The Binding of Quinacrine Mustard to Nucleic Acids

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Quinacrine mustard forms complexes with nucleic acids in solution, as indicated by the yellow precipitation forming when native DNA and high concentrations of quinacrine mustard are mixed. This complex shows different biophysical properties as compared to the nucleic acid alone in solution. The viscosity is elevated, T_m is shifted towards higher temperatures, and the sedimentation constant is reduced. The polymer changes the fluorescence intensity of the dye: a net quench is obtained with all natural DNAs. The bonds between the dye and DNA depend on the pH and ionic strength in the solution. The binding between the dye and nucleic acids is also affected by the strandedness of the polymer. A greater quenching of the fluorescence intensity of quinacrine mustard is obtained with single stranded molecules than with double stranded ones. In a methyl green-DNA complex the methyl green is displaced by quinacrine mustard. This probably reflects competition between the two dyes.

The basic acridine dyes are able to bind nucleic acids in different ways. They are all capable of ionic binding to the phosphate groups in the nucleic acids with or without intercalation.[1-4]

The mode of interaction between acridine dyes and nucleic acid polymers has been studied by Dourlent and Hélène [5] with the dye proflavine. They found two bond species: a cooperative binding along the phosphate backbone and an interaction between the aromatic rings of the dye and nucleic acid bases, i.e. intercalation. These two processes are linked together. Further, the intercalation process is closely correlated to the secondary structure of the polymer. At low ionic strength and low concentration ratios of nucleic acid to proflavine the binding is an electrostatic interaction between the positively charged dye and the negatively charged phosphate groups. At high concentration ratios of nucleic acid to dye, a new complex is formed which suggests a van

der Waals type of interaction between proflavine and the bases of the nucleic acid. Two models have been proposed for this second type of binding: the complete model of Lerman [6] and the partial intercalation model of Pritchard.[7] In the first model the dye is spaced between two bases in the opposite strands of the DNA helix. In the partial intercalation model the dye is spaced between two bases in the same strand of the nucleic acid. A complex formation between acridine dyes and single stranded nucleic acids provides evidence in support of the latter model.

Caspersson et al.[4] introduced the use of quinacrine and quinacrine mustard for staining cytological preparations. They chose quinacrine mustard because of the assumed guanine specificity of the dye. However, Ellison and Barr,[8] Weisblum and de Haseth,[9,10] Pachmann and Rigler,[11] and Michelson et al.[12] all showed that quinacrine and quinacrine mustard preferably stain chromosome regions rich in AT base pairs. Alternatively, proteins associated with (A + T) rich DNA might contribute to strong fluorescence, although this is hardly the case when purified DNAs or nucleotides in solution are tested.

The aim of the present investigation was to study the characteristics of the binding between quinacrine mustard and natural nucleic acids. Changes caused by the dye in some of the biophysical properties of DNA are reported. The results are discussed with reference to findings reported by other investigators using related dyes which also interact with nucleic acids.

EXPERIMENTAL

DNAs. DNA from mouse liver, calf thymus and *Saccharomyces cerevisiae* was isolated according to the method described by Marmur.[13]

The absorbance of 1 mg/ml of DNA from mouse liver and calf thymus was 21 at 257.5 nm in 0.1 × SSC * solution and of DNA isolated from *S. cerevisiae* 25.4 at 260.0 nm. This DNA was denatured, *i.e.* in single stranded form. The (G + C) content was 40 % for the DNA from mouse liver and calf thymus, and 43 % for the DNA isolated from *S. cerevisiae*. Phosphorus analysis gave 11.7 % phosphorus in mouse liver, 10.0 % in calf thymus, and 11.2 % in *S. cerevisiae* DNA. The absorbance of the sodium salt of highly polymerized calf thymus DNA (G + C, 45 %), purchased from the Sigma Chemical, U.S.A, was 21 at 260.0 nm, and the phosphorus content was 11.9 % (0.1 × SSC solution).

Deoxyribonucleic acid from *Escherichia coli*, (G + C, 50 %) and from *Clostridium perfringens* (G + C, 31 %) were purchased from the Sigma Chemical, U.S.A.

Denaturation was achieved by heating DNA (approx. 100 μg/ml in 0.1 × SSC solution) at 100°C for 10 min and then rapidly cooling it to 0°C. The change in absorption was measured spectrophotometrically and the temperature which produced 50 % of the total hyperchromicity resulting from the denaturation was taken to be T_m.

Native and denatured DNA (approx. 1 mg/ml) could be stored for at least 6 months at − 70°C without degradation. The DNA concentration was determined either at the absorption peak at 260.0 nm or with the diphenylamine reaction.[14] The phosphorus analysis was as described by Tuan and Bonner.[15] The phosphorus content for the DNAs as well as the RNAs (see below) was rather high, indicating some contamination during the preparation of the nucleic acids. Deoxyribonuclease I from bovine pancreas was purchased from the Sigma Chemical, U.S.A.

RNAs. RNA from mouse liver and *S. cerevisiae* was isolated according to the method described by Kirby.[16] Stock solutions (approx. 1 mg/ml) were prepared in 0.1 × SSC solution and could be stored for 6 months at − 25°C without degradation. The absorbance of mouse liver RNA at 260.0 nm was 20 and that of *S. cerevisiae* RNA 24.9. The (G + C) content of mouse liver RNA was 40 %. Phosphorus analysis gave 15.2 % phosphorus in mouse liver and 14.2 % in *S. cerevisiae* RNA. The RNA concentration was determined either at the absorption peak at 260.0 nm or with the orcinol reaction.[17]

Quinacrine mustard. Quinacrine mustard * (I) was a gift from the Sterling-Winthrop Research Institute, U.S.A. The fluorescence and absorption spectra and some other characteristics of the dye (anion and pH dependence) have been previously reported.[18] Stock solution of QM (2 mg/ml) in distilled water was stable for two weeks at 4°C in the dark. The solution remained

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* Abbreviations: 0.1 × SSC, 0.015 M NaCl-0.0015 M sodium citrate, pH 7. QM, quinacrine mustard.
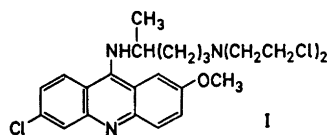
clear the whole time without any changes in the absorption or fluorescence properties.

Fluorescence intensity. A fluorescence attachment ZFM4 to a Zeiss (PMQ II) spectrophotometer equipped with an excitation filter at 436 nm was used for determining the fluorescence intensity of the solution. The spectrophotometer was standardized with a fluorescence standard cuvette F 53 with excitation at 436 nm and with a fluorescence peak at 530 nm and a No. 10 slit arrangement.

The nucleic acids did not interfere with the QM fluorescence at 514 nm.[19] The theory and method of fluorimetric titration have been described by LePecq and Paoletti.[20] The binding parameters could be calculated by measuring the alteration of the fluorescence intensity of the dye under constant conditions of excitation, temperature, solvent composition and concentration. It was established that the fluorescence intensity alterations which occurred after mixing the polymer and the dye were complete within a few seconds and showed no further changes with time or additional mixing.

Absorption studies. Absorption spectra were recorded with a Zeiss (PMQ II) spectrophotometer. The absorption of the polymer did not disturb the determination of QM at 424.0 nm. The theory which was applied to the analysis of QM binding experiments has been described by Peacocke and Skerrett.[21] The association constants and the number of binding sites of the dye can be directly determined from the titration data with the Scatchard equation.[22] Although the curves obtained from the titration experiments with QM and polymers of unknown length deviate from the theoretical curves of Scatchard, his equation has been used in many investigations to determine the number of dye molecules bound to polymers.[5,23-26]

RESULTS

Effect of pH on QM. The effect of pH over the range 2 to 12 (in 0.015 M NaCl) on the ratio of the absorption at 424 nm and 365 nm of unbound QM is shown in Fig. 1. At pH ≥ 9.7 only the nitrogen atom of the diethylamine group of the side chain is protonated, but at pH ≤ 7 the dye is doubly protonated. The increase in the 424 nm to 365 nm ratio between the pH values 9.7 and 7 corresponds to a decrease in the fluorescence intensity of the dye.[18] The corre-
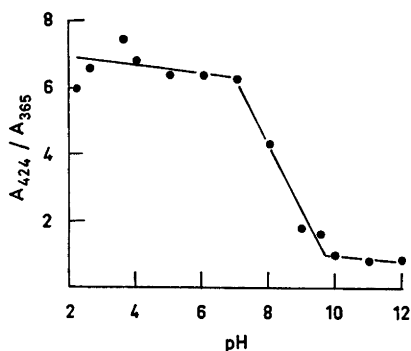
Fig. 1. Effect of pH on QM. Determinations were made in 0.015 M NaCl and the concentration of QM was 156 μM. pH was plotted against the changes in the ratio of absorption at 424 nm and 365 nm.

sponding pK_a values for quinacrine are 10.3 and 7.7, respectively.[27]

Interaction between QM and DNA. The reaction between QM and native DNA was indicated by the precipitation of yellow fibres brought about by the mixing of a solution containing a high concentration of the dye. At lower concentrations of the dye no precipitation occurred. In all the experiments reported below, concentration ranges of polymer and dye were chosen so that precipitation of the complex did not occur.

Effect of DNA on the fluorescence and absorption spectra of QM. In a QM-DNA mixture, quenching of the fluorescence intensity of the dye was noted at 514 nm, but no shift was found in the fluorescence spectrum of QM. However, DNA alters the absorption spectrum of QM as shown in Fig. 2. Increasing amounts of native mouse liver DNA were added to a solution with a constant amount of QM. The shift of the absorption maximum from 424 nm to higher wavelengths and the presence of an isosbestic point at 455 nm indicate the formation of specific complexes of the dye with DNA.[5] The ratio of the absorption at 424 nm and 365 nm decreases as the concentration of DNA increases. At very high concentration ratios of polymer to dye, there is a marked change in the absorption spectrum of QM (Fig. 2).

Fig. 3 depicts the difference spectra of QM in mixtures with native mouse liver DNA and with mouse liver RNA in 0.1 × SSC solution.
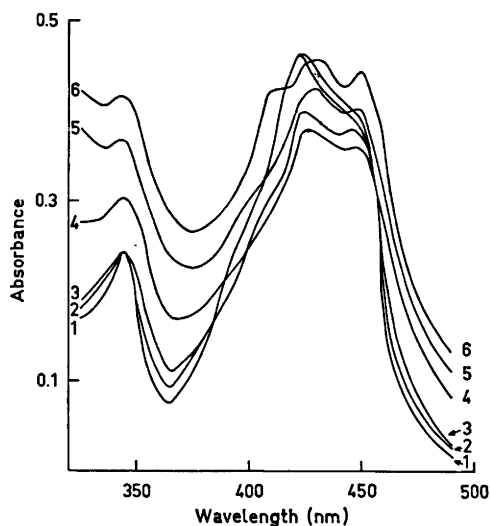
Fig. 2. Absorption spectra of QM partially and completely bound to native DNA. Determinations were made in McIlvaine's buffer, pH 7,[28] and the concentration of QM was (1) 50 μM. Concentrations of native mouse liver DNA phosphorus were: (2) 0.012 mM; (3) 0.129 mM; (4) 0.523 mM; (5) 0.928 mM and (6) 1.230 mM.
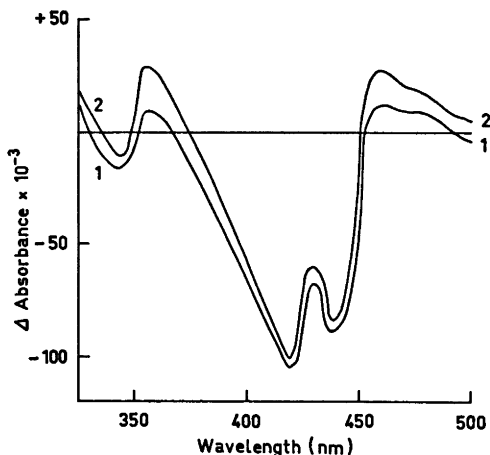

Fig. 3. Difference spectra of QM produced by nucleic acids. Determinations were made in 0.1 × SSC solution, pH 7, with 12.6 μM QM. (1) 0.535 mM RNA phosphorus, and (2) 0.464 mM double stranded DNA phosphorus. Both RNA and DNA were isolated from mouse liver (*cf.* Experimental).

Both nucleic acids depress the absorbance at 424 nm, although there were differences between double and single stranded nucleic acids.

Non-equilibrium dialysis. Since this was not an equilibrium dialysis, the possible dissociation of bound QM as the free QM concentration approached 0 would cause the fluorescence estimation at 514 nm to be too low. Mixtures of 0.5 mg/ml native calf thymus DNA with 0.05 mg/ml QM in 0.05 M Tris solution, pH 7.5, were dialyzed for 50 h at 0°C in viscose cellophane bags. The dialysis solution was changed frequently. After dialysis, the concentration of calf thymus DNA in the dialysis bag was 0.46 mg/ml and that of QM was 0.018 mg/ml which gave n 0.44. Native mouse liver DNA and QM, dialyzed in McIlvaine's buffer at pH 7.0, showed that 0.322 mg/ml DNA bound 0.014 mg/ml QM, *i.e.* n 0.45. The absorption of QM by the dialysis bag was slight as was the loss of nucleic acid to the external solution.

Effect of urea. Native mouse liver DNA (0.032 mg) was mixed with 0.02 mg QM in 0.1 × SSC solution with 3 M urea and 0.025 M sodium sulphate. In a solution without urea the quenching with excess DNA was -22.8 $\varDelta$ %, and with 3 M urea -10.6 $\varDelta$ %, *i.e.* a 50 % enhancement of the fluorescence intensity of QM in the presence of urea. [$\varDelta$ % is the change of the fluorescence intensity of the mixture (in per cent) relative to the fluorescence intensity of QM alone.] The QM-DNA complex was unstable in urea, *i.e.* also some hydrogen bonds might be formed when QM reacts with DNA. The interaction between chloroquine as well as quinacrine and nucleic acids is stable in urea[29] while actinomycin D forms hydrogen bonds with DNA.[30]

Effect of pH on QM-DNA mixtures. The spectrum of bound QM varies considerably within the pH range examined (Fig. 4). Since the protonation of QM is largely unaffected at pH values below 7.0 (*cf.* Fig. 1) the pH-induced changes seen in acidic solutions must result from protonation in the nucleic acid. The ratio of the absorption at 424 nm and 365 nm of QM-calf thymus DNA (double stranded) shows a rapid decline at pH $\leqq 3$ and at pH $\geqq 6$ (Fig. 4). The single stranded molecules show a more irregular dependence on pH. The protonation of DNA begins at pH 3.5−4.[31] Adenine and cytosine protonate first (pK_a values between 5
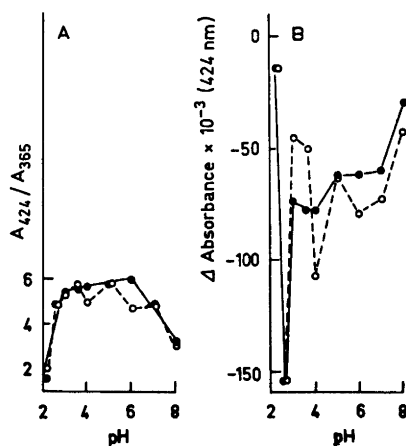

Fig. 4. Effect of pH on QM bound to nucleic acids. The determinations were made with 50 μM QM in McIlvaine's buffer. A: pH was plotted against the changes in the ratio of the absorption at 424 nm and 365 nm; B: pH was plotted against the difference in the absorption at 424 nm. (●) double stranded and (○) single stranded calf thymus NaDNA (Sigma). Concentration of DNA phosphorus was 0.129 mM.

and 4) followed by guanine (p$K_a < 3$), which is almost fully protonated at pH $\leqq 2.4$. The decline in the 424 nm to 365 nm ratio at very acid pH is followed by an enhancement of the fluores-
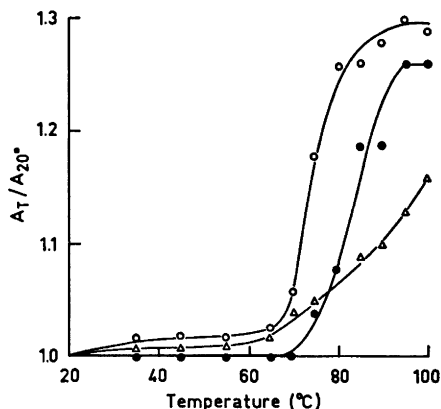

Fig. 5. Effect of QM on thermal denaturation of calf thymus DNA. Determinations were made in 0.05 M Tris solution, pH 7.5. Concentration of calf thymus NaDNA (Sigma) alone was, (○) 0.935 mM DNA phosphorus. (●) was dialyzed complex of 35 μM QM and 3.44 mM DNA phosphorus and, (△) was a mixture of 15 μM QM and 0.925 mM calf thymus DNA phosphorus.

cence at 514 nm and an enhancement of the absorption at 424 nm (Fig. 4). The absorption measurements at low pH were made within 10 min, so that possible depurination due to the acidic solution was negligible.[31] The denaturation of DNA at pH below 3 leads to a change in the binding of the dye through intercalation which leads to an increased binding of QM to the phosphate groups.[5] Since the spectrum of unbound QM varies within the pH range 7 to 8 (Fig. 1) it can be inferred that the changes found at pH above 6 for the bound ligand only depict the pH dependence of the unbound dye. Furthermore the denaturation of DNA at high pH occurs at pH above 11.[20]

Thermal denaturation of QM-DNA mixtures. The interaction between QM and native DNA is further demonstrated by the stabilizing effect of QM on DNA against denaturation by heat, as shown in Fig. 5. The T_m for (Sigma) calf thymus DNA was 73°C and the hyperchromicity 30 %. For a QM-DNA mixture (DNA-P/QM = 100) the T_m value was 83°C and the hyperchromicity 26 %, *i.e.* an increase in T_m of 10 degrees. When the proportion of QM in the mixture was raised (DNA-P/QM = 62), the T_m was increased to 90°C and the hyperchromicity reduced to 16 %. From these data it is evident that an increased stability of DNA was obtained with increased concentration ratios of QM to

Fig. 7. Effect of QM on the methyl green-DNA complex. Increasing amounts of QM were added to 0.05 M Tris solution, pH 7.5, containing 33 mmol native mouse liver DNA phosphorus and 25 µmol methyl green. The mixtures were then allowed to stand for 18 h at room temperature (in the dark) before the absorption at 640 nm was estimated.

DNA. According to Cohen and Yielding [24] the T_m raises 9 degrees at a concentration ratio of chloroquine to DNA equal to 1:10. When the concentration ratios of the dye to DNA approaches 0, the T_m for the dye-DNA complex approaches the T_m for native DNA alone.

Analytical centrifugation. Analytical ultracentrifugation was performed in a Beckman Model E analytical ultracentrifuge equipped with an RTIC unit, a monochromator and a split beam photoelectric scanner as an accessory. The sedimentation of native mouse liver DNA was observed at 260 nm at 20°C and that of QM-DNA mixtures at 424 nm in 0.1 × SSC solution, pH 7. A 12 mm double sector cell with sapphire windows was used in the An-D-rotor at 42040 rpm. The sedimentation coefficients $(S_{20,w}°)$ were calculated from a plot of log r *versus* time and was corrected to the values in a solvent with the viscosity of water at 20°C. In Fig. 6 the sedimentation constant is plotted against the concentration of DNA. This extrapolates to a sedimentation constant of 13.5 S for the QM-DNA complex at zero concentration and to 15.7 S for native mouse liver DNA
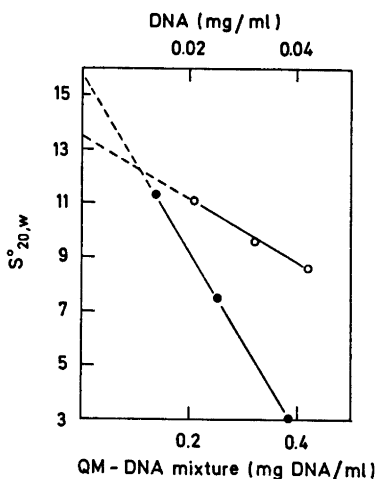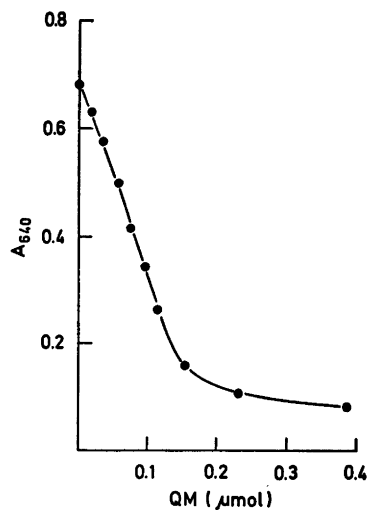

Fig. 6. Sedimentation constant of DNA and of QM-DNA mixtures. (●) DNA alone expressed in mg/ml, and (○) QM-DNA mixtures expressed in mg DNA/ml with 26 µM QM.

alone, *i.e.* a decrease of approximately 14 % in the sedimentation constant. These results agree with those of Lerman [32] and Kurnick and Radcliffe [33] for the quinacrine-DNA complex. The sedimentation constants obtained were 12.4 S for DNA alone and 9.3 S for the quinacrine-DNA complex at zero concentration.[33] The decrease in the sedimentation constant suggests that the QM-DNA complex is more asymmetric than DNA alone.[32]

Displacement of methyl green. The effect of the addition of QM to the methyl green-DNA substrate [34] is presented in Fig. 7. At pH 7.5 free methyl green was converted to leukobase, with a resultant fall in the absorbance at absorption maximum of the methyl green-DNA complex (640 nm).[35] As the QM concentration was increased there was a progressive displacement of methyl green, although complete displacement did not occur. It is possible that the dye methyl green interferes with the approach of QM to the DNA molecule by preventing the intercalation of QM.

Inhibition of the DNase activity by QM. The methyl green-DNA substrate [34] could not be used in this study because of the progressive

Fig. 8. Effect of QM on the viscosity of DNA. The results are presented as the relative viscosity (η_r), *i.e.* the outflow time of the solution divided by the outflow time of water. (O) increasing amounts of native mouse liver DNA (mg/ml), (●) 0.2 mg/ml native mouse liver DNA with increasing amounts of QM (μg/ml). The solution was 0.1 × SSC, pH 7, and each point represents the mean of three determinations.

displacement of the dye from the complex when the concentration of QM was increased (cf. above) DNase assays were therefore performed by viscosimetry. Ostwald Fenske viscosity pipettes were used at 25°C. The pipettes were calibrated with water and gave an outflow time (with water) of 35 s at 25°C.

In Fig. 8 the relative viscosities are plotted against the concentration of QM for the QM-DNA mixtures. The relative viscosities of DNA solutions are also shown in the figure. It was found that as the amount of QM was increased, the relative viscosity also increased up to 60 μg QM/ml, *i.e.* 0.3 mg dye per mg DNA. Kurnick and Radcliffe [33] reported a similar rise in the relative viscosity of a quinacrine-DNA mixture, with the maximum at 0.4 mg quinacrine per mg DNA.

In a series of assays with a DNase concentration of 20 μg/ml, 0.2 mg/ml native mouse liver DNA and varying amounts of QM in 0.1 × SSC solution, progressive reduction in the enzyme activity was observed as the relative concentration of QM increased. After a solution with 0.2 mg QM per mg DNA had been incubated at 37°C for 3 h the relative viscosity of DNA alone decreased by 17 %, while that of the QM-DNA mixture decreased by 12 %. The corresponding values for a solution with 0.1 mg QM per mg DNA was 40 % for DNA alone, and 37 % for the QM-DNA mixture (incubated for 6 h). The inhibition is obviously dependent on the concentration ratios of dye to DNA. A similar inhibition of DNase activity with quinacrine and a complete inhibition of the enzyme activity with chloroquine has been reported.[29,33]

Calculation of binding parameters. When increasing amounts of QM were added to a constant amount of polymer, the fluorescence intensity of the bound dye was quenched (Fig. 9). It is evident that those binding sites resulting in an increase of the quantum yield are occupied first, and that further additions of the dye result in reduction of the fluorescence enhancement. The reverse is illustrated in Fig. 10, where the effect of increasing amounts of native DNA and constant amounts of dye is shown. In Fig. 9 the greatest enhancement of the fluorescence intensity of the dye was obtained with native mouse liver DNA, while the curve of denatured (single stranded) DNA is about the same as
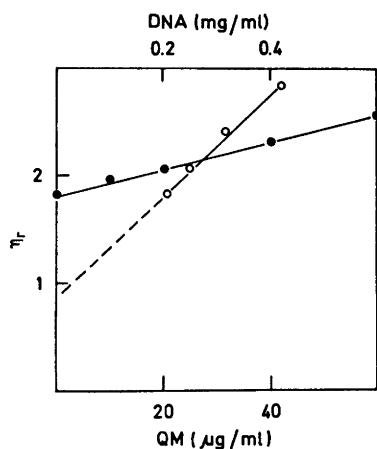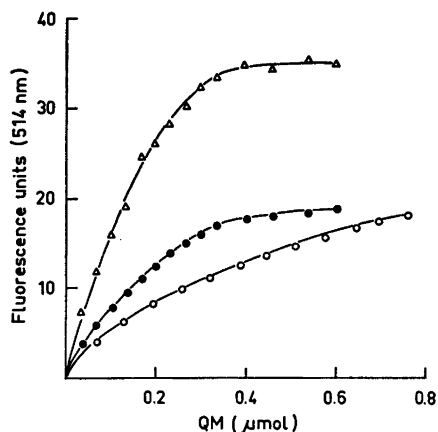
Fig. 9. Fluorescence enhancement of QM bound to nucleic acids. ($\triangle$) to constant amount of native mouse liver DNA (3.77 mmol DNA phosphorus), ($\bullet$) constant amount of denatured (single stranded) mouse liver DNA (3.77 mmol DNA phosphorus) and (O) constant amount of mouse liver RNA (4.91 mmol RNA phosphorus) was added increasing amounts of QM (μmol) in McIlvaine's buffer, pH 7.

that of mouse liver RNA. This quenching agrees with that of the DNAs of quinacrine and QM fluorescence intensity reported by others.[9-12]

The titration of DNAs by the fluorimetric method leads to anomalous binding curves which deviate from linearity in the Scatchard plot. Accordingly, the correct Scatchard plot is a line in which the slope of the first part of the
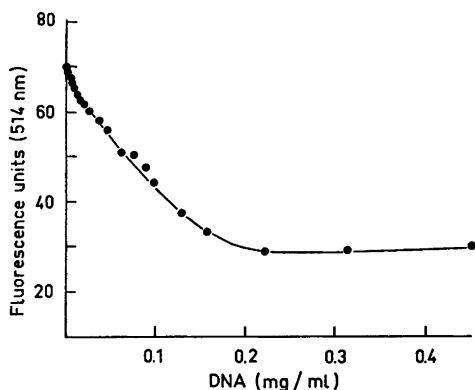

Fig. 10. Effect of DNA on the fluorescence intensity of QM. To a constant amount of QM (71.5 μmol) was added increasing amounts of native mouse liver DNA (mg/ml) in 0.1 × SSC solution, pH 7.

Table 1. Binding of QM to nucleic acids.[a]

Nucleic acid	Buffer system	Binding parameters	
		n_1	n_2
DNA			
native mouse liver	0.1 × SSC, pH 7	0.17	1.11
denatured mouse liver	0.1 × SSC, pH 7	0.13	0.53
denatured S. cerevisiae	McIlvaine, pH 7	0.25	1.10
RNA			
mouse liver	0.1 × SSC, pH 7	0.09	0.19
mouse liver	McIlvaine, pH 7	0.31	0.50
S. cerevisiae	McIlvaine, pH 7	0.17	0.37

[a] Fluorimetric titration was used to estimate the parameter n, which was calculated according to the equation of Scatchard.[22] Each n value represents the mean of three determinations.

curve is negative. The line for titration of DNAs with QM does not conform with the hypothesis of independent binding sites because it is not a straight line. However, the intercept with the r axis (r is the molar ratio of bound dye to DNA phosphorus) can be determined, *i.e.* the value of n (the apparent number of binding sites). These values are given in Table 1. The value of n_1 for native DNA was 0.17 and that of n_2 was 1.11. The high value of n_2 might depend on the high phosphorus content in mouse liver DNA (*cf.* Experimental). For chloroquine-nucleic acid mixtures n values were between 0.15 and 0.75.[24] The n values for native DNA determined by fluorimetric titration agree well with those obtained by dialysis (*cf.* above) and by analytical ultracentrifugation (0.75).

Precipitation of QM-DNA complexes. When a QM-nucleic acid complex is precipitated from a mixture containing an excess of both QM and nucleic acid the value of n increases. After the precipitation, n for native calf thymus DNA was 0.81 and for denatured calf thymus DNA, 0.66. These high n values were partly caused by the high concentration of salt in the solution during precipitation of the yellow QM-nucleic acid complex with alcohol. This high salt concentration may cause aggregation of the dye molecules.[36]
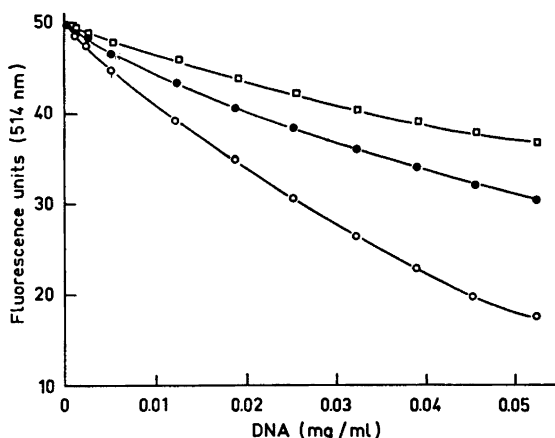
Comparison of different nucleic acids. The

Fig. 11. Effect of different DNAs on the fluorescence intensity of QM. To a constant amount of QM (26 μmol) was added increasing amounts of native DNAs (mg/ml) in 0.1 × SSC solution, pH 7. DNAs: ($\square$) *C. perfringens* (G + C, 31 %); ($\bullet$) mouse liver (G + C, 40 %), and (O) *E. coli* (G + C, 50 %).

fluorimetric titration curves for different DNAs are shown in Fig. 11. The figure depicts the correlation between the (G + C) content of the DNA tested and the quenching effect on the fluorescence intensity of QM. It can be seen that the higher is the (G + C) content, the more readily the DNA quenches the fluorescence intensity of the dye. This finding is in line with the results reported by Weisblum and de Haseth [9,10] and Pachmann and Rigler [11] for the effect of DNAs on the fluorescence intensity of quinacrine. Polynucleotides behave in a similar way.[9,10,12,18]

The same relation between (G + C) content and quenching is also shown with denatured DNAs. The quenching of the fluorescence intensity of QM with excess denatured calf thymus or mouse liver DNA (G + C, 40 %) was approx. − 50 Δ % and for en equal amount of denatured *E. coli* DNA (G + C, 50 %), − 62 Δ %.

Interaction between QM and single stranded nucleic acids. Mixtures of either single stranded DNA or RNA with QM do not give rise to yellow precipitates at any of the concentration ranges of polymer and dye. However, there is an interaction between QM and RNA as is shown by changes in the absorption spectrum of QM when RNA is added to the solution (Fig. 3). This leads to quenching of the fluorescence intensity of QM. The fluorimetric titration with a constant amount of mouse liver RNA and increasing amounts of QM is shown in Fig. 9. The enhancement of the fluorescence intensity of the dye with RNA was about the same as with denatured DNA.

The n values for QM-RNA complexes are summarized in Table 1. They lie between 0.09 and 0.31 for n_1 and between 0.19 and 0.50 for n_2 depending on the ionic strength and pH of the solution. The different n values obtained for RNAs might also be due to the extremely high phosphorus content in the RNAs (*cf.* Experimental). Non-equilibrium dialysis of a mixture containing 0.5 mg/ml mouse liver RNA and 0.05 mg/ml QM in McIlvaine's buffer, gave n 0.27.

The effect of increasing amounts of the dye and a constant amount of denatured mouse liver DNA is also shown in Fig. 9. The enhancement of the fluorescence intensity of the dye was slighter with denatured DNA than with native DNA. A similar effect of the strandedness of the polymer on the binding of acridines has also been reported earlier.[6,24,33,37,38] Fluorimetric titration of denatured DNA (from mouse liver and from *S. cerevisiae*) with QM gave n_1 between 0.13 and 0.25, and n_2 between 0.53 and 1.10 (Table 1).

DISCUSSION

Binding mechanisms. Two different processes are involved in the interaction between dyes and polymers. The first binding (process I) is a weak ionic interaction between positively labelled dye molecules and negatively labelled groups of the polymer. Being an electrostatic interaction, the binding depends on the ionic strength in the solution, *i.e.* increased ionic strength leads to a decrease in process I. At high dye concentrations the absorption maximum of the dye is shifted to higher wavelengths. The isosbestic point suggests the existence of only one kind of binding (process I), *i.e.* the dye molecules are stacked along the phosphate backbone outside the double helix.[5]

The apparent number of binding sites (n) for interaction between QM and DNA in process I are higher than those reported for the interaction between chloroquine and DNA.[24] However, the QM-DNA interaction also shows a dependence on the ionic environment. Thus electrostatic factors are also important in the QM-DNA complex formation.

The second interaction (process II) is a strong binding, where the dye slips into the space between base residues in the helix of the polymer. According to Cohen and Yielding [24] this second type of binding comprises strongly reacting sites associated with bases, *i.e.* intercalation. Although the molecular structure of QM differs from that of chloroquine, the n values for process II were of the same order.[24] Pachmann and Rigler [11] found similar n values for the binding of proflavine and quinacrine with nucleic acids. Thus the side chain at C-9 of the dye does not influence the intercalation (process II) between the dye and the polymer.

Since the interaction between QM and native DNA was affected by urea as shown in this paper, some bindings between QM and DNA are hydrogen bonds. This idea is supported by the observations by Adkisson *et al.*[39] They showed that the bands which are brightly fluorescent after staining of *Drosophila* chromosomes with QM could be removed with acid.

Base-specificity. Several authors have reported base-specificity in the interaction between acridine dyes and nucleic acids.[9-12,40] However, acridines such as acridine orange [9] and 9-aminoacridine [10] show no base-specificity in the interaction with DNA in solution. The base-specificity can be demonstrated in solution with either mono- or polynucleotides containing the base guanine, or with DNAs that differ in the (G + C) content. In two previous papers I presented evidences for base-specificity of QM.[18,41] Quenching of the fluorescence intensity of QM was obtained with polyG, while polyA enhanced the fluorescence intensity of the dye. The relationship between the base content of the DNA and the degree of quenching of the fluorescence intensity of QM is further demonstrated in this paper. The higher the (G + C) content of the DNA the more the nucleic acid quenches the fluorescence intensity of QM in solution. A similar enhancement and quenching of fluorescence is obtained with acridines without a long side chain at C-9 (*e.g.* proflavine.)[9] Thus the side chain at C-9 of QM is not responsible for the base-specific reaction.

Conformational changes of the polymer. When a polymer is mixed with a dye in solution, the polymer undergoes molecular changes. These changes can be seen as altered hydrodynamic properties of the polymer. In the quinacrine-DNA complex, Kurnick and Radcliffe [33] reported an increased viscosity and a reduced sedimentation constant compared to those of native DNA alone. At low dye concentrations, *i.e.* at intercalation, the length of the helix is increased by the dye. At high concentration ratios of dye to polymer, the dye is lined up opposite the phosphate groups, thus increasing the diameter of the helix.

The stabilizing effect of QM on the polymer was shown by increased T_m and increased resistance to DNase depolymerization. The effect on T_m as well as on depolymerization by DNase depends on the concentration ratio of the dye to the polymer. An increased concentration ratio of the dye to polymer increases the protective effect of the dye on the polymer. The protective effect also depends on the salt concentration in the solution. When the salt concentration is increased the protective effect of the dye is reduced.

To stabilize a native nucleic acid against thermal strand separation it is not necessary for the acridine ring to have a side chain at C-9. Ramstein and Leng [40] reported a rise of the T_m for *Micrococcus lysodeikticus* DNA when mixed with proflavine, which does not have any side

chain at C-9. The agent can also be a simple aliphatic compound without any ring structure. Spermine, an aliphatic diaminopentane with two amino groups separated by four carbon atoms, is also active in raising the T_m of nucleic acids.[42] The two exocyclic amino groups in QM are separated by four carbon atoms, hence the dye can be considered a substituted 1,4-diamino-pentane (see formula given above). Thus, it is not surprising that QM was as active as spermine in producing an effect on T_m.

Cytological implications. It is not easy to evaluate the relationship between results obtained with QM and nucleic acids in solution on the one hand and cytological fluorescence investigations on the other. There are several fundamental differences between the two systems. Firstly, metaphase chromosomes contain $50-80 \%$ protein.[43] It is already known that proteins modify several of the fluorescent characteristics of QM in solution.[44] Secondly, the concentration of DNA in the interphase nucleus is greater than that normally used in solution.[10] This is apparent *e.g.* from the extremely rapid reassociation of denatured chromosomal DNA *in situ*.[45-47] Thirdly, the procedures used to obtain metaphase chromosome spreads certainly affect the structure and properties of both DNA and proteins.[48,49] Nevertheless, the base-specificity of the fluorescence intensity of QM with natural DNAs and nucleotides in solution is a striking phenomenon, which may well be related to the occurrence of differential brightness of fluorescence along metaphase chromosomes.

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