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STUDY OF HYBRIDIZATION OF COMPLEMENTARY SINGLE-STRANDED POLYNUCLEOTIDES POLY(rA) AND POLY(rU)

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The study of the interaction of intercalators ethidium bromide (EtBr), methylene blue (MB) and groove binding compound Hoechst 33258 (H33258) with single-stranded synthetic polyribonucleotides poly(rA) and poly(rU) has been carried out by the method of UV-melting, at ionic strengths of the solution 0.04 *M* and 0.1 *M*. Stirring of poly(rA) and poly(rU) by equal-molar concentrations was shown to result in hybridization with formation of double-stranded (ds-) structure poly(rA)-poly(rU). It was revealed that EtBr, with higher degree in comparison with MB, stimulates hybridization and stabilizes the formed ds-structure poly(rA)-poly(rU). It was also found out that the hybridization process and affinity of EtBr and MB depend on the ionic strength of the solution and these processes occur much more effectively at the ionic strength 0.04 *M*. On the other hand, it was shown that the groove binding ligand H33258 practically does not affect the stabilization of the formed ds-structure poly(rA)-poly(rU).

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Keywords: synthetic polynucleotides, hybridization, intercalator, groove binding ligand, ionic strength of the solution, melting curve.

Introduction. Nowadays one of the most actual and important topics of molecular biophysics is the study of the interaction of low-molecular compounds with nucleic acids (NA) [1, 2]. These interactions, along with their fundamental value, lie at the basis of bioanalysis that use NA with various length and sequence for molecular recognition [3, 4]. Bioanalytic methods are based on biosensor technologies, which permit solving number of biological, medical as well as genetic important problems [5, 6]. Genochips and genosensors are referred to these instruments, by which the interaction of NA complementary chains with different length and sequence is possible to detect. It occurs due to the formation of stable adenine-thymine (uracil) and guanine-cytosine pairs, which is called hybridization process and owns high specificity. In fact, using genosensors (genochips) one can register complementary binding of different NA chains and modulate this process varying different factors [7]. Hybridization between various ss-molecules of NA can be assessed by different physical methods – absorption, fluorescent spectroscopies or UV-melting method. It should be mentioned that hybridization is the most important phase of operations on NA-chips or NA-sensors [8, 9].

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NA are important biological targets for numerous compounds. Many of them have applicative value, since they make possible to control transcription processes, DNA reaction with RNA, including formation of hybrid helices DNA–RNA and triplexes of ds-DNA and RNA. Among such compounds intercalators have a special value. It is connected with the fact that intercalation is possible only into ds-DNA, since they are also markers of hybridization process. Particularly, for intercalators ethidium bromide (EtBr), acridine orange (AO), methylene blue (MB) the optic and fluorescent properties change while intercalating, which can be used for registration of signal of biochips and biosensors [10–13].

The present work is aimed at studying the hybridization process between complementary ss-polynucleotides poly(rA) and poly(rU) both in presence and in absence of EtBr, MB as well as non-intercalator Hoechst 33258 (H33258).

Materials and Methods. In the experiments ultrapure synthetic polynucleotides poly(rA) (P9403), poly(rU) (P9528), MB, EtBr, H33258 (all preparations from "Sigma" (USA)), bi-distilled water, NaCl, Na-citrate, Na_2EDTA (in working solutions with concentration 10^{-5} *M*) were used. Concentrations of all used preparations were determined spectrophotometrically, using the following coefficients of extinction: ε_{665}=76000 $M^{-1}cm^{-1}$ for MB [14], ε_{480}=5700 $M^{-1}cm^{-1}$ for EtBr [15], ε_{343}=42000 $M^{-1}cm^{-1}$ for H33258 [16], ε_{257}=10500 $M^{-1}cm^{-1}$ for poly(rA) and ε_{260}=9500 $M^{-1}cm^{-1}$ for poly(rU) [17]. Experiments were carried out at ionic strengths of the solution – 0.04 *M* and 0.1 *M*, pH≈7.0.

UV-melting of hybrid duplexes poly(rA)-poly(rU) and their complexes with the mentioned ligands was carried out on UV-VIS spectrophotometer Unicam SP-8-100 with thermostating cells for cuvettes. Solutions of the samples were placed in hermetically closed quartz cuvettes with 1 *cm* pathway length. Heating of the solutions was realized via Temperature Program Controller SP 876 Series 2. All experimental data and melting curves were treated in Microsoft Excel software. Experimental error was about ~5–6%.

Results and Discussion. Effect of intercalators EtBr, MB and groove binding compound H33258 on the hybridization of synthetic complementary homopolyribonucleotides poly(rA) and poly(rU) was studied by the method of UV-melting. In the Fig. 1, the melting curves of hybrid ds-poly(rA)-poly(rU) and its complexes with MB at the ionic strength of the solution 0.04 *M* (curves 1 and 2, respectively) and 0.1 *M* (curves 3 and 4, respectively) were presented. It was revealed from the obtained data, that while mixing with equal-molar concentrations, poly(rA) and poly(rU) hybridize. Though, hybridization occurs practically completely with formation of ds-structure, as a result of which the hypochromic degree composes 36–40%. It should be mentioned that the hyperchromic degree of ds-poly(rA)-poly(rU) at denaturation is about 35–36%, as it was obtained in [18].

Hybridization process between poly(rA) and poly(rU) takes place both in absence and in presence of MB. Meanwhile, in the result of the formation of ds-structure the melting curves of the complexes poly(rA)-poly(rU)–MB (curve 2) were shifted toward high temperatures as compared to the melting curve of hybrid poly(rA)-poly(rU) (curve 1) at the ionic strength of the solution 0.04 *M*, as it was obvious in Fig. 1. Obviously, MB, binding to hybrid ds-polynucleotide, stabilizes

the structure. Though, at the ionic strength of the solution 0.1 *M*, the melting curve shift of the complexes poly(rA)-poly(rU)–MB toward high-temperature region is small. It indicates that the interaction of MB with poly(rA)-poly(rU) depends on the structural state of this polynucleotide and is more preferable in conditions at which poly(rA)-poly(rU) is in ds-state and is available for the binding of ligand molecules [19]. Being an intercalator, MB, however, not always binds to NA by this mode. The obtained data indicate that at the ionic strength of the solution 0.04 *M* ds-helix of poly(rA)-poly(rU) is more available for MB binding, than at 0.1 *M*. Most apparently, at high ionic strengths of the solution poly(rA)-poly(rU) transits to more compactly wrapped state, as a result of which it becomes less available for binding of this ligand by intercalation mode. This fact permits us concluding that MB can completely intercalate into ds-NA in that case, when their helix is unwrapped.

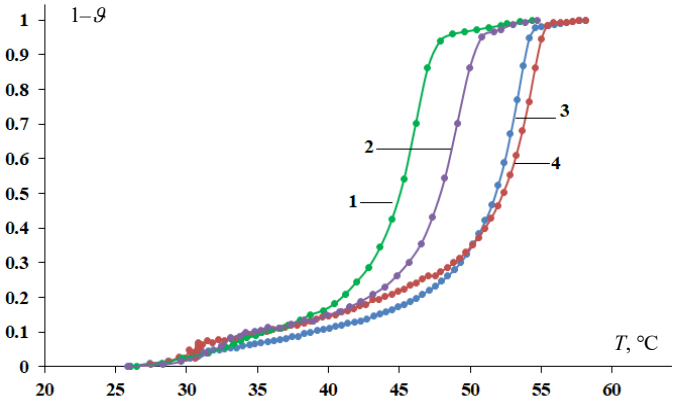

Fig. 1. Melting curves of hybrid ds-poly(rA)-poly(rU) and its complexes with MB at the ionic strength of the solution 0.04 *M* (curves 1 and 2, respectively) and 0.1 *M* (curves 3 and 4, respectively).

Analogously, the melting curves of the complexes of hybrid poly(rA)-poly(rU) with classical intercalator EtBr were obtained and presented in Fig. 2. As it is obvious from the presented Figure, the stabilization effect of ds-structure of poly(rA)-poly(rU) by this ligand is more pronounced, than for MB, because a significant shift toward high temperature region takes place at the ionic strength of the solution 0.04 *M* in relation with the melting curve of poly(rA)-poly(rU). Moreover, the melting curve shift of the complexes poly(rA)-poly(rU)–EtBr at the ionic strength of the solution 0.04 *M* prevails over the analogous shift, taking place at the ionic strength of the solution 0.1 *M*.

Most apparently, in the case of EtBr the structural state of ds-NA plays an important role for the binding. On the other hand, at the ionic strength of the solution 0.1 *M* the shift toward high temperature region for the complexes poly(rA)-poly(rU)–EtBr is much higher, than that for MB. These data are in good accordance with the obtained ones in the work [18], concerning to the interaction of the mentioned ligands with ds-DNA. Our obtained data also allow to conclude that for EtBr intercalation, ds-structure of hybrid poly(rA)-poly(rU) is more available at the ionic strength of the solution 0.1 *M*, than that for MB.

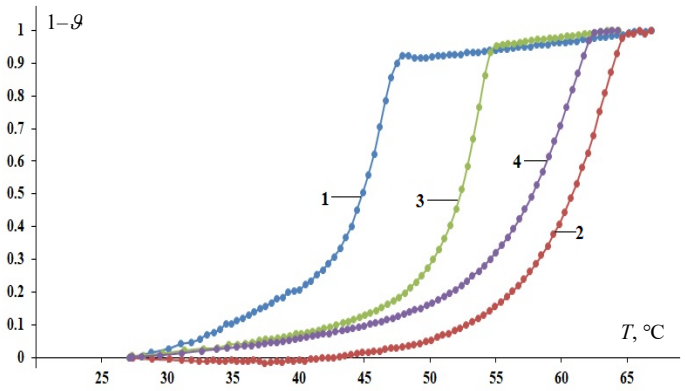

Fig. 2. Melting curves of hybrid ds-poly(rA)-poly(rU) and their complexes with EtBr at the ionic strength of the solution 0.04 *M* (curves 1 and 2, respectively) and 0.1 *M* (curves 3 and 4, respectively).

Melting curves of the complexes of hybrid poly(rA)-poly(rU) with non-intercalator, groove binding compound H33258 were obtained and presented in Fig. 3. This ligand specifically binds to AT-sequences of DNA, though, it preferably interacts with its ds-structure. As it is obvious from the presented Figure, for this ligand radically another scenario is revealed, as compared to intercalators EtBr and MB. Despite the fact that H33258 preferably binds to ds-NA, the melting curves of poly(rA)-poly(rU) and the complexes poly(rA)-poly(rU)–H33258 coincide, which practically does not depend on the ionic strength of the solution. In the work [20] it was shown that H33258 forms a complex with synthetic ds-polynucleotide poly(rA)-poly(rU), which serves as a model for ds-RNA and preserves ds-structure in the interval of the change of the ionic strength of the solution $0.02 \le I \le 0.1$ *M*. Though, our obtained data indicate that H33258, most apparently, does not affect the hybridization between poly(rA) and poly(rU), while EtBr and MB can facilitate this process with following stabilization of ds-structure after hybridization.

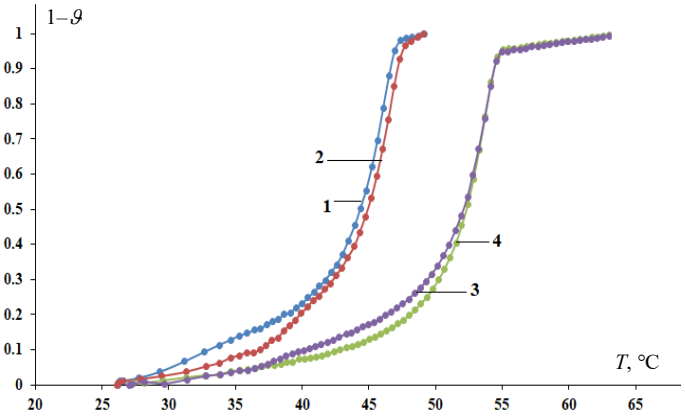

Fig. 3. Melting curves of hybrid ds-poly(rA)-poly(rU) and its complexes with H33258 at the ionic strength of the solution 0.04 *M* (curves 1 and 2, respectively) and 0.1 *M* (curves 3 and 4, respectively).

The results obtained in [20] also indicate the biphasic nature of the melting curves of the complexes of H33258 with poly(rA)-poly(rU), as well as with its deoxy-analogue poly(dA)-poly(dT). In the case of H33258 complexes with hybrid ds-poly(rA)-poly(rU) the melting curves are monophasic, which also indicates that this ligand does not affect the hybridization of single-stranded complementary polynucleotides poly(rA) and poly(rU) and does not invoke conformational reconstructions of hybrid RNA.

Conclusion. The obtained data indicate that the mixing of complementary polynucleotoides poly(rA) and poly(rU) results in hybridization with formation of ds-structure. Though, the classical intercalator EtBr, intercalator MB and groove binding compound H33258 bind to formed ds-structure of synthetic poly(rA) and poly(rU) at ionic strengths of the solution 0.04 *M* and 0.1 *M*. The obtained data show that EtBr with higher degree, than MB, stimulates the hybridization and stabilizes the formed ds-structure poly(rA)-poly(rU), meanwhile, the hybridization process and affinity of EtBr and MB to these polynucleotides depend on the ionic strength of the solution. The obtained data also indicate that these processes take place with much more effectiveness at the ionic strength of the solution 0.04 *M*. On the other hand, it was revealed that the groove binding ligand H33258 practically does not influence the stabilization of forming ds-structure poly(rA)-poly(rU). These data indicate that the intercalators EtBr and MB affect more effectively the hybridization between poly(rA) and poly(rU), while the groove binding H33258 either does not influence or has a weak effect on this process.

Thus, based on the received results, one can conclude that MB and EtBr may become good markers in genosensor technologies. These data can be useful in treatment of biosensors and biochips on the basis of nucleic acids, as well as at the choice of biologically active compounds that affect the hybridization of ss-NA.

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Զ. Հ. ՄՈՎՍԻՍՅԱՆ

ԿՈՄՊԼԵՄԵՆՏԱՐ ՄԻԱՇՂԹԱ ՊՈԼԻՆՈՒԿԼԵՈՏԻԴՆԵՐ POLY(rA)-Ի ԵՎ POLY(rU)-Ի ՀԻԲՐԻԴԱՑՄԱՆ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆԸ

Ուսումնասիրվել է ինտերկալյատորներ էթիդիումի բրոմիդի (ԷԲ), մեթիլենային կապույտի (ՄԿ) և ակոսում կապվող միացություն Hoechst 33258-ի (H33258) փոխազդեցությունը միաշղթա (մշ-) սինթետիկ պոլինուկլեոտիդներ poly(rA)-ի և poly(rU)-ի հետ ՈւՄ-հալման մեթոդով, լուծույթի 0.04 *Մ* և 0.1 *Մ* իոնական ուժերի պայմաններում: Ցույց է տրվել, որ poly(rA)-ի և poly(rU)-ի խառնումը էկվիմոլային կոնցենտրացիաներով հանգեցնում է հիբրիդացման՝ երկշղթա կառուցվածքի առաջացմամբ: Բացահայտվել է, որ ԷԲ-ն ավելի մեծ չափով, քան ՄԿ-ը, խթանում է հիբրիդացումը և կայունացնում է առաջացած poly(rA)-poly(rU) պոլինուկլեոտիդի երկշղթա կառուցվածքը: Ցույց է տրվել, որ հիբրիդացման պրոցեսը և ԷԲ-ի ու ՄԿ-ի խնամակցությունը կախված են լուծույթի իոնական ուժից և առավել արդյունավետ կերպով տեղի են ունենում 0.04 *Մ* իոնական ուժի պայմաններում: Բացահայտվել է, որ ակոսում կապվող լիգանդ H33258-ը գործնականում չի ազդում առաջացած երկշղթա կառուցվածքի poly(rA)-poly(rU)-ի կայունության վրա:

З. О. МОВСИСЯН

ИССЛЕДОВАНИЕ ГИБРИДИЗАЦИИ КОМПЛЕМЕНТАРНЫХ ОДНОЦЕПОЧЕЧНЫХ ПОЛИНУКЛЕОТИДОВ POLY(rA) И POLY(rU)

Проведено исследование взаимодействия интеркаляторов бромистого этидия (БЭ), метиленового синего (МС) и желобкового соединения Hoechst 33258 (H33258) с одноцепочечными синтетическими полирибонуклеотидами poly(rA) и poly(rU) методом УФ-плавления при ионнных силах раствора 0,04 и 0,1 *М*. Показано, что смешивание poly(rA) и poly(rU) в эквимолярных концентрациях приводит к гибридизации с образованием двухцепочечной структуры. Выявлено, что БЭ в большей степени, по сравнению с МС, стимулирует гибридизацию и стабилизирует образовавшуюся двухцепочечную структуру poly(rA)-poly(rU). Выявлено, что процесс гибридизации и сродство БЭ и МС зависят от ионной силы раствора и наиболее эффективны при ионной силе 0,04 *М*. Обнаружено, что желобково связывающийся лиганд H33258 практически не влияет на стабилизацию образовавшейся двухцепочечной структуры poly(rA)-poly(rU).