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THERMODYNAMIC ANALYSIS OF HOECHST 33258 INTERACTION WITH POLY(rA)-POLY(rU)

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Thermodynamic analysis of Hoechst 33258 interaction with poly(rA)-poly(rU) at the ionic strengths of the solution 0.04, 0.02 and 0.1 M has been carried out. Values of both binding constant (K) and number of nucleotides (n) per binding site were determined from Scatchard's curves that were obtained based on spectroscopic and electrochemical measurements. Values of changes of enthalpy (ΔH), entropy (ΔS) and free energy (ΔG) were also determined at the complex-formation. It should be mentioned that the binding of Hoechst 33258 to nucleic acids is accompanied by positive change of enthalpy. Meanwhile depending on the ionic strength of the solution, the values of ΔH for DNA change insignificantly, while for poly(rA)-poly(rU) this parameter is exposed to relevant alterations.

Hoechst 33258 – DNA – poly(rA)-poly(rU) – thermodynamic parameters of complex-formation

Իրականացվել է poly(rA)-poly(rU)-ի հետ Hoechst 33258-ի փոխազդեցության թերմոդինամիկական վերլուծություն լուծույթի 0.04, 0.02 և 0.1 Մ իոնական ուժի դեպքում: Սկետչարդի կորերից, որոնք ստացվել են սպեկտրոսկոպիկ և էլեկտրաքիմիական չափումների հիման վրա, որոշվել են կապման հաստատունի (K) և մեկ կապման տեղին ընկնող նուկլեոտիդների թվի (n) արժեքները: Որոշվել են նաև էնթալպիայի (ΔH), էնթրոպիայի (ΔS) և ազատ էներգիայի (ΔG) փոփոխության արժեքները կոմպլեքսագոյացման դեպքում: Հարկ է նշել, որ Hoechst 33258-ի կապումը նուկլեինաթթուների հետ ուղեկցվում է էնթալպիայի դրական փոփոխությամբ, ընդ որում, կախված լուծույթի իոնական ուժից, ԴՆԹ-ի դեպքում ΔH-ի արժեքները աննշան են փոխվում, մինչդեռ poly(rA)-poly(rU)-ի դեպքում այս պարամետրը ենթարկվում է էական փոփոխությունների:

Hoechst 33258 – ԴՆԹ – poly(rA)-poly(rU) – կոմպլեքսագոյացման թերմոդինամիկական պարամետրեր

Проведен термодинамический анализ взаимодействия Hoechst 33258 с poly(rA)-poly(rU) при ионных силах раствора 0.04; 0.02 и 0.1 М. Определены значения констант связывания K и числа нуклеотидов n, приходящих на одно место связывания, из кривых Скетчарда, полученных на основании спектроскопических и электрохимических измерений. Определены также величины изменений энтальпии (ΔH), энтропии (ΔS) и свободной энергии (ΔG) при комплексообразовании. Необходимо отметить, что связывание Hoechst 33258 с нуклеиновыми кислотами сопровождается положительным изменением энтальпии, при этом, в зависимости от ионной силы раствора, в случае ДНК значения ΔH претерпевают небольшое изменение, в то время как в случае poly(rA)-poly(rU) этот параметр подвергается значительным изменениям.

Hoechst 33258 – ДНК – poly(rA)-poly(rU) – термодинамические параметры комплексообразования

During the recent decades the structural-functional peculiarities of RNA, particularly its double-stranded (ds-) structure that practically plays an important role in realization of encoded genetic information, have been of growing interest [3, 4, 12, 15]. Moreover, new species of RNA (non-coding – nc-RNA) are found, which can be a good instrument for cellular activity modulation. Micro-RNA (mc-RNA) and short interfering RNA (si-RNA) are referred to these species of RNA. They bind to matrix RNA, forming ds-structures that mainly inhibit the translation, but are able to initiate it as well [3, 4, 12, 15]. It indicates that ds-type of RNA can also become a target for many biologically active compounds, which display a specificity to ds-DNA and are hindered in cytoplasm having no chance to get to DNA, which in turn can radically change the cellular activity.

Biologically active compounds that are specific to DNA, including ligands-intercalators or groove binding compounds, form slowly dissociating complexes [2, 7, 11, 14, 17, 19, 20]. In this regard, the specificity of these ligands, especially groove-binding compounds, relevantly depends on DNA structural form. DNA mainly is in ds-form, though at functioning it can be transformed to single-stranded or seldom four-stranded state [19].

Obviously, the studies of the interaction of ds-DNA specific ligands with ds-form of RNA can have an important value for more detailed understanding of mechanisms and ways of modulation of cellular activity throughout biologically active compounds. The recently obtained results have indicated that several intercalators, particularly ethidium bromide (EtBr), methylene blue (MB) can display a specificity to ds-RNA [22, 24]. From this point of view, the studies on the interaction of groove-binding compounds with ds-RNA can be quite interesting and valuable. Among such compounds AT-specific ligand Hoechst 33258 (H33258) is one of well-studied ones. It is localized in minor groove of DNA. Minor and major grooves in B-NA (DNA) and A-forms (RNA as well as DNA) significantly differ from each other [19]. In this regard, the studies dedicated to H33258 interaction with ds-RNA have an important value.

Groove-binding ligands are also interesting because of their ability to affect gene expression, selectively binding to them. From this point of view, some compounds, particularly distamycin A, CC-1065, H33258, being anti-neoplastic agents, possess anti-bacterial, anti-fungal, antivirus properties as well as they are fluorescence dyes for DNA. These compounds are applied with other ligands for cytotoxic purposes as well as for decreasing the toxicity of other drugs [1, 8, 10, 12, 18, 25]. Apart from specific groove-binding and intercalation, H33258 was shown to bind to phosphate groups from external side of DNA due to electrostatic forces [1, 8, 10, 12, 18, 25]. Being localized in minor groove, one ligand molecule covers 5-6 pairs of bases along DNA chain, meanwhile these regions become more "rigid", while adjacent regions remain flexible [1, 8, 10,12, 18, 25]. The ionic strength of the solution is one of chief factors, conditioning the specific binding of H33258 to DNA, since depending on this factor the hydration degree of DNA changes.

Taking into account the above-mentioned, this work is aimed at studying the interaction of H33258 with ds-RNA analogue – poly(rA)-poly(rU) and analyzing thermodynamically the complex-formation depending on the ionic strength of the solution.

Materials and methods. Poly(rA)-poly(rU), calf thymus DNA ("Sigma", USA), Hoechst 33258 ("Sigma", USA) were used in experiments. Binding of H33258 to poly(rA)-poly(rU) and calf thymus DNA was studied in standard solution, containing NaCl, Na-citrate and ethylenediaminetetraacetate, the ionic strengths were equal to 0.02, 0.04 and 0.1 M. Concentrations of poly(rA)-poly(rU), DNA and H33258 were determined spectrophotometrically, using the following values of extinction coefficients ($M^{-1}cm^{-1}$): ε_{260}=7140 for poly(rA)-poly(rU),

ε_{260}=6600 for DNA, ε_{343}=42000 for H33258. Spectrophotometric titration was carried out on spectrophotometer PYE Unicam-SP8-100 (England), fluorescence measurements – on Varian Cary Eclipse Fluorescence Spectrophotometer (Australia). Spectroscopic measurements were carried out in thermostating cells, using quartz cuvettes with optic pathway length 1 cm and hermetically closing caps. Based on the absorption, fluorescence spectra as well as voltammetric curves, the concentration portions of bound and free ligand molecules were obtained and using them the binding curves of H33258 with DNA and poly(rA)-poly(rU) in Scatchard's coordinates were constructed as described in [4]. The binding isotherms of ligands to DNA and poly(rA)-poly(rU) were obtained at three temperatures 20, 30 and 40^0C and the above mentioned ionic strengths of the solution. The theoretical curves were passed through the experimental points by the least square method, using the formula (1) [4]:

$$\frac{r}{C_f} = K\left[\frac{1-nr}{1-(n-1)r}\right]^n (1-(n-1)r) \qquad (1)$$

and the values of binding constants (K) and number of base pairs of nucleic acids (NA) per binding site (n) were determined. These base pairs become unavailable at the binding of one ligand molecule. In formula (1) C_f is concentration of free, C_b – concentration of bound ligand, C_p – concentration of NA and $r = \frac{C_b}{C_p}$.

Values of ΔG were determined by formula ΔG=-RTlnK, the value of ΔH was determined by extrapolation of lnK dependence curve on 1/K to ordinate axis; the point of crossing with it gives a value of ΔH/R. From the equation of ΔG=ΔH-TΔS the entropy change was determined.

Results and Discussion. The rising interest to the interaction of various ligands with nucleic acids is due to the fact that biosensors and biochips on the basis of genetic material (DNA, RNA) are getting wide application. The ligands-intercalators are used as sensors that are inserted into the plane of base pairs of ds-NA, in the consequence of which an analytical signal of sensor or chip is strengthened by several orders. It is conditioned by the fact that the majority of ligands in intercalated state possess high intensity of fluorescence [5,6,13]. Ligands-intercalators, having high intensity of fluorescence, obviously can be good sensors for the signal strengthening of genosensors or genochips. Moreover, some ligands-intercalators, particularly H33258, show a high specificity to many types of DNA nucleotide sequences, which depends on the ionic strength of the solution [21], that is why they can be good analytic sensors. Particularly, at the ionic strengths μ≥0.004 M, H33258, being localized in DNA minor groove, preferably binds to $(AT)_n$ (n=3-6) sequences that are on the boundaries of GC-pairs from both ends; at the low ionic strengths – it preferably binds to GC-rich regions by the intercalation mode [1,8,10,12,18]. The mentioned peculiarities refer to the binding of H33258 to B-form of DNA. Meanwhile, DNA in A-form as well as RNA (that always is in A- or A'-form) structurally differ from B-DNA. Particularly, the distance between ds-NA phosphates in A-form is equal to about ~5.9 Å, in B-form – ~7.0 Å, the minor groove of A-NA is deeper and narrower, than that of B-form. Obviously, such structural differences can have an important value at the interaction of groove-binding ligands to ds-NA [19]. Besides, at the interaction of H33258 with ds-DNA the thermodynamic parameters were obtained and the enthalpy-entropy complexation mechanism of complex-formation was revealed. Though, it was shown that the complex-formation of H33258 with B-form DNA is accompanied by positive change of enthalpy [1, 8, 10, 12, 18].

Analogous data on H33258 interaction with RNA are practically absent. On the other hand, RNA, in contrast to DNA, has less stable ds-structure in physiological conditions [24]. Taking into account the above mentioned, the thermodynamic analysis of the interaction of H33258 with poly(rA)-poly(rU) at the various ionic strengths of the solution has been carried out. Experiments were carried out by absorption and fluorescence spectroscopy methods at the temperatures 293, 303 and 313 K. Absorption and fluorescence spectra of H33258 complexes with poly(rA)-poly(rU) at all ionic strengths of the solution are similar to those obtained in [23] (spectra are not presented). Based on the obtained spectra the adsorption isotherms were constructed and the values of K and n [23] as well as ΔH, ΔS and ΔG (table 1 a, b, c) were determined, as described in experimental part.

Table 1a. Thermodynamic parameters of Hoechst 33258 binding to DNA and poly(rA)-poly(rU) at the ionic strength of the solution μ=0.02 M

T, K	$K \cdot 10^{-8}$, M^{-1}	-ΔG, kcal/mol	ΔH, kcal/mol	ΔS, cal/(mol·K)	n
DNA					
293	0.80±0.05	10.70±1.5	5.0±1.5	53.5±0.5	6.0±0.5
303	1.05±0.05	11.21±1.5			6.0±0.5
313	1.50±0.05	11.80±1.5			5.5±0.5
Poly(rA)-poly(rU)					
293	0.005±0.0005	7.7±1.5	2.2±1.5	33.5±1.0	2.0±0.5
303	0.005±0.0005	7.95±1.5			2.0±0.5
313	0.006±0.0005	8.32±1.5			2.0±0.5

Table 1b. Thermodynamic parameters of Hoechst 33258 binding to DNA and poly(rA)-poly(rU) at the ionic strength of the solution μ=0.04 M

T, K	$K \cdot 10^{-8}$, M^{-1}	-ΔG, kcal/mol	ΔH, kcal/mol	ΔS, cal/(mol·K)	n
DNA					
293	0.65±0.05	10.54±1.5	5.2±1.5	54.0±0.5	5.5±0.5
303	0.85±0.05	11.13±1.5			5.5±0.5
313	1.20±0.05	11.65±1.5			5.0±0.5
Poly(rA)-poly(rU)					
293	0.05±0.005	9.04±1.5	6.5±1.5	53.0±1.0	3.0±0.5
303	0.07±0.005	9.55±1.5			2.5±0.5
313	0.10±0.005	10.09±1.5			2.5±0.5

Table 1c. Thermodynamic parameters of Hoechst 33258 binding to DNA and poly(rA)-poly(rU) at the ionic strength of the solution μ=0.1 M

T, K	$K \cdot 10^{-8}$, M^{-1}	-ΔG, kcal/mol	ΔH, kcal/mol	ΔS, cal/(mol·K)	n
DNA					
293	0.55±0.05	10.5±1.5	6.0±1.5	56.0±0.5	6.5±0.1
303	0.85±0.05	11.0±1.5			6.5±0.1
313	1.05±0.05	11.5±1.5			6.0±0.1
Poly(rA)-poly(rU)					
293	0.20±0.05	9.85±1.5	8.5±1.5	62.65±1.0	5.0±0.2
303	0.33±0.05	10.5±1.5			5.0±0.2
313	0.50±0.05	11.1±1.5			5.0±0.2

In the tables the analogous values of thermodynamic parameters of Hoechst 33258 interaction with DNA are presented for the comparison. As it is obvious from the

presented tables the binding constant of Hoechst 33258 with DNA is higher, than with poly(rA)-poly(rU). At the same time, along with the increase of the ionic strength of the solution from 0.02 M to 0.1 M, the value of K takes a little change in the case of binding to DNA, while in the case of poly(rA)-poly(rU) a significant increase of this parameter takes place. It is due to being of poly(rA)-poly(rU) in unstable ds-form at the ionic strength of the solution 0.02 M, when it is practically denatured at the temperatures T>293 K [24]. At higher ionic strengths ds-structure of poly(rA)-poly(rU) is transformed to stable state, in consequence of which the values of K increase. Though, along with enhancement of the temperature a little increase of K is observed, which results in positive changing of the enthalpy, as in the case of DNA.

However, at Hoechst 33258 interaction with ds-poly(rA)-poly(rU) (at the ionic strengths 0.04 and 0.1 M), the value of K is about an oder less, that that with DNA, which can result from several reasons: 1. being of NA in A-form, due to which the coincidence between ligand molecule and NA decreases that in turn results from deeper and narrower groove, 2. Absence of GC-pairs or 3. two precursor reasons simultaneously [9, 12, 16, 18, 19].

It is also shown from the table data that for DNA, the values of ΔH change less along with the ionic strength increase, than that for poly(rA)-poly(rU). Entropy of the complex-formation of H33258 with ds-poly(rA)-poly(rU) takes much higher change as compared to DNA, which is twice as higher at the ionic strength of the solution 0.1 M. For DNA the entropy change increases by 2-3 cal/(mol·K) with the ionic strength enhancement. Changes of free energy at H33258 complex-formation with DNA are higher, than that with poly(rA)-poly(rU), which indicates the preferance of the binding of this ligand to B-DNA.

It should be mentioned that along with the ionic strength growth, the binding of H33258 to poly(rA)-poly(rU) becomes more and more beneficial from the entropic point of view as compared to DNA, while during DNA-ligand interactions the entropic losses increase. Possible mechanism is the flexibility increase, due to the screening of electrostatic repulsion, which in turn leads to strengthening of geometrical coincidence between ligand and binding center. At the same time, the growth of the entropic losses can be connected to the originally higher flexibility of poly(rA)-poly(rU) that increases with the growth of the solution ionic strength.

Thus, the obtained data show that DNA B-form specific ligand H33258 can also bind to ds-polynucleotides, being in A-form. Moreover, both in the case of DNA and poly(rA)-poly(rU) the interaction occurs according to enthalpy-entropy complex formation mechanism, when the enthalpy change is positive. This fact indicates that one of factors of H33258 specificity to ds-NA is a geometrical coincidence between ligand molecule and minor groove of nucleic acid, which changes depending both on the temperature and on the solution ionic strength.

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REFERENCES

1. *Adhikary A., Buschmann V., Muller Ch., Sauer M.* Ensemble and single-molecule fluorescence spectroscopic study of the binding modes of bis-benzimidazole derivative Hoechst 33258 with DNA. Nucleic Acids Research, *31*, p. 2178-2186, 2003.

2. *Amy R., Smith B.S.* Advances in DNA Binding by Threading Polyintercalation. Dissertation Presented to the Faculty of the Graduate School of The University of Texas at Austin in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy The University of Texas at Austin December, 188 p., 2013.
3. *Andreeva O.E., Krasilnikov M.A.* The phenomenon of RNA interference in oncology: advances, problems and perspectives. Advances in Molecular Oncology, v. 3, p. 8-15, 2016.
4. *Arakelyan V.B., Babayan Yu.S., Potikyan G.* Determination of constant rates of adsorption of ligand on DNA: Analysis of correlation functions. J. Biomol. Struct. Dyn., *18*, 2, p. 231-235, 2000.
5. *Arora K., Prabhakar N., Chand S., Malhotra B.D.* Ultrasensitive DNA hybridization biosensor based on polyaniline. Biosens Bioelectronics, *23*, p. 613-620, 2007.
6. *Berdat D., Marin A., Herrera F., Gijs M.A.M.* DNA biosensors using fluorescence microscopy andimpedance spectroscopy. Sensors Actuators, *118*, p. 53-59, 2006.
7. *Blagoi Y.P.* Interaction of DNA with biologically active compounds. Soross educational J. (In Russian), 10, p. 18-24, 1998.
8. *Breusegem S.Y, Clegg R.M., Lootiens F.G.* Base-sequence specificity of Hoechst 33258 and DAPI binding to five (A/T)4 DNA sites with kinetic evidence for more than one high-affinity Hoechst 33258-AATT complex. J. Mol. Biol., *315*, p. 1049-1061, 2002.
9. *Gowda S.K.R., Mathew B.B., Sudhmani C.N., Naik H.S.B.* Mechanism of DNA binding and Cleavage. Biomedicine and Biothecnology, *2*, 1, p. 1-9, 2014.
10. *Han F., Taulier N., Chalikian T.* Association of the Minor Groove Binding Drug Hoechst 33258 with $d(CGCGAATTCGCG)_2$: Volumetric, Calorimetric and Spectroscopic Characterizations. Biochemistry, *44*, p. 9785-9794, 2005.
11. *Jayaram B., Singh T., Fenley M.* DNA-Drug Interactions. In Methods for Studying Nucleic Acid/Drug Interactions. M. Wanunu and T. Yitzhak, eds. (Boca Raton: CRC Press), p. 315-336, 2011.
12. *Kakkar R., Suruchi L., Grover R.* Theoretical study of molecular recognition by Hoechst 33258 derivatives. J. Biomol. Struct. & Dynam., *23*, p. 37-47, 2005.
13. *Lakowicz J.R.* Principles of Fluorescence Spectroscopy (Third Edition), University of Maryland, School of Medicine Baltimore, Maryland, USA, Printed in Singapore. (KYO), 954 p., 2006.
14. *Leung C.H., Chan D.S.H., Ma V.P.Y., Ma D.L.* DNA-Binding Small Molecules as Inhibitors of Transcription Factors. Med. Res. Rev., *33*, p. 823-846, 2013.
15. *Makarova Y.A., Kramerov D.A.* Non-coding RNA. Biochemistry (In Russian), *72*, 11, p. 1427-1448, 2007.
16. *Palchaudhuri R., Hergenrother P.J.* DNA as a target for anticancer compounds: methods to determine the mode of binding and the mechanism of action. Current Option in Biotechnology, *18*, 6, p. 497-503, 2007.
17. *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., *21*, 5, p. 699-711, 2004.
18. *Rosu F., Gabelica V., Houssier C., De Pauw E.* Determination of affinity, stoichiometry and sequence selectivity of minor groove binder complexes with double-stranded oligonucleotides by electrospray ionization mass spectroscopy. Nucleic Acids Research, *30*, p. 82-88, 2002.
19. Saenger V. Principles of structural organisation of nucleic acids. M., Mir, 584 p., 1987.
20. Strekowski L., Wilson B. Noncovalent interactions with DNA: An overview. Mutation Research, N623, p. 3-13, 2007.
21. *Tavadyan L.A., Minasyan S.H., Kocharyan G.H., Antonyan A.P., Sahakyan V.G., Parsadanyan M.A., Vardevanyan P.O.* Exploring the interaction of ethidium bromide and Hoechst 33258 with DNA by means of electrochemical approach. Biophys. Reviews and Letters, *12*, 3, p. 151-161, 2017.
22. *Vardevanyan P.O., Antonyan A.P., Parsadanyan M.A., Shahinyan M.A., Sahakyan V.G.* Peculiarities of interaction of synthetic polyribonucleotide poly(rA)-poly(rU) with some intercalators. J. of Biomol. Struct. and Dyn., *36*, 14, p. 3607-3613, 2018.

23. *Vardevanyan P.O., Parsadanyan M.A., Antonyan A.P., Sahakyan V.G.* Spectroscopic study of the binding of netropsin and Hoechst 33258 to nucleic acids. J. of Applied Spectroscopy, *85*, 2, p. 335-340, 2018.
24. *Vardevanyan P.O., Antonyan A.P., Parsadanyan M.A., Shahinyan M.A.* Study of the influence of the ionic strength on complex-formation of ethidium bromide with poly(rA)-poly(rU). Journal of Biomolecular Structure and Dynamics, p. 1-6, 2019, DOI: 10.1080/07391102.2019.1630006.
25. *Vekshin N.* Binding of Hoechst with nucleic acids using fluorescence spectroscopy. Journal of Biophysical Chemistry, 2, 4, p. 443-447, 2011.

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