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The Effect of Chloroquine on the Enzymatic Hydrolysis of Nucleic Acids

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SUMMARY

Chloroquine stimulates the hydrolysis of soluble RNA by pancreatic ribonuclease and micrococcal nuclease, enzymes that are primarily endonucleases. The addition of $MgCl_2$, at low concentrations relative to the concentrations of RNA nucleotides, does not appreciably affect the extent of the stimulation. Stimulation by chloroquine does not appear to be due to a change induced by the drug in the tertiary structure of soluble (or transfer) RNA, since the hydrolysis of the single-stranded copolymer of riboadenylate and ribocytidylate is also stimulated by chloroquine. Chloroquine inhibits the hydrolysis of soluble RNA and polyadenylate by spleen phosphodiesterase, an exonuclease. Chloroquine also inhibits the hydrolysis of native (double-stranded) and heat-denatured (single-stranded) DNA by micrococcal nuclease and, in agreement with other reports, hydrolysis by pancreatic deoxyribonuclease.

INTRODUCTION

Chloroquine and quinacrine, two antimalarial drugs which bind to DNA (1–7), inhibit the hydrolysis of DNA by bovine pancreatic DNase and a DNase from rabbit serum (3). Other compounds that bind to DNA, namely, actinomycin D (8–11), acridine orange and acriflavin (12), ethidium bromide (9), isoxanthopterin (13), nogalamycin and U-12241 (14), and chromomycin (15), also inhibit the hydrolysis of DNA by one or more deoxyribonucleases or phosphodiesterases:[2] bovine pancreatic DNase (9, 10, 12–15), micrococcal nuclease (11), hog spleen DNase (DNase II) and snake venom phosphodiesterase (10, 15), and HeLa cell alkaline DNase and poxvirus-induced alkaline DNase in HeLa cells (8, 9). It is generally assumed that the inhibition of the nuclease activity is due to the formation of a DNA-ligand complex resistant to the nuclease rather than to the direct action of the drug on the nuclease. This assumption has been supported in those cases in which the inhibition has been characterized (10, 13).

Chloroquine also binds to RNA of yeast (1, 7) and bacteria (16), and to various poly-

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[2] Enzymes: bovine pancreatic DNase (DNase *I*), deoxyribonucleate oligonucleotidohydrolase, EC 3.1.4.5; micrococcal nuclease or the extracellular phosphodiesterase of *Staphylococcus aureus*, ribonucleate (deoxyribonucleate) 3 -nucleotidohydrolase, EC 3.1.4.7; bovine pancreatic RNase (RNase I), ribonucleate pyrimidine-nucleotido-2′-transferase (cyclizing), EC 2.7.7.16; bovine spleen phosphodiesterase, orthophosphoric diester phosphohydrolase, EC 3.1.4.1.

ribonucleotides (17, 18). The effect of chloroquine on the enzymatic hydrolysis of yeast soluble (or transfer) RNA and various polyribonucleotides by bovine pancreatic RNase and micrococcal nuclease has been examined. The formation of a RNA-chloroquine complex renders the RNA more sensitive to enzymatic hydrolysis by these enzymes. This is in contrast to the formation of a DNA-chloroquine complex, which renders the DNA less sensitive to hydrolysis by pancreatic DNase and micrococcal nuclease.

MATERIALS AND METHODS

Yeast soluble (or transfer) RNA, with amino acid acceptor activity, was obtained from Schwarz BioResearch; calf thymus DNA and pancreatic RNase A, from Sigma Chemical Company; random copolymeric polyribonucleotides poly rAU (62% adenosine) and poly rAC (48% adenosine), from Biopolymers Laboratory; and bovine pancreatic RNase, micrococcal nuclease, and spleen phosphodiesterase, from Worthington Biochemical Corporation.

The conditions of the enzymatic incubations are given in the legends to the tables and figures. Tris base and HCl were used in the preparation of all buffers for enzymatic assays and are designated as Tris. The pH values in Tris buffers reported here were corrected for changes resulting from alterations in temperature (except in Table 1); the pH of a Tris solution measured at 5° and at 37° is 0.5–0.6 greater and 0.3 less, respectively, than the pH measured at 25°.

The hydrolysis of nucleic acids was measured by the formation of orcinol-positive (19) components that were soluble in cold 0.5 N perchloric acid in assays of the hydrolysis of RNA and polyribonucleotides and by the formation of diphenylamine-positive (20) components that were soluble in 0.5 N perchloric acid in assays of the hydrolysis of DNA. In these assays, bovine serum albumin was added as a coprecipitant of the unhydrolyzed polymer; omission of the albumin did not alter the relative rates of hydrolysis but did result in higher values in control and experimental samples.

RESULTS AND DISCUSSION

Data on the stimulation of pancreatic RNase activity by chloroquine are presented in Fig. 1. In each of the two experiments shown, three samples of RNA (over a 2-fold molar concentration range of

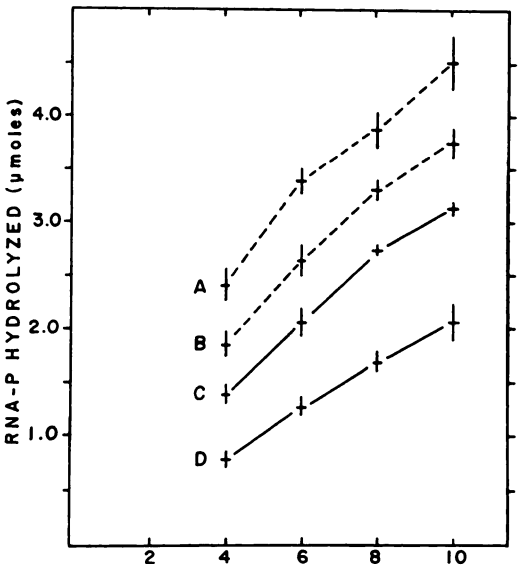

FIG. 1. *Stimulation by chloroquine of RNA hydrolysis by RNase*

Each point represents the average of three samples with various ribonucleic acid phosphorus (RNA-P) and chloroquine concentrations, although the molar ratio of the former to the latter was constant in each experiment. In experiment 1 (- - -), samples contained 0.10 μg of pancreatic RNase (RNase A, Sigma) and 4.2, 6.2, or 8.3 μmoles of RNA phosphorus with (line *A*) or without (line *B*) chloroquine. The chloroquine-stimulated samples contained 0.75, 1.1, or 1.5 μmoles of chloroquine, respectively, equivalent to a constant molar ratio of RNA phosphorus to chloroquine of 5.5. In experiment 2 (——), samples contained 0.10 μg of pancreatic RNase (Worthington) and 3.7, 5.6, or 7.4 μmoles of RNA phosphorus with (line *C*) or without (line *D*) chloroquine. The chloroquine-stimulated samples contained 0.75, 1.1, or 1.5 μmoles of chloroquine, respectively, equivalent to a constant molar ratio of RNA phosphorus to chloroquine of 4.9. In both experiments, incubations were performed at 37° for 4–10 min in a total volume of 6.2 ml of 0.02 M Tris (pH 7.0–7.1). Vertical bars indicate the standard error of the mean.

RNA phosphorus) were hydrolyzed for each period of incubation with a constant, rate-limiting concentration of RNase. In the control, chloroquine-free samples (curves *B* and *D*), at any single interval of incubation, the total amount of RNA hydrolyzed was constant although the total RNA phosphorus concentration in the three samples varied over a 2-fold (in other experiments, a 4-fold) range. The addition of chloroquine (curves *A* and *C*) at a constant molar ratio of RNA phosphorus to chloroquine resulted in an increase in the amount of sRNA hydrolyzed. In the latter samples, the quantity of RNA hydrolyzed and the degree of chloroquine-induced increase in RNA hydrolysis were essentially constant regardless of the total amount of RNA present in the sample. Thus, the extent of stimulation (expressed either as the amount of or as the percentage increase in RNA phosphorus hydrolyzed) by chloroquine was constant when measured at a constant molar ratio of RNA phosphorus to chloroquine, although the absolute quantities or concentrations varied over a 2-fold range.

Since approximately 40–50% of the chloroquine is bound to sRNA under these conditions,[3] the concentration of free chloroquine increases as the molar concentration of total chloroquine increases. Regardless of the increase in free chloroquine concentration, the extent of RNA hydrolysis at each time interval is stimulated to the same extent by the three concentrations of chloroquine at a constant molar ratio of RNA phosphorus to chloroquine. Such a finding is consistent with the stimulation of RNA hydrolysis due to the formation of an RNA-chloroquine complex rather than an RNase-chloroquine complex.

Chloroquine greatly increases the hydrolysis of sRNA by pancreatic RNase at temperatures from 3° to 45° (Table 1), a range which includes the transition temperature (T_m) of sRNA at low ionic strengths. The accentuation of hydrolysis does not appear to be temperature-sensitive if the extent of the hydrolysis of the control, chloroquine-free samples, is maintained at approximately 20% of the total RNA by decreasing the duration of incubation with increasing temperature of incubation. It is generally observed, however, that the greatest stimulation by chloroquine occurs in those samples in which the percentage of hydrolysis of the total RNA is maintained at relatively lower levels (that is, less than 25% hydrolysis of the total RNA in the incubation tube) (Table 1; also Table 2).

TABLE 1

Chloroquine-induced stimulation of RNA hydrolysis by RNase at various temperatures

The incubation mixture consisted of 0.5 μmole of yeast sRNA phosphorus, 0.025 μg of RNase, and 0.14 μmole of chloroquine (where indicated) in a total volume of 1.5 ml of 0.01 M Tris, pH 7.0 (as measured at 25°). In chloroquine-containing incubations, the molar ratio of RNA phosphorus to chloroquine was 3.6.

Incubation conditions	Hydrolysis of total sRNA		Stimulation of RNA hydrolysis by chloroquine
	Control	+ Chloroquine	
	%	%	%
7°, 180 min	22	52	135
17.5°, 40 min	16	49	210
30°, 20 min	14	36	155
37°, 15 min	18	40	120
45°, 8 min	22	30	36
3°, 180 min	11	28	160
16°, 40 min	25	57	125
30°, 20 min	62	74	19
37°, 15 min	42	87	105
45°, 8 min	39	71	82

It was of interest to examine the effect of ionic strength on the chloroquine stimulation of the hydrolysis of sRNA. Increases in ionic strength decrease the binding of chloroquine and other antimalarial aminoquinolines to polynucleotides (1, 4, 7).[4]

[3] L. A. White, unpublished observations.

[4] Equilibrium dialysis studies by L. A. White in this laboratory have shown that at a free chloroquine concentration of 0.03 mM, values of *r* (moles of chloroquine bound per mole of tRNA phosphorus) are 0.07, 0.035–0.040, and 0.010–0.015 in 0.02 M Tris buffer (pH 6.7) supplemented with NaCl to final concentrations of 0, 0.02, and 0.05 M, respectively. Expressed in a different manner—more closely related to the current enzyme studies

The addition of NaCl (0.02–0.20 M) to the buffer (0.02 M Tris, pH 6.7) increases the actual hydrolysis of RNA in both the control and the chloroquine-containing incubations (Table 2). Since the addition of NaCl increases RNA hydrolysis in control incubations to a greater extent than in chloroquine-containing incubations, the percentage stimulation of RNA hydrolysis by chloroquine is decreased. The hydrolysis of poly rAC, to be discussed below, is also greater in the presence of NaCl plus buffer (0.02 M Tris, pH 6.8) than in buffer alone.

Two investigations into the effects of metal ions on the hydrolysis of RNA by pancreatic RNase have been reported recently (21, 22). As discussed by Morrill and Reiss (21), the ratio of concentrations of Mg^{2+} and RNA phosphorus in various mammalian tissues varies from 0.077 to 0.5. Since a portion of the total tissue Mg^{2+} is bound to other cellular constituents, the tissue concentrations of Mg^{2+} that are potentially able to bind to RNA must be considerably less than a ratio of Mg^{2+} to RNA phosphorus of 0.5. In the present study, the effects of Mg^{2+} on the chloroquine-induced stimulation of RNA have been studied, since Mg^{2+} decreases the binding of chloroquine to RNA and other polynucleotides (1, 4, 7) as well as altering RNase activity in chloroquine-free systems (21, 22).

The extent of the RNA hydrolysis (expressed as the percentage hydrolysis of the total RNA) of the control, chloroquine-free samples remained at 30–35% in 0.3 mM EDTA and 0.1–2.0 mM Mg^{2+} (molar ratio of Mg^{2+} to RNA phosphorus of 0.3–6.5) (Fig. 2). Thus, in this range of concentrations, Mg^{2+} did not appreciably affect the hydrolysis of RNA in chloroquine-free incubations. In contrast, higher ratios of Mg^{2+} to RNA phosphorus (20–50) caused a significant decrease in the extent of hydrolysis of RNA in control samples.

The addition of Mg^{2+} (up to 0.3 mM and a ratio of Mg^{2+} to RNA phosphorus of 0.1) does not appreciably alter the percentage (60–100%) of the chloroquine-induced stimulation of RNase activity (Fig. 2). The ratio of the physiological concentrations of Mg^{2+} and RNA phosphorus is well within this concentration range, and chloroquine is potentially able to stimulate RNA hydrolysis by cellular nucleolytic enzymes (nucleases and phosphodiesterases). However, the addition of Mg^{2+} to a final concentration of 0.6–2.0 mM and a ratio of Mg^{2+} to RNA phosphorus of 2.0 or greater abolished the chloroquine-induced stimulation of RNase. Because of the possibility of a trace of Mg^{2+} in the RNA preparation, EDTA, at a final concentration of 0.3 mM (molar ratio of EDTA to RNA phosphorus of 1.0), was added to the incubation system. The resultant chloroquine-induced stimulation of RNase activity (approximately

TABLE 2

Effect of NaCl on chloroquine-induced stimulation of sRNA hydrolysis

The incubation mixtures consisted of 0.50 μmole of RNA phosphorus, 0.025 μg of RNase, and chloroquine (where indicated) in a total volume of 1.5 ml of 0.02 M Tris, pH 6.7. The chloroquine content was 0.10 μmole (molar ratio of RNA phosphorus to chloroquine, 5.0) in experiment A, and 0.069 μmole (molar ratio of RNA phosphorus to chloroquine, 7.3) in the experiment B. Samples were incubated at 37° for 5 min (experiment A) or 8 min (experiment B).

Expt.	NaCl concentration	Hydrolysis of total sRNA		Stimulation by chloroquine
		Control	+ Chloroquine	
	M	%	%	%
A	0	25	43	71
	0.02	44	62	41
	0.05	51	54	<10
	0.10	45	62	38
	0.15	50	67	35
	0.20	53	51	
B	0	29	49	69
	0.02	58	74	28
	0.05	62	70	13
	0.10	64	72	13
	0.15	60	64	<10

—r was 0.055, 0.035, and 0.024 at molar ratios of RNA phosphorus to chloroquine of 10:1 in 0.02 M Tris buffer (pH 6.7) supplemented with NaCl to final concentrations of 0, 0.02, and 0.05 M NaCl, respectively.

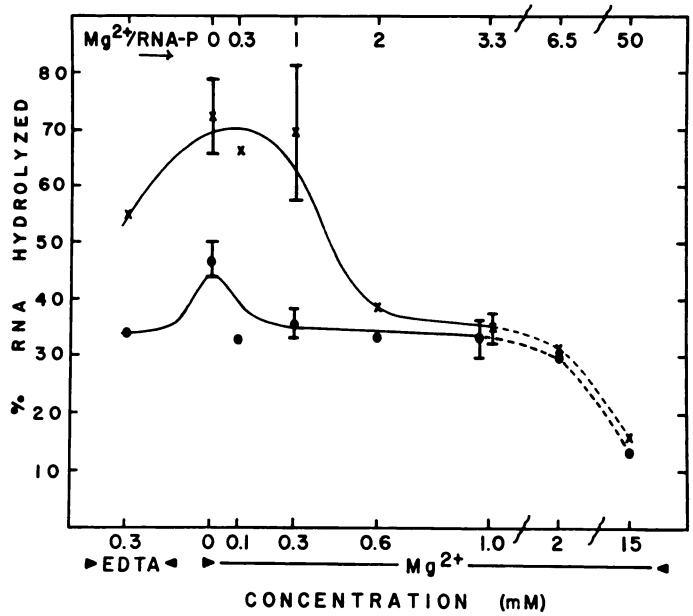

FIG. 2. *Effect of chloroquine on hydrolysis of sRNA at various molar ratios of Mg^{2+} to RNA phosphorus* The incubation mixtures contained 0.5 μmole of ribonucleic acid phosphorus (RNA-P), 0.025 μg of pancreatic RNase, and 0.14 μmole of chloroquine (where indicated) (at a molar ratio of RNA phosphorus to chloroquine of 3.6) in a total volume of 1.5 ml of 0.02 M Tris, pH 6.7. Incubations were performed for 8 min at 37°. Vertical bars indicate the standard error of the mean.

65%) was within the range of the stimulation observed upon addition of Mg^{2+} to a ratio of Mg^{2+} to RNA phosphorus of 0–1.0.

One of the factors considered in this study was that the chloroquine-induced stimulation in the hydrolysis of sRNA might be due to, and specific for, a chloroquine-induced structural change in the tertiary structure of transfer RNA. Therefore, the effect of chloroquine was determined on the RNase activity against two single-stranded polyribonucleotides, poly rAC and poly rAU. Chloroquine also stimulates the hydrolysis of these polymers by RNase; the data for poly rAC are shown in Table 3. From these data it is assumed that the stimulation by chloroquine is not specific solely for the tertiary structure of transfer RNA but is due instead to a general interaction with polyribonucleotides. In addition, the similar chloroquine-induced stimulation of the hydrolysis of poly rAC and poly rAU tends to discount the possibility that chloroquine stimulates the hydrolysis of only a limited region (e.g., either the loops or the double-stranded sections of the proposed cloverleaf structure) of tRNA.

In order to determine whether the stimulation of RNase activity by chloroquine is due to drug-RNA interaction or drug-RNase interaction, an additional endonuclease was studied. Micrococcal nuclease is a phosphodiesterase, with primarily endonuclease activity (23), capable of the hydrolysis of either RNA or DNA (24). As was observed for RNase, the addition of chloroquine greatly stimulates the hydrolysis of sRNA by micrococcal nuclease. Since the activity of micrococcal nuclease requires Ca^{2+}, the incubation mixtures contained a final concentration of 0.20 mM $CaCl_2$, equivalent to a ratio of RNA phosphorus to Ca^{2+} of 1.5–1.6. Upon incubation at 30° and pH 6.9 at a molar ratio of RNA phosphorus to chloroquine of 3.6–3.7, chloroquine markedly stimulates the hydrolysis of sRNA (Table 4, experiments A and B). Micrococcal nuclease exhibits greater enzymatic activities against RNA and DNA above pH 7 (24, 25). The stimulation of sRNA hydrolysis by chloroquine is also observed at pH 7.7 (Table 4, experiments C and D).

TABLE 3

Chloroquine stimulation of hydrolysis of poly rAC by ribonuclease

Incubations were conducted for 5 min at 37° in 0.02 M Tris buffer, pH 6.8. In experiment A, 0.25 mg of poly rAC (equivalent to 0.60 μmole of polynucleotide phosphorus), 5 μμg of bovine pancreatic RNase, and 0.15 μmole of chloroquine (where indicated) were incubated in a total volume of 1.1 ml. In experiment B, the incubation mixture was the same as in experiment A, except that 2.5 μμg of RNase were added. The molar ratio of polynucleotide phosphorus to chloroquine was 4.

Expt.	Hydrolysis of total poly rAC		Stimulation by chloroquine
	Control	+ Chloroquine	
	%	%	%
A	60	80, 89	41
B	64, 61	83, 89	37

TABLE 4

Chloroquine stimulation of hydrolysis of sRNA by micrococcal nuclease

In experiments A and B, incubation mixtures contained 0.25 mg of sRNA (0.5 μmole of RNA phosphorus), 0.14 μmole of chloroquine (where indicated), 5 μg of micrococcal nuclease, and 0.32 μmole of $CaCl_2$ in a total volume of 1.6 ml of 0.01 M Tris buffer, pH 6.9. In experiments C and D, incubation mixtures contained 0.5 μmole of sRNA phosphorus, 0.12 μmole of chloroquine (where indicated), 2.5 μg of micrococcal nuclease, and 0.32 μmole of $CaCl_2$ in a total volume of 1.6 ml of 0.02 M Tris buffer, pH 7.7. Incubations were carried out for 20 min at 30° in experiments A and B, and for 10 min at 37° in experiments C and D. In experiment A, unhydrolyzed sRNA was precipitated with cold perchloric acid (final concentration, 0.48 M); in the other experiments, unhydrolyzed sRNA was precipitated with cold perchloric acid (final concentration, 0.32–0.38 M) together with bovine serum albumin (final concentration 0.10–0.16 mg/ml).

Expt.	RNA phosphorus hydrolyzed		Stimulation by chloroquine
	Control	+ Chloroquine	
	μmole	*μmole*	%
A	0.15, 0.14	0.27, 0.22	65
B	0.16, 0.13	0.20, 0.23	50
C	0.25, 0.24	0.31, 0.34	33
D	0.30, 0.27	0.39, 0.35	30

The observation that chloroquine stimulates the hydrolysis of RNA both by pancreatic RNase and by micrococcal nuclease indicates that the chloroquine effect is due to interaction with the sRNA rather than to an interaction between chloroquine and the two distinct nucleolytic enzymes.

Chloroquine also stimulated the hydrolysis of (single-stranded) poly rA by micrococcal nuclease in systems containing Ca^{2+}. At a polynucleotide phosphorus to chloroquine molar ratio of 4.0, the stimulation by chloroquine exceeded 50%. However, chloroquine (at a polynucleotide phosphorus to chloroquine molar ratio of 5) either did not stimulate or only marginally stimulated the hydrolysis of poly rAC by micrococcal nuclease, presumably because Ca^{2+} decreased the binding of chloroquine to poly rAC to an undetectable level in the nuclease system.

Micrococcal nuclease is also capable of the hydrolysis of native DNA and the more rapid hydrolysis of denatured DNA (23). The interactions of other compounds with DNA have been shown to render it less sensitive to a wide variety of nucleases. In a similar manner, the interaction between chloroquine and native DNA renders the DNA partially resistant to hydrolysis by micrococcal nuclease at pH 7.7 (Table 5) at a molar ratio of DNA phosphorus to chloroquine of 4.0–4.5. Studies at pH 6.9 also show that chloroquine decreases the hydrolysis of native DNA by micrococcal nuclease (data not shown).

Chloroquine inhibits the hydrolysis of heat-denatured DNA by micrococcal nuclease (Table 5) in a manner similar to the inhibition of the hydrolysis of native DNA. The inhibition of micrococcal nuclease by chloroquine does not show specificity for the stranded nature of the DNA; it is observed for both double-stranded (native) and single-stranded (heat-denatured) DNA. Thus, the interaction of DNA with chloroquine renders the nucleic acid partially resistant to the activity of micrococcal nuclease. In contrast, the interaction of sRNA with chloroquine renders the sRNA more sensitive to the activity of the same enzyme.

It also became of interest to re-examine

TABLE 5

Chloroquine inhibition of hydrolysis of native and denatured DNA by micrococcal nuclease

In experiments A and B, incubation mixtures contained 0.37 mg of native calf thymus DNA (equivalent to 0.87 μmole of DNA phosphorus), 0.16 μmole of $CaCl_2$, and 0.20 μmole of chloroquine (where indicated). The molar ratio of DNA phosphorus to chloroquine was 4.3–4.4. In experiment A, the mixture contained 1.25 μg of micrococcal nuclease and was incubated for 10 min; in experiment B, the mixture contained 0.62 μg of micrococcal nuclease and was incubated for 20 min. In experiments C and D, incubation mixtures contained 0.37 mg of heat-denatured DNA, 0.16 μmole of $CaCl_2$, 0.20 μmole of chloroquine (where indicated), and 0.15 μg of micrococcal nuclease. The molar ratio of DNA phosphorus to chloroquine was 3.6. All experiments were conducted in a total volume of 0.80 ml in 0.02 M Tris buffer, pH 7.7, and incubations were carried out at 37°.

Expt.	DNA substrate	Hydrolysis of total DNA		Inhibition by chloroquine
		Control	+ Chloroquine	
		%	%	%
A	Native	60	38	37
B	Native	26	18	31
C	Denatured	22	17	23
D	Denatured	16	11	32

the effect of chloroquine on the hydrolysis of native DNA by pancreatic DNase. Chloroquine inhibits the hydrolysis of native calf thymus DNA by pancreatic DNase (Table 6), in agreement with the report by Kurnick and Radcliffe (3). Considerable inhibition occurs at a molar ratio of DNA phosphorus to chloroquine of 5:1, although some inhibition can be observed at a ratio of 10:1. The DNase reactions were carried out in the presence of Mg^{2+}, which is important for the activity of the enzyme (26). Although Mg^{2+} decreases the binding of chloroquine to DNA, sufficient chloroquine is bound to the DNA under the conditions of the DNase assay to cause a decrease in the activity of DNase.

Pancreatic RNase, micrococcal nuclease, and pancreatic DNase are endonucleases or have primarily endonucleolytic activity and therefore hydrolyze internal phosphodiester bonds at random sites within the nucleic acid or polynucleotide to produce oligonucleotides. Spleen phosphodiesterase, an exonuclease, hydrolyzes the 5′-terminal nucleotide from the polynucleotide chain and releases nucleoside 3′-monophosphates. The effect of chloroquine on the hydrolysis of sRNA and poly rA by bovine spleen phosphodiesterase was examined. In contrast to the results obtained with the endonucleolytic nucleases, the activity of the exonucleolytic spleen phosphodiesterase against sRNA and poly rA is inhibited by 20–35% by chloroquine (Table 7) at molar ratios of RNA phosphorus (or polynucleotide phosphorus) to chloroquine of approximately 4.

The cause of the contrasting effects of chloroquine on the enzymatic hydrolysis of the two types of nucleic acids by endonucleases is unknown at present. However, the cause apparently does not reside in the

TABLE 6

Inhibition of pancreatic DNase by chloroquine

Incubations were conducted for 20 min at 37° in 0.01 M Tris buffer, pH 6.7. In experiment A, 0.75 mg of DNA (equivalent to 1.5 μmoles of DNA phosphorus), 0.32 μmole of $MgCl_2$, 1.0 μg of DNase, and chloroquine (where indicated) were incubated in a total volume of 1.6 ml. In experiment B, 0.75 μmole of DNA phosphorus, 0.32 μmole of $MgCl_2$, 0.5 μg of DNase, and chloroquine (where indicated) were incubated in a total volume of 1.6 ml. In experiment C, 0.75 μmole of DNA phosphorus, 0.16 μmole of $MgCl_2$, 0.5 μg of DNase, and chloroquine (where indicated) were incubated in a total volume of 0.8 ml.

Expt.	Chloroquine	DNA-P: chloroquine	DNA hydrolysis	Inhibition of DNA hydrolysis
	μmole		%	%
A	0		27	
	0.15	10	23	15
	0.30	5	19	30
B	0		55	
	0.075	10	51	<10
	0.15	5	35	36
C	0		27	
	0.075	10	19	30
	0.15	5	11	59

TABLE 7

Inhibition of hydrolysis of sRNA and poly rA by spleen phosphodiesterase

Incubation mixtures contained 0.5 μmole of sRNA phosphorus or 0.5 μmole of poly rA, 0.128 (Worthington) enzyme units of bovine spleen phosphodiesterase, and 0.125 μmole of chloroquine (where indicated) in a total volume of 1.04 ml of 0.02 M Tris buffer, pH 6.8; incubations were conducted for 20 min at 37°. In experiments A and B the molar ratio of RNA phosphorus to chloroquine was 3.9, and in experiments C and D the molar ratio of polynucleotide phosphorus to chloroquine was 4.2.

Expt.	Substrate	Hydrolysis		Inhibition by chloroquine
		Control	+ Chloroquine	
		%	%	%
A	sRNA	55	39.5	28
B	sRNA	43	28	35
C	Poly rA	29	23	21
D	Poly rA	29	21	28

single- or double-stranded nature of the nucleic acid. Chloroquine stimulates the hydrolysis of sRNA (single-stranded with extensive regions of intramolecular double-stranded structure) by RNase and micrococcal nuclease, that of single-stranded poly rAC by RNase, and that of single-stranded poly rA by micrococcal nuclease. In contrast, chloroquine inhibits the hydrolysis of native, double-stranded DNA by DNase and micrococcal nuclease and the hydrolysis of heat-denatured (mostly) single-stranded DNA by micrococcal nuclease. It is also interesting, however, that chloroquine inhibited the hydrolysis of sRNA and poly rA by an exonuclease, namely, spleen phosphodiesterase.

Possible chloroquine-induced changes in the tertiary structure of sRNA (tRNA) do not appear to be a sole determinant (if involved at all) in the stimulation of RNA hydrolysis by the endonucleases, since the hydrolysis of single-stranded poly rAC and of poly rAU is also enhanced by chloroquine.

Ciak and Hahn (27) have found that treatment of a chloroquine-sensitive strain of *Bacillus megaterium* with chloroquine results in rapid degradation of ribosomes, extensive endonucleolytic hydrolysis of the ribosomal RNA to smaller fragments, and the release of a small portion of the components from the RNA into the extracellular fluid. Ladda (28) has examined by electron microscopy the effect of treatment with chloroquine on the erythrocytic forms of *Plasmodium berghei*. The alterations of the ribosomes in the *Plasmodium*, including the dissociation of ribosomes, during exposure to chloroquine were interpreted (28) as correlating with the bacterial response reported by Ciak and Hahn (27). On the basis of present information, it is not yet possible to propose definitely that the chloroquine-induced increase in sensitivity of RNA to nucleases observed *in vitro* is a major determinant in the biological activities (antimalarial activity and toxic effects in animals) of chloroquine. From the present work it appears likely, however, that chloroquine induces a conformational change in soluble RNA which is enzymatically detectable by a measurement of nuclease activity. It will be of interest to determine whether chloroquine-induced alterations in transfer RNA and other RNA species will prove to possess biological importance by an alteration in protein or polypeptide synthesis. The aminoacylation of transfer RNA *in vitro* may (29) or may not (30) be affected by chloroquine, although the observed differences may be caused by experimental conditions (e.g., divalent cation concentration) rather than species differences.

REFERENCES

1. F. S. Parker and J. L. Irvin, *J. Biol Chem.* **199**, 897 (1952).
2. J. L. Irvin and E. M. Irvin, *J. Biol. Chem.* **206**, 39 (1954).
3. N. B. Kurnick and I. E. Radcliffe, *J. Lab. Clin. Med.* **60**, 669 (1962).
4. S. N. Cohen and K. L. Yielding, *J. Biol. Chem.* **240**, 3123 (1965).
5. R. L. O'Brien, J. G. Olenick and F. E. Hahn, *Proc. Nat. Acad. Sci. U. S. A.* **55**, 1511 (1966).
6. D. Stollar and L. Levine, *Arch. Biochem. Biophys.* **101**, 335 (1963).
7. L. P. Whichard, C. R. Morris, J. M. Smith and D. J. Holbrook, Jr., *Mol. Pharmacol.* **4**, 630 (1968).

8. B. R. McAuslan, *Biochem. Biophys. Res. Commun.* **19**, 15 (1965).
9. L. J. Eron and B. R. McAuslan, *Biochim. Biophys. Acta* **114**, 633 (1966).
10. N. K. Sarkar, *Biochim. Biophys. Acta* **145**, 174 (1967).
11. E. Sulkowski and M. Laskowski, Sr., *Biochim. Biophys. Acta* **157**, 207 (1968).
12. J. D. Leith, Jr., *Biochim. Biophys. Acta* **72**, 643 (1963).
13. J. M. Lagowski and H. S. Forrest, *Proc. Nat. Acac. Sci. U. S. A.* **58**, 1541 (1967).
14. L. D. Zeleznick and C. M. Sweeney, *Arch. Biochem. Biophys.* **120**, 292 (1967).
15. W. Behr, K. Honikel and G. Hartmann, *Eur. J. Biochem.* **9**, 82 (1969).
16. K. H. Muench, *Cold Spring Harbor Symp. Quant. Biol.* **31**, 539 (1966).
17. L. W. Blodgett and K. L. Yielding, *Biochim. Biophys. Acta* **169**, 451 (1968).
18. C. R. Morris, L. V. Andrew, L. P. Whichard and D. J. Holbrook, Jr., *Mol. Pharmacol.* **6**, 240 (1970).
19. W. C. Schneider, *Methods Enzymol.* **3**, 680 (1957).
20. K. Burton, *Biochem. J.* **62**, 315 (1956).
21. G. A. Morrill and M. M. Reiss, *Biochim. Biophys. Acta* **179**, 43 (1969).
22. G. L. Eichhorn, P. Clark and E. Tarien, *J. Biol. Chem.* **244**, 937 (1969).
23. L. Wingert and P. H. von Hippel, *Biochim. Biophys. Acta* **157**, 114 (1968).
24. J. N. Heins, J. R. Suriano, H. Taniuchi and C. B. Anfinsen, *J. Biol. Chem.* **242**, 1016 (1967).
25. P. H. von Hippel and G. Felsenfeld, *Biochemistry* **3**, 27 (1964).
26. E. Melgar and D. A. Goldthwait, *J. Biol. Chem.* **243**, 4409 (1968).
27. J. Ciak and F. E. Hahn, *Science* **151**, 347 (1966)
28. R. L. Ladda, *Mil. Med.* **131**, 993 (1966).
29. J. H. Landez, R. Roskoski, Jr., and G. L. Coppoc, *Biochim. Biophys. Acta* **195**, 276 (1969).
30. J. Ilan and J. Ilan, *Science* **164**, 560 (1969).