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PEQULIARITIES OF INTERACTION OF ETHIDIUM BROMIDE WITH DIFFERENT GC-CONTENT DNAS

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In this work the formation of the ligand-DNA complexes by different modes using absorption and fluorescence spectroscopy methods has been investtigated. Comparison of the obtained results has revealed the existence of two “strong” types of binding modes one of which shows fluorescent signal and therefore represents fully intercalated geometry. Second type of “strong” interaction has no fluorescent signal. The nonfluorescent binding corresponds to the ligand hemi intercalative strong binding sites on DNA, where fluorescence of EtBr is quenched. Nonfluorescent complexes are formed in the minor groove of DNA with a significant affinity to GC-pairs.

DNA – ethidium bromide (EtBr) - intercalation – hemi intercalation – fluorescent complex – nonfluorescent complex

Աշխատանքում կլանման և ֆլուորեսցենտային սպեկտրոսկոպիայի մեթոդներով հետազոտվել է ԴՆԹ-լիգանդ կոմպլեքսների տարբեր եղանակներով առաջացումը: Ստացված արդյունքների համեմատությունից բացահայտվել է, որ գոյություն ունի ,ուժեղ կապման եղանակի երկու տիպ, որոնցից մեկն ունի ֆլուորեսցենտային ազդակ և ինտերկալյացիոն երկրաչափություն: ,Ուժեղ փոխազդեցության երկրորդ տիպը չունի ֆլուորեսցենտային ազդակ: Չֆլուորեսցենցող կապումը համապատասխանում է ԴՆԹ-ի վրա լիգանդների կիսաինտերկալյացիոն եղանակով կապման տեղերին, որտեղ ԷԲ-ի ֆլուրեսցենցիան մարում է: Չֆլուորեսցենցող կոմպլեքսները առաջանում են ԴՆԹ-ի փոքր ակոսում և նշանակալից խնամակցություն ունեն GC -զույգերի նկատմամբ:

ԴՆԹ — էթիդիումի բրոմիդ (ԷԲ) — ինտերկալյացիա — կիսաինտերկալյացիա — ֆլուրեսցենցող կոմպլեքս — չֆլուորեսցենցող կոմպլեքս

В данной работе исследовано образование ДНК-лиганд комплексов разными способами с помощью методов абсорбционной и флуоресцентной спектроскопии. Сопоставление полученных результатов выявило существование двух типов «сильного» способа связывания, один из которых имеет флуоресцентный сигнал и представляет собой интеркаляционную геометрию. Второй тип «сильного» взаимодействия не имеет флуоресцентного сигнала. Нефлуоресцирующее связывание соответствует местам связывания лиганда на ДНК полуинтеркаляционным способом, где флуоресценция БЭ тушится. Нефлуоресцирующие комплексы образуются в малом желобке ДНК и обладают значительным сродством к GC-парам.

ДНК – бромистый этидий (БЭ) – интеркаляция – полуинтеркаляция – флуоресцирующий комплекс – нефлуоресцирующий комплекс

The binding parameters of the intercalator etidium bromide (EtBr) with different GC-content DNAs have been determined by absorption and fluorescence methods. The obtained experimental data provided a clear evidence of existence of fluorescent and nonfluorescent "strong" complexes at the low bound EtBr with DNA base pairs ratio ($r< 0.25$). The binding constant (K) and the number of base pairs corresponding to a binding site (binding site size, n) of the first (K_f and n_f) and second (K_{nf}, n_{nf}) interactions have been calculated. The total number of the binding site size $n \approx 1,5$ and $nf \approx 2$ registrated by absorption and fluorescent methods respectively have been independent of the GC-content whereas n_{nf} changed with variation of GC-portion for nonfluorescent mode of interaction. The fluorescent complex corresponds to intercalative model. The nonfluorescent complex is externally bound form of EtBr which exists at bow occupancy ratios and is not the same as the electrostatically stabilized external bound with phosphate group one.

DNA is regulated and affected by many different types of molecules that interact with this biopolymer in various ways. Specifically, intercalative binding by small, planar, cationic aromatic molecules is one of several modes of such binding. The recent investigations showed that majority of intercalators might form more than one type of complexes with DNA (multimodal ligands). Ethidium bromide (EtBr) is one of the prominent representative of the multimodal ligads, which at small drug to DNA base pair ratio (r) values may form two types of "strong" complexes with DNA, one of which has been proposed to be in a stably bound external binding mode and another one is in the well described intercalation mode [2,7,11]. It has been shown that this external binding mode depended on the concentration of counter-ions (Na^+), concentration of the ligand and appeared more frequently in GC-regions of DNA. Existence of the significant affinity to the GC-regions led us to an assumption that different number of binding sites for this ligand on different GC-content DNA are detectable.

In this work absorption and fluorescence spectroscopy methods were employed to provide direct evidence of in formation of different modes of the ligand-DNA complexes. Comparison of the obtained results provided a clear evidence of existence of two "strong" types of binding modes one of which showed fluorescent signal and therefore represented fully intercalated geometry. Second type of "strong" interaction had no fluorescent signal providing the clue for external binding mode. It is suggested that the nonfluorescent strong complex is formed by hemi intercalation of in the minor groove of DNA. This complex is stabilized by hydrogen bonds between the ligand and nucleophilic centers of guanine which is exposed and accessible for the ligand in the minor groove.

Earlier we have shown that at low ligand concentrations EtBr may form two "strong" fluorescent and nonfluorescent complexes with B-form of DNA. The fluorescent complexes were referred to the "strong" one and corresponded to intercalation of this ligand which includes the insertion of the planar phenanthridium ring into the hydrophobic region of double stranded DNA (ds-DNA) base pairs [13]. The nonfluorescent binding corresponded to the ligand hemi intercalative strong binding sites on DNA, where fluorescence of EtBr was quenched. It has been suggested that nonfluorescent complexes formed in the minor groove of DNA with a significant affinity to GC-pairs.

It is revealed the relationship between the GC-content of DNA and binding modes of EtBr we determined the binding parameters of EtBr molecules using the absorption method detecting the total concentration of adsorbed on DNA molecules of the dye and the fluorescence method showing the amount of intercalated in the double helix molecules [6,10].

Materials and methods. Different GC-content DNAs (Cl. perf., 32%GC; calf thymus DNA(CT-DNA) 42%GC and M. lysod., 72%GC) and EtBr were purchased from Sigma Chemical Co. and used without further purification. Other chemicals used were of analytical grade. Solutions were prepared with double distilled water. Experiments were carried out as previously reported [11].

Hydrodynamic and fluoremetric experiments show that intercalation process is realized by hydrophilic transfer of EtBr from solvent to the binding site on ds-DNA. These models of interactions are accomplished exclusively at low ligand concentrations ($r< 0,25$) and referred to the "strong" mode of fluorescent and nonfluorescent complexes, therefore the experiments here were carried out at $0 \leq r \leq 0,25$.

Results and Discussion. The experimental results of investigation of effect of GC-content of DNA on EtBr binding process are represented on Fig. 1.

Parameter r vs. free dye concentrations (C_f) has been calculated using both the absorption method for total concentration of bound molecules (curve1) and fluoremetric method for intercalated EtBr molecules as r_f free ligand concentration C_f (curve 2). The binding curves obtained by two independent methods are different. The discrepancy between these curves can be explained by the presence of nonfluorescent EtBr molecules registered by the absorption technique. The binding curves for the nonfluorescent complex as r_{nf} vs. C_f (curve 3) were obtained by subtracting curve 2 from 1 at equal free dye concentrations C_f.

The binding parameters of EtBr fluorescent and nonfluorescent complexes have been obtained by Scatchard analysis of the absorption and fluorescence spectra (Table 1.).

Table 1. Thermodynamic parameters of the complexes of EtBr with different GC-content DNAs. Ionic strength μ =20mM Na^+; ρ H6,9; t=25^0C

DNA / Mettod	Cl.perfinges		CT		M. lysod	
	$K \times 10^{-5}$, M^{-1}	n, b.p.	$K \times 10^{-5}$, M^{-1}	n, b.p.	$K \times 10^{-5}$, M^{-1}	n, b.p.
Absorption	9,3± 0,10	10,0	1,2,0± 0,15	9	1,1± 0,12	10
Fluorescent	8,9± 0,10	12,0	1± 0,15	11	1± 0,12	12
Nonfluorescent	6,1± 0,06	5,0	6,5± 0,07	5	6,4± 0,08	5

The data of Table 1 show that the binding constants and stoichiometry of the complexes estimated by absorption method are practically independent of the GC-content and correlated with the data published in the literature [6,13]. The binding site of the fluorescent complexes $n \cong 2,2 \pm 0,1$ is also in accordance with experimental data available in literature [1,3].

The binding parameters of the nonfluorescent complexes listed in Table 1 show that the binding constant K_{nf} for low and high GC-content DNAs are the same ($K_{nf} \cong 0,8 \cdot 10^5 M^{-1}$) whereas this parameter noticeable higher ($r=1,2 \cdot 10^5 M^{-1}$) for CT DNA which may reflect the base pair distribution differences in CT DNA (blocks of this DNA can be considered having random base pair distribution) while the Cl.perf and M.lysod DNAs may consist of AT and GC clasters respectively, the structure of which will differ from that of DNA with random base pair distribution.

It is known that sequence specificity may be dominated by ability to select binding sites on the bases of groove width allowing optimum van der Waals' complimentary and hydrophobic surface burial, rather than specific hydrogen bonding interactions with the groove floor [4]. Therefore we registrated weak affinity of EtBr for Cl.perf and M.lysod DNAs.

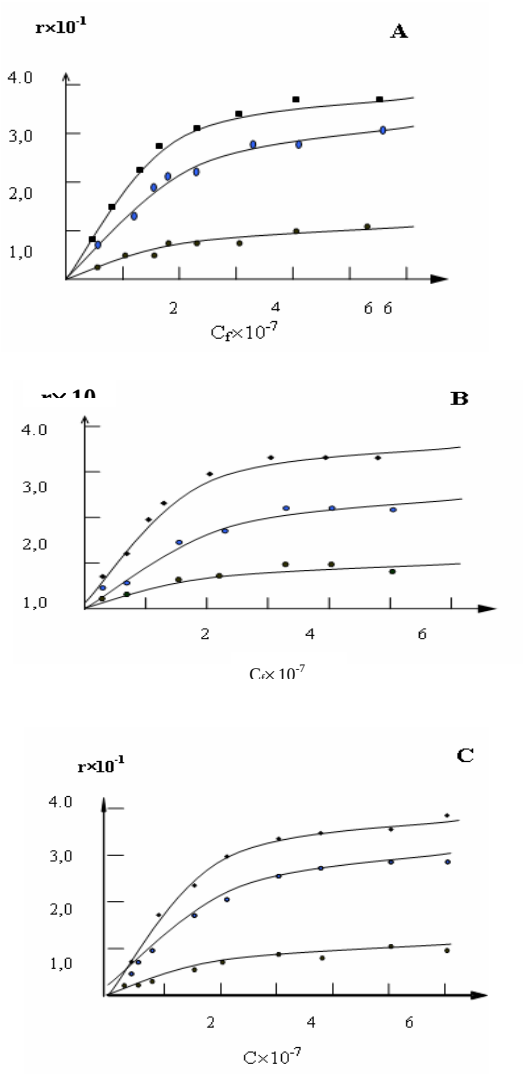

Fig. 1. Adsorption isotherms of EtBr on different GC-content DNAs based on the analyses of absorption (curves 1, r vs. C_f) and fluorescent (curves 2, $r_f$vs.C_f) spectra of the complexes. Curves 3 (r_n vs C_f) were calculated for nonfluorescent complexes by subtraction of curves 2 from curves 1 at the same C_f values.
Cl.perfr.(A); CT (B); Mlys (C). μ =20mMNa^+, ρ H 6,9,4=25^{0}C.

Hydrodynamic and fluoremetric experiments show that at low ligand concentrations intercalation process realized by hydrophobic transfer of EtBr from solvent to binding site where complex stabilized by non covalent molecular interactions (e.g. hydrogen bonds and van der Waals interactions) and is independent on ionic strength of solution [12]. On the contrary according to our experimental data nonfluorescent strong complexes are very sensitive to the environment ionic composition and completely abolished of high concentration of Na^+ (results are not presented).

The above-mentioned experimental data reveal two strong binding sites on DNA, where fluorescent and nonfluorescent complexes are formed. The nature of the fluorescent intercalation complex is well studied. EtBr intercalates in A and B forms of the helix.

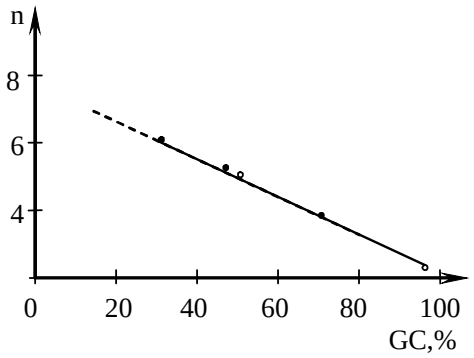

Fig. 2. Dependence of bindings site size (n) on GC-content of DNA. Dark circles for EtBr non-fluorescent complexes. Open circles data for nonintercalative complexes of EtBr from [1].

It is reasonable to suppose that the nonfluorescent strong complex is formed owing either to the EtBr partial (incomplete) intercalation in the double helix or in one on its grooves [7]. In the first and second models, the phenanthridine ring of EtBr molecule will be accessible for H_2O molecules to quench the dye fluorescence [5]. It is well known that EtBr intercalates in the double helix on the side of its minor groove [9], hence the supposition concerning the quenched complex formation in the groove seems justified. The existence of EtBr fluorescent and nonfluorescent strong complexes on DNA eliminates the contradictions arising upon the absorption and fluoremetric studies of the kinetics of its binding to DNA. The absorption technique registered two or three binding kinetics, whereas the fluoremetric one detected only a single kinetics.

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