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Fluorescence Characteristics of Variously Charged Asymmetric Monomethine Cyanine Dyes in the Presence of Nucleic Acids

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Three asymmetric monomethine cyanine dyes bearing one, two, and three positive charges have been synthesized, and their absorption and fluorescence characteristics in the presence of nucleic acids were studied. The maxima of their longest wavelength absorption band lie between 500 and 520 nm. The dyes do not show fluorescence of their own in TE buffer (pH = 7.5), but become strongly fluorescent (Q_F = 0.2–0.6) on binding to double-stranded DNA. The fluorescence maxima of the investigated dye-dsDNA complexes are in the region of 530–550 nm. The influence of the dye/DNA ratio on both the position and intensity of the fluorescence maxima of the complexes is investigated.

KEY WORDS: Fluorescence; fluorescent label; asymmetric monomethine cyanine dyes; nucleic acids.

INTRODUCTION

In recent years, many compounds forming fluorescent complexes with biopolymers have been studied [1–3]. In previous papers [4–6] we reported the spectral characteristics of newly synthesized asymmetric monomethine cyanine dyes (AMCD) and their homodimers for fluorescence detection of nucleic acids. The main advantage of these dyes is that they do not show fluorescence of their own, but become strongly fluorescent after forming complexes with DNA. Two possible types of interaction with nucleic acids—intercalation and electrostatic binding—have been proposed.

Our previous investigations showed that with an increasing number of charges (one and two) in the dye molecules, the fluorescence quantum yield of the dye-DNA complexes increases [4,5]. To elucidate this relationship further, a new dye (TOPY2) bearing three positive charges has been synthesized [7].

The aim of the present paper is to study the influence of the number of charges in the chromophore or side chain on the photophysical properties of the dye-DNA complexes, as well as to investigate the possibilities for their application in quantitative determination of dsDNA in solution.

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ABBREVIATIONS: ds, double stranded; ss, single stranded; Q_F, fluorescence quantum yield, I_F, fluorescence intensity; AMCD, asymmetric monomethine cyanine dyes; DMSO, dimethyl-sulfoxide; TE, 10 m*M* Tris-HCl (pH 7.5) with 1 mM EDTA.

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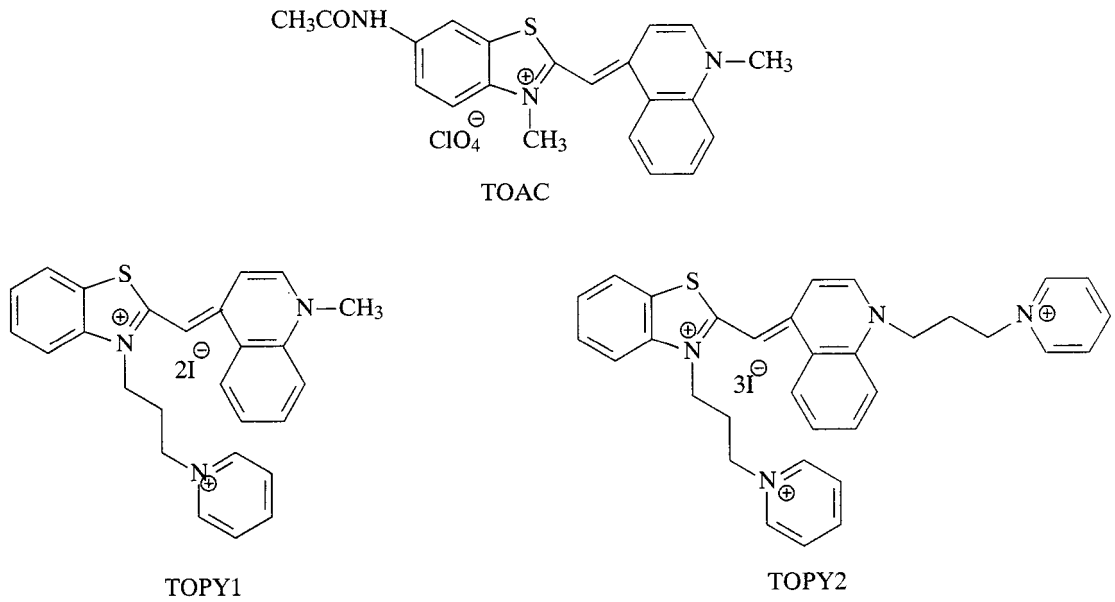

Fig. 1. Structure of the asymmetric monomethine cyanine dyes.

EXPERIMENTAL

The AMCD studied (Fig. 1) have been synthesized according to [7,8]. Stock solutions were prepared by dissolving 1 m*M* of dye in 1 ml DMSO and subsequent dilution with TE buffer (10 m*M* Tris-HCl of pH 7.5, 1 m*M* EDTA) to the final concentrations. The fish sperm dsDNA was purchased from Sigma (USA).

Absorption spectra were scanned on a Specord M40 (Carl Zeiss, Jena) UV-VIS spectrophotometer and the fluorescence spectra (excitation at 480 nm) on a Perkin Elmer MPF44 spectrofluorimeter. The emission spectra were corrected using a standard Tungsten lamp. The fluorescence quantum yield (Q_F) was determined relative to that of the dye thiazole orange ($Q_F = 0.2$) [9]. All measurements were made at room temperature using TE buffer.

RESULTS AND DISCUSSION

The maxima of the longest wavelength absorption bands of the studied AMCD in TE buffer are in the region 505–515 nm (Table I). At concentrations of 1×10^{-5} M and lower, no evidence for dye aggregation is observed in the absorption spectra. A slight bathochromic shift of less than 15 nm takes place on binding to dsDNA. The intensity of the maximum diminishes by 20–40%. It was shown [10] that such effects usually accompany intercalation of the dyes into the base stack. As mentioned above, AMCD do not show fluorescence of their own, but become strongly fluorescent when bound to dsDNA. The experimental results for the fluorescence quantum yields (Table I) confirm our assumption: Increasing the number of charges in the dye molecule leads to significant growth of the quantum yield, Q_F. The fluorescence maxima of the complexes lie between 520 and 550 nm.

To evaluate the effect of the fragment length of DNA on the fluorescence properties of the dye-DNA complexes the dsDNA solutions were sonicated for 5, 10, 15, and 30 sec. The experimental results show that the position of the fluorescence maximum does not depend on the DNA chain length, whereas the fluorescence intensity slightly decreases with time of sonication.

For molecular biology and medicine it is important to have at disposal dyes for qualitative determination of ds and ss DNA. It has been pointed out in the literature [1] that it should be possible to differentiate between ds and ss DNA on the basis of fluorescent lifetimes only.

Table I. Absorption and fluorescence characteristics of the studied AMCD: λ_{abs} (nm): absorption maximum; ε (M^{-1} cm^{-1}): molar absorptivity, λ_F (nm): fluorescence maximum, Q_F: fluorescence quantum yield.

	Without dsDNA		With dsDNA		
Dye	λ_{abs} (nm)	ε	λ_{abs} (nm)	λ_F (nm)	Q_F
TOAC	515	68500	521	544	0.25
TOPY1	505.5	77500	507.9	529.4	0.33
TOPY2	507	78300	521	532	0.55

Earlier we have shown that for some dyes, not only the fluorescence lifetime [6], but also the position of the fluorescence maximum [4–6] depends strongly on the type of nucleic acid, ds or ss. This could be used to distinguish between ds and ss DNA in solution and gel electrophoresis.

The changes in the fluorescence characteristics of dye-DNA complexes after preliminary melting of dsDNA leading to its denaturation have been followed. Upon formation of dye-ssDNA complexes, the fluorescence intensity decreases depending on the number of positive charges in the dye molecule. In the case of TOPY2, which has three positive charges and can bind stronger electrostatically to the phosphate groups, the fluorescence intensity diminishes only 5-fold. When TOPY1 with two positive charges binds to the melted DNA, the fluorescence intensity decreases 10-fold, while for TOAC (one positive charge) the lowering is 30-fold. The fluorescence maximum of the TOAC-ssDNA complex shifts with about 50 nm bathochromically ($\lambda_F = 600$ nm) compared to the TOAC-dsDNA one. The shift of the fluorescence maximum of the TOPY1-ssDNA complex is more than 30 nm, while the fluorescence maximum of TOPY2-ssDNA remains unchanged after melting of dsDNA. Consequently, in contrast to TOAC and TOPY1, the newly synthesized dye TOPY2 bearing three positive charges is not able to distinguish between single- and double-stranded DNA in solution.

We also have studied the relationship between the fluorescence intensity of the complexes and the ratio DNA base pairs/dye. When concentration of the dyes is kept constant (1×10^{-5} M) and the concentration of DNA base pairs is varied between 1×10^{-7} M and 2×10^{-4} M a linear correlation with $r > 0.99$ is observed (Fig. 2A–2C).

Fluorescence arises if dsDNA and TOAC (bearing one positive charge) are at least in equimolar ratio, whereas in the case of TOPY1 and TOPY2 (two and three positive charges) the fluorescence appears even from solutions in which the DNA base pairs/dye ratio is much lower (1:10). This experimental result shows that the sensitivity of the investigated dyes to dsDNA becomes higher with an increasing number of charges in the molecule.

At constant dye concentration (1×10^{-5} M), the relationship between the fluorescence intensity (I_F) and the DNA concentration is linear, 0.5–16 μg/ml for TOAC (Fig. 3), 0.5–64 μg/ml for TOPY1 (Fig. 4), and 0.125–64 μg/ml for TOPY2 (Fig. 5). If the concentration of TOPY2 is decreased to 1×10^{-6} M, the relationship between I_F and [DNA] is linear also at lower concentrations of DNA—from 125 ng/ml to 15 ng/ml, which is the detec-

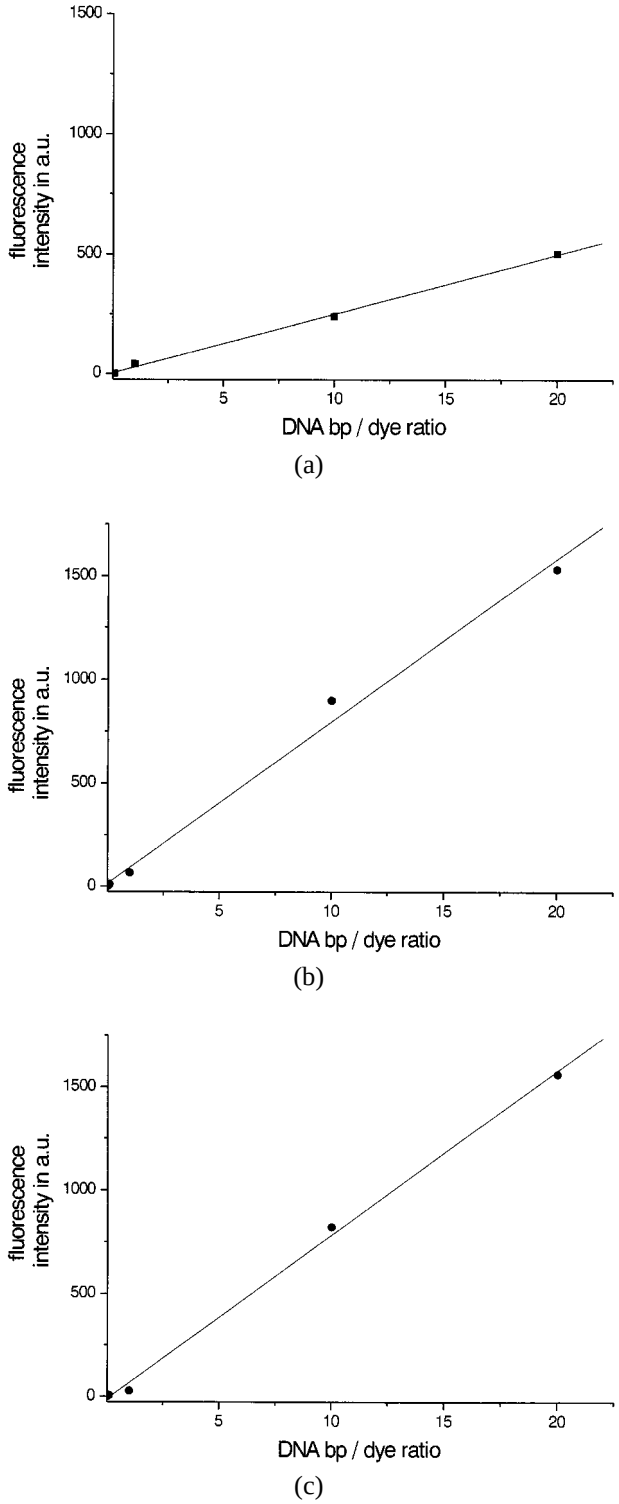

Fig. 2. Fluorescence intensity in arbitrary units as a function of the ratio of DNAbase pairs/dye to (A) TOAC; (B) TOPY1; and (C) TOPY2.

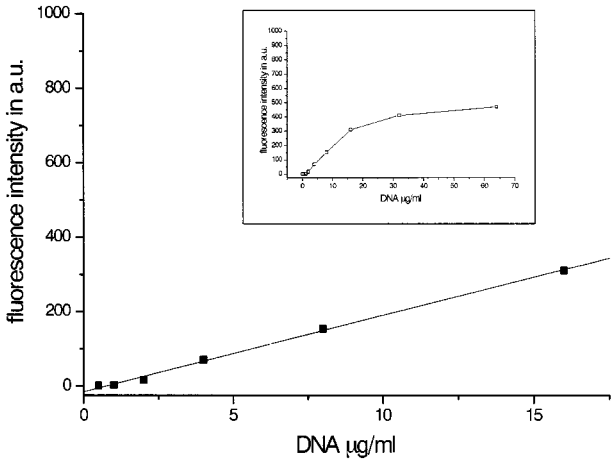

Fig. 3. Fluorescence intensity in arbitrary units of the complex TOAC (1×10^{-5} M)-dsDNA (0.5–16 μg/ml) as a function of the dsDNA concentration; the non-linear dependence I_F/[DNA] in the range 0.5–64 μg/ml dsDNA is given in the insert.

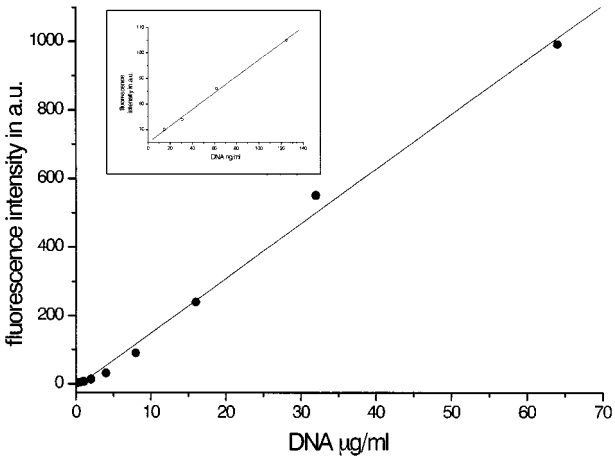

Fig. 5. Fluorescence intensity in arbitrary units of the complex TOPY2 (1×10^{-5} M)-dsDNA (0.125–64 μg/ml) as a function of the dsDNA concentration; in the insert is given the same dependence for concentration of TOPY2 (1×10^{-6} M) in the range 15–125 ng/ml dsDNA.

tion limit of dsDNA with this dye under the given experimental conditions (Fig. 5). The difference in the slopes of the linear plots I_F/[DNA] is evidence for the difference in binding affinity of the dyes toward dsDNA [11]. The slope is lowest in the case of TOAC, a factor of 2 higher in case of TOPY1, and even by a factor of 3 higher for TOPY2. Thus, TOPY2 shows the highest binding affinity of the dyes studied here.

The linear dependence I_F/[DNA] shows that the dyes TOAC, TOPY1, and TOPY2 can be used for the quantitative determination of dsDNA over a broad range of concentrations.

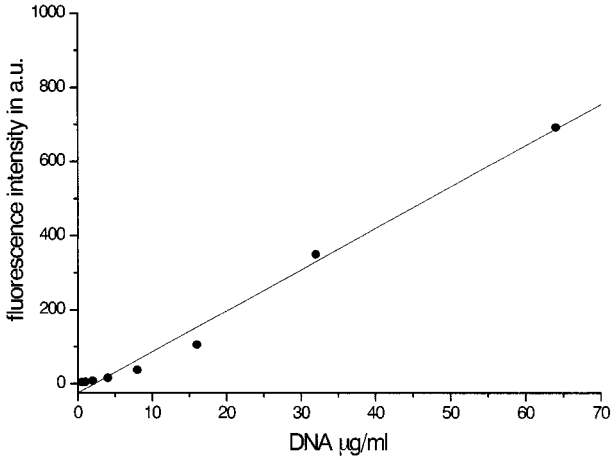

Fig. 4. Fluorescence intensity in arbitrary units of the complex TOPY1 (1×10^{-5} M)-dsDNA (0.5–64 μg/ml) as a function of the dsDNA concentration.

CONCLUSIONS

It was shown that the fluorescent properties of the studied AMCD in the presence of nucleic acids depend on the number of positive charges in their molecules.

The fluorescence quantum yield is highest when TOPY2 (three positive charges) is bound to dsDNA. Therefore this dye provides higher sensitivity in DNA detection compared to TOAC (one positive charge) and TOPY1 (two positive charges). The detection limit of dsDNA using TOPY2 is 15 ng/ml. The fluorescence intensity shows a linear dependence over a broad range of DNA concentrations, allowing quantitative determination of DNA.

The position of the fluorescence maximum of TOPY2 remains unchanged on binding to ds or ssDNA, while that of TOAC shifts 50 nm to the red with ssDNA, making it possible to distinguish between ds and ssDNA.

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