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COMPETITIVE BINDING OF NOVEL CYANINE DYE AK3-5 AND EUROPIUM COORDINATION COMPLEXES TO DNA

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The present study was undertaken to assess the applicability of the novel trimethine cyanine dye AK3-5 as a competitive ligand for the antitumor agents, Eu(III) coordination complexes (EC), in the DNA-containing systems, using the displacement assay as an analytical instrument. The analysis of fluorescence spectra revealed a strong association of AK3-5 with nucleic acids, with the strength of interaction being higher for the double stranded DNA, compared to the single-stranded RNA. The binding parameters of the cyanine dye have been determined in terms of the McGhee & von Hippel neighbouring site-exclusion model and a classical Langmuir model. The AK3-5 association constant in the presence of DNA was found to be equal to 5.1×10^4 M^{-1}, which is consistent to those of the well-known DNA intercalators. In turn, the binding of the cyanine to the RNA was characterized by a significantly lower association constant (~ 3.4×10^3 M^{-1}) indicating either the external or "partially intercalated" binding mode. The addition of the europium complexes to the AK3-5-DNA system was followed by the fluorescence intensity decrease, with a magnitude of this effect being dependent on the EC structure. The observed fluorescence decrease of AK3-5 in the presence of europium complexes V7 and V9 points to the competition between the cyanine dye and antitumor drugs for the DNA binding sites. The dependencies of the AK3-5-DNA fluorescence intensity decrease *vs*. europium complex concentration were analyzed in terms of the Langmuir adsorption model, giving the values of the drug association constant equal to 5.4×10^4 $M^{-1}$and 3.9×10^5 M^{-1} for the europium complexes V7 and V9, respectively. A more pronounced decrease of the AK3-5 fluorescence in the presence of V5 and V10 was interpreted in terms of the drug-induced quenching of the dye fluorescence, accompanying the competition between AK3-5 and Eu(III) complexes for the DNA binding sites. Cumulatively, the results presented here strongly suggest that AK3-5 can be effectively used in the nucleic acid studies and in the dye-drug displacement assays.
KEYWORDS: trimethine cyanine dye, europium coordination complexes, antitumor drug, DNA, association constant

Over the past decade significant research efforts have been devoted to the investigation of the interaction between drugs and nucleic acids, since DNA was found to serve as a prime target for various anticancer drugs and antibiotics [1-3]. A variety of powerful techniques, including an agarose gel based assay, circular dichroism, mass spectrometry, differential scanning calorimetry, surface plasmon resonance, electrophoresis, high-performance or thin-layer chromatography, Raman, fluorescence and absorption spectroscopy, are currently used to monitor the drug-nucleic acid binding. Due to advantages of rapidity and sensitivity, the fluorescence drug displacement assay seems to be an especially promising for the investigation of mechanisms underlying the interaction between nucleic acids and drugs. This method is based on a strong enhancement in the dye emission upon its binding to DNA or RNA, followed by the fluorescence drop upon the dye displacement by the drug [4-7]. Among a variety of commercially available dyes, ethidium bromide is the most widely used in the displacement experiments [7,8]. However, the applicability of ethidium bromide, which is considered as mutagenic and carcinogenic, is complicated by some environmental concerns [9]. Recent studies revealed that cyanine dyes can be effectively used for the high throughput screening of the drug-DNA interactions [10,11]. The applicability of this class of fluorophores is based on the fact that they display a high affinity for nucleic acid double strands and a huge emission enhancement upon DNA binding. A sharp fluorescence increase is supposed to originate from the loss of mobility around the methane bridge between the two heterocyclic moieties as a result of the cyanine-nucleic acid interaction [12]. Due to their excellent staining properties, cyanine dyes are characterized by a wide scope of applications and have been extensively used for sizing and purification of DNA fragments [13,14], fluorescent microscopy [15], DNA damage detection [12], DNA sequencing [16], as well as for the DNA and RNA bioanalytical assays [17,18].

Our previous studies revealed that the novel mono- and pentamethine cyanine compounds can be effectively employed as non-covalent labels for nucleic acids [17, 19, 20]. As a next logical step, herein we directed our efforts towards evaluating the DNA- and RNA-binding ability of the novel trimethine cyanine dye with an emphasis on its use in the drug-displacement studies. More specifically, the aims of the present study were: i) to investigate the sensitivity of the novel trimethine cyanine, referred to here as AK3-5, to the double stranded DNA and single stranded RNA; ii) to estimate the parameters of the cyanine association with nucleic acids; iii) to analyze the binding mode of the novel dye;

iv) to assess the applicability of AK3-5 as a competitive ligand for pharmacological agents, represented here by the novel antitumor compounds, europium coordination complexes.

EXPERIMENTAL SECTION

Materials

Calf thymus DNA and yeast RNA were from Sigma (Sigma, St. Louis, MO, USA). Trimethine cyanine dye AK3-5 [21] and Eu(III) coordination complexes (Fig.1) referred to here as V7, V9 and V10 were synthesized in the University of Sofia, Bulgaria, as described previously [2]. All other materials and solvents were commercial products of analytical grade and were used without further purification.

Preparation of working solutions

The stock solutions of AK3-5 and Eu(III) coordination complexes were prepared in dimethyl sulfoxide. The concentrations of the compounds were determined spectrophotometrically using their molar extinction coefficients (ε) at absorption maxima (λ_{abs}), which are presented in Table 1. The solutions of calf thymus DNA and yeast RNA were prepared in 5 mM sodium phosphate buffer (pH 7.4) at room temperature with occasional stirring to ensure the formation of a homogenous solution. The concentrations of DNA and RNA solutions were determined using their molar absorptivities $\varepsilon_{260} = 6.6\times10^3\,\mathrm{M^{-1}cm^{-1}}$ and $\varepsilon_{260} = 6.9\times10^3\,\mathrm{M^{-1}cm^{-1}}$, respectively.

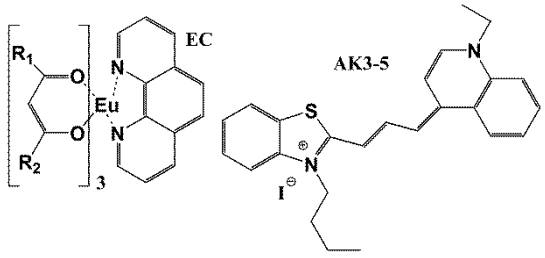

Fig.1. Structures of the AK3-5 and EC

Table 1

Chemical structures and photophysical properties of europium coordination complexes and AK3-5

compound	R_1	R_2	ε, $M^{-1}cm^{-1}$	λ_{abs}, nm
V5	C_6H_5	CH_3	2.6×10^4	266
V7	C_6H_5	CF_3	2.6×10^4	266
V9	C_6H_7O	CF_3	3.4×10^4	271
V10	C_6H_5	C_6H_5	3.4×10^4	167
AK3-5			1.5×10^5	631

Spectroscopic measurements

The steady-state fluorescence spectra were recorded with LS-55 spectrofluorimeter (Perkin-Elmer Ltd., Beaconsfield, UK) at 20°C using 10 mm path-length quartz cuvettes. To measure the fluorescence spectra of the AK3-5-DNA/RNA complexes, the appropriate amounts of the nucleic acid stock solution were added to the dye in 5 mM sodium phosphate buffer, pH 7.4. The fluorescence spectra of AK3-5 were recorded in the range 620 - 800 nm upon excitation at 600 nm. The fluorimetric titrations were carried out by keeping the dye concentration constant at varying the DNA and RNA concentrations. In the case of the dye displacement studies, a solution containing AK3-5 and DNA was titrated with the Eu(III) complexes.

Quantitative analysis of the dye-nucleic acid interactions

The thermodynamic analysis of the cyanine-nucleic acid interactions was performed in terms of the McGhee & von Hippel excluded site model allowing the calculation of the binding constant and stoichiometry [22]:

$$\frac{B}{F}=K_aP\left(1-\frac{nB}{P}\right)\left[\frac{1-(nB/P)}{1-(n-1)(nB/P)}\right]^{n-1}, \quad (1)$$

where B and F are the concentrations of the bound and free dye, respectively, P is the DNA (RNA) phosphate concentration, K_a denotes the association constant, and n represents the site exclusion parameter (i.e. the number of base pairs excluded by the binding of a single ligand molecule). The values of K_a and n were estimated using the nonlinear least-square fitting procedure.

Quantitative analysis of the AK3-5/ EC competitive binding

In order to analyse the competitive binding of AK3-5 and EC to the nucleic acids a simplified model based on the one-site Langmuir adsorption model was employed [23,24]. Assuming that the AK3-5 fluorescence response is proportional to the amount of the DNA-bound fluorophore, B, the DNA-induced change in the probe fluorescence intensity ΔF at the fluorescence maximum can be written as:

$$\Delta F = F - F_0 = \alpha_{bound}B + \alpha_{free}(Z - B) - \alpha_{free}Z = (\alpha_{bound} - \alpha_{free})B = F_{mol}B, \quad (2)$$

where F_0 and F are the fluorescence intensities of the dye in a buffer solution and in the presence of DNA, respectively; F_{mol} is a coefficient proportional to the difference of the dye quantum yields in buffer and when bound to a macromolecule; α_{bound} and α_{free} are the molar fluorescences of the bound and free dye, respectively.

Given that the number of phosphates bound to one dye molecule is n, the association constant (K_a) can be represented as:

$$K_a = \frac{B}{(Z-B)(P/n-B)} = \frac{B}{F(P/n-B)}, \tag{3}$$

where P and Z are total phosphate and total dye concentrations, respectively.

The F_{mol} parameter was calculated from the fluorimetric titration of the dye with the DNA or RNA. Specifically, at high DNA/RNA concentrations, when $P/n \gg B$, from the combination of eqns (2) and (3) one obtains:

$$\frac{1}{\Delta F} = \frac{1}{BF_{mol}} = \frac{1}{K_a PZF_{mol}/n} + \frac{1}{ZF_{mol}}, \tag{4}$$

$$F_{mol} = 1/aZ, \tag{5}$$

where a is the y-intercept of the linear fit of the plot $1/\Delta F(1/P)$ [23].

The parameters K_a and n were estimated from the fluorimetric titration of the DNA by the dye. In this case, when $Z \gg B$, a combination of the eqns (2) and (3) gives:

$$\frac{1}{\Delta F} = \frac{1}{BF_{mol}} = \frac{1}{K_a PZF_{mol}/n} + \frac{1}{PF_{mol}/n}, \tag{6}$$

$$n = bPF_{mol}, \tag{7}$$

$$K_a = -c, \tag{8}$$

where b and c are y- and x-intercepts of the linear fit of the plot $1/\Delta F(1/Z)$. When the drug binds to the AK3-5-DNA complexes, the dye fluorescence in the absence (F_0) and presence (F_i^{corr}) of the drug can be written as:

$$F_0 = B_0 a_{bound} + (Z_0 - B_0) a_{free}, \tag{9}$$

$$F_i^{corr} = B_i a_{bound} + (Z_0 - B_0) a_{free}, \tag{10}$$

where B_0 and B_i are the concentrations of the DNA-bound dye in the absence and presence of a drug, respectively. From the eqns. (9) and (10) one obtains:

$$\Delta F_{calc} = F_0 - F_i^{corr} = (B_0 - B_i)(a_{bound} - a_{free}). \tag{11}$$

The association constants of the dye (K_{dye}) and drug (K_{drug}) binding to the DNA were recovered from the numerical solution of the set of equations under the following conditions: $B_{dye} \le Z_{0\,dye}$; $B_{drug} \le Z_{0\,drug}$; $B_{dye} > 0$; $B_{drug} > 0$; $B_{dye} < N - B_{drug}$ (the total number of binding sites $N = P/n$ is greater than the sum of concentrations of bound dye and drug):

$$K_{dye} = \frac{B_{dye}}{(Z_{0\,dye} - B_{dye})(N - B_{dye} - B_{drug})}, \tag{12}$$

$$K_{drug} = \frac{B_{drug}}{(Z_{0\,drug} - B_{drug})(N - B_{dye} - B_{drug})}, \tag{13}$$

where B_{dye} and B_{drug} are the concentrations of the DNA-bound dye and drug, respectively.

Molecular docking

An interactive molecular graphics program, Hex 8.0.0 was used to study the interaction between the examined europium complexes and the double stranded DNA. The program performs docking using the spherical polar Fourier correlations with the inputs of ligand and receptor in PDB format. The structure of the B-DNA dodecamer d(CGCGAATTCGCG)$_2$ (PDB ID: 1BNA) was downloaded from the Protein Data Bank (http://www.rcsb.org./pdb). The parameters used for molecular docking include: FFT mode – 3D, correlation type – shape only, grid dimension – 0.6, ligand range – 180, receptor range – 180, distance range – 40, and twist range – 360. The docked complexes were visualized by the Visual Molecular Dynamics (VMD) software.

RESULTS AND DISCUSSION

At the first step of the study the AK3-5-nucleic acid binding was characterized using the fluorescence spectroscopy technique. The spectra of AK3-5 in the presence of the double stranded DNA or single stranded RNA are presented in Fig.2. The AK3-5 was found to have a negligible fluorescence in a buffer solution with the emission maximum at 640 nm. In turn, the fluorescence intensity drastically increased upon the dye transfer from the aqueous phase to the dsDNA or RNA environment, with the magnitude of this effect being more pronounced for the dye-DNA complex. Moreover, AK3-5 binding to the RNA caused a 10 nm bathochromic shift in the emission maxima position, whereas no significant change of this parameter was observed in the dye-DNA system. The comparison of the extents of fluorescence enhancement in the presence of the dsDNA (*ca.* ~42.3) and the RNA (*ca.* ~4.3) at the same experimental conditions indicates that the affinity of the AK3-5 to double stranded nucleic acids is higher than that of single stranded one. The difference in the position of the fluorescence maxima in the presence of nucleic acids, as well as different affinities for the RNA and DNA were also reported previously for other dyes of cyanine family [25], and can be used to distinguish between the double and single stranded nucleic acids in solution. Moreover, the 10 nm shift of the AK3-5 fluorescence maximum position in the presence of the RNA with respect to that in the DNA-containing system probably reflects a more polar environment of the dye in a single stranded nucleic acid. This finding is suggestive of the different binding modes of AK3-5 to the single and double stranded nucleic acids.

It is well-known that cyanine dyes can interact with the nucleic acids in the three basic modes: i) electrostatic attraction between the cationic dyes and the anionic phosphodiester groups of DNA/RNA backbone; ii) intercalation between adjacent base pairs, iii) minor groove binding. Intercalation is typically observed for the cationic molecules (with the positive charge preferably located on the ring system) possessing a planar aromatic structure, while the minor groove binders should have at least limited flexibility to be able to adjust to the groove [12]. The structural and physicochemical properties of a fluorophore, a nucleic acid sequence, as well as the phosphate to dye ratio (P/D) were found to determine the molecular mechanism of cyanine complexation with nucleic acid [26,27].

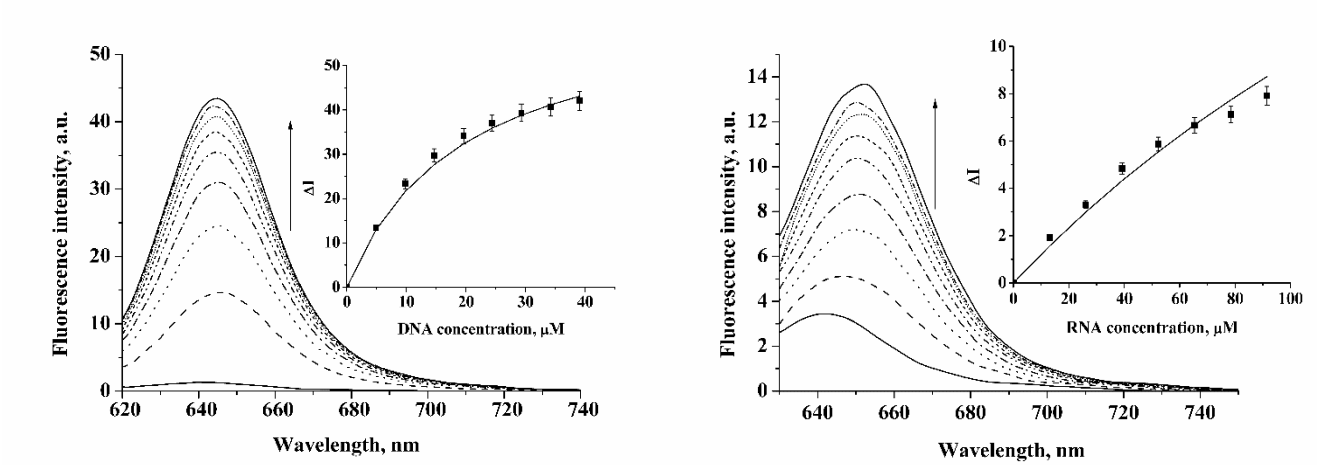

Fig. 2. Fluorescence spectra of the cyanine dye AK3-5 in the presence of increasing concentration of the double stranded DNA (left) and the single stranded RNA (right). Dye concentration was 0.25 μM. DNA concentrations from bottom to top were 0 μM, 4.9 μM, 9.8 μM, 14.7 μM, 19.6 μM, 24.4 μM, 29.3 μM, 34.2 μM, and 39.1 μM, respectively. RNA concentrations from bottom to top were 0 μM, 13.1 μM, 26.2 μM, 39.2 μM, 52.3 μM, 65.4 μM, 78.3 μM, 91.5 μM, and 104.6 μM, respectively. The insets show the isotherms of the dye binding to the DNA or RNA. The experimental data were fitted with the McGhee-von Hippel model.

In order to gain further insight into the binding mode between the AK3-5 and nucleic acids, as well as to calculate the parameters characterizing the stability of the cyanine-nucleic acid complexes, the experimental dependencies of the dye fluorescence increase (ΔI) on the DNA/RNA concentration (insets in Fig. 2) were analyzed in terms of the non-cooperative McGhee & von Hippel model (Eq. (1)) [22]. The results obtained are summarized in Table 2.

Table 2.

The thermodynamic parameters of the AK3-5 binding to nucleic acid

Nucleic acid	$K_a \times 10^4\ M^{-1}$	n	$F_{mol}, \mu M^{-1}$
DNA	5.1±0.9	2	264.3±52.8
RNA	0.34±0.06	2	142.6±29.6

The association constant for the AK3-5-DNA complex was found to be 5.1×10^4 M^{-1}. This value is identical to the association constant observed for the classical intercalating dye, acridine orange [28]. The association constants for other fluorophores possessing intercalating binding mode such as ethidium bromide (~ 1.5×10^5 M^{-1}) [29], EvaGreen

(~ 3.6×10^5 M^{-1}) [30], asymmetric thiazole orange derivatives (0.7 – 7.6×10^5 M^{-1}) [17] and monomethine cyanine dyes [19] do not exceed 10^5 M^{-1}. In view of this, we can suppose that AK3-5 also intercalates between the base pairs of the double stranded DNA. An additional argument in favor of the above assumption comes from the fact that the site exclusion parameter (number of DNA units excluded by the cyanine molecule, n) is equal to 2, that is in a good agreement with the principle of the nearest neighbor exclusion, indicating that the binding of one intercalating molecule between two base pairs hinders an access of the next binding site to another intercalator, so the highest possible dye-base pair ratio for intercalation is 1:2 [12]. As seen in Table 2, the value of association constant for the AK3-5/RNA complex was smaller by an order of magnitude, while the site exclusion parameter was also equal to two. These parameters allowed us to rule out the possibility of AK3-5 binding to the minor groove of RNA and, therefore, we supposed that the AK3-5 binding to the single stranded RNA is presumably driven by the electrostatic attraction between the positively charged group of the dye and negatively charged phosphate backbone. Nevertheless, the possibility of a "partial" intercalation of the AK3-5 between the RNA bases cannot be ruled out. Such a binding mode was previously observed for acridine orange in the presence of ss-nucleic acids [28]. Similarly, the alkaloids berberine and palmatine, which are capable of intercalating between the base pairs of dsDNA, changed their binding mode to the partially intercalating in the presence of tRNA [31].

At the next step of the study an attempt has been made to assess the applicability of the trimethine cyanine dye AK3-5 as a competitive ligand for the potential pharmacological agents Eu(III) tris-β-diketonato coordination complexes. Notably, due to a significantly smaller binding affinity of the AK3-5 to the single stranded RNA, the dye displacement data were quantitatively interpreted only for AK3-5-DNA systems. It is generally accepted that in the competitive displacement assays drug molecules that displace the DNA-bound dye, interact with the DNA in a fashion similar to that of the dye [32]. Such a competition between dye and drug usually manifests itself in a fluorescence intensity decrease of the dye-DNA complex. Recently it has been demonstrated that a series of europium coordination complexes are capable of exerting significant antineoplastic effect, with the abundance of DNA-intercalating motif being the major determinant of cytotoxicity [2]. To confirm that Eu(III) compounds under investigation are capable of intercalating within the DNA helix, the molecular docking studies were performed. A double-stranded DNA dodecamer [PDB ID 1BNA] and the examined drugs were taken as the input structures. The molecular docking indicates that a more energetically favorable binding mode for the EC is a "partial intercalation" (Fig. 3). The 1,10-phenanthroline or 2,2'bipyridine part of the Eu(III) molecule tends to reside between the DNA-bases, while the rest of the complex is placed in the minor groove of the DNA.

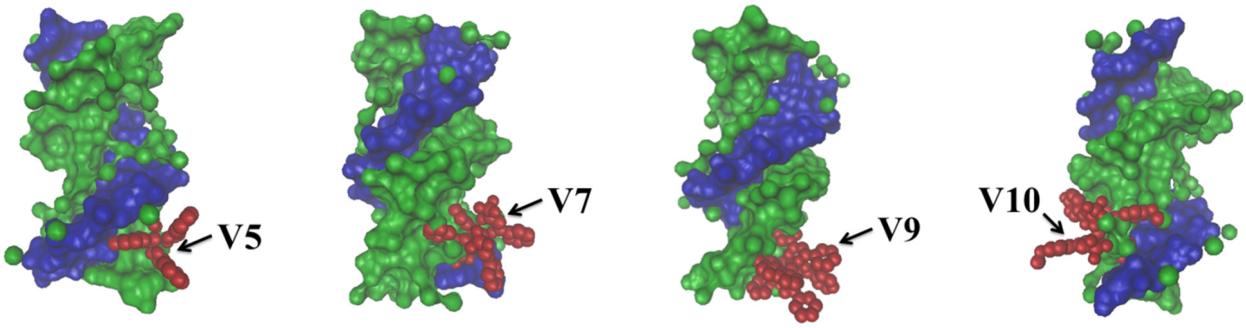

Fig. 3. Schematic representation of the energetically most favourable EC complexes with the double stranded DNA.

Thus, it might be expected that EC as intercalating molecules would replace the AK3-5 from the DNA helix. Fig. 4 shows the emission spectra of the DNA-bound AK3-5 in the absence and presence of europium coordination complexes. As can be seen, increase of the EC concentration was followed by a gradual reduction in the AK3-5 fluorescence intensity, indicating that europium compounds are capable of displacing the AK3-5 from the DNA helix. Notably, the magnitude of the fluorescence intensity decrease was more pronounced for V5 and V10 complexes in comparison to V7 and V9. The most probable explanation for this observation is the different binding affinities of the europium complexes. Similarly, the metal complexes of Cu, Zn and Ni have been reported to possess the distinct DNA binding affinities [5]. Interestingly, V5 and V10 are more bulky in comparison to V7 and V9. So we cannot exclude the possibility of the EC- induced conformational changes in the double stranded DNA. A good wealth of reports indicates that the drug binding to the right-handed B-DNA can produce its conversion to the left-handed Z-DNA [11].

Pursuing a comprehensive picture of the competition between AK3-5 and Eu(III) compounds for the DNA binding sites, our experimental strategy involved collecting the multiple data sets. More specifically, the AK3-5 fluorescence intensity decrease was measured as a function of EC concentration upon simultaneous varying the DNA concentrations. The plots of the fluorescence intensity decrease *vs* EC concentration are presented in Fig.5.

The above dependencies were analyzed in terms of the simplified competition model (Eqs. 2-13) to calculate the association constants of the drug (K_{drug}) binding to the DNA in the presence of AK3-5. The calculated K_{drug} values were equal to 5.4×10^4 $M^{-1}$and 3.9×10^5 M^{-1} for V7 and V9, respectively. Moreover, the association constants of the drug were found to be independent of the DNA concentration in the tested sample.

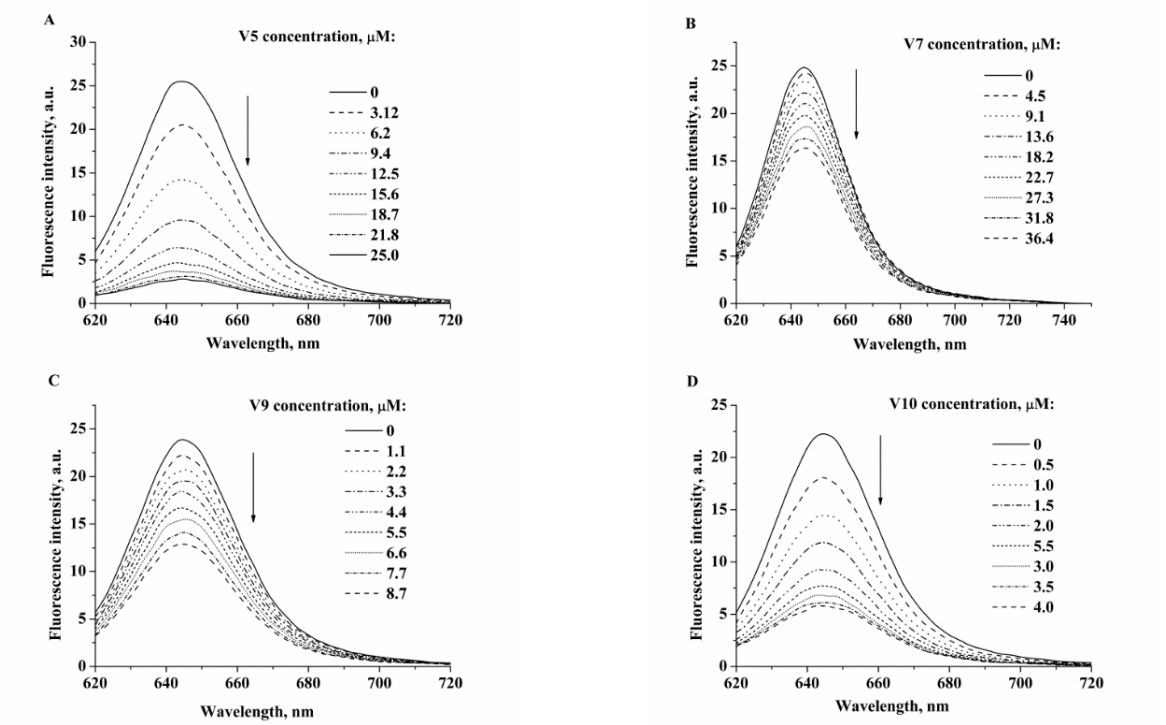

Fig. 4. Competitive displacement assays between the AK3-5 and V5(A), V7(B), V9(C) and V10 (D) in the presence of DNA. Dye concentration was 0.25 μM. DNA concentration was 9.3 μM.

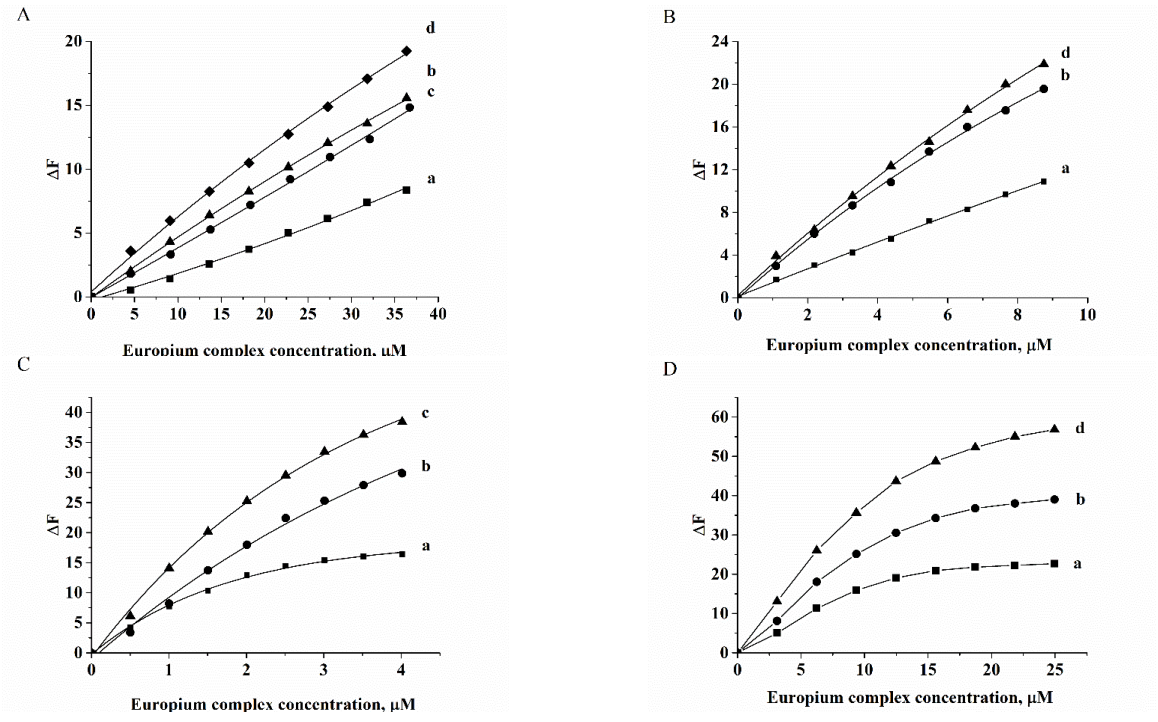

Fig.5. The fluorescence intensity decreases of AK3-5-DNA complexes in the presence of the increasing concentrations of V7 (A), V9 (B), V10 (C) and V5 (D). The concentrations of the DNA were (a) 9.3 μM, (b) 27.9 μM, (c) 37.6 μM, (d) 46.5 μM, respectively.

However, we failed to obtain the realistic value of the association constant K_{drug} for the europium complexes V5 and V10. Moreover, the theoretically calculated ΔF values (eq. 11) were significantly greater than those obtained from the experiment. Most likely, the competition between AK3/5 and Eu(III) complexes for the DNA binding sites is not the only reason for the observed fluorescence decrease in the presence of V5 and V10. In this respect, it seems of interest to draw attention to the shapes of the dependencies of ΔF on the EC concentration: being linear for the V7 and V9, it is almost hyperbolic for V5 and V10. The most probable reason for such a behavior is that besides the competition

between AK3/5 and EC for the DNA binding sites, V5 and V10 are capable of quenching the AK3-5 fluorescence. A more hydrophobic nature of V5 and V10 in comparison to V7 and V9 seems to account for a pronounced drop in the AK3-5 fluorescence intensity in the combined AK3-5/DNA/EC systems [33].

CONCLUSIONS

In conclusion, the present study was focused on the possible application of the novel trimethine cyanine dye AK3-5 in the displacement assay using the potential antitumor agents, europium coordination complexes as competitive ligands. The AK3-5-DNA/RNA binding studies provided evidence for the strong association of the fluorophore to nucleic acids, with the binding affinity being higher for the double-stranded DNA, in comparison with the single-stranded RNA. Upon addition of increasing concentrations of europium complexes, a gradual reduction in the fluorescence intensity of the dye was observed, indicating that EC are capable of displacing the AK3-5 from the DNA helix. The magnitude of the fluorescence intensity decrease was found to be more pronounced for V5 and V10 compared to V7 and V9. The observed effects were interpreted in terms of the different binding affinities of the europium complexes to the DNA. The assumption was made, that a more pronounced fluorescence intensity decrease of in the presence of V5 and V10 results from the EC ability to quench the AK3-5 fluorescence, along with the competition between AK3/5 and Eu(III) complexes for the DNA binding sites. Cumulatively, the results presented here strongly suggest that AK3-5 can be effectively used for the sensitive detection of the nucleic acids, as well as for the drug displacement assays.

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КОНКУРЕНТНЕ ЗВ'ЯЗУВАННЯ НОВОГО ЦІАНІНОВОГО БАРВНИКА АК3-5 ТА КООРДИНАЦІЙНИХ КОМПЛЕКСІВ ЄВРОПІЮ З ДНК

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У даній роботі проведено оцінку можливості використання нового триметинового барвника АК3-5 в якості конкурентного ліганду для протипухлинних препаратів, координаційних комплексів європію (ККЄ), у системах, що містили ДНК. Аналіз спектрів флуоресценції показав високу спорідненість АК3-5 до нуклеїнових кислот, причому ступінь взаємодії з подвійною спіраллю ДНК був вищий, ніж з одноланцюговою РНК. За допомогою моделі Мак-Гі і фон Хіппеля визначено термодинамічні параметри зв'язування ціанінового барвника з нуклеїновими кислотами. Константа асоціації АК3-5 з ДНК дорівнювала 5.1×10^4 M^{-1} , що відповідає значенню цього параметру для відомих ДНК інтеркаляторів. Зв'язування ціанінового зонда з РНК, у свою чергу, характеризувалось значно меншою константою асоціації ~ 3.4×10^3 M^{-1}, що вказує на електростатичне зв'язування або «часткову інтеркаляцію» як можливий механізм комплексоутворення. Додавання комплексів європію до системи АК3-5 – ДНК супроводжувалось падінням інтенсивності флуоресценції, причому величина цього ефекту залежала від структури ККЄ. Зменшення флуоресценції АК3-5, що спостерігалось у присутності комплексів європію V7 і V9, найімовірніше, вказує на конкуренцію між ціаніновим барвником і протипухлинними препаратами за сайти зв'язування з ДНК. Концентраційні залежності змін інтенсивності флуоресценції були проаналізовані в рамках моделі Ленгмюра, що дозволило отримати константи зв'язування 5.4×10^4 M^{-1} та 3.9×10^5 M^{-1} для комплексів європію V7 і V9, відповідно. Більш виражене падіння інтенсивності флуоресценції барвника у присутності V5 і V10, імовірно, свідчить про гасіння флуоресценції АК3-5 комплексами європію, окрім конкуренції між барвником та ККЄ за сайти зв'язування на ДНК, В цілому, отримані результати створюють передумови ефективного використання АК3-5 у дослідженнях нуклеїнових кислот та конкурентних взаємодій фармакологічних агентів і барвників з ДНК.

КЛЮЧОВІ СЛОВА: Триметиновий ціаніновий барвник, координаційні комплекси європію, протипухлинний препарат, ДНК, константа асоціації

КОНКУРЕНТНОЕ СВЯЗЫВАНИЕ НОВОГО ЦИАНИНОВОГО ЗОНДА АК3-5 И КООРДИНАЦИОННЫХ КОМПЛЕКСОВ ЕВРОПИЯ С ДНК

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В данной работе проведена оценка возможности использования нового триметинового красителя АК3-5 в качестве конкурентного лиганда для противоопухолевых препаратов, координационных комплексов европия (ККЕ), в ДНК-содержащих системах. Анализ флуоресцентных спектров показал высокое сродство АК3-5 к нуклеиновым кислотам, причем степень взаимодействия с двухцепочечной ДНК была выше, чем с одноцепочечной РНК. С помощью модели Мак-Ги и фон Хиппеля определены термодинамические параметры связывания цианинового красителя с нуклеиновыми кислотами. Константа ассоциации АК3-5 с ДНК была равна 5.1×10^4 M^{-1}, что соответствует значению этого параметра для известных ДНК интеркаляторов. Связывание цианинового зонда с РНК, в свою очередь, характеризовалось значительно меньшей константой ассоциации (~ 3.4×10^3 M^{-1}), указывая на электростатическое связывание либо «частичную интеркаляцию» как возможный механизм комплексообразования. Добавление комплексов европия к системе АК3-5 – ДНК сопровождалось снижением интенсивности флуоресценции, причем величина этого эффекта зависела от структуры ККЕ. Наблюдаемое уменьшение флуоресценции АК3-5 в присутствии комплексов европия V7 и V9, наиболее вероятно, указывает на конкуренцию между красителем и противоопухолевыми препаратами за сайты связывания с ДНК. Концентрационные зависимости изменения интенсивности флуоресценции были проанализированы в рамках модели Ленгмюра, что позволило получить константы связывания 5.4×10^4 M^{-1} и 3.9×10^5 M^{-1} для комплексов европия V7 и V9, соответственно. Более выраженное падение интенсивности флуоресценции триметинового красителя в присутствии V5 и V10, предположительно, обусловлено тушением флуоресценции АК3-5 комплексами европия, наряду с конкуренцией между красителем и ККЕ за сайты связывания на ДНК. В целом, представленные результаты создают предпосылки для эффективного использования АК3-5 как для исследования нуклеиновых кислот и конкурентного связывания фармакологических агентов и красителей с ДНК.

КЛЮЧЕВЫЕ СЛОВА: Триметиновый цианиновый краситель, координационные комплексы европия, противоопухолевый препарат, ДНК, константа связывания