

ABSORPTION AND FLUORESCENCE SPECTRA OF THE COMPLEXES
OF METHYLENE BLUE AND ACRIDINE ORANGE
WITH POLY(rA)-POLY(rU)M. A. SHAHINYAN *, Z. H. MOVSISYAN, G. G. HOVHANNISYAN,
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The interaction of methylene blue (MB) and acridine orange (AO) with poly(rA)-poly(rU) was studied using absorption and fluorescence spectroscopy methods. The absorption and fluorescence spectra of complexes of these ligands with a polynucleotide were obtained, similar to those of complexes of these ligands with double-stranded (ds-) DNA. It was revealed that the isosbestic point in the spectra of the AO-poly(rA)-poly(rU) and MB-poly(rA)-poly(rU) complexes is not formed, although the binding of AO and MB with ds-RNA by intercalation mode is not excluded.

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Keywords: synthetic polynucleotide, methylene blue, acridine orange, absorption spectrum, fluorescence spectrum.

Introduction. Interaction of different biologically active compounds with nucleic acids (NA) nowadays is of big interest. Many of these compounds, including methylene blue (MB), acridine orange (AO) (Fig. 1) and others are multi-functional from the point of both the interaction with biomacromolecules and the effect on a cell, which conditions its practical application. MB has a powerful antioxidant property, since it can block various oxidative processes in organism as well as shows an anti-inflammatory property. Recently MB has been shown to be able to inhibit the activity of Ebola virus and MERS-CoV (COVID, which invokes severe respiratory disease) in blood plasma. MB as well as AO are known to be photosensibilizers, possessing a high photosensitivity, and absorbing the light energy, they transfer the latter to oxygen, which transits into singlet activated state. In this state the oxygen acquires a chemically high activity and initiates oxidative damages in the covalent structure of biomolecules, as a result of which cells become non-survivable and die. This property leads to their practical application in, so called, photodynamic therapy at the curing of oncological diseases [1–3]. MB possesses a vessel-contracting property as well as it is applied in therapy of different psychoses at bipolar and neuron-degenerative disorders, particularly, against dementia, Alzheimer’s disease, besides, MB can significantly suppress an anti-depressant monoamine-oxidase [1–5].

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AO also has a number of unique biological properties, such as anti-tumorous, photosensibilizing, pH-determining activities, fluorescence emission and dyeing activity in sperm, bacteria, viruses, parasites and fungi, as well as metachromasia. AO and MB have a planar chemical structure (Fig. 1), they easily can penetrate into cytoplasm through plasmatic membrane and bind to various RNA [6–10].

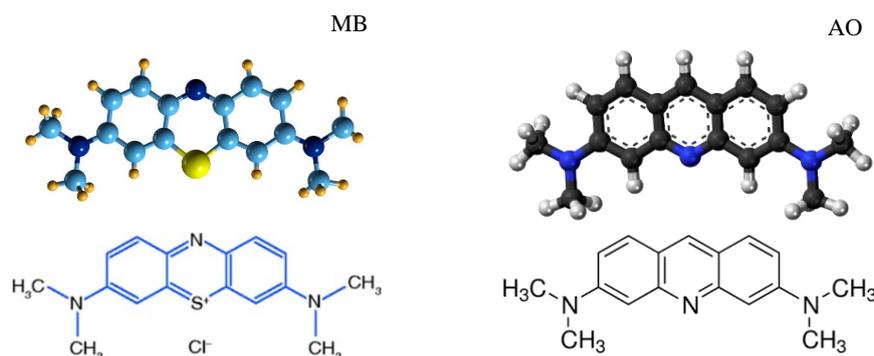


Fig. 1. Chemical structures of methylene blue and acridine orange.

AO and MB preferably bind to double-stranded (ds-) DNA by intercalation mode, but they can bind to single-stranded (ss-) nucleic acids (NA) as well. However, the interaction mechanism of these ligands with ss-NA is still under discussion [11–14].

The presented work is aimed at spectroscopic (absorption and fluorescence) studying of MB and AO complexes with ds-RNA as a model of which poly(rA)-poly(rU) serves.

Materials and Methods. Poly(rA)-poly(rU) (“Sigma”, USA), AO, MB (“Sigma”, USA), NaCl, Na-citrate, EDTA (ethylenediaminetetraacetate) (chemically pure), physiological solution (sterile apyrogenic) for injection (0.154 M NaCl) (Liquor Pharmaceuticals, Armenia), deionized water, resistivity R equal $18.2 \text{ M}\Omega \cdot \text{cm}$ (H₂O Economy, LLC, Armenia–US JC) were used in experiments. All preparations were used without any purification.

Spectroscopic studies were carried out in the solution with ionic strength 0.04 M at which poly(rA)-poly(rU) is in stable ds-form.

Concentrations of poly(rA)-poly(rU) and ligands were determined spectrophotometrically, using the following values of absorption coefficients: $\varepsilon_{260} = 7140 \text{ M}^{-1} \cdot \text{cm}^{-1}$ for poly(rA)-poly(rU), $\varepsilon_{490} = 35,000 \text{ M}^{-1} \cdot \text{cm}^{-1}$ for AO; $\varepsilon_{664} = 76,000 \text{ M}^{-1} \cdot \text{cm}^{-1}$ for MB.

Spectrophotometric measurements were carried out on double-beam spectrophotometers UV-VIS Unicam SP-8-100 (England) and Perkin Elmer UV/VIS Lambda 365. Fluorescence studies were carried out on spectrofluorometer Varian Cary Eclipse Fluorescence Spectrophotometer (Australia). Fluorescence spectra in the case of MB were registered in the interval of wavelengths 600–800 nm, at the excitation wavelength 610 nm; in the case of AO – 450–600 nm, at the excitation wavelength 450 nm.

Absorption measurement was realized in quartz cuvettes with width 1 *cm*, volume 1 *mL* and hermetically closing caps and with similar optic parameters. Titration of solutions was realized by micropipettes with whole volume 10 μL (“Hamilton”, USA).

Results and Discussion. Among various analytical methods the absorption and fluorescence spectroscopies are sufficiently informative and their combination permits revealing some physicochemical properties of different compounds on the basis of optical or fluorescence properties of the latter. Thus, the absorption or fluorescence spectra of many ligands are exposed to noticeable changes at their complex-formation with proteins or nucleic acids. Moreover, the changes in the absorption spectra of biologically active compounds at the binding to biomacromolecules can reflect the peculiarities of molecular mechanisms of these interactions [4–6]. Taking this fact into account we have studied the complexes of MB and AO with poly(rA)-poly(rU) and the absorption spectra of these complexes were obtained and presented in Fig. 2.

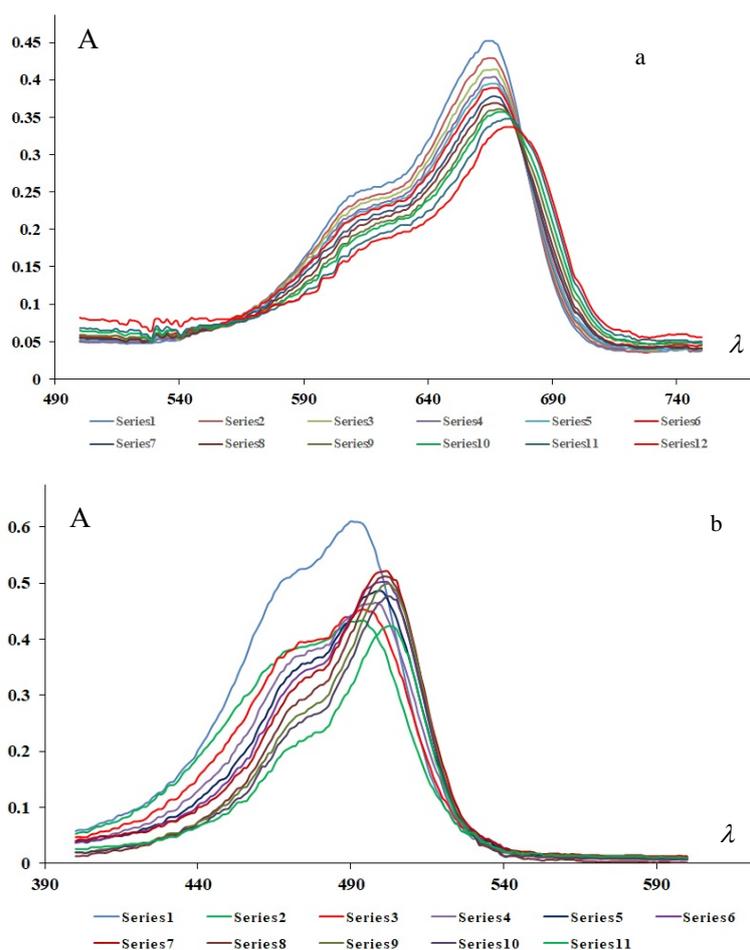


Fig. 2. Absorption spectra of the complexes of MB (a) and AO (b) with poly(rA)-poly(rU).

It is obvious from Fig. 2, that the absorption spectra of MB in the presence of poly(rA)-poly(rU) (Fig. 1, a) decrease monotonously along with increasing of polynucleotide concentration. Analogous effect takes place at the binding of this ligand with ds-DNA [15, 16]. This fact allows us to conclude that MB also binds to ds-RNA. It is also obvious from Fig. 2, that an isosbestic or pseudo-isosbestic points are not formed in the absorption spectra of the complexes MB-poly(rA)-poly(rU). It should be mentioned that from this point of view, the absorption spectra of the complexes MB-poly(rA)-poly(rU) and MB-ds-DNA differ from each other, since a pseudo-isosbestic point is formed in the spectra of the complexes of this ligand with DNA, resulted from the binding semi-intercalation mode of MB. In the Fig. 2, b, the absorption spectra of the complexes of AO-poly(rA)-poly(rU) are shown, which along with some similarity, also have some differences compared to those of the complexes AO-ds-DNA. We should also mention that although AO is a classical intercalator, an isosbestic point is not formed in the spectra of AO complexes with ds-NA (both DNA and RNA).

Fluorescence spectra of the complexes of MB (a) and AO (b) with poly(rA)-poly(rU) were obtained and presented in Fig. 3.

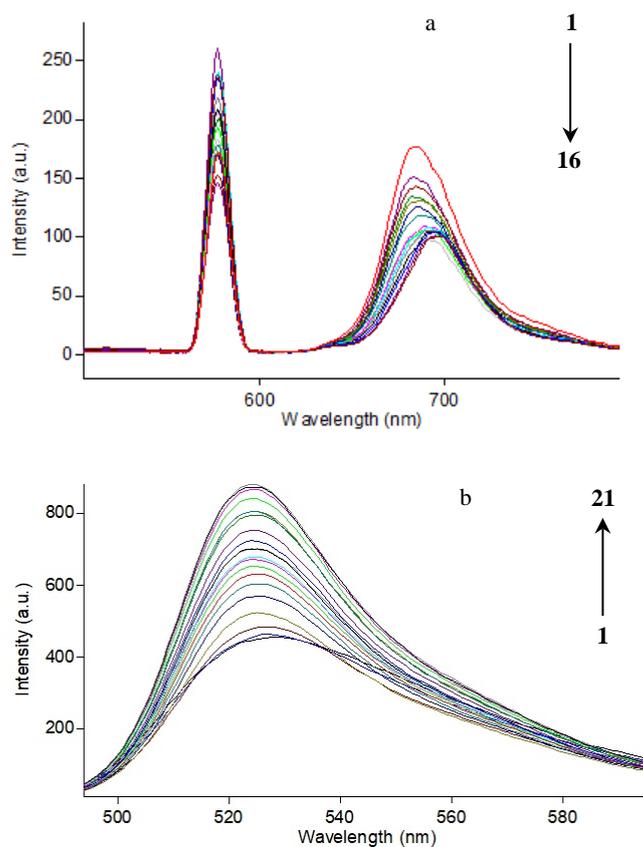


Fig. 3. Fluorescence spectra of the complexes of MB (a) and AO (b) with poly(rA)-poly(rU).

At fluorescence measurements (as at absorption ones) the ligand concentration remained constant, while the polynucleotide concentration increased in the solution. Though, the fluorescence intensity of the complexes MB–poly(rA)–poly(rU) decreased (Fig. 3, a) as compared to that of MB. Analogous effect was revealed for MB complexes with ds-DNA as well [16]. At the same time the spectra of the complexes AO–poly(rA)–poly(rU) increase at the enhancing of the ds-polynucleotide concentration in the solution. It is known that acridine ring (AO) is inserted into the plane between DNA base pairs and enters into stacking interactions with it. As a result, the hydrophobic aromatic rings of the ligand are screened from water and dissolved oxygen (active quenchers of fluorescence) that is why the fluorescence intensity increases [17]. The observed reverse scene in the case of MB comes from the sulfur atom presence in acridine nucleus (Fig. 1), which with positive charge of this ligand molecule leads to unfavorable effects for entire intercalation. Consequently, MB molecules do not fully intercalate into ds-regions of NA and the negative phosphate groups of the latter induce a quenching, which results in decreasing of the fluorescence intensity of the complexes MB–ds-NA as compared to the fluorescence intensity of free MB.

Conclusion. Thus, the obtained experimental results indicate that both MB and AO actually exhibit similar optic and fluorescence properties, while interacting with ds-DNA and ds-RNA. However, absorption characteristics of MB complexes with ds-RNA and ds-DNA are not completely identical: in the case of MB–ds-DNA a pseudo-isosbestic point is appeared in the spectra [16], while in the case of MB–poly(rA)–poly(rU) the spectra of the complexes come close to each other, but do not cross as for the isosbestic point formation and do not contact as for the pseudo-isosbestic point formation. In the case of AO, structural analogue of MB, the absorption and fluorescence spectra of the complexes of this ligand with poly(rA)–poly(rU) and ds-DNA are in fact identical.

Based on the results we assume that some difference between optic characteristics of the complexes MB–poly(rA)–poly(rU) and MB–ds-DNA results from NA conformation, while the NA conformation do not affect the spectral characteristics of AO complexes with ds-RNA and ds-DNA.

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POLY(rA)-POLY(rU)-Ի ՀԵՏ ՄԵԹԻԼԵՆԱՅԻՆ ԿԱՊՈՒՅՏԻ
ԵՎ ԱԿՐԻՂԻՆԱՅԻՆ ՆԱՐՆՁԱԳՈՒՅՆԻ ԿԼԱՆՄԱՆ ԵՎ
ՖԼՈՒՈՐԵՍԵՆՑԻԱՅԻ ՍՊԵԿՏՐՆԵՐԸ

Իրականացվել է poly(rA)-poly(rU)-ի հետ մեթիլենային կապույտի (ՄԿ) և ալիլիդինային նարնջագույնի (ԱՆ) փոխազդեցության հետազոտություն

կլանման և ֆլուորեսցենցիայի սպեկտրադիտման մեթոդներով: Ստացվել են պոլինուկլեոտիդի հետ այս լիգանդների կոմպլեքսների կլանման և ֆլուորեսցենցիայի սպեկտրները, որոնք նման են երկշղթա ՌՆԹ-ի հետ դրանց կոմպլեքսների համանուն սպեկտրներին: Բացահայտվել է, որ ԱՆ-poly(rA)-poly(rU) և ՄԿ-poly(rA)-poly(rU) կոմպլեքսների սպեկտրներում իզոբեստիկ կետ չի ձևավորվում, ինչը սակայն չի բացառում ԱՆ-ի և ՄԿ-ի կապումը երկշղթա ՌՆԹ-ի հետ ինտերկալյացիոն մեխանիզմով:

М. А. ШАГИНЯН, З. О. МОВСИСЯН, Г. Г. ОГАННИСЯН, П. О. ВАРДЕВАНЯН

СПЕКТРЫ ПОГЛОЩЕНИЯ И ФЛУОРЕСЦЕНЦИИ КОМПЛЕКСОВ
МЕТИЛЕНОВОГО СИНЕГО И АКРИДИНОВОГО ОРАНЖЕВОГО
С POLY(rA)-POLY(rU)

Проведено исследование взаимодействия метиленового синего (МС) и акридинового оранжевого (АО) с poly(rA)-poly(rU) методами абсорбционной и флуоресцентной спектроскопии. Получены спектры поглощения и флуоресценции комплексов этих лигандов с полинуклеотидом, которые схожи с аналогичными спектрами их комплексов с двухцепочечной (дц-) ДНК. Выявлено, что изобесстическая точка в спектрах комплексов АО-poly(rA)-poly(rU) и МС-poly(rA)-poly(rU) не образуется, что, однако, не исключает связывание АО и МС с дц-РНК интеркаляционным механизмом.