA–nucleotides; the corresponding levels are 9, 7 and 6 for pentaquine binding to RDNH-0·5, RDNH-1·0 and RDNH-1·2 respectively. Pentaquine, like primaquine, shows no significant interaction with free calf thymus histone.

The binding of the predominantly divalent chloroquine to DNA and to each of the DNA–protein complexes exceeds the binding of the monovalent 8-aminoquinolines under these experimental conditions (0·01 M potassium phosphate buffer, pH 7·0;

ionic strength, 0·018) (Fig. 1c). The levels of binding of chloroquine to the various polymers, in decreasing order, are native DNA ⩾ RDNH-0·5 > RDNH-1·0 > RDNH-1·2 > chromatin; this is essentially the same order observed with the pentaquine and primaquine. At a free chloroquine concentration of $1·0 \times 10^{-4}$ M, the chloroquine binding to native DNA and to chromatin amounts to 19 and 7 molecules, respectively, per 100 DNA–nucleotides; the corresponding levels are 18, 14 and 11 for RDNH-0·5, RDNH-1·0 and RDNH-1·2, respectively.

The concentration of the DNA and DNA–protein complexes in Figs. 1a, b and c is expressed as millimolar quantities of DNA–P. If the level of binding (r) is expressed as micromoles of aminoquinoline bound per milligram of polymer (sum of DNA plus protein), the level of binding decreases, in order, as follows: DNA > RDNH-0·5 > RDNH-1·0 > RDNH-1·2 > free histone. At a free aminoquinoline concentration of $1·5 \times 10^{-4}$ M, the r values of primaquine are 0·20, 0·14, 0·08, 0·07 and <0·004 for free DNA, RDNH-0·5, RDNH-1·0, RDNH-1·2 and free histone, respectively; the corresponding r values of pentaquine are 0·26, 0·18, 0·10, 0·08 and <0·004, respectively. At a free chloroquine concentration of $1·0 \times 10^{-4}$ M, the r values of chloroquine are 0·55, 0·35, 0·20, 0·14 and <0·004 for free DNA, RDNH-0·5, RDNH-1·0, RDNH-1·2 and free histone, respectively.

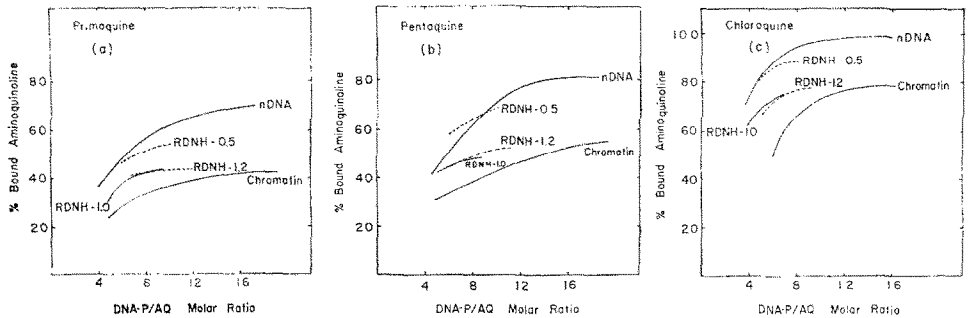

FIG. 2. Percentage of aminoquinoline bound to DNA and to DNA–protein complexes at various DNA–P to aminoquinoline (AQ) ratios. The DNA–P concentrations in the polymers ranged from 0·9–4·3 mM.

The percentages of the aminoquinolines bound to free DNA and the DNA–protein complexes are given in Figs. 2a, b and c. The relative order of binding of the aminoquinolines to the various polymers is generally the same as observable in Figs. 1a, b and c. The lower level of binding to free DNA in these experiments at pH 7·0, compared to the binding measured at pH 6·0,[8,9] is due to the increase in ionic strength of 0·01 M potassium phosphate with increase in pH. It previously has been demonstrated that the level of the total binding of the aminoquinolines to free DNA does not change with increase in pH if constant ionic strength is maintained.[8] At a DNA–P to aminoquinoline molar ratio of 10, approximately 60 and 35 per cent of the primaquine, and 70 and 40 per cent of the pentaquine bind to free native DNA and to chromatin, respectively; at the same molar ratio of 10, approximately 95 and 75 per cent of the chloroquine binds to free native DNA and to chromatin respectively. For each of the aminoquinolines, the percentage binding to the RDNH samples is intermediate between the binding to free native DNA and to chromatin.

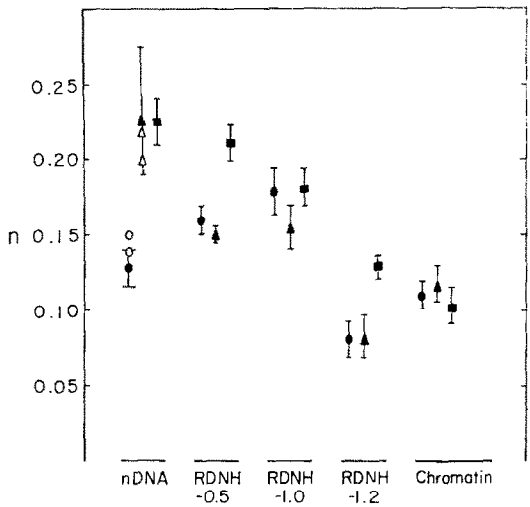

FIG. 3. Maximum number of binding sites (n) for interaction of aminoquinolines with DNA and DNA–protein complexes. The range of n indicated is calculated from the observed vertical intercept (equal to $1/n$) $\pm$ the median percentage difference between observed and calculated (from linear regression line) $1/r$ in the plot of $1/r$ vs. $1/c$. Values measured in these experiments (ionic strength, 0·018): ●, primaquine; ▲, pentaquine and ■, chloroquine. Values previously measured at ionic strengths of 0·011 and 0·022 and reported (ref. 8): ○, primaquine; △, pentaquine.

The values of the maximum number of binding sites, n (mole of aminoquinoline per mole of DNA–nucleotide), are given in Fig. 3 for the various DNA-containing polymers. At low ionic strengths, the n values generally decrease as the relative proportion of protein in the polymer increases. The n values of those polymers with high protein content (i.e. chromatin and RDNH-1·2) are usually about one-half of the values of free DNA for each aminoquinoline. These results imply that some of the potential binding sites are masked by the histones and by other proteins of chromatin or that the proteins induce a conformational change in the macromolecule making it unavailable to interact with the aminoquinolines.[14] The association constant for the binding of an aminoquinoline to chromatin is somewhat less than the constant for binding to native DNA (Table 1); for chloroquine and primaquine, the association constants for binding to the RDNH-0·5 and RDNH-1·0 are intermediate between the constants for binding to native DNA and to chromatin. The association constants for chloroquine binding to the various polymers are 3–5 $\times$ 10^4 M^{-1}, approximately 8-fold greater than the association constants for the binding of primaquine and pentaquine. The greater association constant of chloroquine presumably reflects the larger contribution of ionic binding by the predominantly divalent chloroquine (compared to the ionic binding by the monovalent primaquine and pentaquine) to the anionic polymers.

Data on the binding of the aminoquinolines to isolated rat liver ribosomes are presented in Fig. 4. At a low ionic strength and at a concentration of free aminoquinoline of 1–2 $\times$ 10^{-4} M, the ribosomes bind approximately twice as much chloroquine as primaquine or pentaquine. The addition of Mg^{2+} ions to final concentrations of 1–2 mM decreases the binding of chloroquine to ribosomes by 30–50 per cent and the binding of primaquine and pentaquine by nearly the same amount.

TABLE 1. ASSOCIATION CONSTANTS OF AMINOQUINOLINE BINDING TO DNA AND NUCLEOPROTEINS

Polymer	Association constants, K ($M^{-1} \times 10^{-3}$) ($\pm$ Standard error)		
	Chloroquine	Primaquine	Pentaquine
Native DNA	52·6 $\pm$ 5·2	9·5 $\pm$ 0·8	5·6 $\pm$ 0·5
RDNH-0·5	40·6 $\pm$ 5·2	5·9 $\pm$ 0·7	5·5 $\pm$ 0·2
RDNH-1·0	35·5 $\pm$ 5·0	3·0 $\pm$ 0·2	6·4 $\pm$ 0·4
Chromatin	25·1 $\pm$ 2·5	2·7 $\pm$ 0·8	3·7 $\pm$ 0·2

DISCUSSION

Parker and Irvin[1] previously have found that the association constant for chloroquine interaction with deoxyribonucleoprotein is similar to or somewhat less than for the interaction with free DNA. Studies on the interaction of ethidium with DNA and with deoxyribonucleoprotein (or chromatin) show that the maximum number of binding sites on the DNA is decreased by the presence of protein.[14–16] However, the association constant for ethidium binding to DNA may[14] or may not[15,16] decrease in the presence of chromosomal proteins. The decreasing binding of aminoquinolines observed with increasing protein content of the polymers is due predominantly to a decrease in the number of potential binding sites and is also attributable to a small decrease in the strength of binding, i.e. in the association constants.

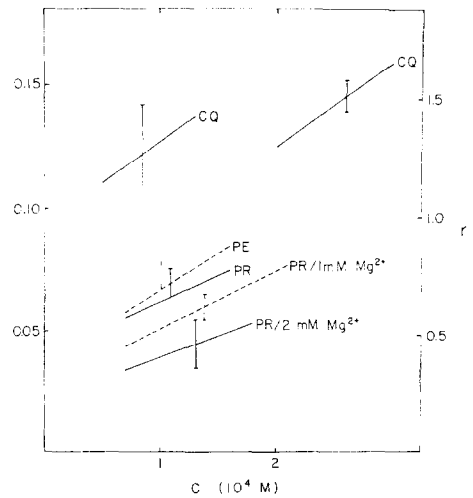

FIG. 4. Binding of aminoquinolines to isolated rat liver ribosomes. The values of r are expressed on the left scale as the millimoles of aminoquinoline bound per millimole of RNA–P and on the right scale as the millimoles ($\times 10^4$) per milligram of ribosomes. Each line is drawn from the calculated least-squares regression line. The binding studies were conducted in the absence of Mg^{2+} unless otherwise indicated. The free aminoquinoline concentrations were within the range of 0·7 to 2·1 $\times$ 10^{-4} M for the primaquine and pentaquine studies, and 0·5–1·3 $\times$ 10^{-4} M and 2·0–3·0 $\times$ 10^{-4} M for the chloroquine experiments. There were 9–29 samples for each aminoquinoline–polymer combination denoted.

For each of the antimalarial aminoquinolines, little or no difference is observed in the binding to free native DNA and to RDNH-0·5; thus, the presence of some histone does not appreciably decrease the binding of these aminoquinolines. Further, the levels of binding of the aminoquinolines to RDNH-1·0 and RDNH-1·2 do not differ significantly from each other but are generally considerably less than the binding to RDNH-0·5. However, the binding to each RDNH was considerably greater than to chromatin. The non-histone proteins of chromatin, which are absent in the RDNH, may block a portion of the interaction of the aminoquinolines with the DNA component. Alternatively, the formation of the DNA–histone complexes by gradient dialysis may not result in a completely normal association and may leave additional sites in the complex for interaction with the aminoquinolines.

The binding of each of the aminoquinolines to isolated chromatin is comparable to the binding to isolated ribosomes. Nonetheless, because of the differences involved during the isolation procedures and the uncertainty of the amounts of residual divalent cation remaining within each polymer, rigid quantitative comparisons should not be made between the two polymers.

The interaction of chloroquine and the 8-aminoquinolines with free nucleic acids has previously been established. Although the presence of protein in significant amounts in the nucleoproteins tends to decrease the interaction of the nucleic acid component with the aminoquinolines, considerable binding of the aminoquinolines to chromatin and to ribosomes does occur. Thus, the interaction of these compounds with such nucleoproteins logically may be expected to produce significant effects in the proper functioning of the nucleoproteins in nucleic acid and protein synthesis in various intact cellular systems. Chloroquine, primaquine and other nucleic acid-binding antimalarial drugs inhibit the biosynthesis of DNA, RNA or protein in intact malarial parasites,[17–19] in cultured mammalian cells[20] and in bacterial cells.[21] It has been observed in this laboratory that chloroquine and primaquine inhibit DNA and RNA synthesis *in vivo* and protein synthesis *in vitro* in rodent systems; the synthesis of DNA and RNA by bacterial polymerases is also inhibited[22] (see also references 4 and 23). It generally has been assumed that the interaction of the nucleic acid-binding drugs with nucleoproteins (or nucleic acids) accounts for the inhibition of precursor incorporation into nucleic acids and proteins. It must be considered, however, that besides the putative inhibition of nucleic acid polymerases and the various proposed site(s) of inhibition of protein synthesis,[24–27] other reactions and processes such as transport across cellular membranes, energy production or intracellular metabolism may also be affected by these compounds and may alter the results of precursor incorporation studies in intact cells.[19,26]

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