Journal of Fluorescence, Vol. 12, No. 1, March 2002 (© 2002)

Spectroscopic Studies of α,γ-Disubstituted Trimethine Cyanine: New Fluorescent Dye for Nucleic Acids

I. V. Valyukh,[1] V. B. Kovalska,[2] Y. L. Slominskii,[3] and S. M. Yarmoluk[2,4]

Received September 27, 2001; revised January 4, 2002; accepted January 4, 2002

Spectral properties of 3-methyl-2-{3-[3-methyl-1,3-benzothiazolo-2(3*H*)-ylidene]-1,4-cylopentadien-1-yl}-1,3-benzothiazolo-3-ium tosylate (Cyan-Cpentd) in a free state and in the complexes with nucleic acids and synthetic polynucleotides have been investigated by absorption and fluorescence spectroscopy. Significant fluorescence intensity enhancement of dye-nucleic acids complexes is observed. For the first time Cyan-Cpentd is proposed as a new probe for nucleic acid detection. Binding mechanism of Cyan-Cpentd is discussed in view of the NA-ligand interaction models.

KEY WORDS: Nucleic Acid Fluorescent Detection, Cyanine Dyes.

INTRODUCTION

Fluorescent detection and chemical labeling of nucleic acids (NA) are ones of the powerful tools of the molecular biology. Asymmetrical carbocyanines are widely used for these purposes, as well as monomethines [1]. Recently we proposed symmetrical trimethine cyanines with β-substitution in polymethine chain for the qualitative and quantitative detection of biomolecules in homogeneous assays. These dyes have high extinction coefficients, wide absorption bands, and increased quantum yields, up to 2 orders of magnitude in the presence of nucleic acids [2].

For the first time, symmetrical trimethine cyanine with vinylene bridge in the polymethine chain, Cyan-Cpentd, has been investigated as a possible fluorescent probe for NA detection. Spectral-luminescent properties of this dye in a free state and in the complexes with nucleic acids and synthetic polynucleotides are presented in this paper.

[1] Physical Department of Kyiv Taras Shevchenko National University, 64 Volodymyrs'ka St., 03033 Kyiv, Ukraine.
[2] Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, 150 Zabolotnogo St., 03143 Kyiv, Ukraine.
[3] Institute of Organic Chemistry, National Academy of Sciences of Ukraine, 5 Murmanska St., 02094 Kyiv, Ukraine.
[4] To whom correspondence should be addressed. Tel.: 38 (044) 252 23 89; Fax: 38 (044) 252 24 58; E-mail: otava@carrier.kiev.ua

EXPERIMENTAL

Cyanine dyes were synthesized according to the procedure described in [3]. The dye stock solutions were prepared in DMSO. Concentration of the dye in working solutions was $2.0 \cdot 10^{-5}$ M. The 0.05 M Tris-HCl (pH = 7.5) buffer was used for measurements. Chicken erythrocyte DNA and the total yeast RNA, synthetic polydeoxyribonucleotides poly(dA)poly(dT) and poly(dGdC)$_2$ (Sigma) were used for the studies. The concentrations of NA in working solutions were $6 \cdot 10^{-5}$ M b.p. for DNA or synthetic polynucleotides and $1.2 \cdot 10^{-4}$ M b. for RNA. Fluorescence emission and excitation spectra were obtained with Cary Eclipse fluorescence spectrophotometer (Australia).

RESULTS AND DISCUSSION

The maxima of absorption band of the trimethine cyanine in DMSO at 471 nm, and short-wave shoulders

1053-0509/02/0300-0105/0 © 2002 Plenum Publishing Corporation

at 449 nm are observed. Wide non-structured absorption band with the maximum at 454 nm is typical for the dye in aqueous solution. At increasing dye concentration (C) from 10^{-6} M to 10^{-5} M the shapes of absorption spectra do not change in both solutions. Emission maxima of Cyan-Cpentd are detected at 498 nm (in DMSO) and 490 nm (in buffer) in this case (Table I).

It should be noted that the shape of the dye-DNA complex absorption band is similar to that of the dye in DMSO (monomeric form). Upon the increase of dye concentration from 10^{-6} M to 10^{-5} M the intensity of the short-wavelength absorption band (λ = 450 nm) grows more intensive (0.05–0.45 a.u.) than that of monomeric band (λ = 470 nm, from 0.08–0.48 a.u.). Such changes in spectra are the evidence of corresponding of the short wavelength band to H-aggregates absorption. We consider that in DNA presence H-aggregates formation occurs preferentially on the biopolymer surface. A similar phenomenon is observed in the presence of RNA and synthetic polynucleotides (Table I).

The dye (at C = 10^{-6} M) significantly increases its emission intensity in the DNA and RNA complexes (up to 100 times in the presence of DNA and 22 times in the presence of RNA). It should be noted that Stokes' shift (ΔS) values are close in both cases: ΔS_{DNA} = 14 nm, ΔS_{RNA} = 15 nm. As noted above the increase in the dye concentration leads to the formation of H-aggregates and fluorescence quenching takes place as a result (Table I). Analysis of the fluorescence excitation spectra of the dye-NA complexes also confirms our assumption about H-aggregates formation in the presence of the biopolymers.

To obtain binding stoichoimetry of Cyan-Cpentd with DNA we studied the dependence of dye-NA complexes fluorescence intensity from the dye/DNA ratio at the constant dye concentration C = 10^{-5} M (Fig. 1). There is only one break in the titration curve with DNA at the ratio of 1 ligand molecule to 16 base pairs. Further

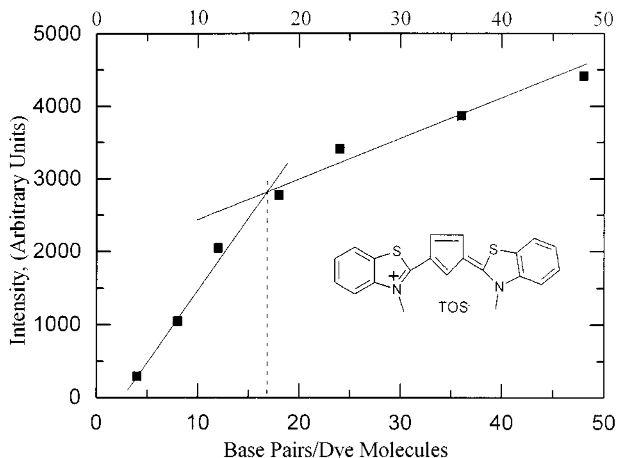

Fig. 1. Dependence of fluorescence intensity of the dye-DNA complex (■) on DNA b.p./dye molecules ratios. Dye concentration was 10^{-5} M.

addition of DNA molecules does not lead to the essential enhancement of the fluorescence intensity because of the formation of aggregates.

We have investigated interaction of Cyan-Cpentd with synthetic polynucleotides poly(dGdC)$_2$ and poly (dA)poly(dT) to study binding specificity of the dye (Table I). The emission intensity enhancement (ΔQ) is higher for the dye-poly(dA)poly(dT) complex (ΔQ = 72 times) compared with the dye-poly(dGdC)$_2$ complex (ΔQ = 25 times). Decrease in fluorescence intensity is also observed at increasing dye concentration. Stokes' shifts are equal to 15 nm and 23 nm in the presence of poly(dA)poly(dT) and poly(dGdC)$_2$, respectively.

It is believed that the movement and oscillations of bound dye molecules are restricted in the presence of nucleic acids. As a result, non-radiative excitation energy deactivation is less likely to occur, and, thus, significant enhancement in emission intensity is observed. We remarked that Stokes' shifts are nearly identical for Cyane-Cpentd in a free state and in the presence of nucleic

Table I. Spectral-Luminescent Characteristics of Dye Alone and in Complexes with Nucleic Acids

Dye concentration, M	DMSO	Buffer			DNA			RNA			poly(dA)poly(dT)			poly(dGdC)$_2$		
	λ_{abs}	λ_{abs}	λ_{em}	I_0	λ_{abs}	λ_{em}	I	λ_{abs}	λ_{em}	I	λ_{abs}	λ_{em}	I	λ_{abs}	λ_{em}	I
10^{-6}	449(*sh*), 471	454	489	29	450(*sh*), 474	489	2958	458(*sh*), 482	503	521	446(*sh*), 471	486	2090	458(*sh*), 484	517	662
10^{-5}	449(*sh*), 471	451	491	126	458(*sh*), 474	501	1307	452, 482(*sh*)	506	526	447(*sh*), 471	495	1119	450	515	157

λ_{em} and λ_{abs}: wavelength of emission and absorption maxima, respectively (in nm); I_0: fluorescence intensity of dye in a free state (in arbitrary units); I: fluorescence intensity of the dye in the presence of corresponding biopolymer (in arbitrary units); sh: shoulder. Dye was excited at absorption maximum (454 nm in buffer). For dye-DNA, -RNA, -poly(A)poly(T) and poly(GC)$_2$ complexes excitation wavelengths were 471 nm, 474 nm, 474 nm, and 482 nm, respectively.

acids, despite the differences in the dye molecule environment. Cyan-Cpentd also demonstrates a certain preference to interact with AT-sequence. It is known that existence of AT binding preference is the evidence of groove-binding mode. Thus, we believe that studied cyanine dye interact with DNA through the fixation in the groove.

Significant enhancement of emission intensity in the NA presence is observed. For the first time, α-, γ-substituted in polymethyne chain cyanine is proposed as a fluorescent probe for NA labeling and detection.

ACKNOWLEDGMENTS

This work was sponsored by the United States Department of Energy, with funding from Initiatives for Proliferation Prevention (IPP) Program and was performed under the terms and conditions of Material Support Agreement No.B507077 between Institute of Molecular Biology and Genetics, Kyiv, Ukraine and the University of California, Lawrence Livermore National Laboratory, Livermore, California.

REFERENCES

1. R. P. Haughland (1996) *Molecular Probes: Handbook of Fluorescent Probes and Research Chemicals*, Molecular Probes, Inc.
2. S. M. Yarmoluk, V. B. Kovalska, S. S. Lukashov, and Y. L. Slominsky (1999) *Bioorg. Med. Chem. Lett.* **9**, 1677–1678.
3. F. M. Hamer (1964) in *The Chemistry of Heterocyclic Compounds*, XVIII, J. Willey & Sons, New York, London.