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Influence of the Aggregation of Homo-N-Mer Cyanine Dyes on the Nucleic Acids Detection Sensitivity

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Novel benzothiazolopyridinium homo-n-mer cyanine dyes are proposed for nucleic acid fluorescent detection. Dependence of the sensitivity of detection in solution from the dye molecules/DNA base pairs ratio was studied. It was shown that the presence of the dye excess could significantly decrease the detection limit. We believe this could be explained by the formation of the dye aggregates on DNA surface.

KEY WORDS: Cyanine dyes; aggregation; fluorescence; and nucleic acids detection.

INTRODUCTION

Cyanine dyes are among the most widely used fluorescent probes for nucleic acids (NA) detection. Chemically conjugated dyes have noticeably increased binding affinity to DNA and fluorescence intensity in nucleic acids complexes compared to the monomeric ones [1]. The aggregate formation in aqueous solutions is well known for cyanine dyes and causes significant changes in their absorption and fluorescence spectra [2]. It was established that n-mere cyanines show an increased ability to form aggregate structures [3]

Earlier we investigated spectral-luminescent properties of novel benzothiazolopyridinium homodimers and homotrimers and their nucleic acid complexes [3,4] (Fig. 1). We also studied aggregation processes that accompany interaction of homo-n-mere cyanines with DNA. It was shown that the excess of the dye causes formation of the weakly fluorescent dye associates directly on the DNA surface [3,4].

The present work aimed to study dependence of the DNA detection sensitivity on the dye molecules/DNA base pairs ratio. In other words, we wanted to establish if the excess of the dye in the reaction solution decreases sensitivity of the nucleic acids detection.

EXPERIMENTAL

Cyanine dye K-6 was synthesized according to the procedure described in [5]. The dye stock solutions were prepared in DMSO. The 0.05-MTris-HCl (pH = 7.5) buffer for measurements and total DNA from chicken erythrocytes were used. Fluorescence emission and excitation spectra were obtained with Cary Eclipse fluorescence spectrophotometer (Australia).

Two procedures were used for the preparation of the dye–DNA complex. According to the first, the aliquot containing $1.2*10^{-4}$ M base pairs DNA was mixed with the aliquot containing 10^{-5} M of the homodimer dye. Fluorescence spectra of the obtained complex were measured. Then solution was diluted two times following by the recording of it's emission spectra. We repeated this procedure up to the disappearance of the clear fluorescence band. According to the second procedure, all work solutions were obtained by mixing of the dye of the fixed concentration (10^{-5} M) and different concentrations of DNA. Concentration of DNA in the mixture was

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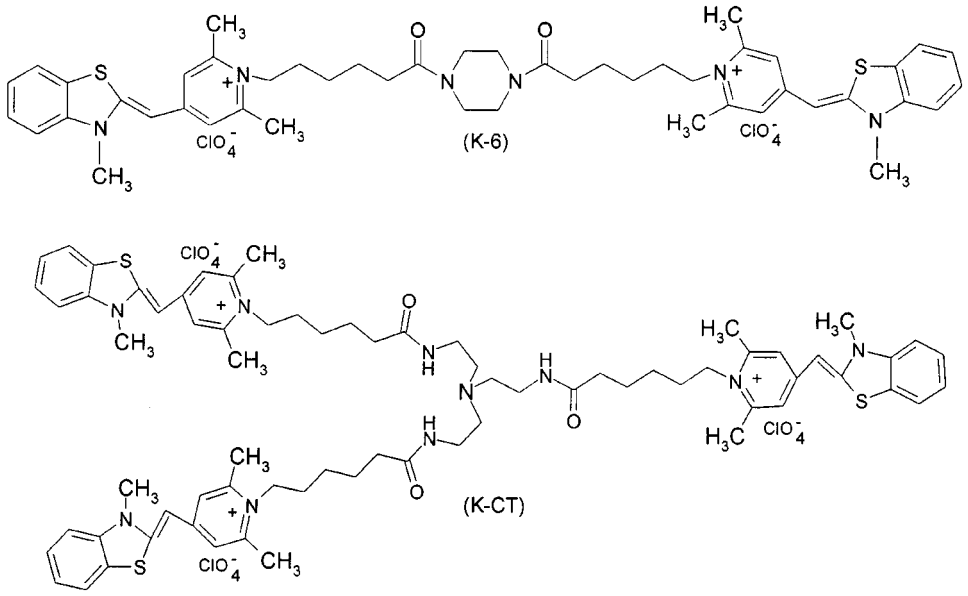

Fig. 1. Structures of the studied homodimer and homotrimer cyanine dyes.

decreased up to the disappearance of the band of "monomer" fluorescence.

RESULTS AND DISCUSSION

Fluorescence maximum of homodimer dye K-6 in organic solvent DMSO is observed near 485 nm and corresponds to the "monomer" emission. Whereas in the aqueous solution, a broad low-intensity band with maximum near 570 nm appears, which corresponds to aggregates emission. Interaction of the dyes with nucleic acids leads to the partial destruction of associates. Non-aggregated dye binds to the DNA strand, and a significant increase in the monomer band intensity (near 485 nm) is observed. At the same time the addition of the excess dye leads to the formation of aggregates on the DNA surface that results in a noticeable decrease of the monomer fluorescence intensity and appearance of the clear red-shifted aggregate emission band [3–5].

It is proposed that even though cyanine dyes bind to the double-stranded (ds) DNA through intercalation, fixation of the dye in the DNA groove is also possible [6]. Taking into account the stochiometry of the complexes typical for the groove binders and intercalators, we used the ratio dye molecule/12 base pairs for the preparation of homodimer dye–DNA complex (first procedure). At this ratio the clear monomer fluorescence band (near 485 nm) was observed for the solutions containing down to 5 μg DNA (Fig. 2).

At the same time, the presence of the dye excess allows observation of the dye monomer fluorescence only at the ratios lower than 1 dye molecule/0.1 DNA base pair, ($5*10^{-7}$ M DNA base pairs in the presence of $5*10^{-6}$ M of dye). In other words, when the second procedure of complex preparation was used, we could detect ds DNA amount only down to 200–300 μg. At the dye molecule/0.1 DNA base pair ratio, only the red-shifted band with the maximum near 570 nm corresponding to

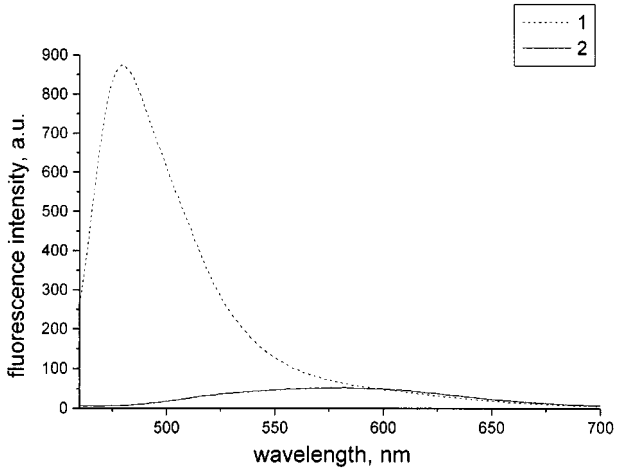

Fig. 2. (1) Emission spectra of the solution containing $5*10^{-7}$ M DNA base pairs and $4*10^{-8}$ M dye (ratio 12/1). (2) Emission spectra of the solution containing $5*10^{-7}$ M DNA base pairs $5*10^{-6}$ M dye (ratio 1/10). Spectra were obtained using 450-nm excitation wavelength. The spectrum 2 overlaps with the spectrum of the free dye fluorescence.

the aggregate emission was observed in corresponding spectra. This band had similar shape and intensity to those of the free dye emission spectra.

Fig. 2 shows fluorescence spectra of the dye-DNA complexes containing the same amount of DNA with different amounts of dye. An intense band corresponding to the monomer fluorescence is observed for the complex of the 1 dye molecule/12 base pair ratio (lack of the dye). At the same time in the spectra of the complexes with 1 dye/0.1 base pair ratio (excess of the dye), only the band that corresponds to the aggregate emission is present.

Thus, the presence of the excess of the fluorescent homo-n-mer dyes in NA detection systems can noticeably decrease the detection limit. We consider that when the dye with the pronounced tendency to aggregation is used in the detection system, it is necessary to take into account both nucleic acid and dye concentrations to obtain accurate results.

CONCLUSIONS

Increased ability of homo-n-mer cyanine dyes to form aggregate structures on DNA surface could significantly influence fluorescence properties of the dye–nucleic acid complexes, shifting emission maxima and decreasing fluorescent intensity. The excess of the dye in DNA/dye–containing solution leads to the intensive formation of low fluorescence associates and plays the role of "fluorescence quencher". We propose that using of a correct dye/DNA ratio could help to avoid the intensive formation of such aggregates and thus obtain appropriate detection sensitivity during in-solution detection experiments with the use of homo-n-mer cyanine dyes.

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