• *Փորձարարական և տեսական հոդվածներ* •*Экспериментальные и теоретические статьи*•
•*Experimental and theoretical articles*•

PECULIARITIES OF ACRIDINE ORANGE BINDING WITH DNA

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Absorption and fluorescence spectra of acridine orange (AO) complexes with DNA at 0.02 M ionic strength of the solution have been obtained. It was revealed that maximums of absorption spectra of AO complexes with DNA at low concentrations of DNA decrease and are shifted to longer wavelength region. At further enhancement of DNA concentration the absorption spectra of AO-DNA complexes start increasing. Moreover, in the spectra of AO-DNA complexes an isosbestic or pseudo-isosbestic point does not emerge. At the same conditions the fluorescence spectra of the complexes enhance as compared to AO fluorescence spectrum, which indicates the intercalation binding mode of AO with DNA.

DNA – acridine orange – AO-DNA complexes – absorption spectra – fluorescence spectra

Ստացվել են ԴՆԹ-ի հետ ակրիդին նարնջագույնի (ԱՆ) կոմպլեքսների կլանման և ֆլուո-րեսցենցիայի սպեկտրները լուծույթի 0.02 Մ իոնական ուժի պայմաններում: Ցույց է տրվել, որ ԴՆԹ-ի հետ ԱՆ-ի կոմպլեքսների կլանման սպեկտրների մաքսիմումները նվազում են ԴՆԹ-ի փոքր կոնցենտրացիաններից դեպքում և շեղվում են դեպի երկարալիք տիրույթ: ԴՆԹ-ի կոնցենտրացիայի հետագա աճի դեպքում ԱՆ-ԴՆԹ կոմպլեքսների կլանման սպեկտրները սկսում են աճել: Ընդ որում, ԱՆ-ԴՆԹ կոմպլեքսների սպեկտրների վրա իզոբեստիկ կամ պսևդոիզոբեստիկ կետեր չեն հայտնվում: Միևնույն պայմանների դեպքում կոմպլեքսների ֆլուորեսցենցիայի սպեկտրները աճում են ԱՆ-ի ֆլուորեսցենցիայի սպեկտրի համեմատ, ինչը վկայում է ԴՆԹ-ի հետ ԱՆ-ի կապման ինտերկալյացիոն եղանակի մասին:

ԴՆԹ – ակրիդին նարնջագույն – ԱՆ-ԴՆԹ կոմպլեքսներ – կլանման սպեկտրներ – ֆլուորեսցենցիայի սպեկտրներ

Получены спектры поглощения и флуоресценции комплексов акридинового оранже-вого (АО) с ДНК при ионной силе раствора 0.02 М. Выявлено, что максимумы спектров по-глощения комплексов АО с ДНК при низких концентрациях последней уменьшаются и пре-терпевают сдвиг в длинноволновую область. При дальнейшем увеличении концентрации ДНК спектры поглощения комплексов АО-ДНК начинают увеличиваться. При этом в спект-рах АО-ДНК изобестическая или псевдоизобестическая точка не образуется. При тех же условиях спектры флуоресценции комплексов возрастают по сравнению со спектром флуо-ресценции АО, что указывает на интеркаляционный способ связывания АО с ДНК.

ДНК – акридиновый оранжевый – комплексы АО-ДНК – спектры поглощения – спектры флуоресценции

Nowadays biomacromolecules (DNA, proteins) are targets for numerous studies connected to designing of nano-particles and nanotechnologies on their basis. DNA molecule is one of the main elements in the development of new nano-materials [7, 11].

It is connected to DNA physical-chemical peculiarities and ability to self-assemble and form highly ordered structures from separate nano-particles. Besides, DNA

carries negative charge (is poly-anion), possesses certain rigidity (in short areas) [5, 7, 11, 12, 18].

Many organic dyes (acridine orange (AO), ethidium bromide (EtBr), methylene blue (MB) etc.) are biologically active compounds, since they can perform a photo-dynamic effect. These phenomena can occur due to binding of the mentioned dyes with DNA [5, 7, 11, 12, 18]. Particularly, AO (scheme 1) is applied as a fluorescence dye and is intensively used for DNA staining in cell to study apoptosis in the latter [8]. The interest to acridine dyes is conditioned by the fact that these compounds have different properties. They have anti-bacterial or mutagenic activity. Their structure and ability to bind with other biological molecules, including DNA, condition their cancerogenic properties [7, 11]. Besides, several dyes, including AO, show metachromism, which lies in the basis of wide application of this ligand in cytochemistry [4]. These properties of AO can be the result of different binding modes with DNA.
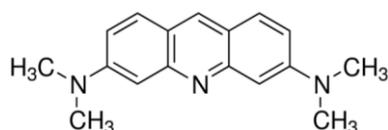
Scheme 1. Structure of AO.

The aim of this work is to study the binding of AO to DNA by absorption and fluorescence spectroscopy methods for revelation of interaction molecular mechanisms.

Materials and methods. Calf thymus DNA – D-1501 ("Sigma", USA), AO ("Sigma", USA), NaCl, Na-citrate, EDTA (ethylenediamintetraacetate) (chemically pure) were used in experiments. Preparations were used without further purification. Concentrations of DNA and AO were determined spectrophotometrically using the following values of extinctions: ε_{260}=6600 $M^{-1}cm^{-1}$ for DNA c.t. and ε_{490}=35000 $M^{-1}cm^{-1}$ for AO. Experiments were carried out at t=25^{0}C and pH=7.0, I=0.02 M.

Spectrophotometric measurements were carried out on double-beam spectrophotometer UV-VIS Unicam-SP8-100 (England) and single-beam spectrophotometer UV-VIS Jenway 6715. Absorption measurements were realized in quartz cuvettes with 1 cm optic pathway length and similar optic parameters. Spectrophotometric titration of solutions was realized by 1 μl total volume micropipette ("Hamilton", USA). Fluorescence measurements were carried out on Varian Cary Eclipse Fluorescence spectrophotometer (Australia).

Results and Discussion. Alterations in the absorption spectra of ligands at their interaction with DNA really reflect those peculiarities that lie in the basis of molecular mechanisms of these interactions. Particularly, at complex-formation of some intercalators with DNA, one or more isosbestic points (IP) can be formed in the absorption spectra [13-15]. Though the isosbestic point formed in spectra of different compounds, in majority of cases, is rarely applied for qualitative or quantitative analysis of information contained in studying system [2, 3, 9, 10, 16, 17]. It is accepted to consider that IP emerges when one existing compound in the solution, which has certain absorption, transits to other one with other absorption; moreover, the spectra of these forms cross in one point (or points) which is called isosbestic [2, 3, 9, 10, 16, 17]. From this point of view, in the case of classical intercalator EtBr all the mentioned representations are entirely maintained, because this ligand along with intercalation and electrostatic modes, binds to DNA by semi-intercalation mode as well [13-15]. Simultaneous exhibition of these modes conditions the presence of the isosbestic point in the spectra of DNA-EtBr complexes.

From this point of view, it was considered earlier that the presence of IP in the absorption spectra of DNA-ligand complexes is a sufficient argument for benefit of the intercalation binding mechanism of the given ligand with DNA. Moreover, our studies show that the presence of IP in the spectra of DNA-ligand complexes cannot be considered as a sufficient condition for revelation of the intercalation mode. Particularly, AO is an intercalator. It is indicated by the fluorescence spectra of the complexes of this ligand with DNA that are presented in fig. 1.
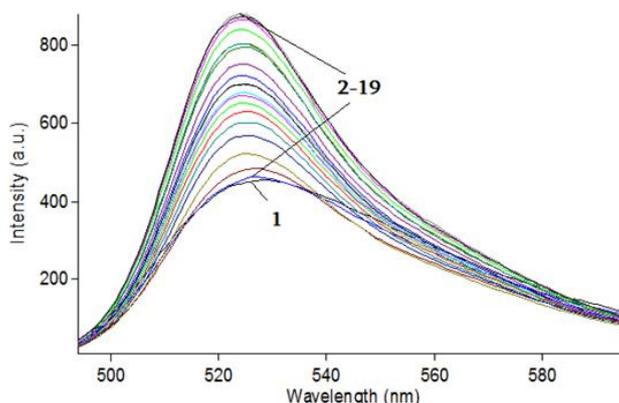

Fig.1. Fluorescence spectra of AO (1) and its complexes (2-19) with DNA at 0.02 M ionic strength of the solution.

It is obvious from the presented figure that the fluorescence spectra of AO-DNA complexes (curves 2-19) rise with enhancement of DNA concentration in the solution as compared to AO spectrum (curve 1). This fact is conditioned both by parallel orientation of this ligand molecules to planes of DNA base pairs (intercalation between planes of these pairs) and by interaction through stacking contacts. Moreover, in intercalated state molecules of AO are thoroughly screened from water (fluorescence quencher) [6]. It should be mentioned that in the case of MB, which also belongs to acridine dyes, analogous phenomenon is not observed [1], which in turn is conditioned by the fact that MB is not entirely intercalated into DNA. We assume that the increase of AO fluorescence intensity at complex-formation with DNA is the result of intercalation binding mode of this ligand.

Absorption spectra of AO-DNA complexes in the wavelength interval $220 \leq \lambda \leq 600$ nm are presented in fig. 2. It is obvious from fig. 2 that IP is not formed in the absorption spectra of these complexes.

It should be mentioned that the absorption spectra of AO-DNA complexes were obtained against DNA spectra, moreover, its concentration in the presence and absence of AO is similar (differential spectra). From the presented figure it is obvious that with DNA concentration enhancement in the solution the absorption spectra of the complexes (curves 2-26) decrease in minimums at $400 \leq \lambda \leq 600$ nm and are shifted to longer wavelength region as relative to free AO spectrum (curve 1). It should be also noted that at DNA low concentrations a monotonous decreasing of maximums of the spectra of complexes take place, moreover, in these conditions a relevant shift to longer wavelengths does not occur (this effect is not clearly reflected in the spectra due to covering). With further enhancement of DNA concentration in the solution a reverse image is observed – maximums in the absorption spectra of the complexes start increasing and shifting to longer wavelengths by 15-20 nm in the mentioned interval of changes.
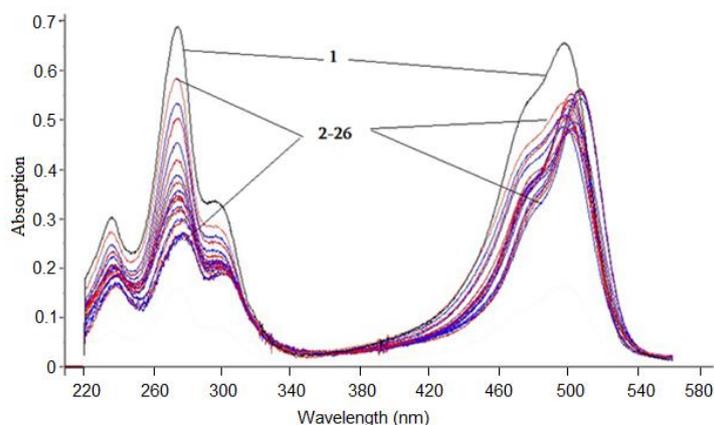
Fig.2. Absorption spectra of AO (1) and its complexes with DNA (2-26)
at 0.02 M ionic strength of the solution.

At the same time in the region of λ=220-340 nm, at λ=275-280 nm the absorption spectra of the complexes decrease monotonously. This effect emerges along whole interval of DNA concentration enhancement and indicates that the forming complexes have less absorption as compared to free AO absorption. We assume that the displayed hypochromism effect at short wavelengths (in UV region) is a result of parallel arrangement (intercalation) of chromophore groups of AO molecules bound to DNA. In the absence of DNA only dilution of AO solution takes place, which results in less decreasing of maximums of the spectra that is not comparable to hypochromism effect (spectra are not presented). On the basis of behavior of the absorption spectra of AO complexes with DNA it is concluded that the absorption intensity decreasing at λ=275-280 nm and enhancement of maximums at λ=500-520 nm are conditioned by intercalation binding mode.

Based on the fact that in λ=400-600 nm interval maximums of the spectra of the complexes decrease at relatively low concentrations of DNA, we assume that AO binds to DNA by more than one mode. Being a cationic in the solved state, the more probable mechanism of this mode is electrostatic. Moreover, the other – for instance, stack-like mode (this binding type corresponds to dimerization) by external side of DNA helix is not excluded, at which in the fluorescence spectra minor peaks at longer wavelengths relative to the main peak should appear. Such minor peaks are absent in the fluorescence spectra presented in fig. 1 [6]. Consequently we assume that the external stack-like binding does not appear in our experiment conditions, but the second – non-intercalation mode is electrostatic mechanism at which positively charged ligand molecules interact with negatively charged phosphate groups of DNA.

Therefore, the obtained data indicate that AO binds to DNA by two modes – intercalation and electrostatic. It is also revealed that in the absorption spectra of AO-DNA complexes IP is not formed despite the intercalation binding mode of this ligand. Based on this it is concluded that this mode is not a reason of IP formation in the spectra of some intercalators at binding with DNA. Most probably, in the spectra of these intercalators the peaks emerging at longer wavelengths compared to the main peak are the result of intercalation mode performance.

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