МОЛЕКУЛЯРНА БІОФІЗИКА

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FLUORIMETRIC STUDY OF INTERACTION BETWEEN EUROPIUM COORDINATION COMPLEXES AND DNA

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Lanthanide coordination complexes have found numerous applications in a number of areas, including laser techniques, fluorescent analysis, biomedical assays. Likewise, they exhibit antitumor properties. Eu(III) tris-β-diketonato complexes (EC) are newly synthesized compounds with high anticancer activity. Despite extensive studies, the detailed mechanism of their biological effects is far from being resolved. Examining the interactions between EC and biological molecules in model systems is essential for deeper understanding of the mechanisms behind their biological activity. In the present work we employed fluorescent probe acridine orange (AO) to investigate EC-DNA interaction. AO-DNA binding was followed by the marked fluorescence increase detected at 530 nm. EC addition suppressed this fluorescent changes. EC were found to differ in their ability to modify AO-DNA interactions. EC4 and EC6 have demonstrated the most pronounced effect on AO-DNA binding. AO-DNA complexation occurs predominantly via intercalation mode. EC are large planar structures, whose DNA intercalating ability was reported to increase with the planarity of ligands. It seems likely that AO and EC can compete for the binding sites on DNA molecule.

KEY WORDS : DNA, lanthanide complexes, acridine orange

ФЛУОРИМЕТРИЧЕСКОЕ ИЗУЧЕНИЕ ВЗАИМОДЕЙСТВИЯ КООРДИНАЦИОННЫХ КОМПЛЕКСОВ ЕВРОПИЯ И ДНК

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Координационные комплексы европия широко применяются в различных областях, включая лазерную технику, флуоресцентный и биомедицинский анализ. Кроме того, они имеют антиопухолевые свойства. Eu(III) трис-β-дикетонато комплексы (ЕС) – новые препараты, имеющие высокую противораковую активность. Несмотря на многочисленные исследования, молекулярные аспекты их биологического действия до сих пор не охарактеризованы. Выяснение молекулярных механизмов взаимодействия ЕС с ДНК может открыть новые пути для создания эффективных лекарственных препаратов. В данной работе ЕС-ДНК комплексы исследовали с помощью флуоресцентного зонда акридинового оранжевого (АО). Добавление препаратов в систему, содержащую связанный с ДНК АО в разной степени повлияло на спектральные свойства зонда – наибольший эффект проявили EC4 и EC6. В связывании АО с ДНК важную роль играет механизм интеркаляции. ЕС – большие плоские структуры, для которых интеркаляция – наиболее вероятный способ связывания с нуклеиновыми кислотами. Следовательно, можно сделать вывод, что АО и ЕС конкурируют за места связывания на ДНК, причем наибольшим сродством обладает EC6.

КЛЮЧЕВЫЕ СЛОВА: ДНК, координационные комплексы европия, акридиновый оранжевый.

ФЛУОРИМЕТРИЧНЕ ДОСЛІДЖЕННЯ ВЗАЄМОДІЇ КООРДИНАЦІОННИХ КОМПЛЕКСІВ ЕВРОПІЮ ТА ДНК

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Координаційні комплекси європію широко використовуються в різноманітних областях, включаючи, лазерну техніку, флуоресцентний та біомедичний аналіз. Крім того, вони мають антипухлинні властивості. Eu(III) трис-β-дікетонато комплекси (ЕС) – новітні препарати, які мають високу протиракову активність. Незважаючи на численні дослідження, молекулярні аспекти їх біологічної дії до цього часу ще не охарактеризовані. З'ясування молекулярних механізмів взаємодії ЕС з ДНК може відкрити нові шляхи для створення ефективних лікарських препаратів. В даній роботі взаємодію ЕС з

ДНК досліджували за допомогою флуоресцентного зонду акридинового оранжевого (АО). Додавання препаратів до системи АО, зв'язанного з ДНК, по-різному вплинуло на спектральні властивості зонду – найбільший ефект проявили ЕС4 та ЕС6. У зв'язуванні АО з ДНК важливу роль відіграє механізм інтеркаляції. ЕС – це великі планарні структури, для яких інтеркаляція – найбільш вірогідний спосіб зв'язування з нуклеїновими кислотами. Отже, можна зробити висновок, що АО та ЕС конкурують за місця зв'язування на ДНК, причому найбільшу спорідненість має ЕС6.
КЛЮЧОВІ СЛОВА: ДНК, координаційні комплекси європію, акридиновий оранжевий.

Lanthanide coordination complexes have found numerous applications in a number of areas, including laser techniques, fluorescent analysis, biomedical assays [1-3]. Likewise, they currently attract increasing attention due to their pharmacological properties.

Eu(III) tris-β-diketonato complexes (EC) are newly synthesized compounds with high anticancer activity [4]. Despite extensive studies, the molecular details of their biological action are far from being resolved. Examining the interactions between EC and biological molecules in model systems is essential for deeper understanding of the mechanisms behind their biological activity. Interest in this area arises, in part, as a result of several advantages of small molecules as potential drugs, including the economics of their synthesis and their comparatively efficient delivery to cells [5]. Since the interaction between small molecules and DNA is in close relationship with their potential biological and pharmaceutical activities, elucidating the nature of EC association with DNA is very important in the development of new therapeutic agents for numerous diseases. Recent progress in the structure-based design of small molecules targeted toward specific DNA sequences show that the promise of this area as a source of novel therapeutic agents is beginning to be realized.

To characterize EC-DNA complexes fluorescent probe acridine orange has been employed.

MATERIALS AND METHODS

Cattle spleen DNA was from Reakhim (Russia). Eu(III) coordination complexes (Fig. 1) were synthesized as described previously [4]. Acridine orange (AO) was purchased from Sigma (St. Louis, USA). All other chemicals were of analytical grade.

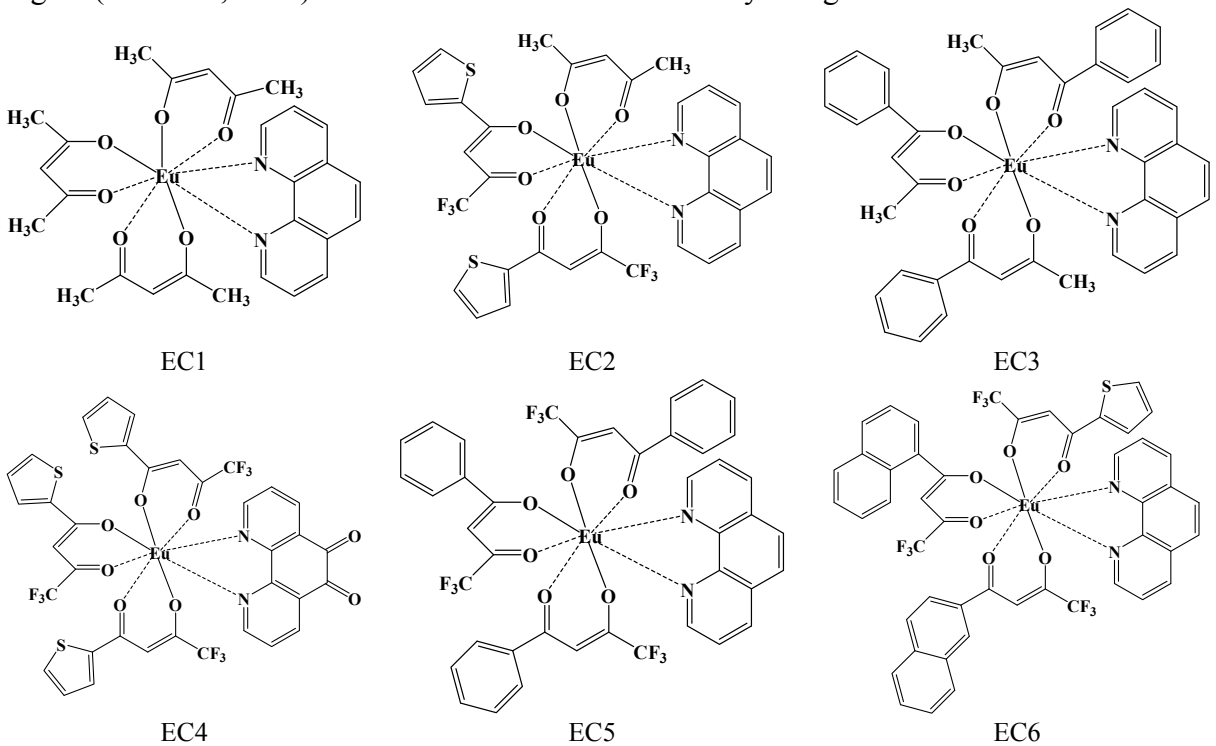


Fig. 1. Structure of lanthanide complexes

Adsorption isotherms were obtained using fluorescence spectroscopy techniques. Fluorescence measurements were performed with a CM 2203 (SOLAR, Belarus) and Perkin Elmer LS55 (Beaconsfield, UK) spectrofluorimeters. Absorption measurements were conducted using an SF-46 spectrophotometer (LOMO, Russia) against solvent blanks. All experiments were performed in 5 mM sodium phosphate buffer, pH 7.4, at room temperature. Fluorescence spectra of acridine orange were recorded at the excitation wavelength of 490 nm. The AO concentration was determined spectrophotometrically using extinction coefficient $\varepsilon_{494}^{AO} = 5.6 \times 10^4$ $M^{-1}cm^{-1}$.

Quantitative parameters of AO binding to DNA were determined by analyzing the dependencies of probe fluorescence changes upon varying DNA concentration. It was assumed that the observed fluorescence changes ΔF is proportional to the concentration of bound probe B_{dye}:

$$\Delta F = aB_{dye}, \tag{1}$$

where a is a coefficient of proportionality equal to difference between molar fluorescence of AO bound to DNA and free in solution. If one phosphate contains n probe binding sites, the association constant (K_b) can be written as:

$$K_b = \frac{B_{dye}}{Z_f\left(nP - B_{dye}\right)} = \frac{B_{dye}}{\left(Z_0 - B_{dye}\right)\left(nP - B_{dye}\right)}, \tag{2}$$

where P is the total DNA phosphate concentration, Z_f is the concentration of free probe, Z_0 is total probe concentration.

Accordingly, Eq. (1) can be rearranged to give:

$$\Delta F = 0.5a\left(Z_0 + nP + \frac{1}{K_b} - \sqrt{\left(Z_0 + nP + \frac{1}{K_b}\right)^2 - 4Z_0 nP}\right), \tag{3}$$

The binding parameters (K_b and n) were derived by the fitting procedure involving minimization of the function:

$$f = \frac{1}{N}\sum_{i=1}^{N}\left(\Delta F_{\exp} - \Delta F_t\right)^2 \tag{4}$$

where $\Delta F_{\exp}$ is experimental value, ΔF_t is ΔF calculated according to Eq. (3), N is the number of experimental points.

In the case, when there are two competitive binding sites, determination of binding parameters comes to solving the system of two following equations:

$$K_b - \frac{B_{dye}}{\left(Z_0^{dye} - B_{dye}\right)\left(nP - B_{dye} - B_{drug}\right)} = 0,\ K_{drug} - \frac{B_{dye}}{\left(Z_0^{drug} - B_{drug}\right)\left(nP - B_{dye} - B_{drug}\right)} = 0, \tag{5}$$

where K_{drug} is association constant of EC binding to DNA, B_{dye} and B_{drug} are concentrations of DNA-bound AO and EC, respectively. Parameters K_b and n were determined as described previously, K_{drug} was derived by the fitting procedure involving minimization of the function:

$$f = \frac{1}{N}\sum_{i=1}^{N}\left(\Delta F_{\exp}^{EC} - \Delta F_t^{EC}\right)^2, \qquad \Delta F_t^{EC} = a\left(B_{dye}^0 - B_{dye}^t\right) \tag{6}$$

where $\Delta F_{\exp}^{EC}$ is experimental value (decrease of AO fluorescence in the presence of EC).

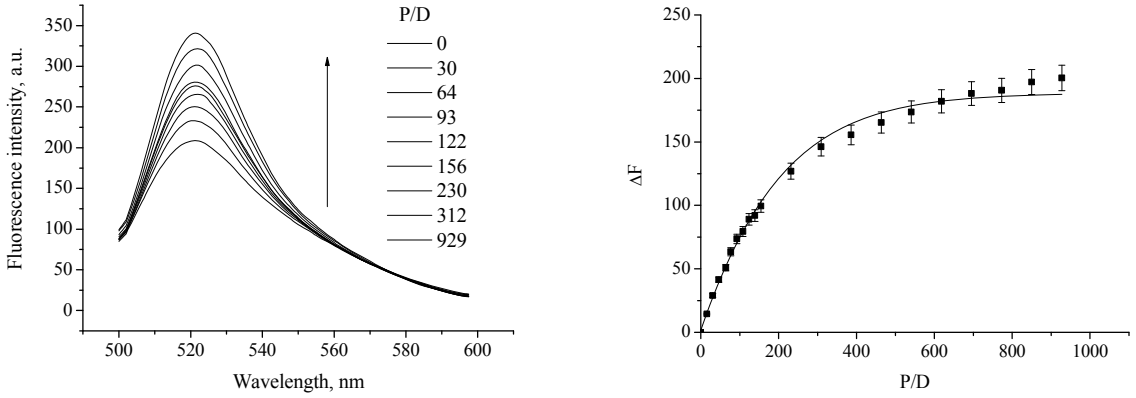
where B_{dye}^t is B_{dye} calculated according to Eq. (5), B_{dye}^0 is B_{dye} calculated according to Eq. (2) with K_b and n obtained for the system containing only AO and DNA.

To investigate EC effect on AO-DNA binding two experimental schemes were applied: i) AO was incubated with DNA and this mixture was subsequently titrated with EC

(experiments at high P/D ratios), ii) the dye was mixed with EC solutions prior to DNA addition (low P/D ratios). EC concentrations in this case were 53 μM (EC1), 38 μM (EC2), 41 μM (EC3), 33 μM (EC4), 34 μM (EC5), 33 μM (EC6).

RESULTS AND DISCUSSION

Small molecules can interact with the double helical DNA via three binding modes: intercalation, groove binding, and external ion pairing. Among these, intercalation is one of the most important DNA-binding mode as it invariably leads to cellular degradation.



A B

Fig. 2. Fluorescence spectra of AO in the presence of DNA (A) and the isotherm of AO binding to DNA (B). AO concentration was 0.0275 μM.

AO is a commonly used DNA probe, it can specifically bind to DNA [6] and cause DNA degeneration [7]. AO binds to DNA to form two types of complexes depending on the DNA phosphate-to-dye ratio (P/D) [8]. Complex 1, formed at high P/D ratios, corresponds to intercalation of the AO planar aromatic ring between the base pairs of double helical DNA [9]. This strong binding mode is stabilized from stacking interactions between the intercalator and base pairs [10]. Complex 2 results from external binding where electrostatic forces between AO and DNA phosphates or guanine bases play an important role [11]. Bound dye molecules predominantly intercalate into DNA resulting in alteration of DNA helix. This DNA alterations can prevent intercalation of subsequent AO molecules into DNA. Under these conditions some part of AO molecules can be externally bond to DNA. Likewise, some part of AO molecules can be partially intercalated into DNA. In this case either one of two dimethylamino residues of AO molecule can partially intercalates between base pairs and the acridine ring of AO molecule remained outside of DNA helix [12]. Decreasing P/D values causes AO monomers to associate into dimers and higher order aggregates [13].

Table 1

EC-DNA binding parameters	
	K_{drug}, M^{-1}
EC1	$(5.0\pm1.0)\times10^3$
EC2	$(5.0\pm1.0)\times10^3$
EC3	$(1.0\pm0.2)\times10^4$
EC4	$(2.9\pm0.6)\times10^4$
EC5	$(1.1\pm0.2)\times10^4$
EC6	$(4.3\pm0.8)\times10^4$

AO-DNA binding was followed by the marked fluorescence increase detected at 520 nm, ΔF (Fig. 2). The complexation of AO to DNA was analyzed in terms of Langmuir binding model (Eq. 1 – 4). Since intercalation seems to be predominant mechanism in AO-DNA binding, while analyzing the results obtained we restricted ourselves only to this mode of dye – nucleic acid association. The obtained association constant was $K_b = (1.28 \pm 0.26) \times 10^5$ M^{-1}. Our results are in a good accordance with other estimates of AO-DNA binding constant [14]. It should be noted that data analysis in terms of McGhee & Hippel adsorption model yields association constant of 1.2×10^5, the value comparable to the above estimates [15].

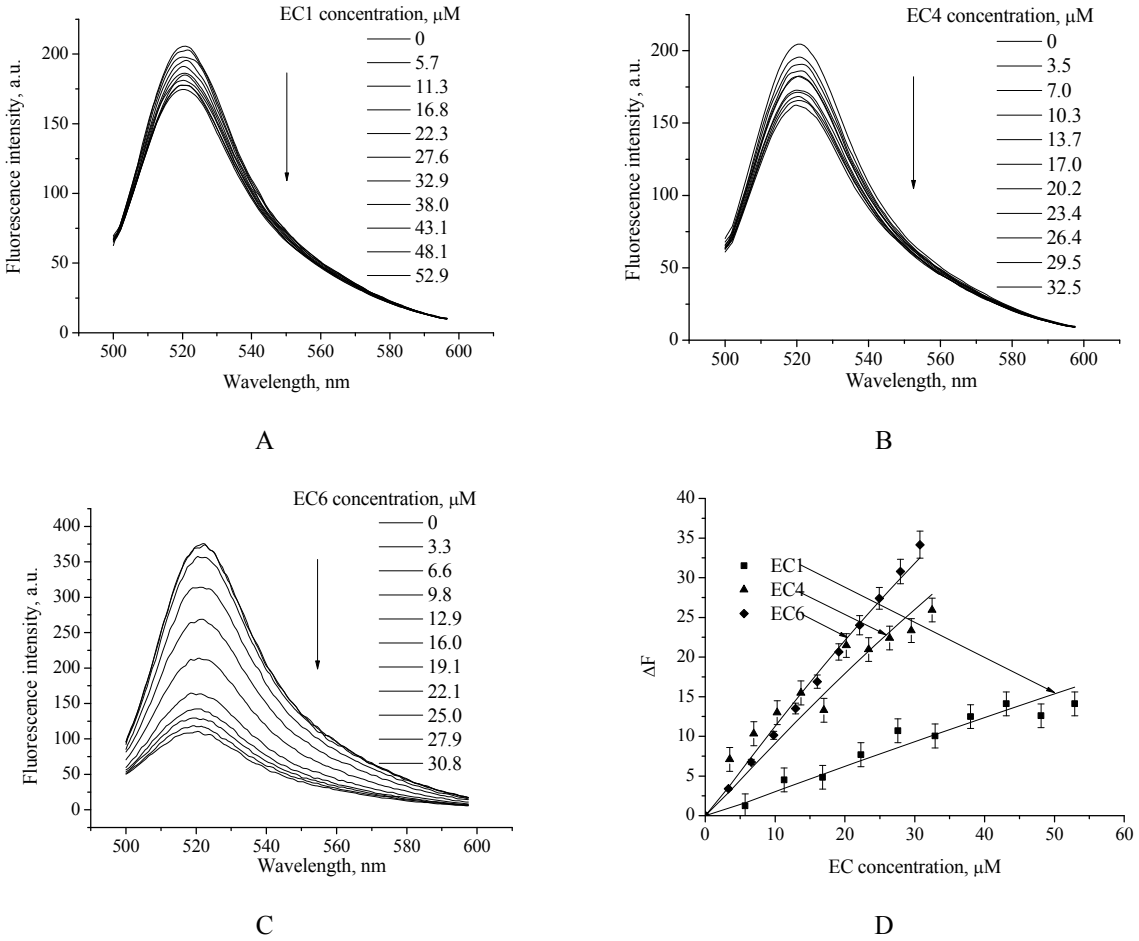


Fig. 3. Fluorescence spectra of AO-DNA systems in the presence of EC (A, B, C). EC effect on AO-DNA binding (D). DNA and AO concentrations were 17.18 μM and 0.025 μM, respectively.

EC addition to AO-DNA systems (P/D = 688) results in decrease of AO fluorescence (Fig. 3A, B, C). Obtained EC-DNA binding isotherms (Fig. 3D) were analyzed in terms of Langmuir binding model for two competitive binding sites on DNA molecule. Thermodynamic binding parameters are presented in Table 1. As seen from Table 1, the compounds under study were found to differ in their ability to bind to DNA. EC4 and EC6 have demonstrated the most pronounced affinity to DNA. EC are large planar structures, whose DNA intercalating ability was reported to increase with the planarity of ligands. It seems likely that AO and EC can compete for the binding sites on DNA molecule.

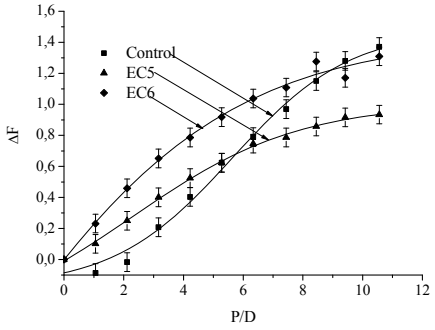


Fig. 4. The isotherms of AO binding to DNA in the presence and absence of lanthanide complexes. AO concentration was 4.4 μM.

Notably, the association of AO with DNA at low P/D value (P/D < 10) was featured by sigmoidal adsorption isotherms (Fig. 4). In this case AO binds to DNA via electrostatic mode and tends to associate into dimers. The formation of AO dimers on DNA matrix may be the reason for the observed sigmoidal shape of AO-DNA binding isotherms. EC5 and EC6 have demonstrated the most marked effect on AO-DNA binding. Moreover, in the presence of EC the binding curve proved to change its shape from sigmoidal to

hyperbolic. Since EC are uncharged molecules, they unlikely to occupy the binding sites for cations on DNA.

CONCLUSIONS

The key findings of the present study can be summarized as follows:

1. The acridine orange associating with DNA via intercalation mechanism at P/D > 10 is capable of competing with Eu(III) complexes for DNA binding sites.

2. EC can modify AO-DNA electrostatic interaction at P/D < 10 possible via alterating DNA structure during drug intercalation into DNA.

3. Eu(III) compounds display ability to intercalate between DNA base pairs, with the binding affinity being dependent on EC chemical structure. EC with the greatest number of adjacent aromatic rings (EC6) was featured by the highest DNA-associating propensity.

The results obtained may prove useful for understanding EC pharmaceutical activity and design of new promising drugs.

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