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COMPARATIVE STUDY OF ETHIDIUM BROMIDE INTERACTION WITH SYNTHETIC POLYNUCLEOTIDES ds-POLY(rA)-POLY(rU) AND ds-POLY(dA)-POLY(dT)

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A comparative study on the interaction of classical intercalator ethidium bromide (EtBr) with different nucleic acids (NA) has been carried out. EtBr was revealed to preferentially bind to ds-NA belonging to A-family, rather than to B-family, because the stabilizing effect of this ligand decreases in the following order: ds-poly(rA)-poly(rU), ds-poly(dA)-poly(dT), ds-DNA.

***Keywords*:** DNA, synthetic polynucleotide, intercalator, complex, melting temperature.

Introduction. Nowadays the studies on the reversible interaction of various low-molecular compounds – ligands with different forms of nucleic acids (NA) are of great interest, which is conditioned by development of new methods of bioanalysis, on the one hand, and by the screening and creating of new efficient and less toxic drug preparations, on the other hand [1–4].

Despite the fact that the conditions of NA (DNA, RNA) interaction with low-molecular regulators and effectors of different nature differ from those in a living cell, particularly, via DNA-sensors it is possible to estimate the binding specificity, the regulator and damaging action mechanism and transformation of biologically active compounds in biochemical reactions involving DNA. DNA-sensors are promising for screening and pharmacokinetic studies of new drugs and nutrients [5–8]. It should be mentioned that NA are biological targets for a wide group of drug preparations, first of all, for those with anti-tumorous action. Their action on NA is due to control of transcription processes or activity of polymerases; due to the effect of DNA reaction with RNA, due to direct interaction – intercalation into DNA helix. In the last case a flat aromatic molecule or its fragment is inserted into the plane between the pairs of complementary nucleic bases of DNA helix. Taking into account that the intercalation is possible only into double-stranded DNA, the intercalators can be applied as markers of hybridization process between different strands of deoxyribonucleotides or ribonucleotides [8–10].

Intercalative NA-sensors are constructed on the principle of insertion of some compound into double helix structure of DNA or RNA. Consequently, the hybridization efficacy between separate strands of different NA, as well as the

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interaction of studying bioactive compounds with them can be estimated by their intercalation degree, which directly determines level of the biosensor signal (change of fluorescence intensity, absorption in visible region of spectrum or CD (circular dichroism) signal) [10–13]. It should be noted that the intercalation, on the one hand, depends on the NA structural state (the preferably double-stranded structure of NA) and, on the other hand, contributes to establishment of such a structure (hybridization) [14].

To study the effect of intercalating compounds on the hybridization between separate strands of DNA/RNA or on the stabilization of NA double-stranded structures, the model experiments with application of well-known intercalators are considered to be informative. Among such compounds the classical intercalator, also known as a classical multimodal ligand, ethidium bromide (EtBr) is an appropriate one, because its interaction with NA is well-studied both theoretically and experimentally and can be used to raise the efficacy of the biosensors [14–16]. Taking it into account the work is aimed at assessing the stabilization effect of the classical intercalator EtBr on the ds-structure of different forms of NA (A- and B-).

Materials and Methods. Ultrapure synthetic polynucleotides poly(rA)-poly(rU), poly(dA)-poly(dT), Calf Thymus DNA ("Sigma", USA), EtBr ("Serva", Germany), EDTA (ethylenediaminetetraacetate), NaCl, trisodium citrate (chemically pure) were used in this work. All preparations were used without additional purification. The concentrations of the preparations used were determined spectrophotometrically, using the following extinction coefficients: ε_{260}=7140 $M^{-1}cm^{-1}$ for poly(rA)-poly(rU), ε_{260}= 6000 $M^{-1}cm^{-1}$ for poly(dA)-poly(dT), ε_{260}= 6600 $M^{-1}cm^{-1}$ for DNA and ε_{260}=5800 $M^{-1}cm^{-1}$ for EtBr. The experiments were carried out at the ionic strength of the solution 0.02 *M*, containing only monovalent Na^+ cations.

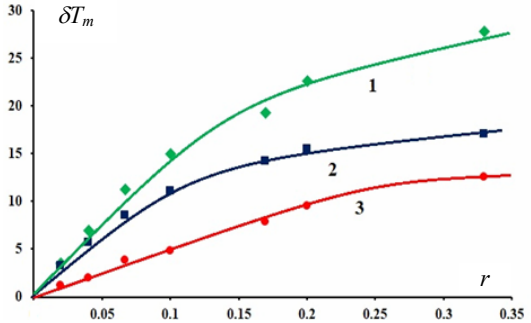
UV-melting of EtBr complexes with DNA and polynucleotides was carried out on spectrophotometer UV/VIS PYE Unicam-SP-8-100 (England). The thermostating cells was heated at the wavelength of maximal absorption of polynucleotides (DNA) 260 *nm*. The concentration ratio r = [ligand] / [RNA] was varied in the interval $0 \le r \le 0.33$ (per nucleotide). At the melting, the heating rate was 0.5°*C*/*min*, registration was done automatically every 60 *min*. Data were displayed on PC monitor in LabVIEW software. The values of both temperatures and absorptions of the samples were transformed and saved via program software Excel Office 13. All calculations of the experimental data as well as the melting curves were obtained in Excel [14]. From the melting curves of the complexes the values of the melting temperature change (δT_m) were determined and the dependency curves of δT_m on r were constructed in Excel software.

Results and Discussion. Being in a cell, nucleic acids (DNA, RNA) at the functioning can perform a structural multiformity, therefore, taking part in various processes of the cellular cycle. DNA mainly has a double-stranded (ds-) structure, though it can be transformed into single-stranded (ss-) or, rarely, into four-stranded (fs-, quadruplex) state. Meanwhile, DNA may be either in A- or in B-forms. RNA is able to take more diverse structures, but it is always in A-form. The synthetic homopolynucleotide poly(rA)-poly(rU) is a good model for ds-RNA. However, at the relatively high ionic strengths of the solution or in the presence of divalent and polyvalent cations it transformes into three-stranded poly(rA)-2poly(rU) and

ss-poly(rA) state, which, in turn, at low values of pH self-associates with the formation of ds-structure [17, 18].

Poly(dA)-poly(dT) at low salt concentrations and high relative humidity of chains has a structure typical to B'-DNA. In solution this polynucleotide is more hydrated, than poly[d(A-T)]-poly[d(A-T)] or DNA, though it is the only case, when the structural characteristics of poly(dA)-poly(dT) in fibers and in solutions completely coincide. There are 10.1 ± 0.1 pairs of bases per turn of the helix of this polynucleotide [19], besides the helix poly(dA)-poly(dT) is not transformed into other structural forms. Particularly, the decrease of water activity upon the addition of ethanol to the solution does not induce a conformational transition from B- to A-form, as in the case of poly[d(A-T)]-poly[d(A-T)] or DNA. Poly(dA)-poly(dT) owns a special CD spectrum and this polynucleotide does not form nucleosome when interacting with histone octamers [19]. The minor groove of this polynucleotide is narrower and the molecule generally has a more rigid structure than B-DNA.

NA in cells are surrounded by various non-organic and organic low- or high-molecular compounds (ligands), with which they form various complexes. Obviously, the structure or conformational state of DNA or RNA can be decisive for the interaction with this or other ligand. In response to this, the model studies of the interaction of different NA with one ligand can be informative. Proceeding from this, the binding of the classical intercalator and multimodal EtBr ligand with ds-poly(rA)-poly(rU), poly(dA)-poly(dT) and DNA was studied by the UV-melting method at the ionic strength of the solution of 0.02 *M*. The melting curves (curves were smooth, indicated the transition from ds- to ss-state and are not presented) were obtained and the values of the melting temperature (T_m) were determined from them. Along with the increase in ligand concentration, T_m of the complexes rises which is a result of EtBr stabilizing effect on ds-structure of the mentioned NA. The values of δT_m, which is a difference between values of T_m of NA and NA–EtBr complexes ($\delta T_m = T_m - T_0$, where T_0 is a melting temperature of NA in absence of EtBr), were calculated, and the dependencies of δT_m on r are presented in Figure.



Dependence curves of δT_m on r, obtained for EtBr complexes with poly(rA)-poly(rU) (1), poly(dA)-poly(dT) (2) and DNA (3) at the ionic strength of the solution of 0.02 *M*.

From the presented Figure it is obvious that the highest change of T_m is achieved at EtBr binding to ds-poly(rA)-poly(rU) (A-form) and the lowest when binding to DNA. In the case of structural analogue of poly(rA)-poly(rU) – poly(dA)-poly(dT) (B-form) the change of T_m is higher than in the case of DNA, despite the fact that this polynucleotide and DNA belong to B-family of nucleic acids. It should be mentioned that as compared to poly(dA)-poly(dT) and DNA, ds-structure of poly(rA)-poly(rU) at the room temperatures and ionic strength of

the solution of 0.02 *M* is relatively unstable. Nonetheless, the highest stabilizing effect of EtBr on the native structure of poly(rA)-poly(rU) may be due to becoming of A-form of nucleic acids more preferable, than B-form. On the other hand, the preference of EtBr to poly(dA)-poly(dT) may be the result of the fact that this polynucleotide has more rigid ds-structure as compared to DNA.

It was stated that poly(rA)-poly(rU) has an unusually wide melting interval width ($\Delta T \approx 13–15^{o}C$), which is peculiar for DNA with random nucleotide sequences (including Calf Thymus DNA), while in the case of poly(dA)-poly(dT) this parameter has a much lower value ($\Delta T \approx 0.5–1.0^{o}C$) [14]. This fact, apparently, is conditioned by the fact that in A-form both nitrogen bases in pair enter in stacking contacts, while in the case of B-form mainly one of nitrogen bases in the nucleotide pair takes participation in stacking interactions [19]. At the intercalation the ligand molecules are inserted into the plane between adjacent nucleotide pairs of NA along its molecule and participate in stacking interaction with them [19]. With this in mind, we suggest that the above fact underlines the EtBr preference to A-form of NA. This is maintained by the fact that poly(dA)-poly(dT) has a more rigid ds-structure than DNA, that is why the EtBr intercalation into this polynucleotide prevails over the intercalation into DNA. Besides, in intercalated state the hydrophobic aromatic group of the ligand is more screened from water medium, which also has a certain contribution to the stabilization of the ds-structure of NA.

Conclusion. The obtained data reveal that in the line of poly(rA)-poly(rU), poly(dA)-poly(dT) and DNA, EtBr performs the highest preference to the polynucleotide belonging to A-family – ds-poly(rA)-poly(rU). From this point of view, ds-DNA is the least preferable one, despite the fact that it has more conformational mobility, than poly(rA)-poly(rU) and poly(dA)-poly(dT) [19]. We suggest that the interaction peculiarities of EtBr with the mentioned NA may have an important value for the elaboration of more effective biosensors, on the one hand, and for screening and synthesis of new bioactive compounds, on the other hand.

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REFERENCES

1. Anjum V., Pundir C.S. Biosensors: Future Analytical Tools. *Sensors & Transduccers Journal*, **76** (2007), 937–944.
2. Kavita V. DNA Biosensors – A Review. *Journal of Bioengineering & Biomedical Science*, **2** : 7 (2017), 5 p. doi : 10.4172/2155-9538.1000222
3. Malhotra B.D., Chaube A. Biosensors for Clinical Diagnostics Industry. *Sensor Actuators B*, **9** (2003), 117–126.
4. Drummond T.G. Electrochemical DNA Sensors. *Nature Biotechnol.*, **21** (2003), 1192–1199.
5. Bagni G., Osella D., Sturchio E., Mascini M. Deoxyribonucleic Acid (DNA) Biosensors for Environmental Risk Assessment and Drug Studies. *Anal. Chim. Acta*, **574** (2006), 81–89.
6. Berney H., West J., Haefele H., Alderman J., Lane W., Collins J.K. DNA Diagnostic Biosensor Development, Characterization and Performance. *Sens. Actuators B*, **68** (2000), 100–108.

7. Chikashi N., Teruaki K., Masato M., Makoto S., Jun M. Usage of a DNA Aptamer as a Ligand Targeting Microcystin. *Molecular Crystals and Liquid Crystals*, **371** (2001), 369–374.
8. Arora K., Prabhakar N., Chand S., Malhotra B.D. Ultrasensitive DNA Hybridization Biosensor Based on Polyaniline. *Biosens Bioelectronics*, **23** (2007), 613–620.
9. Prabhakar N., Arora K., Singh S.P., Pandey M.K., Singh H., Malhotra B.D. Polypyrrole-polyvinylsulphonate Film Based Disposable Nucleic Acid Biosensor. *Anal. Chim. Acta*, **589** (2007), 6–13.
10. Berdat D., Marin A., Herrera F., Gijs M.A.M. DNA Biosensors Using Fluorescence Microscopy and Impedance Spectroscopy. *Sensors Actuators*, **118** (2006), 53–59.
11. Sharma K., Sehgal N., Kumar A. Biomolecules for Development of Biosensors and Their Application. *Curr. Appl. Phys*., **3** (2003), 307–316.
12. Prabhakar N., Arora K., Singh S.P., Singh H., Malhotra B.D. DNA Entrapped Polypyrrole-polyvinyl Sulfonate Film for Application to Electrochemical Biosensor. *Anal. Biochem*., **366** (2007), 71–79.
13. www.motorola.com/Lifesciences 25. ww.dna-chip.toshiba.co.jp/eng/
14. Vardevanyan P.O., Antonyan A.P., Parsadanyan M.A., Shahinyan M.A. Study of the Influence of the Ionic Strength on Complex-formation of Ethidium Bromide with poly(rA)-poly(rU). *J. of Biomol. Struct. & Dyn*. (2019). doi : 10.1080/07391102.2019.1630006
15. Vardevanyan P.O., Antonyan A.P., Parsadanyan M.A., Shahinyan M.A., Sahakyan V.G. Peculiarities of Interaction of Synthetic Polyribonucleotide poly(rA)-poly(rU) with Some Intercalators. *J. of Biomol. Struct. & Dyn*., **36** : 14 (2018), 3607–3613.
16. Vardevanyan P., Antonyan A., Parsadanyan M., Shahinyan M., Melqonyan G. Behavior of Ethidium Bromide-Hoechst 33258-DNA and Ethidium Bromide-Methylene Blue-DNA Triple Systems by Means UV Melting. *International Journal of Spectroscopy*, **2015** (2015), 1–5.
17. Kankia B.I. Mr^{2+}-induced Triplex Formation of an Equimolar Mixture of poly(rA) and poly(rU). *Nucl. Acids Res*., **31** : 17 (2003), 5101–5107.
18. Andrushchenko V., Blagoi Yu., van de Sande J.H., Wieser H. Poly(rA)-Poly(rU) with Ni^{2+} Ions at Different Temperatures: Infrared Absorption and Vibrational Circular Dichroism Spectroscopy. *J. of Biomol. Struct. & Dyn*., **19** : 5 (2002), 889–906.
19. Saenger V. *Principles of Structural Organization of Nucleic Acids*. M., Mir (1987), 584 p.