BINDING PECULIARITIES OF POLY(rA)-POLY(rU) WITH MINOR GROOVE LIGAND HOECHST 33258

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Study on the interaction of DNA-specific ligands – classical intercalator acridine orange (AO) and groove binding compound Hoechst 33258 (H33258) with poly(rA)-poly(rU), being a model for double-stranded (ds-) RNA, has been carried out. The absorption and fluorescence spectra of the complexes of these ligands with ds-polynucleotide were obtained. It was revealed that the optic and fluorescent characteristics of the complexes of both ligands with ds-RNA are similar with those at the complex-formation with DNA.

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Introduction. Structure of double-stranded (ds-) RNA has important regulating effects in a cell, since it can serve as a target for modulation of cellular processes [1]. From this point of view, molecular recognition of ds-RNA by various small molecules can be a key phase of regulation of some processes: transport, editing and maturation of cellular RNA, antiviral response of interferon, RNA-interference etc. Taking this fact into account, for rational design of new RNA-binding molecules, detailed basic knowledge is needed about main aspects of interaction – mode, mechanism, affinity, specificity and selectivity of already existing molecules [2]. One of possible paths in this direction is a study of interaction of DNA-specific ligands with ds-form of RNA. Among various small molecules non-covalently binding compounds, possessing different interaction mechanisms with nucleic acids, are of great interest, particularly, intercalators, as well as groove binding materials [3].

Intercalators can bind not only with DNA, but also with RNA or proteins. Nucleic acids (NA) are polyanions and their negatively charged nucleotides permit binding cationic dyes, such as acridine orange (AO-3,6-dimethylaminoacridine). AO interacts with ds-DNA by intercalation, while with single-stranded (ss-) RNA it mainly binds electrostatically, which leads to different fluorescence with maximal irradiation at $\lambda = 530 \text{ nm}$ and $640 \text{ nm}$ for DNA and RNA respectively. Basophilic AO also binds to other anions, for example, to proteins, which sometimes makes hard DNA dying in vivo [4–8]. Interaction of AO is a complicated process, which includes

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different states of ligand in equilibrium – free and bound monomers of AO with DNA, free and bound aggregates of AO, bound to monomeric molecules or with aggregates of DNA. Aggregation decreases lifetime and quantum yield of excited states of AO. On the other hand, disaggregation of AO at excess of DNA increases the lifetime of the ligand quantum yield in excited states. AO binding with DNA decreases a probability of its contact to other molecules as well. It, particularly, can reduce a transition probability of energy from excited AO molecules to molecular oxygen. All these effects can change the efficiency of AO as photosensibilizer in photodynamic therapy or fluorescent diagnosis [9].

One of known groove binding compounds is Hoechst 33258 (H33258) – derivative of N-methypiperazine with two benzimidazole groups and one phenyl group. This is a long flexible molecule with positively charged end and many donors and acceptors of proton, which contributes to formation of hydrogen bonds between ligand and macromolecules. Interest to H33258 is high due to its clinical value, for example, it has an antimicrobial and antitumor as well as radioprotector action [10, 11]. As a basis for some drug preparations, H33258 can easily be absorbed by cells and show a selectivity to ds-DNA, ds-RNA, A-DNA and NA in other conformations [3].

The presented work is aimed at studying of binding peculiarities of AO and H33258 binding to synthetic polynucleotide poly(rA)-poly(rU), which is a model for ds-RNA to reveal the interaction modes.

Materials and Methods. In this work poly(rA)-poly(rU), AO, H33258 (“Sigma”, USA), physiological solution (sterile apyrogenic) for injection (0.154 $M$ NaCl) (Liqvor Pharmaceuticals, Armenia), deionized water, resistivity $R$ equal to $18.2$ $M\Omega\cdot cm$ (H2O Economy, LLC, Armenia – US JC) were used. All preparations were used without additional purification. Concentrations of poly(rA)-poly(rU) and ligands were determined spectrophotometrically, using the following coefficients of absorption – $\varepsilon_{260}=7140$ $M^{-1}\cdot cm^{-1}$ for poly(rA)-poly(rU), $\varepsilon_{490}=35000$ $M^{-1}\cdot cm^{-1}$ for AO, $\varepsilon_{343}=42000$ $M^{-1}\cdot cm^{-1}$ for H33258.

Spectrophotometric measurements were carried out on double-beam spectrophotometers UV-VIS Unicam-SP-8-100 (England) and Perkin Elmer UV/VIS Lambda 365. Absorption measurements were realized in quartz cuvettes with optic pathway length 1 cm and the same optic parameters. Fluorescent studies were carried out on fluorospectrometer Varian Cary Eclipse Fluorescence Spectrophotometer (Australia).

All measurements were carried out at the solution ionic strength 0.04 $M$, at which poly(rA)-poly(rU) is in only ds-state [12–14].

Results and Discussion. Interaction of cationic compounds with polyanions (particularly, with NA) can be studied using electronic spectroscopy method in visible region, since usually the binding leads to hypochromic and bathochromic effects in absorption bands of respective ligands. Thus, AO has maximal absorption at the wavelength 490 $nm$, the absorption maximum of H33258 out of NA absorption band corresponds to the wavelength 343 $nm$ (absorption spectra of AO and H33258 are presented in Fig. 1). From the presented Figure, it is obvious that along with concentration increasing of poly(rA)-poly(rU), the absorption spectra of AO exhibit a hypochromism and bathochromic shift to longer wavelength region. Proceeding from the fact that dilution of the solutions of ligands was neglecting, obviously the
appeared hypochromism is a result of complex-formation between ligand and NA. It is important to mention that in AO absorption spectra in visible region besides a maximum, a shoulder emerges near the wavelength 475 nm. It was shown that this shoulder increases at high concentrations of AO in solutions, which is a result of dimerization of ligand molecules. Though, at the interaction of this ligand with DNA the absorption of the complexes at both 490 and 475 nm decreases at low concentrations of NA [4].

**Fig. 1.** Absorption spectra of the complexes of AO (a) and H33258 (b) with poly(rA)-poly(rU).

As it is obvious from Fig. 1,a, such an effect was revealed at the titration of AO with poly(rA)-poly(rU) (curve 2), which indicates the formation of the complexes between ligand and NA. Moreover, the spectra, presented in Fig. 1, a, show a similar behavior as those of the complexes AO–ds-DNA. From this point of view, it should be mentioned that the absorption spectra of the complexes AO–poly(rA)-poly(rU), at relatively high concentrations of homopolynucleotide, increase. We assume that as for ds-DNA, molecules of poly(rA)-poly(rU) destroy dimers of AO, resulting in transition of this ligand molecules to monomeric state and binding to NA. It results in bathochromic shift to longer wavelength region (curves 3–5), as well as to the increase of maximum in relation to the shoulder. Though, the absorption spectra of the complexes of AO monomeric forms AO–poly(rA)-poly(rU) also show hypochromism along with increasing of NA concentration. Meanwhile, in contrast to the complexes AO-DNA, at further increasing of NA concentration, the enhancement of maxima and hypochromic shift [4] toward short-wave region are not revealed in the spectra of the complexes AO–poly(rA)-poly(rU). On the other hand, in the spectra of the complexes of intercalator with poly(rA)-poly(rU), an isosbestic point is not formed as in the spectra of the complexes of other intercalators with NA [15–17].

Hypochromism with a small bathochromic shift takes place at the interaction of H33258 with poly(rA)-poly(rU). Analogously to AO, in the case of H33258 a similarity between optic characteristics of the complexes of this ligand with ds-DNA and poly(rA)-poly(rU) is also revealed (Fig. 1, b). This fact can be explained by the binding of DNA-specific (B-conformation) benzimidazole dye H33258 to ds-RNA (A-form of NA).
Fluorescence spectra of the complexes of AO and H33258 with poly(rA)-poly(rU) are presented in Fig. 2. It is obvious from figure that the fluorescence intensity, along with AO binding to poly(rA)-poly(rU), significantly increases as compared to the intensity of non-bound ligand molecules. Analogous effect is observed at the interaction of this ligand with DNA as well. Meanwhile, AO binds to ds-form of DNA by intercalation mechanism and at the saturation of these centers – by electrostatic one [4–6].

As a result of complete intercalation, the fluorescence occurs in green band of visible light. Based on the obtained results we assume that for poly(rA)-poly(rU) the intercalation of AO takes place, since the fluorescence maxima are registered in green band.

It is obvious from Fig. 2, b, that H33258 dye binds to poly(rA)-poly(rU) in a sufficiently effective way, because a sharp enhancement of the fluorescence intensity takes place along with increasing of concentration of the synthetic polynucleotide in the solution.

The observed increase of the luminescence intensity of H33258 indicates the formation of significantly stable complexes, most apparently, due to the fixation of ligand molecules on NA (free molecules of H33258 are flexible and possess an ability of free oscillations, which results in low intensity of their fluorescence) [18]. In this regard, the shift of the maxima to shorter or longer wavelength region practically does not occur, which indicates that the polarity in the surrounding of the ligand bound molecules does not change. Obviously, the hydration degree in the surrounding of free and bound molecules of H33258 is practically the same. It is known that H33258 is a typical AT-specific ligand and is localized in DNA minor groove, which usually is in B-form [19, 20]. By this reason the minor groove of RNA that is in only A-form, becomes wider and not deeper, the major one – narrower and deeper, while in B-DNA – vice versa. Therefore, structural differences of these conformations should affect the affinity of ligands to A- or B-forms of NA [21, 22]. Taking this into account, we assume that H33258 is localized mainly in major groove of poly(rA)-poly(rU), where the polarity practically is the same as in the solution.

**Conclusion.** Thus, the obtained data indicate that AO and H33258, being DNA-specific ligands and possessing various mechanisms of interaction with DNA, practically bind to poly(rA)-poly(rU) (ds-RNA) with the same probability. Moreover, we can assume that these fluorescent dyes are specific not only for DNA, but also for RNA. In this regard, it is revealed that these ligands bind to both B- and
A-forms of NA with the equal probability. We also assume that the obtained data can be useful for application of the mentioned ligands as potential therapeutic measures as well as can serve as a basis for design of new drug or bioactive preparations.

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ОСОБЕННОСТИ СВЯЗЫВАНИЯ POLY(rA)-POLY(rU)
С МАЛО-ЖЕЛОБКОВЫМ ЛИГАНДОМ HOECHST 33258

Проведено исследование по взаимодействию ДНК-специфических лигандов, классического интеркалятора акридинового оранжевого (АО) и желобково связывающегося соединения Hoechst 33258 (H33258) с poly(rA)-poly(rU), являющегося моделью двухцепочечной (дц-) РНК. Получены спектры поглощения и флуоресценции комплексов этих лигандов с дц-полинуклеотидом. Выявлено, что оптические и флуоресцентные характеристики комплексов обоих лигандов с дц-РНК схожи с таковыми при их комплексообразовании с ДНК.