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INTERACTION OF CYANINE DYES WITH NUCLEIC ACIDS. XII.β-SUBSTITUTED CARBOCYANINES AS POSSIBLE FLUORESCENT PROBES FOR NUCLEIC ACIDS DETECTION.

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ABSTRACT. Results of investigations of fluorescent properties of a β-substituted carbocyanine and its complexes with nucleic acids in comparison with those for the unsubstituted dye are presented. Carbocyanine substituted in polymethine chain has shown promising properties for use as a fluorescent probe in homogeneous systems of nucleic acids detection.

Fluorescent cyanine dyes as nonradioactive probes for labeling and detection of nucleic acids are increasingly used for fluorescent microscopy, DNA sequencing, staining nucleic acids in gel electrophoresis [1]. Majority of cyanine dyes used in those techniques are monomethine cyanines, that as a rule can be excited with Ar laser line, whereas cyanines that can be excited with another semiconductor lasers lines are less numerous. Polymethine cyanines present an opportunity to use this and other additional laser lines, thus facilitating preparing fluorescent probes for multicolor analysis in cytometry and diagnostics [2,3].

Typically, symmetric polymethine cyanines in free state exist in all-*trans* conformation that possesses high intrinsic fluorescence [4]. Carbocyanines substituted in polymethyne chain in solution present as mixture of different *trans*- and *cis*- conformers that lead to decreasing of emission intensity of free dye [5,6].

A representative of β-substituted polymethine carbocyanines with the commercial name Stains-all was already used as an absorption probe for biopolymer detection [7], but a dye of this class has been studied as a fluorescent probe for nucleic acids detection for the first time in this paper. Spectral-luminescent properties of β-substituted carbocyanine ***Cyan 2*** (Fig.1) and its complexes with nucleic acids in comparison with those for unsubstituted ***Cyan 46*** are presented below.

Dyes were synthesized according to methods described [8] and their structures were confirmed by H^1 NMR spectroscopy. Nucleic acids and dye solutions were prepared and spectroscopic measurements were carried out according to [9].

Carbocyanine ***Cyan 2*** had relatively low level of intrinsic fluorescence while fluorescence of cyanine ***Cyan 46*** in unbound state is rather significant. Both dyes have medium Stokes shifts (25-30nm) (Tab.1). Dye ***Cyan 2*** forms highly fluorescent complexes with DNA and RNA, but fluorescent intensity of ***Cyan 46*** slightly decreases in the presence of nucleic acids. Presence of nucleic acids slightly shifts positions of absorption and emission maxima of the dye complexes and Stokes shift values (Tab.2).

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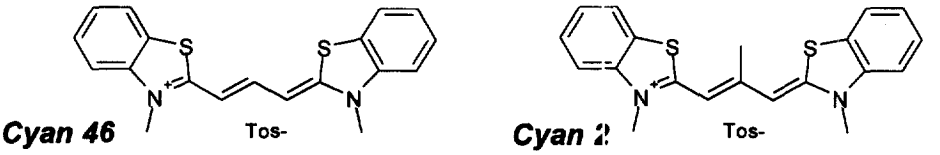

Figure 1. Chemical structures of carbocyanine dyes.

Table 1. Spectroscopic properties of free carbocyanine dyes.

Dye	λ abs buffer, nm	λem, nm	ΔS, nm	I_0, r.f.u.
Cyan 2	541	570	29	0.36
Cyan 46	553	577	25	13.7

λ abs buffer – absorption maximum of unbound dye in Tris buffer; λ em – fluorescence maximum of unbound dye in Tris buffer; I_0 – fluorescent intensity of unbound dye, r.f.u. – relative fluorescent units; ΔS – Stokes shift.

Table 2. Spectroscopic properties of carbocyanine dyes in complexes with nucleic acids.

Dye	λ abs DNA, nm	λem DNA, nm	ΔS, nm	I DNA, r.f.u.	ΔQ DNA	λ abs RNA, nm	λex RNA, nm	ΔS, nm	I RNA, r.f.u.	ΔQ RNA
Cyan 2	545	572	27	28	78	545	575	30	37	103
Cyan 46	555	578	23	12.4	0.9	555	580	25	12	0.89

λ abs (DNA/RNA) – absorption maximum of dye-DNA/RNA complex in Tris buffer. λ em (DNA/RNA) – fluorescence maximum of dye-DNA/RNA complex in Tris buffer. ΔS – Stokes shift. I (DNA/RNA) – fluorescent intensity of dye-DNA/RNA complex, r.f.u. – relative fluorescent units. ΔQ (DNA/RNA) – fluorescence intensity enhancement relatively to unbound dye, I (DNA/RNA)/ I_0.

We believe that the described spectral properties of the present β-substituted carbocyanine showed this class of dyes to be promising fluorescent probes for nucleic acids detection.

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