EVIDENCE FOR THE QUINACRINE FLUORESCENCE ON THREE AT PAIRS OF DNA

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1. Introduction

Fundamental microfluorometric investigations with quinacrine (Q) ('atebrine') or quinacrine iprite-stained chromosomes, carried out by Casperson et al. [1–3] have made it possible to identify chromosomes on the basis of the fluorescent band distribution along the chromosome. It was found [4,5], that the fluorescence quantum yield is high for a Q–poly (A, T) complex and low for a Q–poly (G, C) complex. The fluorescence intensities of Q–DNA complexes decrease as the G-content increases [4, 6–8].

Therefore the fluorescent bands along chromosomes were attributed to the AT sequence rich regions [4]. Pachman and Rigler [5] suggested that a sequence of four or more AT pairs in a binding site is required for the Q–DNA complex to fluoresce with a high quantum yield. But it is well known [9,10], that proflavin and acriflavin DNA complexes fluoresce with a high quantum yield on being bound with two or more AT pairs.

In this work we have made an attempt to determine a minimum site of DNA and the nucleotide content required for a Q–DNA complex to fluoresce with a high quantum yield.

2. Materials and methods

All measurements were performed in 0.33 SSC buffer (pH 6.8; 20°C). DNA species with different GC content (*Propionibacterium schermanii* – 67% GC pairs; *Photobacterium* sp. 42–47%; *Listeria monocytogenes* – 42%; *Bacillus subtilis* – 38%; *Eleginus navaga* – 48%; *Tinca tinca* – 39,7%; *Esox lucinus* – 43.2%; *Cyprinus cartio* – 38.8%; T5 phage – 39.0%; *Escherichia coli* (B/r) – 50,3%; Calf thymus – 42,4%) were kindly supplied by Drs L. C. Popov and N. I. Alexandruschkina of Laboratory of Bioorganic Chemistry of Moscow State University. Quinacrine (George T. Gurr Ltd., London) was used without further purification. Poly (A, U) was prepared from poly(A) and poly(U) (Reanal, Hungary) by mixing their equimolar solutions in 0.33 SSC buffer (pH 6.8). The nucleic acids and dye concentrations were determined spectrophotometrically, for that the native DNA $E_{260} = 6.65 \cdot 10^3$; the free dye $E_{424} = 7270$ [9,11]; the poly(A) $E_{257} = 10.5 \cdot 10^3$ and the poly(U) $E_{260} = 9.2 \cdot 10^3$ [12]. The concentration and adsorption isotherm measurements were performed using a Cary 16 spectrophotometer. Absorption spectra were taken with a Specord UV-VIS spectrophotometer, fluorescence spectra – with a Hitachi model MPF-2A spectrophotofluorometer. Fluorescence lifetime measurements were carried out with a ORTEC 9200 nanosecond fluorescence spectrometer and with a phase fluorometer described in [13].

3. Results and discussion

Visible absorption and fluorescence spectra of the solutions containing Q and native double-stranded DNA are given in fig. 1. Similar spectra were obtained for a Q–poly (A, U) complex. These spectra were used to estimate the energy transfer radius R., between the dye molecules bound to poly (A, U) as being equal to 31 Å.

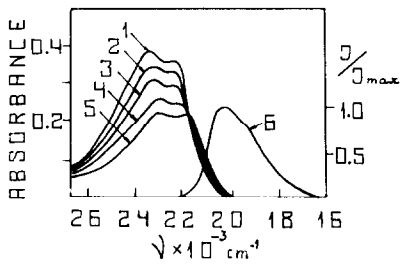

Fig. 1. Absorption spectra of the solutions containing Q and native calf-thymus DNA in SSC buffer (pH 6.8; ionic strength 0.01; 20°C). Dye concentration 5.3×10^{-5} M; DNA concentrations for curves 1–5 were equal to 0; 3.3×10^{-5}; 6×10^{-5}; 13.7×10^{-5}; 22×10^{-5} M. Curve 6 – normalised fluorescence spectrum of Q–DNA or Q–poly(A,U) complexes.

The binding isotherms in Scatchard's graphic form for the Q complex with three DNA species having different GC contents give one straight line within the experimental error in the region of $7 < P/\mathrm{Co} > 50$, as is shown in fig. 2. Here P is the total nucleic acid

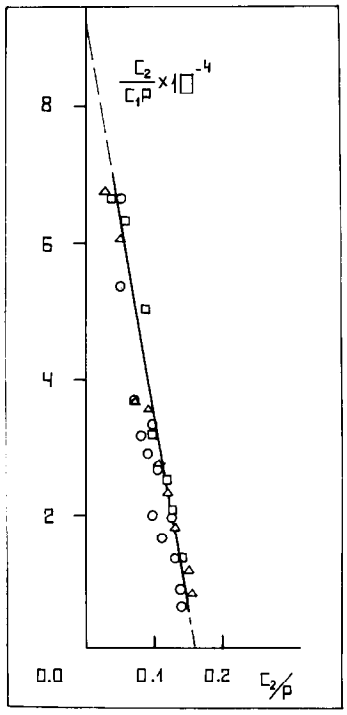

Fig. 2. Absorption isotherm of Q with DNAs of different GC content in SSC buffer (ionic strength 0.08; pH 6.8; 20°C). (○) – T5 phage DNA (39%GC); (□) – *E. coli* DNA (50%GC); (△) – *Propionibacterium shermonii* DNA (67% GC).

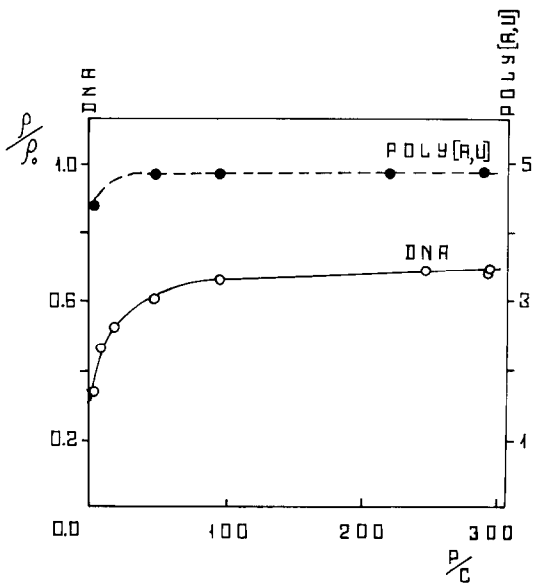

Fig. 3. The dependence of the fluoresence quantum yield of Q–DNA solutions on the concentration of DNA in SSC buffer (ionic strength 0.01; pH 6.8; 20°C; λ_{ex} = 436 nm). (○) – Q–native calf thymus DNA; (●) – Q–poly(A,U); ρ=0.17.

concentration expressed as moles of phosphorus. The data were treated in terms of the theory of one dimensional dye adsorption on a regular DNA type homopolymer [14,15] and the following conclusions were made. Quinacrine has one type of binding site on different GC content DNAs. Each bound dye molecule occupies a sequence of two pairs of DNA nucleotides. The dye molecules, when bound, are distributed in a random fashion over all the binding sites without any GC content dependence. The binding constant is the same for the three DNA species studied and is equal to 9.5×10^4. According to [16], it is a strong type of Q–DNA binding.

The fluorescence quantum yields, ρ, of the solutions containing Q and native DNA or poly(A,U) are shown in fig. 3. The quantum yield of the Q-native DNA complex tends to 0.12 ± 0.01; Q–poly(A,U) – to 0.83 ± 0.02, when the P/Co ratio exceeds 100. This agrees with the Pachman and Rigler data [5].

All the fluorescence lifetime, τ, measurements for the Q–nucleic acid solutions were made at a P/Co-ratio of above 100. The fluorescence decay curves consisted of two components both for Q–native DNA and Q–poly(A,U) solutions. The short-lived component had

$\tau = 4\pm1$ nsec for both solutions and was the same as τ for the free dye solution (phase fluorometer – 4.3±0.1 nsec; ORTEC – 4.3±0.2 nsec). The long-lived component of the Q–DNA and Q–poly(A,U) solutions had $\tau = 20.7\pm0.6$ nsec and 21.8±0.3 nsec, respectively, and was attributed to the Q–DNA and Q–poly(A,U) complex fluorescence.

It was thus established that τ and ρ for the Q–poly (A,U) complex increase in parallel about 5-fold as compared to τ and ρ for free Q. This indicates that all bound dye molecules fluoresce with a high quantum yield which is close to unity. The fluorescence lifetime τ, for the Q–DNA complex increases about 5 times but decreases about 1.5-fold. The difference in the ρ and τ behavior should be interpreted as showing the existence of two types of bound dye molecules on DNA – one type fluoresces with a high quantum yield and the other is quenched. The quantum yields for the Q complexes with all DNA species studied were considerably lower than ρ for Q–poly(A,U) or Q–poly(A,T) [5] complexes and decreased as the GC content in DNA increased (fig. 4). Our data are in good agreement with those of other authors [4–6], and confirm their conclusion about the Q–DNA complex fluorescence quenching on GC pairs. This quenching is not complete. Pachman and Rigler [5] have shown Q–poly (G,C) complex to have a weak fluorescence with ρ of about 0.04.

One can derive a relationship between the Q–DNA solution fluorescence intensities and the DNA nucleotide content at P/Co ratios above 100. If the complex fluorescence is highly quenched on GC pairs (about 30 times in our case), then

$$\rho \sim (AT)^n \qquad (1)$$

where (AT) is the DNA AT content; n is the minimum number of AT pairs required for a high fluorescence yield of a bound dye molecule.

The quantum yields of the complexes Q–DNA of different nucleotide composition measured at a P/Co of about 100 and used as a function of the AT content of DNAs, are shown in fig. 4. The continuous lines show the theoretical curves of ρ vs. $(AT)^n$ dependence for n = 1,2,3,4 and 5. It may be seen that the experimental points fit the theoretical curve at $n = 3$.

The evidence for $n = 3$ may also be found by comparing the experimental τ (phase fluorometer) for a

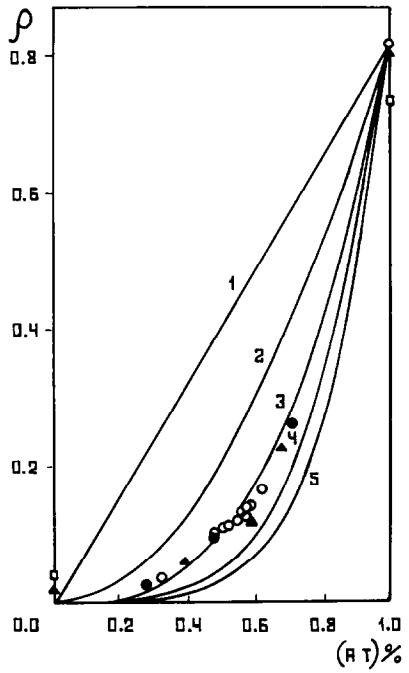

Fig. 4. The dependence of the fluorescence quantum yield of Q–DNA complexes on the AT content of DNA in SSC buffer (pH 6.8; ionic strength 0.08; 20°C; P/C~100; λ_{ex}=436 nm). (○) our data; (▲), (●) – the data, which were calculated based on [4] and [5] respectively; (□) – the data from [5]. The continuous lines show the theoretical curves of $\rho \sim (AT)^n$ dependence for n=1,2,3,4 and 5.

Q–DNA solution with the τ calculated by using the formula analogous to (81) in [17], but for a three-component system (a Q–DNA complex with a high quantum yield, a quenched Q–DNA complex and a free dye remaining in the solution). Measured τ equals 10 nsec. The semiquantitative calculations give τ = 15.2 for $n = 2$; τ= 9.7 nsec for $n = 3$ and $\tau = 5.8$ nsec for $n = 4$.

It may be concluded therefrom that the Q–DNA complex fluoresces with a high quantum yield if there is a sequence of at least three AT pairs in the binding site of the dye. Q markedly differs from proflavin and acriflavin, which fluoresce with a high quantum yield on two AT pairs. This accounts for the fluorescent band along the chromosome being more contrast when stained with Q as compared to proflavin and acriflavin.

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