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Quinacrine, A Chromosome Stain Specific for Deoxyadenylate–Deoxythymidylate-Rich Regions in DNA

(double-stranded DNA/heterochromatin/proflavine/acridine orange/fluorescence)

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ABSTRACT **Fluorescence of quinacrine in the presence of different polynucleotides was studied to attempt to identify the specific nucleotides responsible for the fluorescence of stained chromosome preparations. A marked enhancement of fluorescence was seen in the presence of bihelical polynucleotides, such as poly(dA-dT), poly(dA)·poly(dT), and poly(rA)·poly(rU), but not in the presence of single-stranded polynucleotides, such as poly(dA), poly(dT), poly(rA), or poly(rU) alone. The higher was the GC content of natural DNAs, the more they quenched. Quenching was also seen with poly(dG) or poly(rG) alone, but not with poly(dC) or poly(rC) alone. Native and denatured DNA were both effective in quenching fluorescence. Thus, a bihelical conformation is *not* required for fluorescence quenching. Nearly all of these properties are shared with proflavine. In contrast, acridine orange, which stains most areas of chromosome preparations, shows enhanced fluorescence in the presence of all members of a series of natural DNAs. These data suggest that base-pairs composed of AT (rather than GC) residues are responsible for the observed fluorescence of specific chromosome regions after treatment with quinacrine, and support the proposal of Ellison and Barr (*Chromosoma*, in press) that the highly localized quinacrine fluorescence in their cytological preparations reflects the presence of DNA that has a high (A + T)/(G + C) ratio.**

Quinacrine mustard was shown by Caspersson and his colleagues (1) to stain certain regions of chromosomes with a very brilliant intensity. It was proposed that the mustard group might react with high specificity with the reactive *N*-7 position of guanine, thereby providing an affinity label for GC base-pairs in DNA. It was subsequently observed by Vosa (2) and others, however, that quinacrine itself possessed the same specificity as its mustard derivative. This finding suggested that the mustard function was not critical for the specificity of the staining reaction.

The base specificity of this reaction was not further elucidated until Ellison and Barr (3) showed that a large quinacrine-bright area in the nucleus of *Samoaia leonensis* (a drosophilid fly) could be labeled with [^{3}H]thymidine, but not with [^{3}H]deoxycytidine, whereas the remainder of the nucleus incorporated both [^{3}H]thymidine and [^{3}H]deoxycytidine into DNA. They therefore proposed that the quinacrine-bright area, at least in *S. leonensis*, was characterized by a high (A+T)/(G+C) ratio—a fact that correlates with the presence of a large quantity of DNA that bands in CsCl solution as a low density satellite. Since this conclusion is at variance with that of Caspersson *et al.* (1), we undertook the present investigation with purified DNAs and synthetic polynucleotides in an attempt to simulate chromosome staining behavior with model compounds that might exhibit similar staining properties with fluorescent dyes, and from which the base specificity of the quinacrine staining reaction might be extrapolated.

MATERIALS AND METHODS

Ribopolynucleotides, *Clostridium perfringens* DNA, *Escherichia coli* DNA, and *Micrococcus luteus* DNA were purchased from the Sigma Chemical Co. Poly(dA), poly(dT), poly(dG), and poly(dC) were generously donated by R. D. Wells (Biochemistry Dept., Univ. of Wis.). Poly (dA-dT) was synthesized (4). *Agrobacter tumefaciens* DNA was prepared from *A. tumefaciens* (strain B6) by the method of Schilperoort (5). Chicken DNA was prepared from erythrocyte nuclei by treatment with Pronase and sodium dodecyl sulfate, by the same method used to purify *A. tumefaciens* DNA. Quinacrine·HCl was obtained from the Sigma Chemical Co., and acridine orange was a product of the Chroma-Gesellschaft, Schmid and Co.

All reactions were performed in 0.1 M Na phosphate buffer (pH 6.8). Fluorescence was measured in an Aminco-Bowman fluorimeter.

RESULTS

Quinacrine staining produces highly intense fluorescence in *localized* regions of chromosome preparations (1). In contrast, staining with acridine orange produces a generalized fluorescence of nearly all regions of the chromosome.

If we examine the effects of various DNA samples, over a range of concentrations, on the fluorescence of a constant concentration of quinacrine, the results shown in Fig. 1 are obtained. Fluorescence was measured at 494 nm, the maximum wavelength for emission; quenching or enhancement of fluorescence were not accompanied by shifts in the wavelength of the emission maximum. All natural DNA samples quench fluorescence, apparently in relation to their GC content: DNAs with higher GC content show higher quenching. Poly (dA-dT) enhances fluorescence. If we test poly(dA) and poly(dT) alone, no marked effect on fluorescence is seen; however, a 1:1 mixture strongly enhances fluorescence, Fig. 2. We therefore conclude that AT base-pairs are responsible for enhanced fluorescence in our test system, and that a bihelical conformation is required for this effect. Quinacrine mustard was also tested, and results similar to those shown in Fig. 1 were obtained.

Quenching is seen if poly(dG) or poly(rG) alone is used in the assay, whereas neither poly(dC) nor poly(rC) quenches. The results shown in Fig. 3 refer to the ribopoly-

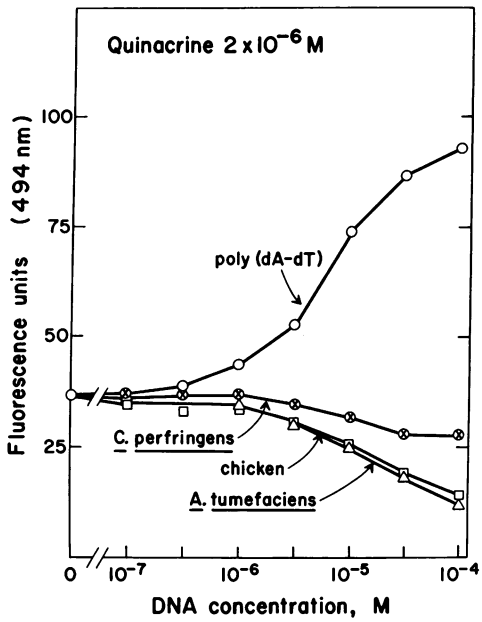

FIG. 1. Effects of various DNA samples on quinacrine fluorescence. The fluorescence of a quinacrine sample (2 μM) was determined in the presence of various concentrations of four different DNA samples (*C. perfringens*, 32% GC; chicken, 41% GC; *A. tumefaciens*, 61% GC). DNA concentration expressed in mol/liter of bases. Excitation at 424 nm.

nucleotides, because only very limited amounts of poly(dG) and poly(dC) were available; only incomplete, but nevertheless confirmatory, data could be obtained. These results suggest that fluorescence quenching is due to G residue, alone. These data are consistent with observations that natural DNAs quench in relation to their GC content, as shown in Fig. 1, and that both native and denatured DNAs quench, as shown in Fig. 3.

For reasons to be discussed below, we also tested the fluorescence of proflavine in the presence of DNA samples (as in Fig. 1). The data, shown in Fig. 4, indicate that proflavine exhibits a pattern of fluorescence similar to that of quinacrine. In other published studies (6, 7), it has been reported that proflavine fluorescence is unaffected by poly(rU), poly(rC), or poly(rA), but is quenched by poly(rG). Thus, proflavine is qualitatively similar to quinacrine; however, the enhancement of fluorescence by poly(dA-dT) is not as great.

In contrast to these findings, fluorescence of acridine orange is enhanced by all DNA samples tested over the same range of polynucleotide concentrations as used in Figs. 1-4 (Fig. 5).

In view of the striking base-specificity of fluorescence enhancement and quenching seen for quinacrine *in vitro*, we suggest that a similar specificity may exist in cytological preparations as well, and that quinacrine-bright areas of the chromosome may also represent AT-rich regions of DNA.

DISCUSSION

The use of quinacrine as a specific chromosomal stain by Caspersson and his colleagues (1) has been one of the major recent developments in cytogenetics (for reviews, see refs. 8 and 9). Despite the importance of this reaction, the chemical basis for its high specificity has not been completely elucidated. Recent studies on the interaction between DNA and acridine dyes *in vitro* have provided some important insights into this problem.

Löber and Achtert (10) and Thomes *et al.* (7) have distinguished two types of fluorescence interactions that occur between acridines and DNA. One type is exhibited by proflavine

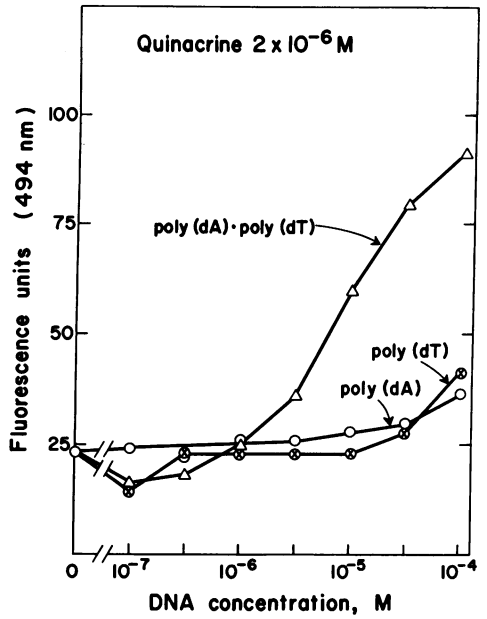

FIG. 2. Effect of poly(dA), poly(dT), and poly(dA)·poly(dT) on quinacrine fluorescence. The fluorescence of a quinacrine solution (2 μM) was determined as in Fig. 1.

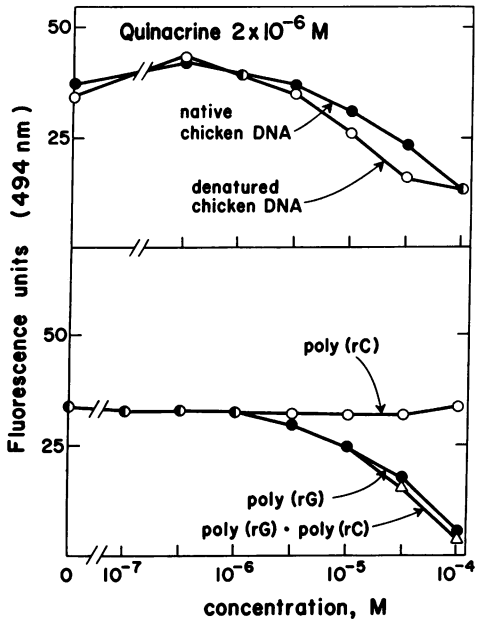

FIG. 3. Effect of native or denatured chicken DNA on quinacrine fluorescence. The fluorescence of a quinacrine solution (2 μM) was determined in the presence of various concentrations of native or denatured chicken DNA, as indicated (*upper panel*), and in the presence of poly(rG), poly(rC), and poly(rG)·poly(rC), (*lower panel*).

and the other by acridine orange. From these works, the following patterns emerge:

The proflavine type of interaction is characterized by fluorescence quenching in the presence of calf-thymus DNA. Two types of binding were distinguished as contributing to this interaction—a strong binding involving intercalation into AT sites that results in enhanced fluorescence, and a weaker binding that involves GC base pairs (or G alone) in which G is responsible for quenching. Tubbs *et al.* (11) (using acriflavin) showed a correlation between increased quenching and increased GC content.

The acridine-orange type of interaction is characterized by enhancement of fluorescence in the presence of calf-thymus DNA; moreover, from our own studies described above, several other DNA samples also enhance the fluorescence of acridine orange. No quenching is found at high DNA to dye ratios for any of the DNA samples tested.

From this comparison, quinacrine unmistakably interacts with DNA with a specificity similar to proflavine; however, the enhancement of fluorescence with poly (dA-dT) is relatively greater with quinacrine than with proflavine. On the basis of the similarity between proflavine and quinacrine, it is reasonable to ask whether proflavine has similar staining properties in cytological preparations. Such a similarity was, in fact, reported by Caspersson *et al.* (12). Moreover, they noted that the fluorescence of proflavine-stained chromosomes was relatively weak. These observations accord precisely with the expectations from the data we have presented. Proflavine gives weaker fluorescence in the "quinacrine-bright" bands and also weaker fluorescence in the presence of poly(dA-dT).

In addition to the cytological studies of Ellison and Barr (3), Blumenfeld and Forrest (13), in studies with *Drosophila melanogaster*, have recently used a series of flies that contain different doses of Y-chromosome material. Associated with increased doses of Y-chromosome material, they were able to show an increasing amount of a satellite DNA of low buoyant density in Cs_2SO_4–$HgCl_2$ solution. In this connection, it has been reported by Vosa (14) that the Y-chromosome in *D. melanogaster* fluoresces brightly with quinacrine, and Ellison and Barr (3) have suggested that these observations may be correlated with the presence of a low-density satellite.

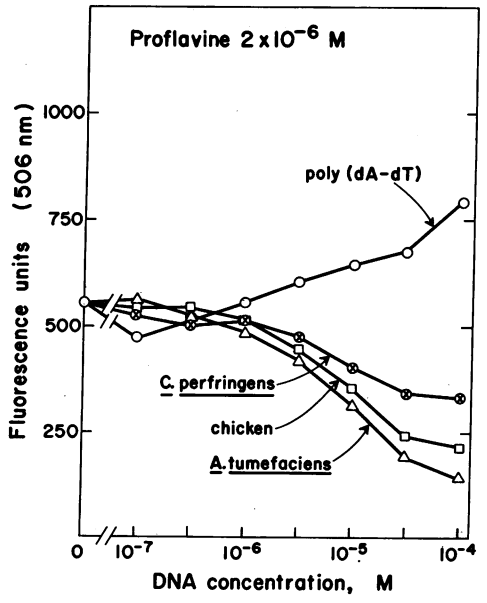

FIG. 4. Effect of various DNA samples on proflavine fluorescence. The fluorescence of a proflavine sample (2 μM) was determined in the presence of various concentrations of four different DNA samples as in Fig. 1. Excitation at 447 nm.

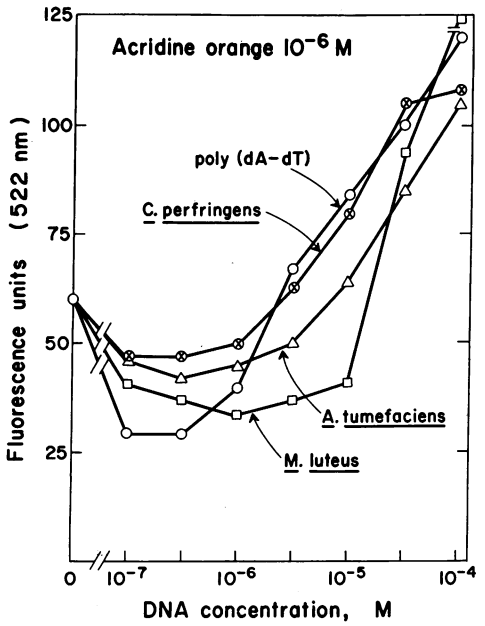

FIG. 5. Effect of various DNA samples on acridine-orange fluorescence. The fluorescence of an acridine-orange sample (1 μM) was determined in the presence of various concentrations of four different DNA samples as in Fig. 1. (*M. luteus* DNA, 72% GC). Excitation at 470 nm.

A role for quinacrine-bright material has recently been described by Barr and Ellison (15, 16). They reported that ectopic pairing in polytene chromosomes of *D. melanogaster* occurs frequently (though not exclusively) between quinacrine-bright bands. This observation suggests more generally that such regions may represent sites on the chromosome for facilitated insertion or excision of DNA; complementarity between AT-rich DNAs provides a chemical basis for such phenomena. An intriguing possibility is that the AT-rich, or other reiterated sequences, may represent sites of integration for *exogenous* agents as well—e.g., oncogenic and "slow" viruses.

Inherited variations involving quinacrine-bright material in human chromosomes have been observed by Patau, as well as by other investigators (K. Patau, personal communication). In connection with these observations, it would be of interest to correlate inherited variations in amounts and distribution of quinacrine-bright material or of other forms of heterochromatin, [e.g., see Craig-Holmes and Shaw (17)] with inherited susceptibility to diseases caused by oncogenic or other agents that are believed to be capable of integration into the cellular genome. Such patterns of inheritance might deviate from those commonly associated with the better-known inherited deficiencies of enzymatic functions.

In the experiments reported here, we have attempted to use conditions similar to those used by Ellison and Barr (3). We feel that it has been possible in these studies to simulate with acridine dyes and purified nucleic acids in solution the interactions observed in cytological preparations. From these

studies, the inescapable conclusion is that the quinacrine-bright material in cytological preparations consists mainly, if not solely, of AT base-pairs.

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