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An Investigation of Tricarbocyanines "Stains-All" and "iso-Stains-All" as Fluorescent Nucleic Acids Probes

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Luminescent properties of carbocyanine dyes Stains-All and its isomer iso-Stains-All were studied in the presence of nucleic acids. Both dyes show sufficient fluorescent intensity increase in the presence of DNA and RNA and may be used as fluorescent probes for nucleic acids (NA) detection in homogeneous assays. It was supposed that Stains-All and iso-Stains-All bind with nucleic acids through different interaction modes.

KEY WORDS: Nucleic acid fluorescent detection, cyanine dyes, Stains-All.

INTRODUCTION

In recent years there has been increasing interest in the application of highly fluorescent cyanine dyes in homogenous assays for nucleic acids (NA) detection in solution, agarose gel electrophoresis, and capillary gel electrophoresis [1].

β-Substituted thiacarbocyanine dye Stains-All (Fig. 1) is well known as a stain for nucleic acids, proteins, polysaccharides, and lipids [2–5]. In the presence of biopolymers, significant changes in absorption spectra of this dye occur [5]. Nevertheless, the influence of the NA on the fluorescent properties of Stains-All was not studied.

In previous studies we proposed the use of β-substituted trimethine cyanine dyes as fluorescent probes for nucleic acids detection [6]. It is known that these dyes show significantly lower intrinsic fluorescence in different solutions than their unsubstituted analogues, but in the presence of NA the emission intensity of β-substituted carbocyanines increases several orders of magnitude [6].

Studies of spectral-luminescent properties of β-substituted carbocyanines Stains-All and its structural isomer iso-Stains-All as fluorescent probes for the detection of nucleic acids are presented.

EXPERIMENTAL

The cyanine dyes were synthesized according to the procedure described in [7]. The dye stock solutions were prepared in DMS. Concentration of dye in working solutions was $2.0 \cdot 10^{-5}$ M. A 0.05 M Tris-HCl (pH = 7.5) buffer was used for all measurements. Total DNA from chicken erythrocytes, total yeast RNA, synthetic polynucleotides poly(dAdT)/poly(dAdT), and poly(dGdC)/poly(dGdC) were purchased from Sigma. The NA concentrations in the working solutions were $6 \cdot 10^{-5}$ M (base pairs) for DNA and synthetic polynucleotides and $1.2 \cdot 10^{-4}$ M (base pairs) for RNA. Fluorescence excitation and emission spectra were obtained with a Cary Eclipse fluorescence spectrophotometer (Australia).

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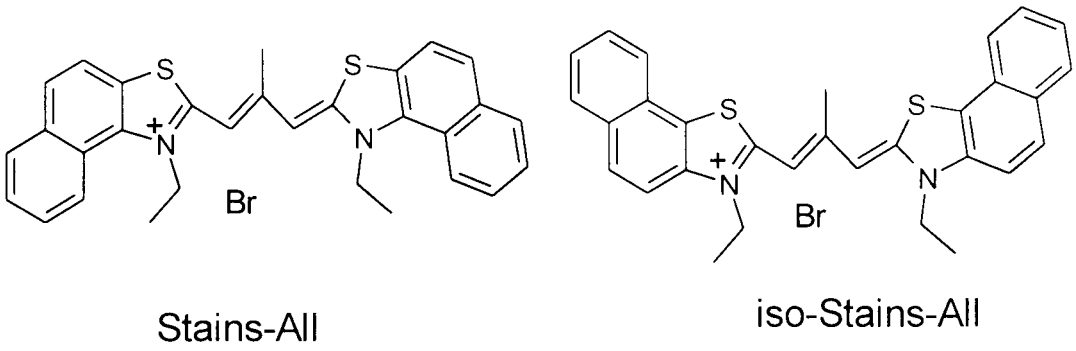

Fig. 1. Structures of studied carbocyanine dyes.

RESULTS AND DISCUSSION

The characteristics of the absorption and fluorescence spectra of the free dyes and their complexes with biopolymers are presented in Table 1. The profiles of absorption spectra of both dyes in DMS are close (maxima situated near 540 and 580 nm) (Fig. 2). In buffer solution, Stains-All and iso-Stains-All show absorption maxima at 572 and 645 nm and at 530 and 637 nm, respectively (Table 1). According to literature data, the 570-nm maximum in Stains-All can be attributed to a monomer absorption, while the maxima near 470, 510, 535 nm are attributed to the different H-aggregates absorption and the long wavelength maximum at 650 nm corresponds to the J-aggregate absorption [8]. In aqueous medium, both dyes exhibit intensive emission from J-aggregates at 656 nm (Stains-All) and 650 nm (iso-Stains-All) (Fig. 2).

The interaction with NA leads to the partial destruction of dye aggregates followed by the rigid fixation of the dye monomer in the NA backbone. As a result, a significant increase (1–3 orders of magnitude) of monomer fluorescence (at 590–600 nm) and the disappearance of the J-aggregate band (near 650 nm) are observed. While iso-Stains-All shows the highest value of fluorescence intensity increase in complexes with dsDNA and poly(dAdT)/poly(dAdT) (near 1350 and 2250 times, respectively), Stains-All exhibits the highest fluorescence intensity enhancement (near 700 times) in the presence of RNA (Fig. 3).

Both dyes show increased fluorescence enhancement when they are bound to AT compared to GC-containing polynucleotides. However, with poly(dG/dC)poly(dG/dC) iso-Stains-All interacts with significant enhancement of its monomer fluorescence (over 150 times increase at 593 nm), while for Stains-All the J-aggregate emission is clearly pronounced in presence of poly(dG/dC)poly(dG/dC).

Presence of a 0.2-M NaCl concentration significantly increases fluorescent intensity of iso-Stains-All–DNA complex, while the fluorescent intensity of Stains-All–DNA complex is not affected by the salt concentration (data not presented). Based on the obtained results,

Table I. Spectral Data of Studied Dyes and Their Complexes with Nucleic Acids

	DMS		Buffer			DNA			RNA			poly(dAdT)/(dAdT)			poly(dGdC)/(dGdC)		
Dye	λ_{abs} nm	λ_{em} nm	λ_{abs} nm	λ_{em} nm	I_0 a.u.	λ_{abs} nm	λ_{em} nm	I a.u.	λ_{abs} nm	λ_{em} nm	I a.u.	λ_{abs} nm	λ_{em} nm	I a.u.	λ_{abs} nm	λ_{em} nm	I a.u.
Stains-All	541 (*sh*) 580	611	572, 645	596 656	2 242	576, 645	596	246	540, 580, 643	600	1386	545, 582, 642	596	558	545, 580, 637	602 650	31 350
iso-Stains-All	538 (*sh*) 578	608	530, 637	592 650	0.77 185	537, 637	592	1045	533, 635 (sh)	596	385	540, 580, 636	592	1727	536, 636	593	287

λ_{em} and λ_{abs}: Wavelength of emission and absorption maxima respectively; I_0: fluorescence intensity of free dye; I: fluorescence intensity of the dye in the presence of corresponding biopolymer, a.u.: arbitrary units; sh: shoulder. Dyes were excited at "monomer" absorption maxima (575 nm).

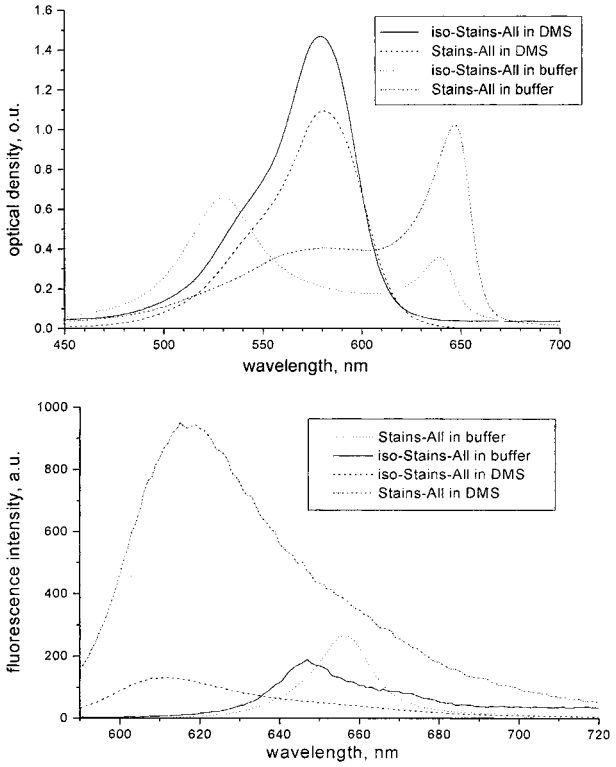

Fig. 2. Absorption and fluorescence spectra of carbocyanine dyes Stains-All and iso-Stains-Al.

we expect essential differences in the binding mechanisms of these two dyes.

Preliminary experiments on NA visualization in agarose gel plates have shown that iso-Stains-All has approximately the same detection sensitivity as ethidium bromide. It was shown that for the studied dyes the presence of the proteins only has a slight influence on their luminescent properties (data not presented).

Stains-All and its analogue iso-Stains-All could be successfully used for the fluorescent detection of NA. The mechanism of interaction with NA and their applicability as stains for NA visualization in gel-electrophoresis are under investigation.

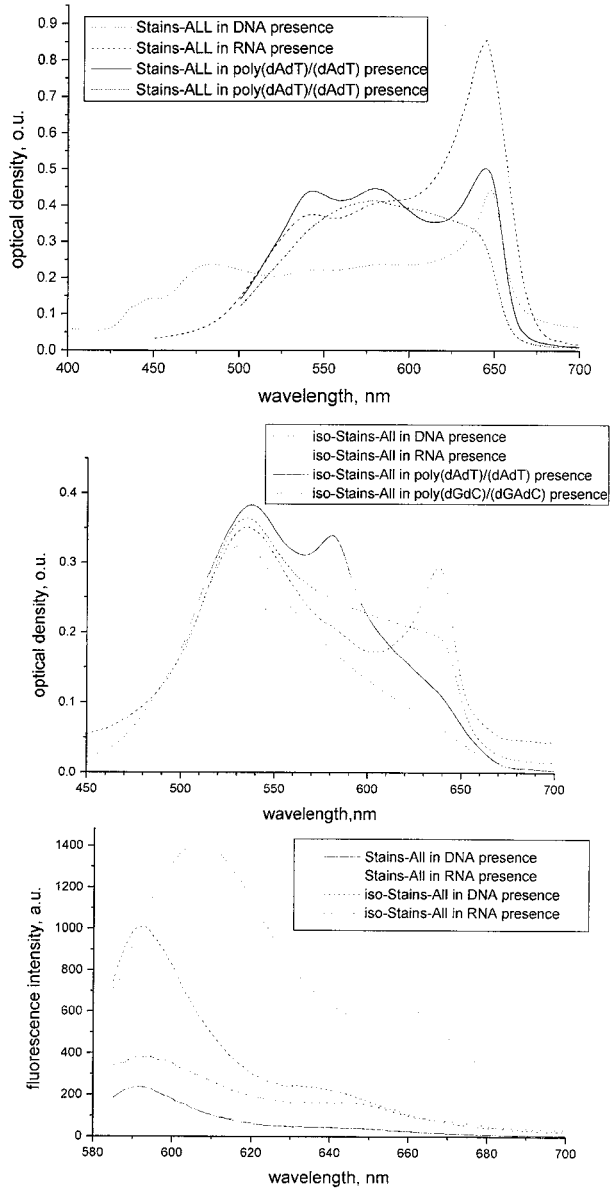

Fig. 3. Absorption and fluorescence spectra of carbocyanine dyes Stains-All and iso-Stains-All in the presence of nucleic acids.

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REFERENCES

1. R. P. Haughland (1996) *Molecular probes: Handbook of fluorescent probes and research chemicals*, Molecular Probes Inc., Eugene, Oregon.
2. L. Saso, G. Bonanni, and E. Grippa *et al.* (1999) *Res. Commun. Mol. Pathol. Pharmacol.* **104**, 277–284.

3. D. C. Dominguez, H. Adams, J. H. Hageman (1999) *J. Bacteriol*, **181**, 4605–4610.
4. A. P. Valaitis, J. L. Jenkins, and M. K. Lee *et al*. (2001) *Arch. Insect Biochem. Physiol*. **46**, 186–200.
5. M. R. Green, J. V. Pastewka, A. C. Peacock (1973) *Anal. Biochem.* **56**, 43–51.
6. S. M. Yarmoluk, V. B. Kovalska, S. S. Lukashov, and Yu. L. Slominsky (1999) *Bioorg. Med. Chem. Lett.* **9**, 1677–1678.
7. F. M. Hamer (1964) in *The Chemistry of Heterocyclic Compounds*, J. Willey & Sons, New York, London.
8. R. E. Kay, E. R. Walwick, and C. G. Gifford (1964) *J. Phys. Chem.* **68**, 1896–1906.