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SPECTROPHOTOMETRIC STUDY OF COMPLEXES OF SOME INTERCALATORS WITH DOUBLE-STRANDED AND SINGLE-STRANDED NUCLEIC ACIDS

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Interaction of ethidium bromide (EtBr), acridine orange (AO) and non-intercalator methylene blue (MB) with DNA as well as interaction of EtBr and MB with single-stranded synthetic polynucleotides poly(rA) and poly(rU) have been studied. It was revealed that in absorption spectra of EtBr and MB with DNA or single-stranded polynucleotides a real- or pseudo-isosbestic point appears. Particularly, in absorption spectra of the complexes of classical intercalator EtBr with DNA the real isosbestic point is formed, but in the case of non-classical intercalator MB a pseudo-isosbestic point is formed, while in the absorption spectra of their complexes with single-stranded polynucleotides a pseudo-isosbestic point is formed. It was found out that for another classical intercalator AO there is no a real or pseudo-isosbestic point.

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***Keywords*:** DNA, synthetic polynucleotides, classical and non-classical intercalators, absorption spectra, binding mode.

Introduction. Nucleic acids (NA) as well as proteins are polymer compounds and compose the most important constituents of a cell that are permanently in the surrounding of various ligands and can form different chemical bonds with them. Remarkably, in the absorption spectra of the complexes of numerous compounds in the absorption spectra there can appear one or several isosbestic points (IP). However, as a spectral characteristic IP seldom is applied for information quantitative or qualitative analysis [1–7]. Indeed, for the given concentrations, IP is an important feature for characterizing of qualitative and quantitative properties of one compound chromophore or the mixture of such compounds. It is considered that IP is expressed only in that case, when the given compound in the solution, which has the certain absorption, is transited into another one, the absorption of which is not the same as compared to the initial compound. But the spectra of these two forms are crossed at one point, which is called isosbestic one. IP is also expressed, when there exist, at least, two compounds in the solution with various spectral properties, which, in turn, can have the same absorption in one or several points [1–7].

According to the accepted idea, IP formation in the absorption spectra of ligand complexes with DNA resulted from exhibition of intercalation mechanism.

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Particularly, IP is formed in the absorption spectra of the complexes of classical intercalator ethidium bromide (EtBr) as well as methylene blue (MB) with DNA. Studies of the interaction of these ligands with NA are important due to their biological meaning [8–14]. The received data in recent years have indicated that the formation of IP in the absorption spectra of the complexes NA-intercalator ligand is not the interaction result by intercalation mechanism [8].

The work is aimed at analyzing of IP in the absorption spectra of the complexes of EtBr, MB and AO with double-stranded (ds-) and single-stranded (ss-) NA.

Materials and Methods. In the experiments ultrapure DNA, poly(rA), poly(rU) ("Sigma", USA), ethidium bromide (EtBr) ("Serva", Germany), MB, acridine orange (AO) ("Sigma", USA), NaCl, Na-citrate, ethylenediaminetetraacetate (EDTA) were used. All preparations were used without additional purification. DNA, extracted from calf thymus cells, is high-molecular, average molecular mass of ss-polynucleotides poly(rA) and poly(rU) is equal to 700–800 *kDa*.

Concentrations of used preparations were determined by absorption spectroscopy, using the following values of extinction coefficient: ε_{260}=6600 $M^{-1}cm^{-1}$ for calf thymus DNA, ε_{480}=5700 $M^{-1}cm^{-1}$ for EtBr, ε_{664}=76000 $M^{-1}cm^{-1}$ for MB, ε_{490}=35000 $M^{-1}cm^{-1}$ for AO, ε_{257}=10500 $M^{-1}cm^{-1}$ for poly(rA) and ε_{260}=9500 $M^{-1}cm^{-1}$ for poly(rU) [15]. The studies were carried out in standard salt-citrate solution (1×SSC), which contains 0.15 *M* NaCl, 0.015 *M* three-substituted sodium citrate (10^{-5} *M* EDTA). The ionic strength of the solution was equal to 0.1 *mol*. To get the necessary ionic strength the solution of 1×SSC was diluted by bi-distilled water.

Spectrophotometric measurements were carried out, using UV-VIS double-beam spectrophotometer Perkin Elmer Lambda 365 (USA), in hermetically closed quartz cuvettes with optic pathway 1 *cm*.

During spectroscopic measurements, the concentration of ligands remained constant and the concentration of NA (DNA, polynucleotides) increased during titration. The absorption spectra of the complexes NA–EtBr and NA–AO were registered in the interval $400 \le \lambda \le 600$ *nm*, those for the complexes NA–MB – in the interval $500 \le \lambda \le 750$ *nm*.

EtBr absorption maximum was registered at $\lambda = 480$ *nm*, in the case of AO at $\lambda = 490$ *nm* and in the case of MB at $\lambda = 665$ *nm*. During spectrophotometric titration, the maxima of the absorption spectra under the mentioned wavelengths decreased. In the case of DNA, a shift toward long wavelength interval took place as well.

All absorption spectra and analytic figures were obtained through software Microsoft Excel 13. The experimental error does not exceed 5%, hence the error-bar on analytic curves is not presented.

Results and Discussion. The studies of molecular mechanisms of the interaction of different ligands with DNA will be more effective, if they are based on fundamental models. From this point of view, the obtained results for classical intercalator EtBr complexes with DNA can be a convenient tool to get additional information about the interaction of other ligands with DNA. Particularly, in the case of this ligand interaction with DNA IP appears in the absorption spectra and to prove this fact a mathematical model has been suggested [6]. Absorption spectra of DNA–EtBr complexes are presented in Fig. 1, a, where there are A_i titration spectra

with number N in $1 \le I \le N$ interval. Moreover, the spectrum i=1 corresponds to EtBr absorption in the case of absence of DNA. According to the suggested model, the values of absorption dispersion – σ_i, depending on λ were determined and the respective curve was constructed, which is presented in Fig. 1, b. $\sigma(\lambda)$ function minimum in this dependence curve corresponds to the wavelength (λ_{IP}) of the IP. It is necessary to reveal the IP and its position in case, when the latter exists. As it is obvious from the given Fig. 1, there is a pronounced minimum point in the σ dependence curve on λ at the wavelength 515 *nm*. It is obvious that the formation of IP, most apparently, resulted from the fact that, at least, one interaction mode of EtBr with DNA occurs by intercalation mechanism.

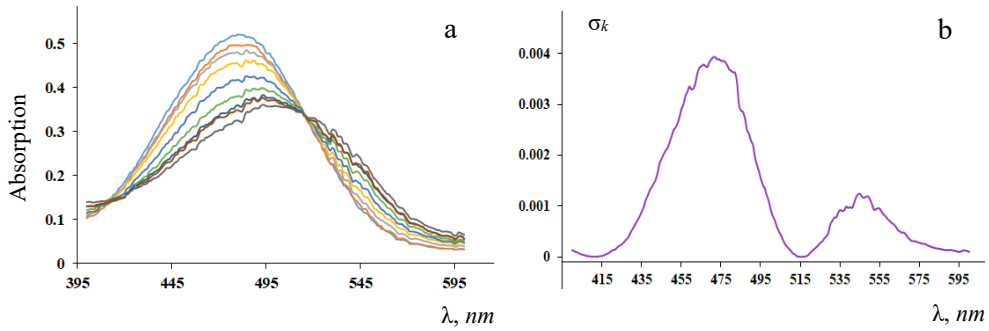

Fig. 1. (a) – Absorption spectra of the complexes DNA–EtBr.
(b) – Dependence of σ_k on λ, in which there is minimum point, which corresponds to IP.

To check this fact the absorption spectra of the complexes DNA–AO were analyzed as well. For the complexes DNA–EtBr, DNA–AO and DNA–MB the absorption spectra were received, from the analysis of which the curves $\sigma(\lambda)$ are presented in Fig. 2. It is obvious from the Fig. 2 that in the absorption spectra of the complexes AO–DNA there is no IP, although in the dependence curve of σ_k on λ a minimum is formed as well at λ = 500 *nm*. However, the minimal value of this function – $\sigma_k = 2 \cdot 10^{-3}$, which is higher by three orders, than for the complexes EtBr–DNA (λ = 515 *nm*, $\sigma_k = 5.34 \cdot 10^{-6}$).

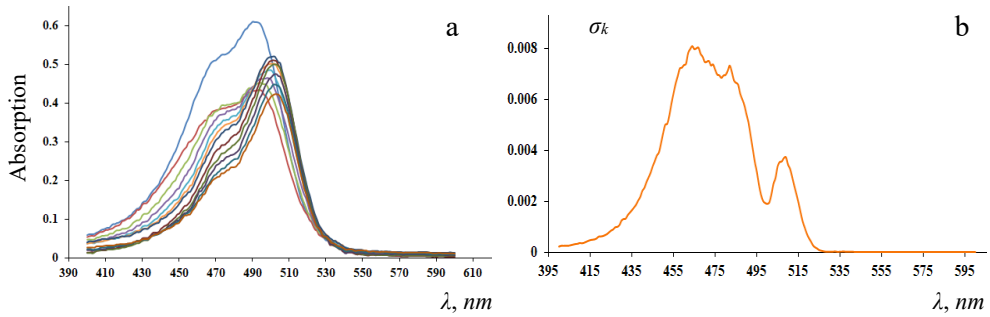

Fig. 2. (a) – Absorption spectra of the complexes DNA–AO. There is no an IP in the absorption spectra.
(b) – Dependence of σ_k on λ.

Absorption spectra of the complexes MB–DNA were analyzed and presented in Fig. 3. MB differs from AO insignificantly by its structure, but as it is shown in Fig. 3, non-exact IP was appeared in the absorption spectra of this ligand complexes with DNA by the following characteristics $\lambda = 677\ nm$, $\sigma_k = 3.53 \cdot 10^{-5}$ (Fig. 3).

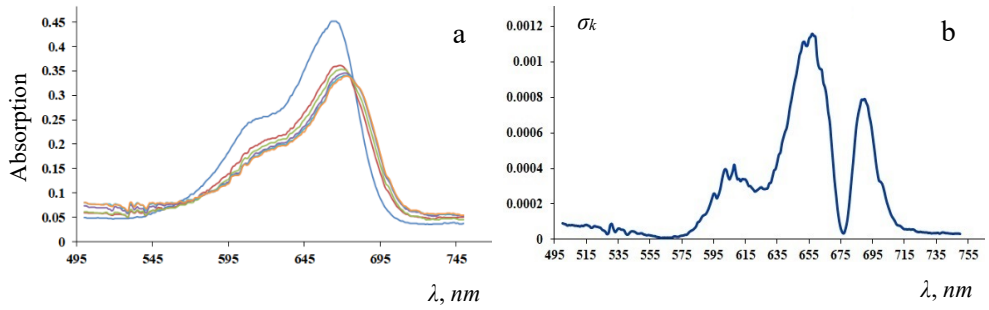

Fig. 3. (a) – Absorption spectra of the complexes DNA–MB. There is non-clear IP in the absorption spectra. (b) – Dependence of σ_k on λ.

It is remarkable that the minimum point in σ_k dependence on λ is sufficiently exact, but it is almost an order higher, than the forming one in the absorption spectra of the complexes EtBr–DNA and is a pseudo-isosbestic point [8].

Thus, analysis of the absorption spectra of various intercalators with DNA via mathematical model, suggested in the work [6], permits revealing and characterizing real or pseudo-isosbestic point presence in the absorption spectra of these complexes.

Absorption spectra of the complexes of EtBr and MB with single-stranded synthetic homopolynucleotides poly(rA) and poly(rU) were obtained. In the Fig. 4 EtBr absorption spectra with ss-poly(rU) (a) and dependence curve of σ_k on λ (b) were presented. It is obvious that in the absorption spectra of the complexes of EtBr–poly(rU) there is non-exact IP (a), which is expressed in σ_k dependence curve on λ through the minimum at $\lambda = 520\ nm$. This fact indicates that classical intercalator EtBr binds to ss-polynucleotides as well, though, in all appearances, the interaction takes place by intercalation mechanism. Based on literature data, we assume that EtBr molecules form semi-intercalation complexes with ss-polyribonucleotides [16, 17]. Analogous data were obtained for EtBr–poly(rA) complexes as well (absorption spectra and σ_k dependence curve on λ are not presented).

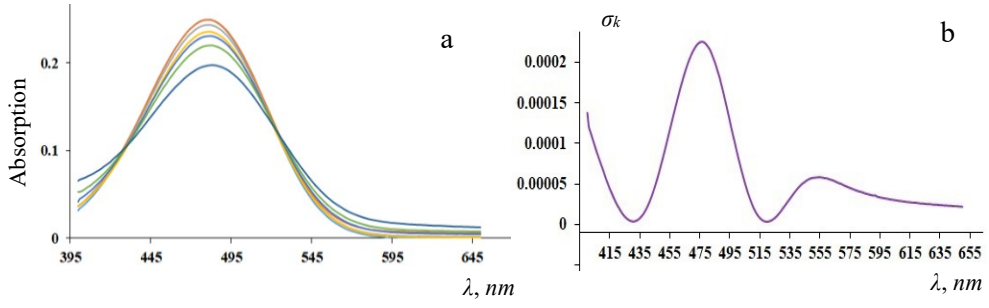

Fig. 4. (a) – Absorption spectra of the complexes EtBr–poly(rU). There is non-exact IP. (b) – Dependence of σ_k on λ.

Absorption spectra of MB complexes with homopolynucleotides poly(rA) and poly(rU) and σ_k dependence curve on λ were obtained as well. Absorption spectra of the complexes MB–poly(rU) is presented in Fig. 5, a, and as it is seen, there is non-exact IP, to which a minimum point corresponds in the dependence curve of σ_k on λ at $\lambda = 675$ *nm* were presented.

It is remarkable that in the absorption spectra of the complexes MB–poly(rA) there is no a formed IP. The fact that there exists an IP the absorption spectra of the complexes MB–poly(rU) as for those of MB complexes with ds-nucleic acids, indicates that MB presumably binds to this polynucleotide by the same mechanism. Earlier it was shown that MB interacts with ds-DNA and ds-RNA through semi-intercalation and electrostatic mechanisms [8], hence, we conclude that the interaction of MB with poly(rU) occurs by the same modes.

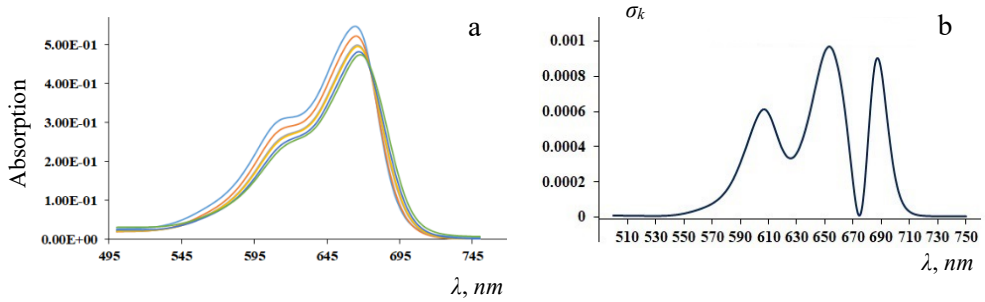

Fig. 5. (a) – Absorption spectra of the complexes MB–poly(rU). In these spectra there is non-exact IP. (b) – Dependence of σ_k on λ.

IP analysis was realized through the titration of ligand–ss-poly(rA) and ligand–ss-poly(rU) complexes with the solution of complementary polynucleotides (respectively, poly(rU) and poly(rA)), which results in formation of the complexes ligand–poly(rA)-poly(rU). In the case of EtBr, a decrease of the absorption maxima and shift toward long wave interval take place, as a result of complex-formation of this ligand with ds-NA (spectra are not presented). It is remarkable that the shift of IP took place toward short wavelength interval as well. As it was mentioned above, in the absorption spectra of EtBr complexes with ds-DNA IP again was formed at $\lambda = 515$ *nm*. This fact permits us concluding that the titration of EtBr–poly(rU) complexes with poly(rA) results in hybridization of ss-polyribonucleotides as well as formation of ds-poly(rA)-poly(rU), due to which IP is shifted toward short wavelength interval. Such a result was obtained also for the contrary titration, when the solution of poly(rU) was added to EtBr–poly(rA) complexes by equimolar concentrations of polynucleotides. Similarly, titration of MB–poly(rU) complexes by the solution of poly(rA) leads to the significant decrease of the absorption spectra maxima and long wavelength shift, which is less expressed in the absorption spectra of the complexes MB–poly(rU). However, it was not expectable that there is no IP in the absorption spectra of the complexes MB–poly(rU)-poly(rA) and MB–poly(rA)-poly(rU), which is obvious in the σ_k dependence curve on λ (Fig. 6). The obtained data indicate that there is no a real IP in the absorption spectra of MB complexes with both ds- and ss-NA, as for EtBr. Numerous studies indicate that

although MB binds to ds-NA by intercalation mechanism, it does not totally intercalated, like EtBr, AO and other intercalators. MB mainly binds by not entire intercalation (semi-intercalation) mode, which in turn results in pseudo-IP formation [8, 18]. Taking into account the aforementioned facts, we consider that in the absorption spectra of the complexes ligand–NA there is formed a real IP, when the ligand can simultaneously bind by both entire intercalation and semi-intercalation modes, as EtBr, while in the cases of the performance of only intercalation or semi-intercalation modes, a real IP is not formed.

Conclusion. Thus, the obtained data indicate that in the cases of several intercalatores, binding to ds- or ss-NA, there appear real- or pseudo-isosbestic points in their absorption spectra. Particularly, in the absorption spectra of the complexes of a classical intercalator EtBr to DNA, a real IP is formed, while in the case of non-classical intercalator MB a pseudo-IP is formed, on the other hand, for another classical intercalator AO there is no such a point. This fact indicates that the existence of intercalation mechanism of the binding of ligands to DNA is necessary, but not enough condition for appearing of IP.

The obtained data also find out that for revelation and determination of IP in the absorption spectra the applied method allows to find out and differentiate exact and inexact IPs in the absorption spectra of the complexes of ligand–NA.

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ԵՐԿՇՂԹԱ ԵՎ ՄԻԱՇՂԹԱ ՆՈՒԿԼԵԻՆԱԹԹՈՒՆԵՐԻ ՀԵՏ ՈՐՈՇ ԻՆՏԵՐԿԱԼՅԱՏՈՐՆԵՐԻ ՀԱՄԱԼԻՐՆԵՐԻ ՍՊԵԿՏՐՈՖՈՏՈՄԵՏՐԻԿ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆԸ

Ուսումնասիրվել է երկշղթա ԴՆԹ-ի հետ դասական ինտերկալյատորներ էթիդիումի բրոմիդի (ԷԲ), ակրիդինային նարնջագույնի (ԱՆ) և ոչ դասական ինտերկալյատոր մեթիլենային կապույտի (ՄԿ), ինչպես նաև միաշղթա սինթետիկ poly(rA) և poly(rU) պոլինուկլեոտիդների հետ ԷԲ-ի և ՄԿ-ի փոխազդեցությունը: Հայտնաբերվել է, որ ԴՆԹ-ի կամ միաշղթա պոլինուկլեոտիդների հետ ԷԲ-ի և ՄԿ-ի համալիրների կլանման սպեկտրներում ի հայտ է գալիս իրական կամ կեղծ իզոբեստիկ կետ: Մասնավորապես, ԴՆԹ-ի հետ դասական ինտերկալյատոր ԷԲ-ի կոմպլեքսների կլանման սպեկտրներում ձևավորվում է իրական, իսկ ոչ դասական ինտերկալյատոր ՄԿ-ի դեպքում՝ կեղծ իզոբեստիկ կետ, մինչդեռ միաշղթա պոլինուկլեոտիդների հետ դրանց

համալիրների կլանման սպեկտրներում ձևավորվում է կեղծ իզոբեստիկ կետ: Հայտնաբերվել է, որ մեկ այլ դասական ինտերկալյատոր՝ ԱՆ-ի դեպքում իրական կամ կեղծ իզոբեստիկ կետ չի առաջանում:

А. П. АНТОНЯН, З. О. МОВСИСЯН, А. О. КАРАПЕТЯН, П. О. ВАРДЕВАНЯН

СПЕКТРОФОТОМЕТРИЧЕСКОЕ ИССЛЕДОВАНИЕ КОМПЛЕКСОВ НЕКОТОРЫХ ИНТЕРКАЛЯТОРОВ С ДВУХЦЕПОЧЕЧНЫМИ И ОДНОЦЕПОЧЕЧНЫМИ НУКЛЕИНОВЫМИ КИСЛОТАМИ

Исследованы взаимодействия классических интеркаляторов бромистого этидия (БЭ), акридинового оранжевого (АО) и неклассического интеркалятора метиленового синего (МС) с двухцепочечной (дц-) ДНК, а также взаимодействие БЭ и МС с одноцепочечными (оц-) синтетическими полинуклеотидами poly(rA) и poly(rU). Выявлено, что на спектрах поглощения комплексов БЭ и МС с ДНК и оц-полинуклеотидами появляется реальная или псевдоизобестическая точка. В частности, на спектрах поглощения комплексов классического интеркалятора с ДНК формируется реальная изобестическая точка, тогда как в случае неклассического интеркалятора МС формируется псевдоизобестическая точка; однако, в случае комплексов лигандов БЭ и МС с оц-полинуклеотидами на спектрах поглощения формируется псевдоизобестическая точка. Показано, что в случае другого классического интеркалятора – АО, реальная или псевдоизобестическая точка не образуется.