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Thermodynamic analysis of DNA complexes with methylene blue, ethidium bromide and Hoechst 33258

P. O. Vardevanyan[1], A. P. Antonyan[1], L. A. Hambardzumyan[1], M. A. Shahinyan[1], A. T. Karapetian[2]

[1]Yerevan State University
1, Alex Manoogian Str., Yerevan, Armenia, 0025

[2]Yerevan State University of Architecture and Engineering
301, Teryan Str., Yerevan, Armenia, 0009

biophys_dep@ysu.am

Aim. *To investigate the thermodynamic characteristics of complexes of calf thymus double-stranded DNA with methylene blue (MB), ethidium bromide (EtBr) and Hoechst 33258 (H33258).* ***Methods***. *The binding of MB with double-stranded DNA was observed by UV-melting method.* ***Results***. *Several types of MB binding to DNA-intercalating, semi-intercalating and electrostatic with DNA phosphate backbone, have been revealed at low concentrations of* Na^+ *(2 mM). At high concentrations of cations and low ratios of* r_b *= [ligand]/[DNA] (≤ 0.05), the molecules of ligand semi-intercalate into the space between adjacent bases. At higher concentrations of ligand the main mode becomes electrostatic binding of MB to DNA phosphate groups.* ***Conclusions***. *The comparison of thermodynamic characteristics of DNA-MB complexes with those of EtBr and H33258 indicates that there is more than one mode of binding ligands to DNA: besides nonspecific, external electrostatic binding with phosphate groups, intercalation and semi-intercalation modes of interaction coexist.*

Keywords: UV-spectrophotometry of DNA melting, methylene blue, intercalation, semi-intercalation.

Introduction. Investigation of peculiarities of the complex formation of natural and artificially synthesized ligands with DNA is of actual importance since it permits to reveal the mechanisms of interaction and their specificity in certain regions of nucleic acid [1–7]. Nowadays the ligands noncovalently binding with DNA are divided into two classes – intercalators and groove binding compounds [8–16]. However, the results of theoretical and experimental investigations show that depending on the external medium conditions several ligands may bind to double-stranded (ds-) or single-stranded (ss-) DNA in more than one mode (multimodal ligands) [17–25]. From this point of view methylene blue (MB, Fig. 1), which is considered to be an alternative to the classical intercalator ethidium bromide (EtBr), represents a certain interest. This ligand is a photosensitizer and initiates the formation of singlet oxygen in solution invoking different damages in DNA molecule [20].

A large number of works are devoted to interaction of MB with DNA. It has been shown that depending on the Na^+ concentration, nucleotide sequences, ligand concentration, MB may bind with DNA by different mechanisms but at this moment there is no unambiguously identified dominant type of binding with the biopolymer and this problem remains an interesting matter of discussion [3, 20].

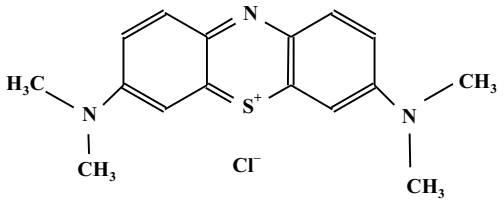

Fig. 1. The structure of methylene blue

In the present work the interaction between MB and calf thymus DNA (ctDNA) has been investigated using UV-melting method. The comparison of thermodynamic parameters of DNA-MB complexes with those of EtBr and Hoechst 33258 (H33258) revealed more than one binding type with DNA [8, 10, 20–26].

Materials and methods. The extra pure ctDNA («Sigma», USA), MB («Aldrich», USA), NaCl, Na-citrate (s. p.) were used in experiments. All preparations were used without additional purification.

The concentrations of DNA and MB were determined by absorption method taking into account the following extinction coefficients: calf thymus – ε_{260} = = 6600 $M^{-1}cm^{-1}$ (the concentration of DNA in solution was ≈ 50–60 μM), MB-ε_{664} = 76000 $M^{-1}cm^{-1}$. The solutions of preparations were prepared in SSC (1 × SSC contains 0.15 M NaCl and 0.015 M Na-citrate (three-substituted)). Investigations were carried out at 2 and 20 mM Na^+, pH ≈ 7.0.

Equipment. The melting of DNA complexes with ligands as well as spectrophotometric measurements were carried out on spectrophotometer PYE-Unicam-SP8-100 (England). The heating of solutions of complexes was performed with the Temperature Programme Controller SP-876 Series 2. The quarts cuvettes with hermetically closed teflon plug with 3 ml volume and 1 cm optic pathway length were used for spectrophotometric measurements. The melting was realized at λ = 260 nm wavelength. The data were displayed on PC monitor via a program elaborated in Lab VIEW medium. The curves of melting were obtained as described in [21].

Results and discussion. The simple method of investigation of the interaction of different compounds with DNA is the melting in ultraviolet region of light. Applying this method the investigations of MB interaction with DNA were carried out at 2 and 20 mM Na^+ concentrations in $0 < r_b \le 0.33$ (r_b = [ligand]/[DNA]) interval of change at λ = 260 nm. The melting curves (not represented here) were obtained and the values of melting temperature – T_m of complexes were determined. The plot of experimentally estimated values of the changes of melting points $\delta(1/T_m)$ ($\delta(1/T_m) = 1/T_0 - 1/T_m$, where T_0 and T_m are the melting temperatures of DNA and ligand-DNA complexes respectively) represented in Fig. 2 shows, that this parameter increases monotonously with ligand concentration enhancement indicating the

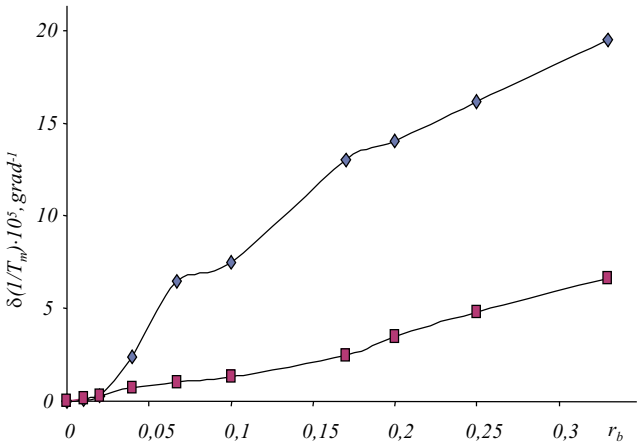

Fig. 2. Dependence of the change in melting temperature $\delta(1/T_m)\cdot10^5$ of the DNA-MB complexes on r_b at: 2 (*1*) and 20 mM (*2*) Na^+

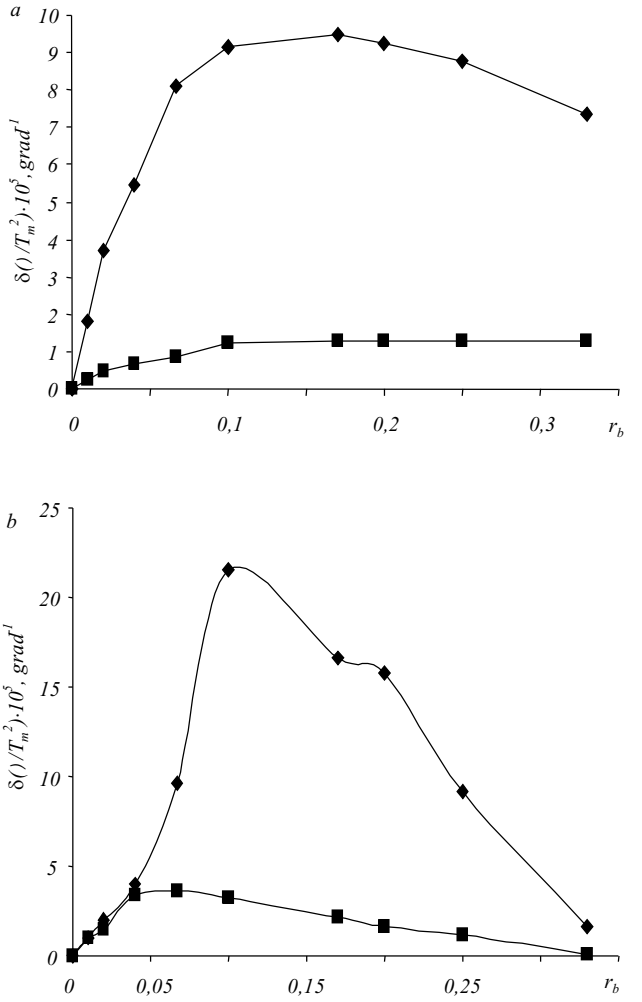

Fig. 3. Dependence of the change $\delta(\Delta T/T_m^2)\cdot10^5$ of the DNA-MB complexes (*a*) on r_b and DNA-EtBr complexes (*b*) at: 2 (*1*) and 20 mM (*2*) Na^+

stabilizing effect of MB of ds-DNA structure at both Na^+ concentrations.

The dependence of other parameter – change of melting interval width – ($\delta(\Delta T/T_m^2) = \Delta T/T_m^2 - \Delta T_0/T_0^2$, where ΔT_0 is the values of melting temperature of DNA; ΔT is that of MB complexes with DNA respectively) on ligand concentration is represented in Fig. 3. The dependence of ($\delta(\Delta T/T_m^2)$ on r_b represented in Fig. 3, *a* (curves *1* and *2*) corresponds to DNA-MB complexes at 2 and 20 mM Na^+. For comparison the analogous curves for EtBr are also represented in Fig. 3, *b*, (curves *1* and *2* at 2 and 20 mM Na^+ concentrations) [24]. Fig 3 shows that for both EtBr and MB the ($\delta(\Delta T/T_m^2)$ (curves *1*) increases at relatively low values of r_b and reaches its maximal values at $r_b \le 0.1$. At further increasing of ligand concentration this parameter starts to decrease in both cases, for EtBr a decrease being sharper [10, 21–27].

We have shown earlier that at low concentrations in case of EtBr the ligand molecules are mainly intercalated into ds-regions of DNA and with the melting they are redistributed from denaturized (ss-regions) to still non denaturized ds-regions as a consequence of enhancement of the dependence of ($\delta(\Delta T/T_m^2)$ on r_b. MB molecules behave in analogous way, since at low concentrations of salt the main mode becomes the intercalation of these ligand molecules into ds-DNA [28].

At subsequent increasing of r_b in case of EtBr the main binding mode is semi-intercalation at which the ligand molecules show practically a similar affinity to both ds- and ss-regions. As a result, the redistribution of bound ligand molecules stops and the dependence of ($\delta(\Delta T/T_m^2)$ on r_b passing through the maximum decreases. Based on the revealing of analogous behavior in case of MB we assume that in these conditions this ligand binds to DNA by semi-intercalation mode as well. This conclusion is in good agreement with the reported data [3, 29, 30].

On the other hand, curve *2* (Fig. 3, *a*) shows that ($\delta(\Delta T/T_m^2)$ of DNA-MB complexes at 20 mM Na^+ increases at low values of r_b and reaches the saturation at $r_b \le 0.1$. Such radical change in the dependence indicates that this ligand binds to DNA in other modes. The data in literature indicate that at low ionic strengths of solution MB binds to GC-rich regions of DNA by intercalation as well. However, the basic interaction mode is the AT-specific binding in one of DNA grooves which practically does not depend on solution ionic strength [20]. It should be mentioned that among ligands binding to DNA, the fluorescent dye for DNA and chromosomes – H33258 shows the pronounced AT-specificity. Moreover this ligand, like netropsin and other lexitropsines, is preferably localized in DNA minor groove, *i. e*. it is a groove binding ligand [31]. Our studies on the melting reveal that at 20 mM Na^+ concentration the dependence of ($\delta(\Delta T/T_m^2)$ on r_b decreases getting negative values (Fig. 4, *b*, curve *3*) [8]. This is conditioned by the fact that at binding to AT sequences the melting temperature of that sequences increases as a consequence of which at relatively low concentrations of ligand ($0 < r_b \le$ $\le 0{,}1$) as well as at the melting DNA-H33258 complexes behave themselves like double-stranded homopolynucleotide the melting interval width of which is usually much less than in case of DNA with quasi-random sequences [27]. This is connected with the fact that DNA is sufficiently heterogeneous system alike aperiodic one-dimensional crystal that melts in wide temperature interval ($\Delta T \approx 10$–15 °C) [32, 33]. At saturation of binding sites of H33258, a decrease in ΔT of complexes stopped and the ($\delta(\Delta T/T_m^2)$ dependence curve gets out of plateau at $r_b > 0.1$, while the $\delta(1/T_m)$ dependence on r_b in these conditions continues to increase indicating that at 20 mM Na^+ concentration H33258 binds to DNA in at least two modes – AT specific in minor groove at low concentrations and electrostatically with phosphate backbone groups at relatively high concentrations [8]. It is also obtained that at 2 mM Na^+ concentration H33258 binds to DNA non specifically, moreover at $0 <$ $< r_b \le 0.1$ ($\delta(\Delta T/T_m^2)$ increases (getting positive values) and at $r_b > 0.1$ gets out of plateau (Fig. 4, *b*, curve *1*) [8]. This is conditioned by the fact that at low ionic strengths of solution the DNA double helix is more untwisted and its diameter is longer than at relatively high ionic strengths of solution [32, 33]. As a result, AT-specific binding of H33258 in DNA minor groove becomes thermodynamically non profitable. In turn it results in radical change in the interaction mechanism of this ligand, and the intercalation of hydrophobic bisbenzimidazole groups of H33258 into the plane of base pairs becomes preferable, since these groups are screened from water [8]. Moreover, the piperazine and phenol groups of H33258 are in polar surrounding that also promotes the stabilization of complexes at low ionic strengths of

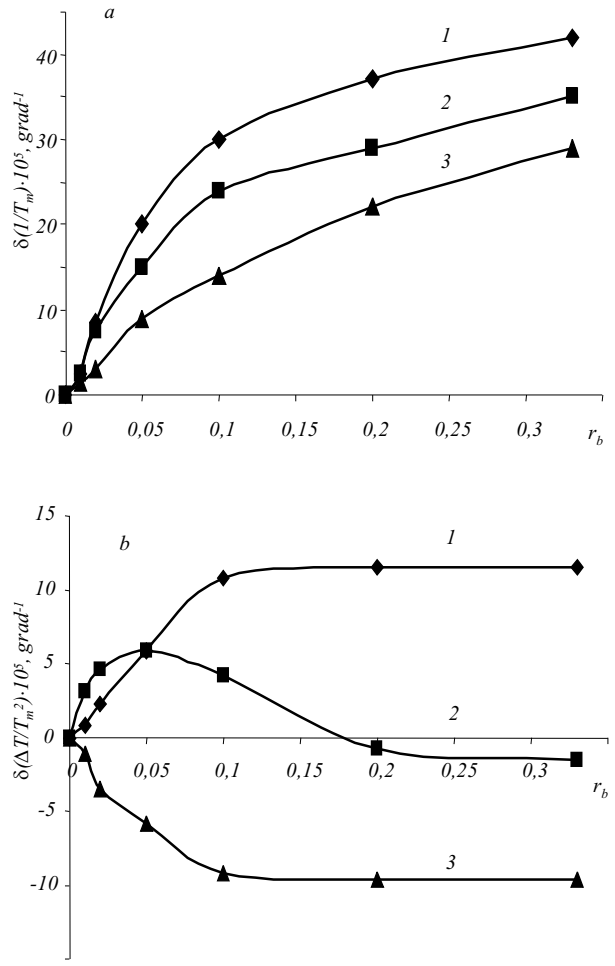

Fig. 4. Dependence of the change in melting temperature $\delta(1/T_m)\cdot 10^5$ (*a*) and $\delta(\Delta T/T_m^2)\cdot 10^5$ (*b*) of the DNA-Hoechst 33258 complexes on r_b at: 2 (*1*), 4 (*2*) and 20 mM (*3*) Na^+

solution. Therefore at non specific (intercalation) binding mode of H33258 to DNA, ΔT of complexes increases, at AT specific binding mode – decreases, moreover in both cases the binding sites for these modes are complicated and at their saturation ligand molecules start interacting with DNA by the second, electrostatic, mode. From this point of view the dependence of $(\delta(\Delta T/T_m^2)$ on r_b in case of MB interaction with DNA at 20 mM Na^+ concentration also may be the result of non specific binding of this ligand to DNA. Meanwhile the incomplete intercalation (semi-intercalation) of ligand molecules into one of DNA chains becomes the most preferable binding mode. This is indicated by the fact that the change in $\delta(\Delta T/T_m^2)$ is significantly less than in case of MB intercalation into DNA. At relatively high salt concentrations, the DNA structure is more compact but at full ligand intercalation the DNA helix should locally untwist to form an intercalation chamber [34]. The geometry of this chamber in case of EtBr is sufficient to intercalate while in case of MB it does not occur. At the same time the semi-intercalation is thermodynamically permitted, since in this case DNA structural reconstructions are not as scaled as in the case of full intercalation [12, 34]. The fact that curve *2* in Fig. 3, *a*, gets out of plateau at $r_b > 0.1$, indicates the limitation of MB binding sites on DNA in semi-intercalation mode and after their saturation the ligand molecules start interacting with phosphate backbone groups of nucleic acid in electrostatic mode.

Therefore the obtained data allow to make the conclusion that ligands preferably interact with DNA in intercalation mode, bind to DNA in minor groove or semi-intercalation mode, moreover in certain cases this mode may be preferable. At the same time ligands interacting with DNA in non intercalation mode and showing specificity to certain types of bases also may intercalate into double helix if the conditions for specific binding are not suitable. It may be also concluded from the obtained data that EtBr is a classic intercalator as well as multimodal ligand and the mechanisms of its binding to DNA do not depend on external medium factors [21, 24, 35] while for both MB and H33258 a certain dependence of interaction mechanisms of these ligands with DNA on external medium factors is revealed (see [3]). The above obtained results may be a good addition to the literature data being practically applicable at the screening of compounds directly binding to DNA and influencing its structural and functional properties.

Conclusions. The interaction of MB with ds-DNA has been characterized in the course of thermodynamic studies. The obtained results show that the mechanisms of MB binding to DNA are similar to those of EtBr: the binding modes of these ligands depend on the molar ratio r_b and the concentration of cations in solution. It was shown that besides nonspecific external electrostatic binding with DNA backbone phosphate groups such interaction modes as intercalation or semi-intercalation binding also existed in the DNA-MB system. At low Na^+ concentrations (2 mM) the possible binding of MB with ds-DNA is intercalation. At increasing cation concentration to 20 mM Na^+ and small r_b ratios ($r_b \leq 0.1$) the ligand molecules semi-intercalate into the nucleic acid base pairs.

П. О. Вардеванян, А. П. Антонян, Л. А. Амбарцумян, М. А. Шагинян, А. Т. Карапетян

Термодинамический анализ комплексов ДНК с метиленовым синим, бромистым этидием и Hoechst 33258

Резюме

Цель. *Изучить термодинамические характеристики комплексов двухцепочечной ДНК тимуса теленка с метиленовым синим (МС), бромистым этидием (БЭ) и Hoechst 33258 (H33258).* ***Методы***. *Связывание МС с двухцепочечной ДНК исследовали методом УФ-плавления.* ***Результаты***. *Обнаружено несколько типов связывания МС с ДНК: интеркаляционный, полуинтеркаляционный и электростатический с фосфатным остовом ДНК при низких концентрациях Na^+ (2 мМ). При больших концентрациях катионов и низких соотношениях r_b = [лиганд]/[ДНК] (≤ 0,05) молекулы лиганда полуинтеркалируют в пространство между соседними основаниями. При более высоких концентрациях лиганда основным способом становится электростатическое связывание МС с фосфатными группами ДНК.* ***Выводы***. *Сравнение термодинамических параметров комплексов ДНК–МС с таковыми для БЭ и H33258 указывает на наличие более чем одного способа связывания лигандов с ДНК. Установлено, что, кроме неспецифического, внешнего электростатического связывания с фосфатными группами, существуют интеркаляционный и полуинтеркаляционный типы взаимодействия.*

Ключевые слова: УФ-спектрофотометрия плавления ДНК, метиленовый синий, интеркаляция, полуинтеркаляция.

П. О. Вардеванян, А. П. Антонян, Л. А. Амбарцумян, М. А. Шагинян, А. Т. Карапетян

Термодинамічний аналіз комплексів ДНК з метиленовим синім, бромистим етидієм і Hoechst 33258

Резюме

Мета. *Дослідити термодинамічні характеристики комплексів дволанцюгової ДНК тимусу теляти з метиленовим синім (МС), бромистим етидієм (БЕ) і Hoechst 33258 (H33258).* ***Методи***. *Зв'язування МС з дволанцюговою ДНК вивчали методом УФ-плавлення.* ***Результати***. *Визначено декілька типів зв'язування МС з ДНК: інтеркаляційний, напівінтеркаляційний і електростатичний з фосфатним остовом ДНК за низьких концентрацій Na^+ (2 мМ). За великих концентрацій катіонів і низьких співвідношеннях r_b = = [ліганд]/[ДНК] (≤ 0,05) молекули ліганда напівінтеркалюють у простір між сусідніми основами. За вищих концентрацій ліганда переважаючим способом є електростатичне зв'язування МС з фосфатними групами ДНК.* ***Висновки***. *Порівняння термодинамічних параметрів комплексів ДНК–МС з такими для БЕ і H33258 виявило більш ніж один спосіб зв'язування лігандів з ДНК. Встановлено, що, окрім неспецифічного, зовнішнього електростатичного зв'язування з фосфатними групами, існують інтеркаляційний і напівінтеркаляційний типи взаємодії.*

Ключові слова: УФ-спектрофотометрія плавлення ДНК, метиленовий синій, інтеркаляція, напівінтеркаляція.

REFERENCES

1. *Hurley L. H.* Secondary DNA structures as molecular targets for cancer therapeutics // Biochem. Soc. Trans.–2001.–**29**, Pt 6.–P. 692–696.
2. *Hurley L. H.* DNA and its associated processes as targets for cancer therapy // Nat. Rev. Cancer.–2002.–**2**, N 3.–P. 188–200.
3. *Hossain M., Giri P., Kumar G. S.* DNA intercalation by quinacrine and methylene blue: a comparative binding and thermodynamic characterization study // DNA Cell Biol.–2008.–**27**, N 2.–P. 81–90.
4. *Denison L., Haigh A., D'Cunha G., Martin R. F.* DNA ligands as radioprotectors: molecular studies with Hoechst 33342 and Hoechst 33258 // Int. J. Radiat. Biol.–1992.–**61**, N 1.–P. 69–81.
5. *van Iperen H. P., Beijersbergen van Henegouwen G. M.* Clinical and mechanistic aspects of photopheresis // J. Photochem. Photobiol. B: Biology.–1997.–**39**, N 2.–P. 99–109.
6. *Osashi M., Oki T.* Ellipticine and related anticancer agents // Expert. Opin. Therp. Patents.–1996.–**6**, N 12..–P. 1285–1294.
7. *Bartulewicz D., Markowska A., Wolczynski S., Dabrowska M., Rozanski A.* Molecular modeling, synthesis and antitumor activity of carbocyclic analogues of netropsin and distamycin – new carriers of alkylating elements // Acta Biochim. Pol.–2000.–**47**, N 1.–P. 23–35.
8. *Vardevanyan P. O., Antonyan A. P., Parsadanyan M. A., Pirumyan K. V., Muradyan A. M., Karapetyan A. T.* Influence of ionic strength of Hoechst 33258 binding with DNA // J. Biomol. Struct. Dyn.–2008.–**25**, N 6.–P. 641–646.
9. *Bugs M. R., Cornelio M. L.* Analysis of the ethidium bromide bound to DNA by photoacoustic and FTIR spectroscopy // Photochem. Photobiol.–2001.–**74**, N 4.–P. 512–520.
10. *Vardevanyan P. O., Antonyan A. P., Parsadanyan M. A., Davtyan H. G., Karapetyan A. T.* The binding of ethidium bromide with DNA: Interaction with single- and double-stranded structures // Exp. Mol. Med.–2003.–**35**, N 6.–P. 527–533.
11. *Vardevanyan P. O., Antonyan A. P., Parsadanyan M. A., Davtyan H. G., Boyajyan Z. R., Karapetian A. T.* Complex-formation of ethidium bromide with poly [d(A-T)]-poly[d(A-T)] // J. Biomol. Struct. Dyn.–2005.–**22**, N 4.–P. 465–470.
12. *Brana M. F., Cacho M., Gradillas A., de Pascual-Teresan B., Ramos A.* Interacalators as anticancer drugs // Curr. Pharm. Des.–2001.–7, N 17.–P. 1745–1780.
13. *Martinez R., Chacon-Garcia L.* The search of DNA-intercalators as antitumoral drugs: what if worked and what did not work // Curr. Med. Chem.–2005.–**12**, N 2.–P. 127–151.
14. *Zhang Z., Yang Y., Liu F., Qian X., Xu Q.* Study on the interaction between 4-(2-diethylamino-ethylamino)-8-oxo-8H-acenaphtho[1,2-b]pyrrole-9-carbonitrile and DNA by molecular spectra // Int. J. Biol. Macromol.–2006.–**38**, N 1.–P. 59–64.
15. *Wheate N. J., Brodie C. R., Collins G. J., Kemp S., Aldrich-Wright J. R.* DNA intercalators in cancer therapy: organic and inorganic drugs and their spectroscopic tools of analysis // Mini Rev. Med. Chem.–2007.–**7**, N 6.–P. 627–648.
16. *Hendry L. B., Mahesh V. B., Bransome E. D. Jr., Ewing D. E.* Small molecule intercalation with double stranded DNA: implications for normal gene regulation and for predicting the biological efficacy and genotoxicity of drugs and other chemicals // Mutat. Res.–2007.–**623**, N 1–2.–P. 53–71.
17. *Ghosh R., Bhowmik S., Bagchi A., Das.D., Ghosh S.* Chemotherapeutic potential of 9-phenyl acridine: biophysical studies on its binding to DNA // Eur. Biophys. J.–2010.–**39**, N 8.–P. 1243–1249.
18. *Strekowski L., Wilson B.* Noncovalent interactions with DNA: an overview // Mutat. Res.–2007.–**623**, N 1–2.–P. 3–13.
19. *Ismail M. A., Rodger P. M., Rodger A.* Drug self-assembly on DNA: sequence effects with trans-bis-(4-N-methylpyridiniumyl)diphenyl porphyrin and Hoechst 33258 // J. Biomol. Struct. Dyn.–2000.–**17**, Suppl. 1.–P. 335–348.

20. *Rohs R., Sklenar H.* Methylene blue binding to DNA with alternating AT base sequence: minor groove binding is favored over intercalation // J. Biomol. Struct. Dyn.–2004.–**21**, N 5.–P. 699–711.
21. *Vardevanyan P. O., Antonyan A. P., Manukyan G. A., Karapetyan A. T.* Study of ethidium bromide interaction peculiarities with DNA // Exp. Mol. Med.–2001.–**33**, N 4.–P. 205–208.
22. *Wadkins R. M., Jares-Erijman E. A., Klement R., Rudiger A., Jovin T. M.* Actinomycin D binding to sigle-stranded DNA: sequence specificity and hemi-intercalation model from fluorescence and ^{1}H NMR spectroscopy // J. Mol. Biol.–1996.–**262**, N 1.–P. 53–68.
23. *Veselkov A. N., Dimant L. N., Bolotin P. A., Baranovskii S. F., Parkes Ch., Devis D*. Investigation of ethidium bromide interaction with deoxitetraribonucleozidetriphosphate 5'-d(GCGC) by ^{1}N MMR spectroscopy method // Mol. Biol. (Mosk.).– 1995.–**29**, N 2.–P. 326–338.
24. *Karapetian A. T., Mehrabian N. M., Terzikian G. A., Vardevanian P. O., Antonian A. P., Borisova O. F., Frank-Kamenetskii M. D.* Theoretical treatment of melting of complexes of DNA with ligands having several types of binding sites on helical and single-stranded DNA // J. Biomol. Struct. Dyn.–1996.–**14**, N 2.–P. 275–283.
25. *Karapetian A. T., Terzikian G. A., Megrabyan N. M., Arutyunyan S. G., Vardevanian P. O.* Theory of cooperative transitions in DNA complexes with multimodal ligands // Mol. Biol. (Mosk.).–1995.–**29**, N 4.–P. 841–847.
26. *Karapetian A. T., Vardevanian P. O., Terzikian G. A., Frank-Kamenetskii M. D.* Theory of helix-coil transition on DNA-ligand complexes: the effect of two types of interaction of ligand on the parameters of transition // J. Biomol. Struct. Dyn.–1990.–**8**, N 1.–P. 123–130.
27. *Karapetyan A. T., Vardevanyan P. O., Frank-Kamenetskii M. D.* The effect of [Na^{+}] ions concentration on the enthalpy of DNA helix-coil transition // Biopolym. Cell.–1989.–**5**, N 5.–P. 31–37.
28. *Vardevanyan P. O., Antonyan A. P., Parsadanyan M. A., Shahinyan M. A., Hambardzumyan L. A.* Mechanisms for binding between methylene blue and DNA // J. Appl. Spectrosc.–2013.–**80**, N 4.–P. 595–599.
29. *Nafisi S., Saboury A. A., Keramat N., Neault J.-F., Tajmir-Riahi H.-A.* Stability and structural features of DNA intercalation with ethidiume bromide, acridine orange and methylene blue // J. Mol. Struct.–2007.–**827**, N 1–3.–P. 35–43.
30. *Tong C., Hu Z., Wu J.* Interaction between methylene blue and calf thymus deoxyribonucleic acid by spectroscopic technologies // J. Fluoresc.–2010.–**20**, N 1.–P. 261–267.
31. *Lane A. N., Jenkins T. C.* Thermodynamics of nucleic acids and their interactions with ligands // Q. Rev. Biophys.–2000.–**33**, N 3.–P. 255–306.
32. *Saenger V*. The principles of structural organization of nucleic acids.–Moscow: Mir, 1987.–584 p.
33. *Cantor Ch., Shimmel P*. Biophysical chemistry.–Moscow: Mir, 1985.–Vol. 3.–534 p.
34. *Chaires J. B.* Energetics of drug-DNA interactions // Biopolymers.– 1997.–**44**, N 3.–P. 201–215.
35. *Monaco R. R.* A novel major groove binding site in B form DNA for ethidium cation // J. Biomol. Struct. Dyn.–2007.–**25**, N 2.–P. 119–125.

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