Հայաստանի Գիտությունների Ազգային Ակադեմիա
Национальная Академия Наук Армении
National Academy of Sciences of Armenia

Հայաստանի Կենսաբանական Հանդես
Биологический Журнал Армении
Biological Journal of Armenia

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

Biolog. Journal of Armenia, 1 (70), 2018

INTERACTION OF DIFFERENT INTERCALATORS WITH DNA IN THREE-COMPONENT SYSTEMS

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The study of the joint binding of the intercalating compounds ethidium bromide (EtBr) and mitoxantrone (MTX) with DNA has been carried out. Melting parameters of the three-component system EtBr-DNA-MTX were obtained. Changes in the melting parameters of the complexes revealed that EtBr binds by several modes with ds-DNA both in the presence and in the absence of another intercalator. In the absence of EtBr, MTX binds to ds-DNA by semi-intercalation mode. At the joint binding of EtBr and MTX with ds-DNA a competition between ligands emerges, though the effect of EtBr on the melting parameters of the complexes becomes dominant.

DNA – ethidium bromide – mitoxantrone – joint binding – melting parameters

Իրականացվել է ԴՆԹ-ի հետ ինտերկալացվող միացությունների՝ էթիդիումի բրոմիդի (ԷԲ) և միտոքսանտրոնի (ՄՏՔ) համատեղ կապման ուսումնասիրություն: Ստացվել են ԷԲ-ԴՆԹ-ՄՏՔ եռակի համակարգերի հալման պարամետրերը: Կոմպլեքսների հալման պարամետրերի փոփոխությունները բացահայտել են, որ ԷԲ-ն եշ-ԴՆԹ-ի հետ կապվում է մի քանի եղանակներով ինչպես այլ ինտերկալյատորի առկայության, այնպես էլ բացակայության պայմաններում: ԷԲ-ի բացակայության դեպքում ՄՏՔ-ը եշ-ԴՆԹ-ի հետ կապվում է կիսաինտերկալյացիոն եղանակով: Եշ-ԴՆԹ-ի հետ ԷԲ-ի և ՄՏՔ-ի համատեղ կապման դեպքում լիգանդների միջև ի հայտ է գալիս մրցակցություն, սակայն ԷԲ-ի ազդեցությունը կոմպլեքսների հալման պարամետրերի վրա դառնում է դոմինանտ:

ԴՆԹ – էթիդիումի բրոմիդ – միտոքսանտրոն – համատեղ կապում – հալման պարամետրեր

Проведено исследоваие совместного связывания интеркалирующих соединений бромистого этидия (БЭ) и митоксантрона (МТК) с ДНК. Получены параметры плавления комплексов тройных систем БЭ-ДНК-МТК. Изменения параметров плавления комплексов выявили, что БЭ связывается несколькими способами с дц-ДНК как в присутствии, так и в отсутствие другого интеркалятора. В отсутствие БЭ, МТК связывается с дц-ДНК полуинтеркаляционным способом. При совместном связывании БЭ и МТК с дц-ДНК возникает конкуренция между лигандами, однако доминирующим является влияние БЭ на параметры плавления комплексов.

ДНК – бромистый этидий – митоксантрон – совместное связывание – параметры плавления

Study of RNA interaction with ligands that immediately bind to DNA nowadays is of big interest and is connected to the possibility to regulate thereby the processes taking place with RNA participation. It is known that some ligands significantly affect the biological functions of DNA, since they are mutagens (for instance, acridine dyes) or cancerogens, as well as inhibitors of transcription (for example, actinomycin and other antibiotics).

It is established that this effect is determined by the ability of these compounds to form slowly dissociating complexes with DNA. Inhibition of transcription is created by both difficulties for DNA untwisting and practical irreversibility of the complex formation in type of DNA-ligand [1,6,7,12-14]. From this point of view the interaction of these compounds with different RNA (being in single-, double-, triple- or tetra-stranded state) may have an important value.

Among ligands immediately binding to DNA the intercalators, including ethidium bromide (EtBr), acridine orange (AO), mitoxantrone (MTX) etc. are of special interest and have a wide application in the medicine as well [5,8-11,17,18].

One of the important problems is the study of the interaction of DNA with several ligands simultaneously. These problems are particularly valuable from that point of view that DNA in a cell is surrounded by different compounds that bind to it both by similar and by various mechanisms. Nowadays some data on simultaneous interaction of different ligands (in particular intercalator EtBr and groove binding compound Hoechst 33258, as well as two intercalators EtBr and methylene blue (MB)) with DNA are obtained [6,13]. Taking this fact into account the comparative studies of the interaction of wide range intercalators with ds-DNA are informative. The aim of the present work is to investigate the binding peculiarities of EtBr and MTX as well as EtBr and AO to ds-DNA

Materials and methods. Calf thymus DNA, poly(rA)-poly(rU), MTX, ("Sigma", USA), EtBr ("Serva", Germany) were used in experiments. All preparations were used without additional purification. Concentrations of the used preparations were determined by absorption method, using the following extinction coefficients: ε_{260}=6600 $M^{-1}cm^{-1}$ for calf thymus DNA, ε_{480}=5800 $M^{-1}cm^{-1}$ for EtBr, ε_{659}=25000 $M^{-1}cm^{-1}$ for MTX. The experiments were carried out in water medium, containing 0.01 M Tris buffer, 0.1 M NaCl, pH=7.4.

Melting of the complexes of DNA with ligands as well as spectrophotometric measurements of absorption of the solutions of the preparations were carried out using PYE Unicam-SP-8-100 spectrophotometer (England). Heating of the solutions of the complexes was realized using program device SP 876 Series 2.

For spectrophotometric measurements quartz cuvettes were used with hermetically closing Teflon caps, 3 ml volume, and 1 cm optic pathway length. The melting was carried out at λ=260 nm wavelength for DNA corresponding to its maximal absorption. The values of the absorptions of the complexes at the melting were performed on PC monitor using the program elaborated in LabVIEW medium. The melting curves of the complexes were constructed as described [13].

DNA complexes with ligands were prepared taking into account the concentration ratio r=[C]/[P], where C is ligand concentration (EtBr or MTX or joint EtBr-MTX), P – concentration of DNA phosphate groups. The values of r change in interval $0<r\leq0.33$. In the case of the joint binding of two intercalators to DNA the concentration of each ligand was taken twice less to prevent the similarity of the values of r with those values corresponding to the complexes of one ligand with DNA in the absence of another one.

Results and Discussion. Among biologically active compounds, interacting with DNA and significantly affecting its structural-functional characteristics, a special place belongs to classical intercalator EtBr, which binds not only in vitro, but also in vivo and inhibits the replication and transcription processes [5,8-11,14,17,18]. This ligand is an appropriate object for modeling of molecular mechanisms of the interaction of various

compounds with DNA, since the theoretical model of DNA helix-coil transition in the complex with EtBr elaborated in [6] allows to calculate the value of heat – ΔH via dependence of the change of T_m and ΔT on the ligand concentration. This theory also allows to carry out the complete thermodynamic analysis of DNA formed complexes with ligands that cannot bind by several modes [6]. This theoretical model revealed that EtBr forms three types of complexes with ds-DNA – intercalation, semi-intercalation and electrostatic. However, these binding modes of EtBr with DNA are universal, since their performance does not depend on the solution ionic strength, pH or other external factors [6,13].

This fact is important for DNA-EtBr system application as a fundament in studies of the interaction of different ligands both at separate and at joint interaction with NA.

To find out the peculiarities of EtBr and MTX joint binding with ds-DNA, the studies of DNA-ligand complexes have been carried out by UV melting method. Melting curves (the curves are not presented) were obtained based on which the melting parameters of DNA-ligand complexes were determined – melting temperature T_m and melting interval width ΔT. Change of these parameters depending on r (r=ligand/DNA) permits revealing the peculiarities of the binding of ligands to DNA. From this point of view the change of the melting interval width ($\delta(\Delta T/T_m^2)$) depending on ligand/DNA concentration ratio is certainly informative. Particularly, in the number of cases this parameter allows to find out the specificity of ligand to the certain sequences of DNA [15].

Dependence curves of $\delta(\Delta T/T_m^2)$ on r obtained for EtBr and MTX complexes with ds-DNA are presented in fig. 1. It is obvious from the presented figure that this dependence acquires a bell-like shape in the case of EtBr-DNA complexes (curve 1), since it enhances at ligand low concentrations and attaining to its maximal value at r=0.1 starts decreasing. In the case of MTX-DNA complexes an increase of $\delta(\Delta T/T_m^2)$ dependence on r at the ligand low concentrations ($0<r\leq0.05$) takes place, at further increase of this ligand concentration the dependence of $\delta(\Delta T/T_m^2)$ on r comes up to plateau (curve 2).

Another situation is observed in the case of EtBr and MTX joint binding to DNA. Particularly, it is obvious from the presented figure that the sum curve of $\delta(\Delta T/T_m^2)$ dependence on r practically has the same form as that for EtBr-DNA complexes (curve 4). The dependence of $\delta(\Delta T/T_m^2)$ on r corresponding to the complexes of EtBr-DNA-MTX (curve 3) qualitatively differs from the rest, since in this curve two regions are obvious: the first – in $0<r\leq0.17$ interval, the second – $0.17<r\leq0.33$. Moreover, it is obvious from the presented figure that in the interval of $0<r\leq0.1$ the dependence of $\delta(\Delta T/T_m^2)$ on r in the case of EtBr-DNA-MTX complexes increases, in the interval of $0.1<r\leq0.17$ it passes through the weakly expressed maximum, decreases, in the interval of $0.17<r\leq0.33$ it acquires a bell-like shape. Analogous effect was revealed at the joint interaction of EtBr and semi-intercalator methylene-blue (MB) with DNA [13]. Similarity of the obtained data allows to conclude that the main binding mechanism of MTX with ds-DNA as in the case of MB is semi-intercalation [2-4].

In [16] the dependencies of $\delta(\Delta T/T_m^2)$ on r of the complexes EtBr-DNA, AO-DNA and EtBr-DNA-AO were obtained. It becomes obvious from the obtained dependencies that in the case of EtBr-DNA-AO three-component system, the dependence of $\delta(\Delta T/T_m^2)$ on r is bell-like shaped as the curve corresponding to the complexes EtBr-DNA. Bell-like shape was also obtained at mathematical summation of the values of $\delta(\Delta T/T_m^2)$ for DNA-EtBr and DNA-AO complexes at corresponding values of r [16]. These data indicate that the intercalation mechanism mainly contributes to the stabilization of DNA ds-structure.

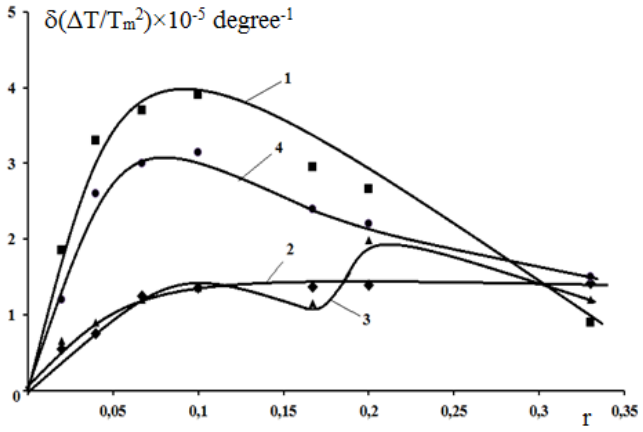

Fig. 1. Dependence curves of $\delta(\Delta T/T_m^2)$ on r of the complexes EtBr-DNA (1); MTX-DNA (2), EtBr-DNA-MTX (3) and DNA-EtBr + DNA-MTX (4).
The curve 4 is a mathematical sum of the values of $\delta(\Delta T/T_m^2)$ of the complexes DNA-EtBr and DNA- MTX at corresponding values of r.

At low concentrations of the ligand the sites for intercalation are not saturated and along with the melting a redistribution of the binding molecules from denatured regions of DNA to still non-denatured ones takes place. It results in increasing of the melting interval width of the complexes as compared to that of DNA. Along with ligand concentration enhancement with the saturation of the intercalation sites the redistribution ends due to which $\delta(\Delta T/T_m^2)$ attains to its maximum. In the case of AO and MTX (as well as MB) this effect is performed as the dependence curve of $\delta(\Delta T/T_m^2)$ on r comes up to plateau, while in the case of EtBr at further increase of the concentration this dependence starts decreasing [14]. This fact is conditioned by beginning of EtBr molecules to bind to DNA by semi-intercalation and electrostatic modes. From this point of view the result obtained at EtBr and AO joint binding to DNA is unexpected, since the values of $\delta(\Delta T/T_m^2)$, which are obtained for three-component system DNA-EtBr-AO and DNA-AO complex, less differ from each other in the interval $0.05<r\leq0.2$. We assume that in the case of the joint binding of AO and EtBr, the intercalated molecules of AO block the semi-intercalation binding sites for EtBr molecules. However, at further increasing of the concentration of both ligands ($0.2\leq r$) the EtBr molecules begin binding with ss-regions of DNA, which in turn facilitates the helix-coil transition process. On the other hand, the obtained data indicate that the semi-intercalation binding type of AO to ds-DNA is not revealed (in the case of MTX as for MB the intercalation mode is not found out at analogous conditions). By the virtue of this we assume that the bell-like change of $\delta(\Delta T/T_m^2)$ dependence on r of DNA-EtBr complexes in the absence of other ligands is conditioned by the simultaneous performance of three – intercalation, semi-intercalation and electrostatic modes. In the presence of other ligands depending on their type (intercalator, semi-intercalator or non-intercalator), not all binding modes, characteristic for EtBr, may appear [13,14,16]. For performance or suppression of one or another binding mode of separate ligands in the presence of the other, the binding constant K plays an important role: in the case of AO and EtBr the values of K corresponding to the intercalation mode differ from each other by almost 5-10 times, due to which the effect of EtBr on the melting parameters of the complexes prevails at the high concentrations of ligands. On the other hand, the values of K, corresponding to the

semi-intercalation mode for EtBr are less than that corresponding to the intercalation for AO that is why this mode is suppressed in the interval $0.05<r\leq0.2$ at the joint binding of EtBr and AO with DNA. From this point of view, the dependence curve peculiarities of $\delta(\Delta T/T_m^2)$ on r for EtBr-DNA-MTX three-component system (as well as EtBr-DNA-MB [14]) are conditioned by the performance or suppression of the respective modes depending on values of K.

Thus, the obtained data indicate that the joint binding of different ligands with DNA is not a mathematical sum of their separate binding. Our obtained data also indicate that MTX binds to DNA by the mechanisms similar to MB, i.e. in the conditions of relatively high ionic strengths, at low concentrations MTX semi-intercalates into ds-structure of DNA, at high concentrations – it binds electrostatically from outside of the helix.

In the case of binding of intercalators, particularly EtBr and AO, it is revealed that these ligands differently affect the melting interval width of DNA. Though, in the presence of AO the semi-intercalation of EtBr molecules into ds-regions of DNA is suppressed, while into ss-regions it is performed at high concentrations. This fact indicates that at relatively high concentrations of both ligands and the joint binding to DNA, a competition between them emerges.

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Received on 14.11.2017