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STUDY OF COMPARATIVE INTERACTION OF ETHIDIUM BROMIDE AND MITOXANTRONE WITH NUCLEIC ACIDS

A. P. ANTONYAN, M. A. TOROSYAN, M. A. PARSADANYAN *

Chair of Biophysics YSU, Armenia

The study of joint binding of intercalating compounds – ethidium bromide (EtBr) and mitoxantrone (MTX) with double-stranded DNA (ds-DNA) and poly(rA)-poly(rU) (ds-RNA) has been carried out by UV-melting method. The melting parameters of the complexes of both EtBr–ds-DNA–MTX and EtBr–ds-RNA–MTX triple systems were obtained. Changes of the melting parameters of these complexes revealed that EtBr binds to both ds-DNA and ds-RNA by several modes, though these binding modes of EtBr with nucleic acids (NA) were performed in the presence of MTX as well. MTX binds to ds-DNA and ds-RNA by semi-intercalation mode although this ligand shows more pronounced affinity to DNA than to RNA. It was found that at the joint binding of both ligands to NA a competition emerges between them and the effect of EtBr on the melting parameters of NA prevails.

Keywords: DNA, ethidium bromide, mitoxantrone, poly(rA)-poly(rU), melting parameters.

Introduction. The study of interaction of RNA with ligands that immediately bind to DNA, nowadays acquires huge interest which is connected with the regulation of processes taking place by RNA participation. It is known that several ligands significantly affect the biological function of DNA, since they are mutagens (for instance, acridine dyes) or cancerogens, as well as transcription inhibitors (for example, actinomycins and other antibiotics). It has been established that this effect is determined by the ability of these compounds to form slowly dissociating complexes with DNA. Inhibition of the transcription is created not only by difficulties of DNA untwisting, but also by practical irreversibility of the formation of DNA–ligand complexes [1–6]. From this point of view, the interaction of such compounds with different RNA (being in single-, double-, triple-, tetra-strand state) can have an important value as well.

Among ligands immediately binding to DNA, intercalators represent a certain interest, including ethidium bromide (EtBr), methylene blue (MB), mitoxantrone (MTX) etc., which have a wide application in medicine as well [7–12].

The problems concerning the simultaneous interaction of several ligands with nucleic acids (NA) are important. These topics are especially worthy from that point of view that NA, particularly, RNA in cytoplasm is surrounded by various compounds that may bind to it by both similar and different mechanisms. Nowadays,

* E-mail: marine.parsadanyan@ysu.am

using spectral as well as electrochemical methods, some data are obtained on simultaneous interaction of different ligands (particularly, intercalator EtBr, groove binding compound Hoechst 33258, as well as intercalators MB and MTX) with DNA [1, 4, 5, 13, 14]. From this point of view the studies of the interaction of mentioned ligands with double-stranded (ds-) RNA and their comparison to those obtained for ds-DNA become informative. Taking this fact into consideration the aim of this work is to study the binding peculiarities of EtBr and MTX with ds-DNA and ds-RNA.

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_{230}=7140 $M^{-1}cm^{-1}$ for poly(rA)-poly(rU), 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 NA with ligands as well as spectrophotometric measurements of absorption of the solutions of the preparations was 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 and poly(rA)-poly(rU) corresponding to maximal absorption of these NA. The values of the absorptions of the complexes at melting were performed on PC monitor using the program elaborated in LabVIEW medium. The melting curves of the complexes were constructed as described [4].

NA 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 and MTX), P is concentration of NA phosphate groups. The values of r change in the interval $0<r\leq0.33$. In the case of EtBr and MTX joint binding to NA, the concentration of each ligand was taken twice less to prevent the similarity of the values of r with those values corresponding to EtBr–NA and MTX–NA complexes.

Results and Discussion. Among biologically active compounds, interacting with DNA significantly affecting its structural-functional characteristics, a special place belongs to EtBr, which binds not only in vitro, but also in vivo and inhibits the replication and transcription processes [6–12]. This ligand is a classical intercalator and an appropriate object for modeling molecular mechanisms of the interaction of various compounds with DNA. Thus, the theoretical model of DNA helix-coil transition in the complex with EtBr elaborated by Vardevanyan et al [1] allows to calculate the value of heat – ΔH via the change of T_m and ΔT dependencies on the ligand concentration and to carry out the thermodynamic analysis of DNA formed complexes with non-covalently binding ligands [1]. This theoretical model revealed the possibility of binding of EtBr and other ligands with DNA by different modes, i.e. intercalation, semi-intercalation and electrostatic. Though, 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 [1, 4].

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

To find out the peculiarities of EtBr and MTX joint binding with ds-DNA and ds-RNA a comparison of the experimental results on the interaction of one of these ligands in the absence of another with the analogous data obtained for the triple system EtBr–NA–MTX has been carried out. For this aim the studies of NA–ligand complexes were realized by UV-melting method. Melting curves of the complexes were obtained shifting them to high-temperature region comparing with NA melting curve in the absence of ligands.

Moreover, the shift is revealed both in the case of one ligand and in the case of two ligand binding. The values of temperature (T_m) and melting interval width (ΔT) of NA and its complexes with ligands were determined from the melting curves. The values of changes of these parameters, δT_m and $\delta\Delta T$, were obtained.

The dependence curves of $\delta\Delta T$ on r obtained for the complexes of the ligands with ds-DNA are presented in Fig. 1, it is obvious that this dependence acquires a bell-like form for EtBr–DNA complexes, because it enhances at low concentrations of ligand and attaining to its maximal value at r=0.1, starts decreasing. In the case of MTX–DNA complexes an increase of $\delta\Delta T$ dependence on r occurs at low concentrations of the ligand ($0<r\leq0.05$), along with further enhancement of the ligand concentration the dependence curve of $\delta\Delta T$ on r comes up to plateau.

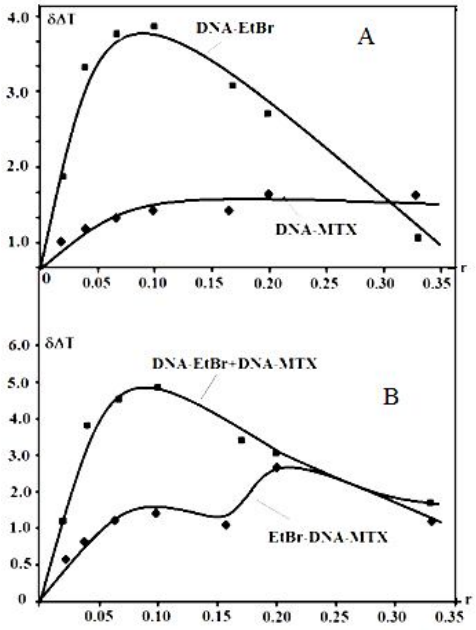

Fig. 1. Dependence curves of $\delta\Delta T$ on r for the complexes of EtBr–DNA and MTX–DNA (A); for the complexes of DNA–EtBr+DNA–MTX and EtBr–DNA–MTX (B). The dependence curve of $\delta\Delta T$ on r for DNA–EtBr+DNA–MTX is a mathematical sum of the values of $\delta\Delta T$ of DNA–EtBr and DNA–MTX complexes at the corresponding values of r.

Another situation is observed in the case of EtBr and MTX joint binding to NA. The dependence curves of $\delta\Delta T$ on r for the complexes of EtBr–DNA–MTX and the sum curve obtained at mathematical summation of $\delta\Delta T$ values for the complexes of DNA–EtBr and DNA–MTX at respective values of r are presented in Fig. 1, B, as it is seen the sum curve practically has the same form as the dependence of $\delta\Delta T$ on r for EtBr–DNA complexes. The dependence of $\delta\Delta T$ on r corresponding to the complexes of EtBr–DNA–MTX qualitatively differ from the rest, since in this curve it is possible to separate two regions: the first one is in $0<r\leq0.17$ interval,

the second one is in $0.17<r\leq0.33$ interval. It is clear from the Fig. 1, B, as well that in the interval $0<r\leq0.1$ the dependence of $\delta\Delta T$ on r for EtBr–DNA–MTX complexes enhances in the interval $0.1<r\leq0.17$ it passes through weakly pronounced maximum decreases, but in the interval $0.17<r\leq0.33$ acquires a bell-like shape.

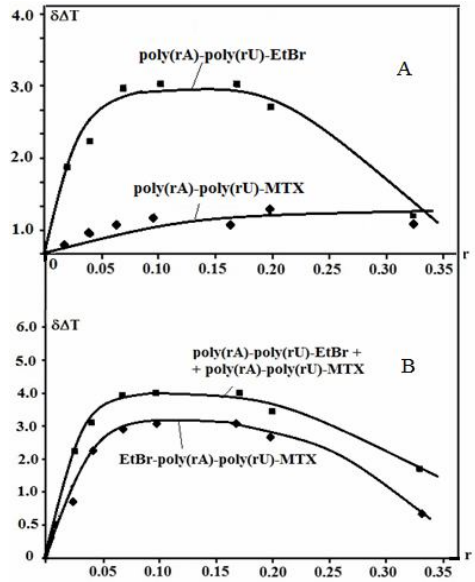

Fig. 2. Dependence curves of $\delta\Delta T$ on r for the complexes EtBr–poly(rA)-poly(rU) and MTX–poly(rA)-poly(rU) (A); for the complexes poly(rA)-poly(rU)–EtBr+poly(rA)-poly(rU)–MTX and EtBr––poly(rA)-poly(rU)–MTX (B). $\delta\Delta T$ dependence curve on r for DNA–EtBr+poly(rA)-poly(rU)–MTX is the mathematical sum of the values of $\delta\Delta T$ for the complexes of poly(rA)-poly(rU)–EtBr and poly(rA)-poly(rU)–MTX at respective values of r.

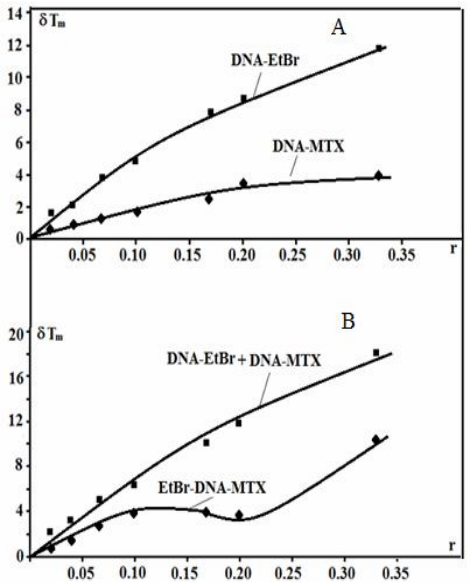

Fig. 3. Dependence curves of δT_m on r for the complexes of EtBr–ds-DNA and MTX–ds-DNA (A); for the complexes of DNA–EtBr+ds-DNA–MTX and EtBr+ds-DNA–MTX (B). The dependence curve of δT_m on r for DNA–EtBr+ds-DNA–MTX is a mathematical sum of the values of δT_m of the complexes of ds-DNA–EtBr and ds-DNA–MTX at respective values of r.

The analogous effect was revealed at the joint interaction of EtBr and semi-intercalator MB with DNA [4]. Similarity of the obtained data permits us concluding that the main mechanism of MTX binding to ds-DNA is semi-intercalation as for MB [15, 16]. In the case of EtBr and MTX interaction with poly(rA)-poly(rU) the analogous results with the above mentioned data are obtained for separate binding of each of these ligands with the mentioned polynucleotide (Fig. 2). It is necessary to mention that MTX binds to RNA much weaker (almost by an order) than to DNA, while EtBr shows practically similar affinity to both ds-DNA and ds-RNA. This fact conditions the practical correspondence with the form of $\delta\Delta T$ dependence curve on r at EtBr and MTX joint binding to poly(rA)-poly(rU) to the same curve for the complexes of EtBr–poly(rA)-poly(rU).

Dependence curves of δT_m on r for complexes of EtBr–DNA and MTX–DNA (A) as well as EtBr–DNA–MTX and EtBr–DNA+MTX–DNA (B) have been obtained and presented in Fig. 3. From the presented figure it is obvious that in cases of both EtBr and MTX δT_m enhances with an increase of r. At the same time for EtBr–DNA–MTX the dependence of δT_m on r again consists of two regions: the

first increases in the interval $0<r\leq0.1$, reaches to plateau in the interval $0.1<r\leq0.2$, then at $r>0.2$ sharply enhances. Mathematical sum of the dependence of δT_m on r obtained by the summation of δT_m values for EtBr–DNA and MTX–DNA complexes increases monotonously with the enhancement of the values of r and by its form coincides to δT_m dependence curve for the complexes of EtBr–DNA.

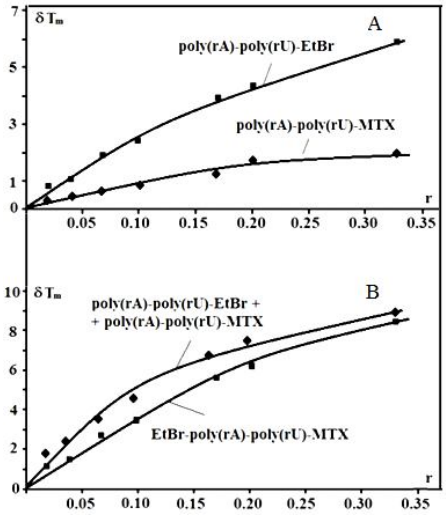

Fig. 4. Dependence curves of δT_m on r for the complexes of EtBr–ds-RNA and MTX–poly(rA)-poly(rU) (A); for the complexes of poly(rA)-poly(rU)–EtBr+poly(rA)-poly(rU)–MTX and EtBr–poly(rA)-poly(rU)–MTX (B). The dependence curve of δT_m on r for poly(rA)-poly(rU)–EtBr+poly(rA)-poly(rU)–MTX is the mathematical sum of δT_m values of the complexes of poly(rA)-poly(rU)–EtBr and poly(rA)-poly(rU)–MTX at the respective values of r.

More possible explanation of such behavior of the dependence curve of δT_m on r obtained for EtBr–DNA–MTX triple system may be the result of the fact that at low concentrations, despite the stabilizing effect of both ligands on DNA ds-structure, their joint effect is not a sum of the separate effects. Moreover, a mutual weakening of separate effects of each of ligands takes place due to the competition between them. The maintenance of such conclusion is that δT_m of the complexes acquires the constant value in the interval $0.1\leq r\leq0.167$ and decreases in the interval $0.167\leq r\leq0.2$. Most apparently, in these conditions the triple system structure of EtBr–DNA–MTX becomes more relaxed, than that of the complexes EtBr–DNA or MTX–DNA, which contributes to denaturation.

Further enhancement of the ligand concentration results in a sharp increase of δT_m, which is observed in the case of the separate effect of EtBr at relatively high concentrations ($r>0.2$) (respective curves on Fig. 3, A and B), while for MTX the dependence curve of δT_m on r comes up on plateau at high concentrations of the ligand. This fact permits concluding that at the increasing of the concentrations of both ligands the competition between them becomes more real and EtBr effect becomes prevailing. This fact also insists on the fact the main mode of MTX binding with ds-DNA, as in the case of MB is semi-intercalation.

Another significant result is obtained at joint interaction of EtBr and MTX with poly(rA)-poly(rU) since the dependence curve of δT_m on r (as $\delta\Delta T$) practically coincides with analogous curve by its form obtained for the complexes of EtBr with poly(rA)-poly(rU) (Fig. 4, A and B). Though, the analogous dependence is obtained for the mathematical sum of δT_m values for the complexes of poly(rA)-poly(rU)–EtBr and poly(rA)-poly(rU)–MTX at corresponding values of r.

Therefore, the obtained data indicate that the joint binding of EtBr and MTX with NA is not the mathematical sum of their separate binding. The obtained data indicate as well that MTX binds to DNA by similar MB mechanisms, i.e. in relatively high conditions of the ionic strengths and low concentrations MTX semi-intercalates in ds-structure of NA; at high concentrations it binds externally to the helix electrostatically. Analogous results are obtained at the separate interaction of EtBr and MTX with poly(rA)-poly(rU). Though, the data comparison obtained for the triple system of EtBr–poly(rA)-poly(rU)–MTX indicates that MTX binds to RNA much weaker than to DNA, while EtBr shows to DNA or RNA practically the similar affinity (Fig. 4). This fact is maintained by the fact that at EtBr and MTX joint binding to poly(rA)-poly(rU) the effect of EtBr on the melting parameters of these helices is mainly performed. It is also shown that the number of modes of EtBr binding with the mentioned NA does not depend on the absence or presence of MTX.

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