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STUDY OF METHYLENE BLUE INTERACTION WITH SYNTHETIC POLYNUCLEOTIDE POLY(rA)-POLY(rU)

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A study of the interaction of methylene blue (MB) with poly(rA)-poly(rU) by the method of fluorescence spectroscopy has been carried out. The data obtained revealed that MB, being a DNA-specific ligand, can bind to double-stranded regions of RNA. In this regard, as in the case of DNA, semi-intercalation was the most preferable mechanism for the binding of this ligand to poly(rA)-poly(rU). On the other hand, non-linear curves of dependence of F_0/F on concentration of polynucleotide might result from two binding modes, the second of which was probably of an electrostatic nature. Proceeding from the data obtained, the value of K_{SV} was revealed to be almost an order of magnitude less than for DNA, which may indicate that RNA is a less preferable target for MB.

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***Keywords*:** synthetic polynucleotide, methylene blue, binding, fluorescence spectra, fluorescence quenching.

Introduction. There exist many experimental works on the interaction of methylene blue (MB) with DNA that is due to the high biological activity of this ligand, and nowadays it is applied as a pharmacological compound [1–4]. MB may bind to nucleic acids (NA), proteins or ligands and, being a photosensitive compound, absorbs light energy, then transferring it to resolved oxygen. The resolved oxygen is transformed into an activated singlet state, and binding to macromolecules causes photo-oxidative damages within them.

Studies on the interaction of MB with DNA or proteins are based on spectroscopy methods that have shown specific binding of MB to DNA (through the intercalation mechanism) and non-specific binding with proteins [1–4]. However, the mechanisms of MB binding to DNA are still discussed in the literature. Particularly, according to some data, MB molecules intercalate into the plane between nitrogenous bases. Moreover, various data also indicate that MB molecules can be localized in both minor and major grooves of DNA [1–4].

Recently, the application sphere of MB has expanded due to the development of genosensor (NA-biosensors, NA-chips) technologies. Particularly, MB has

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demonstrated utility in the development of aptamer-based electrochemical biosensors for both diagnostics and basic research applications [5, 6].

It has been shown that the interference of RNA via different types of short RNAs (i-RNA, regulatory system occurring within eukaryotic cells) is one of the important components of regulation of cellular processes, through which a cell controls the activity of gene expression. These short-stranded molecules of i-RNA bind to m-RNA and form double-stranded (ds-) regions [7–9]. Obviously, ds-regions and ds-RNA can become targets for DNA-specific ligands as well. From this point of view, the studies of the interaction of ligands-intercalators with ds-RNA are of certain interest. Based on this, the work is aimed at studying the interaction of MB with ds-RNA. For this purpose, a synthetic polynucleotide poly(rA)-poly(rU) was chosen as a ds-RNA model.

Materials and Methods. Synthetic polynucleotide poly(rA)-poly(rU), MB ("Sigma", USA, ultrapure), EDTA (ethylenediaminetetraacetate), NaCl, trisodium citrate (chemically pure) were used in experiments. All preparations were used without additional purification. The concentrations of the used preparations were determined spectrophotometrically, using the following extinction coefficients: ε_{260}=7140 $M^{-1}cm^{-1}$ for poly(rA)-poly(rU), ε_{668}=76000 $M^{-1}cm^{-1}$ for MB. Experiments were carried out at ionic strengths of the solution of 0.02, 0.04 and 0.1 *M*, containing only monovalent Na^+ cations.

Spectrophotometric measurements of the concentrations of poly(rA)-poly(rU) and MB were carried out on a UV/VIS PYE Uncam-SP8-100 spectrophotometer, fluorometric measurements on a Varian Cary Eclipse Fluorescence Spectrophotometer. Spectroscopic measurements were done using quartz cuvettes with a volume of 3 *mL* and an optical path length of 1 *cm*.

Results and Discussion. The fluorescence spectra of MB and its complexes with poly(rA)-poly(rU), obtained at a solution ionic strength of 0.04 *M*, are presented in Fig. 1. Analogous spectra were obtained at solution ionic strengths of 0.02 and 0.1 *M* (not shown). The spectra of MB and its complexes with poly(rA)-poly(rU) are similar to those obtained for complexes of this ligand with ds-DNA [10]. Along with an enhancement of the polynucleotide concentration in the solution, the fluorescence intensity decreases due to the change of average local environment of the dye chromophores. Quenching is observed up to the certain concentrations of the polynucleotide, after which the saturation is attained. These changes in the fluorescence spectra of MB indicate the formation of this ligand complex with ds-RNA. Fluorescence quenching of the ligand in the presence of NA is considered as a result of formation of stacking contacts between the aromatic chromophore of the dye and the nitrogenous bases of NA. In consequence of this fact, the frequency of collisions of ligand molecules with solvent molecules decreases when a transition of the ligand chromophore groups to intercalated state – into internal hydrophobic environment of NA occurs. Meanwhile, quenching of the fluorescence of bound dye molecules by poly(rA)-poly(rU), apparently, does not occur due to the energy transfer, because electronic absorption by nucleic acids takes place at shorter wavelengths with respect to dye emission band [11, 12].

This fact suggests that MB interacts with poly(rA)-poly(rU), though binds to the latter by similar mechanisms as with DNA: the fluorescence quenching takes

place due to the fact that the chromophore group of the ligand does not entirely intercalated into the plane between base pairs of NA and becomes available for quenchers (molecules of water and solved oxygen) [10]. This is indicated by the fact that, in the case of EtBr and other intercalators (which entirely intercalate into DNA), the fluorescence intensity significantly increases [13]. Qualitative analysis of the changes of the fluorescence spectra of MB and its complexes with poly(rA)-poly(rU) results in large dispersion of points at the evaluation of portion of bound and free ligand molecules, which ultimately makes it impossible to construct the binding curve of MB with polynucleotide in Scatchard's coordinates. This, in turn, does not permit to estimate the binding parameters of this ligand with poly(rA)-poly(rU), as in the case of DNA [10]. That is why the binding parameters of MB with poly(rA)-poly(rU) were evaluated on the basis of quenching of MB fluorescence according to the Stern–Folmer relationship [11]:

$$\frac{F_0}{F} = 1 + K_{SV}[\mathrm{RNA}]$$

where F_0 and F are the fluorescence intensities of MB in the absence and presence of the quencher (RNA), respectively, K_{SV} quenching constant of Stern–Folmer.

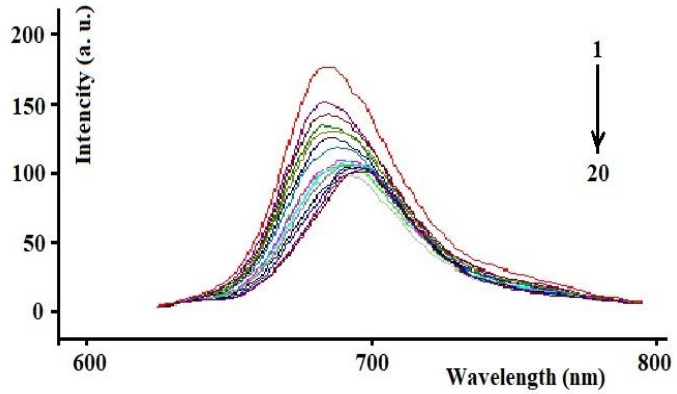

Fig. 1. Fluorescence spectra of MB in the absence (1) and presence (2–20) of synthetic polynucleotide poly(rA)-poly(rU). The ionic strength of the solution was 0.04 *M*, pH 7.0, t=25°C.

The dependence curves of F_0/F on the polynucleotide concentration at solution ionic strengths of 0.02 (curve 1), 0.04 (curve 2) and 0.1 *M* (curve 3) are presented in Fig. 2. It is obvious from the presented figure that the obtained curves are non-linear, especially the curve 1 obtained at an ionic strength of the solution of 0.02 *M*. The analogous curve, obtained earlier at the interaction of MB with DNA, was linear and practically characterized the binding of the ligand with DNA and made it possible to determine the value of the binding constant [10]. The non-linear dependence of F_0/F on the polynucleotide concentration can result from the fact that, along with the binding of MB to polynucleotide, a structural change of the latter occurs, due to which the value of the fluorescence quenching of the ligand by polynucleotide alters. This is indicated by the fact that the synthetic homopolynucleotide poly(rA)-poly(rU), although being a good model for ds-RNA, has an unstable ds-structure at low ionic strengths of the solution, particularly at

μ<0.04 *M*, as was revealed in the work [13]. MB is a stabilizer of the ds-structure of NA, since, by binding to poly(rA)-poly(rU), it can induce structural changes in the polynucleotide, as a result of which, at high ratios of ligand to polynucleotide, the fluorescence quenching increases linearly, then tends to saturation at low ratios. Possibly, at high concentrations of the polynucleotide, MB binds to it by one mode, while at relatively low concentrations of poly(rA)-poly(rU), two binding modes are realized. Two binding modes were earlier revealed at the interaction of MB with ds-DNA, though one of them corresponded to the semi-intercalation type, and the other to the electrostatic type [10]. Based on this we assume that MB binds to poly(rA)-poly(rU), most probably, by two modes.

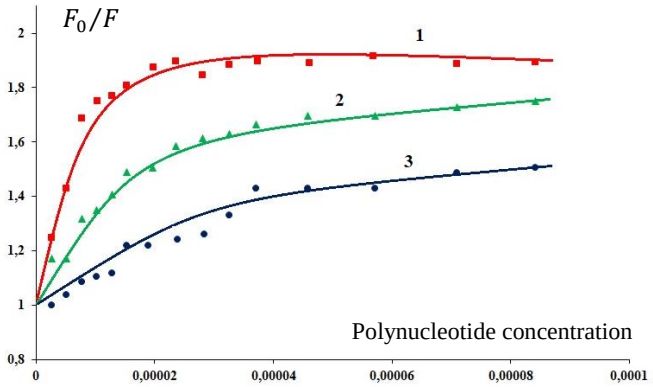

Fig. 2. Curves of F_0/F versus polynucleotide concentration (curves of fluorescence quenching of MB by molecules of poly(rA)-poly(rU)) at the ionic strengths of the solution 0.02 (curve 1), 0.04 (curve 2) and 0.1 *M* (curve 3).

From the curves of the dependence of F_0/F on the polynucleotide concentration, the values of the quenching constants were determined at the respective ionic strengths of the solution: $K(0.02)=5.95\cdot10^3\ M^{-1}$; $K(0.04)=7.7\cdot10^3\ M^{-1}$ and $K(0.1)=6.3\cdot10^3\ M^{-1}$. From the data obtained, it was revealed that K_{SV} took the highest value at the ionic strength of the solution of 0.04 *M*. This fact also is a result of the structural state of the polynucleotide, since at the indicated ionic strength of the solution poly(rA)-poly(rU), on the one hand, is in a stable ds-state and, on the other hand, has such a conformation that is most available for intercalation or semi-intercalation of ligands [13]. At the ionic strength of 0.1 *M*, the polynucleotide structure becomes more compact and closed for incorporation of ligand molecules into the hydrophobic inter-nucleotide space. This results in a situation when a significantly smaller number of ligand molecules pass into a bound state that is why the value of K_{SV} decreases, since this parameter is concentration-dependent, characterizing the interaction of ligands with macromolecules.

Conclusion. Thus, the data obtained indicate that MB, being a DNA-specific ligand, may bind to ds-regions of RNA. In this regard, as in the case of DNA, semi-intercalation is a more preferable mechanism for the binding of this ligand to poly(rA)-poly(rU). On the other hand, the non-linearity of the curves of the dependence of F_0/F on the polynucleotide concentration can be the result of the

performance of two binding modes, the second of them, apparently, is of an electrostatic nature. From the data obtained, it is also revealed that the value of K_{SV} is about an order of magnitude less than in the case of DNA [10], which may indicate that RNA is a less preferable target for MB. However, the data obtained indicate that MB, like another intercalator, EtBr, may be applied as an analyzer of various structures of NA in genosensor technologies.

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ՍԻՆԹԵՏԻԿ ՊՈԼԻՆՈՒԿԼԵՈՏԻԴ POLY(rA)-POLY(rU)-Ի ՀԵՏ ՄԵԹԻԼԵՆԱՅԻՆ ԿԱՊՈՒՅՏԻ ՓՈԽԱԶԴԵՑՈՒԹՅԱՆ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆԸ

Իրականացվել է poly(rA)-poly(rU)-ի հետ մեթիլենային կապույտի (ՄԿ) փոխազդեցության ուսումնասիրություն ֆլուորեսցենտային սպեկտրոսկոպիայի մեթոդով: Ստացված տվյալները բացահայտել են, որ ՄԿ-ն, լինելով ԴՆԹ-ին կապվող սպեցիֆիկ լիգանդ, կարող է կապվել նաև ՌՆԹ-ի երկշղթա հատվածների հետ: Ընդ որում, ինչպես ԴՆԹ-ի դեպքում, ՄԿ-ի համար poly(rA)-poly(rU)-ի հետ կապման առավել նախընտրելի մեխանիզմ է կիսաինտերկալյացիան: Մյուս կողմից, պոլինուկլեոտիդի կոնցենտրացիայից F_0/F-ի կախվածության կորերի ոչ գծայնությունը կարող է լինել կապման երկու եղանակների ի հայտ գալու արդյունք, որոնցից երկրորդը, ամենայն հավանականությամբ, ունի էլեկտրաստատիկ բնույթ: Ելնելով ստացված տվյալներից նաև ցույց է տրվել, որ K_{SV}-ի արժեքը գրեթե մեկ կարգով ավելի փոքր է, քան ԴՆԹ-ի դեպքում, ինչը կարող է վկայել այն մասին, որ ՌՆԹ-ն պակաս նախընտրելի թիրախ է ՄԿ-ի համար:

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ИССЛЕДОВАНИЕ ВЗАИМОДЕЙСТВИЯ МЕТИЛЕНОВОГО СИНЕГО С СИНТЕТИЧЕСКИМ ПОЛИНУКЛЕОТИДОМ POLY(rA)-POLY(rU)

Методом флуоресцентной спектроскопии проведено исследование взаимодействия метиленового синего (МС) с poly(rA)-poly(rU). Полученные данные выявляют, что МС, являющийся ДНК-специфическим лигандом, может связываться и с дц-участками РНК. При этом, как и в случае ДНК, наиболее предпочтительным механизмом связывания этого лиганда с poly(rA)-poly(rU) является полуинтеркаляция. С другой стороны, непрямолинейные кривые зависимости F_0/F от концентрации полинуклеотида могут быть результатом проявления двух способов связывания, второй из которых скорее всего имеет электростатическую природу. Исходя из полученных данных также выявлено, что значение K_{SV} примерно на порядок меньше, чем в случае ДНК, что может указывать на то, что РНК является менее предпочтительной мишенью для МС.