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# STUDY OF STRUCTURAL TRANSITIONS OF COMPLEXES OF DIFFERENT LIGANDS WITH DNA AND BSA

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Structural transitions of DNA and bovine serum albumin (BSA) and their complexes with methylene blue (MB) and Hoechst 33258 (H33258) by UV-denaturation method have been studied. The obtained results revel that MB stabilizes both protein dimensional structure and DNA double-stranded structure, while H33258 stabilizes double-stranded structure of DNA and destabilizes protein structure. It was also revealed that the stabilizing effect of MB on native structure of DNA is more pronounced at relatively low ligand concentrations, than on protein.

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***Keywords*:** biomacromolecule, ligand, binding modes, UV-denaturation method, transition temperature.

**Introduction.** Study of structural transformations of biomacromolecules (nucleic acids and proteins) and their complexes with ligands has an important value for solution of a number of questions, lying at the basis of organism functioning. One of approaches is the study of denaturation of DNA and proteins and their complexes with ligands. For revelation of molecular basis of protein denaturation, one should explain why they are divided by free-energetic barrier (which creates the transition of the type “all or nothing”) [1, 2]. From this point of view, denaturation of nucleic acids (NA) occurs otherwise by intermediate states, than of protein. Particularly, when DNA water solution is heated up to 100℃ or pH changes up to extreme values, its secondary structure is broken down and double-helix chains, which are held together, are separated [3–6] (Figs. 1, a and b).

Structural state of biomacromolecules plays a prominent role as well as at their interaction with different biologically active compounds [7–16]. Among various ligands the compounds can be important that showing specificity to NA are able to interact with proteins as well. Such compounds are methylene blue (MB), which is a feno-thiazine cationic aromatic dye or bis-benzimide compound Hoechst 33258 (H33258) that can stain NA. MB possesses anti-malaria action and is widely used as a photosensibilizing agent for photodynamic inactivation of RNA-viruses, including HIV, virus hepatitis B and virus hepatitis C in plasma, intracellular viruses B-CoV and SARS-CoV2. Though, the fact is considered more important

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that MB also shows therapeutic properties toward the rest viruses from coronavirus family [10–14, 16].

Bisbenzimidazole compound H33258 also has a practical application in medicine as an anti-tumorous preparation. This ligand enhances irradiation cytotoxicity in some lines of tumors and therefore improves radiotherapy effect. H33258 also possesses radioprotector properties, since it decreases a bad influence of irradiation on normal cells [17].

The present work is aimed at comparatively studying of structural transitions of MB and H33258 complexes with DNA and BSA.

**Materials and Methods.** In this work Calf Thymus DNA, bovine serum albumin (“Sigma”, USA), MB, H33258 (“Sigma”, USA), physiological solution, bidistilled water were used in experiments. Concentrations of MB, H33258 and DNA were determined spectrophotometrically, using the following coefficients of extinction: $\varepsilon_{664}$=76000 $M^{-1}cm^{-1}$ for MB; $\varepsilon_{342}$=43000 $M^{-1}cm^{-1}$ for H33258; $\varepsilon_{260}$=6600 $M^{-1}cm^{-1}$ for Calf Thymus DNA, $\varepsilon_{280}$=43824 $M^{-1}cm^{-1}$ for BSA [18]. Denaturation of the complexes of MB and H33258 with DNA and BSA was carried out on double-beam spectrophotometer PYE Unicam-SP8-100 (England). Heating of thermostating cells was carried out with program device SP-876 Series 2. Absorption changes of the complexes were registered at DNA absorption wavelength $\lambda$ = 260 *nm* and at BSA absorption wavelength $\lambda$ = 280 *nm*. In the interval $220 \le \lambda \le 300$ *nm* these ligands absorb intensively, though this effect significantly does not affect analysis of the obtained data. Data on absorption were displayed on PC monitor with the application of LabVIEW software.

**Results and Discussion.** Structural transitions of the complexes of DNA and BSA with MB and H33258 were studied by UV-denaturation method, increasing the temperature of sample solutions. On the basis of absorptions, the denaturation curves were constructed [12], from which the values of denaturation temperatures were determined and generalized in Tabs. 1 and 2. MB, like many ligands-intercalators, as well as groove-binding compound H33258 are stabilizers of double-stranded (ds-) structure of NA that is why at the binding of these ligands to ds-DNA an increase of NA transition temperature takes place in single-stranded (ss-) state [12–14]. This effect depends on ligand concentration and enhances with the increasing of ligand/DNA ratio, as it is obvious from Tabs. 1 and 2 data. As it is obvious from presented data for MB at relatively low values of $r$ (≈1/5), $\Delta T_m \approx 6$°C (Tab. 1), for H33258 $\Delta T_m \approx 11$°C (Tab. 2).

*Table 1*

*Values of denaturation temperature of DNA–MB and BSA–MB complexes*

| $r$ = [ligand]/[biomacromolecule] | Transition temperature, $T_m$,°C | |
|---|---|---|
| | DNA | BSA |
| 0 | 70.0±0.2 | 79.5±0.2 |
| 0.02 | 71.5±0.2 | – |
| 0.05 | 73.0±0.2 | 81.0±0.2 |
| 0.2 | 76.0±0.2 | 82.0±0.2 |
| 1.0 | – | 84.0±0.2 |

Transition temperature shift to high temperatures occurs at MB binding to BSA, which is reasoned by stabilizing effect of this ligand on protein structure. It is known that albumin has two regions for drug preparation binding, which are localized in subdomains IIA and IIIA and called sites 1 and 2. Although, site 1 is more hydrophobic, but site 2 is preferable for MB binding [15].

*Table 2*

*Values of denaturation temperature of DNA–H33258 and BSA–H33258 complexes*

| $r$ = [ligand]/[biomacromolecule] | Transition temperature, $T_m$, °C | |
|---|---|---|
| | DNA | BSA |
| 0 | 70.0 ± 0.2 | 79.5 ± 0.2 |
| 0.02 | 73.2 ± 0.2 | – |
| 0.05 | 77.8 ± 0.2 | 78.1 ± 0.2 |
| 0.2 | 81.0 ± 0.2 | 77.2 ± 0.2 |
| 1.0 | – | 73.8 ± 0.2 |

On the basis of the aforementioned we assume that MB binding to albumin has an electrostatic nature. However, the localization of aromatic ligand in hydrophobic chamber of protein cannot be excluded. There are hydrophobic and hydrophilic regions in DNA that are adsorption centers for ligands. As compared to albumin, hydrophobic adsorption centers (inter-plane spaces between adjacent nucleotides or nucleotide pairs) for majority of intercalators, including MB, can be considered specific binding sites, with which this ligand interacts by intercalation mechanism [12–14]. On the other hand, ligands-intercalators can also bind to ds-DNA non-specifically: this mechanism corresponds to electrostatic one, as a result of which ligand molecules bind to sufficiently hydrophilic sugar-phosphate skeleton of NA. Taking into account a single-direction of MB effect on native structure of BSA and DNA as well as forces, conditioning known molecular binding mechanisms of this ligand to DNA, we assume that MB also binds to BSA under the effect of these forces, especially by hydrophobic and electrostatic interactions. It results in stabilization of DNA ds-structure and additional wrapping of 3D structure of albumin [12–14].

DNA-specific ligand H33258 binds to albumin as well, meanwhile in contrast to MB, it invokes loosening of dimensional structure of macromolecule. Though, along with increasing of $r$, the denaturing effect of H33258 on albumin strengthens, attaining to its maximum at the ratio $r$=1. At further enhancement of $r$, this effect is not observed at all (that is why the data are not brought). It can be the result of the fact that the binding stoichiometry of H33258 binding to albumin, as for MB, corresponds to 1:1.

On the basis of $T_m$ the values of denaturation temperatures of the complexes at different concentration ratios $r$=[MB]/[biomacromolecule] were obtained and the dependence curves of $\Delta T_m$ on $r$ were constructed (Figs. 1 and 2). It is obvious from Fig. 1, that the curve 1, corresponding to the complexes MB–DNA, is significantly sharper, than the curve 2, corresponding to the complexes MB–BSA. Practically, at the same concentration ratios the increase of $T_m$ of the complexes MB–DNA is more by four-times, as compared to the complexes MB–BSA. This fact can be explained proceeding from the presence of at least two types of adsorption centers

for MB on DNA (as for other ligands-intercalators or groove-binding compounds), with which MB binds by various constants of association – $K_1$ and $K_2$. Moreover, the literature data indicate that $K_1/K_2 \approx 10$ [12–14], since one of the modes, the stronger one is realized by intercalation mode, another one – weaker mode is electrostatic binding that stabilize ds-DNA. For BSA, as it is shown from Tab. 1 data, the biggest effect of stabilization is obtained at $r \approx 1/1$, which indicates that albumin contains one adsorption center for this ligand. It is not excluded that the ligand association constant with this center is less, than that of MB with by the mentioned modes.

Analogously, for H33258 a big shift of the transition temperature toward DNA native structure stabilization is observed, that is why the curve 1 in Fig. 2 has a big slope. Though, $T_m$ of the complexes of H33258–DNA is much more higher, than for MB (Fig. 2). From this point of view, for BSA a radically another scene is observed: the dependence curves of the complexes are shifted to low temperatures, which indicates the denaturing effect of H33258 on protein structure.

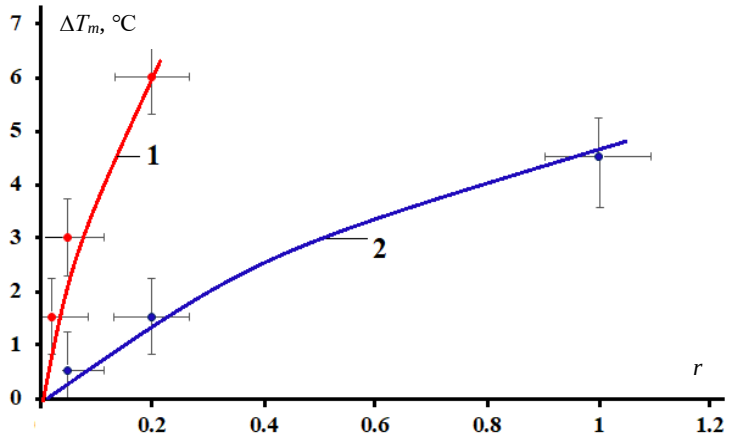


Fig. 1. Dependence curves of denaturation temperature changes ($\Delta T_m$) on concentration ratio $r$ = [MB]/[biomacromolecule].

It is known that H33258 also binds to DNA by more, than one mode, which together significantly stabilize its ds-structure [12]. Moreover, the interaction of this ligand with DNA by different modes is characterized by high values of association constant. On the other hand, the destabilizing effect of H33258 on dimensional structure of albumin cannot be conditioned by low value of the association constant and the denaturing effect can be the result of force breakup, stabilizing the protein dimensional structure as well as by changes in micro-surrounding of protein surface. Consequently, from our data one can conclude that the effect of various ligands on structure of biomacromolecules can have a differently directed character, which lies at the basis of their biological activity.

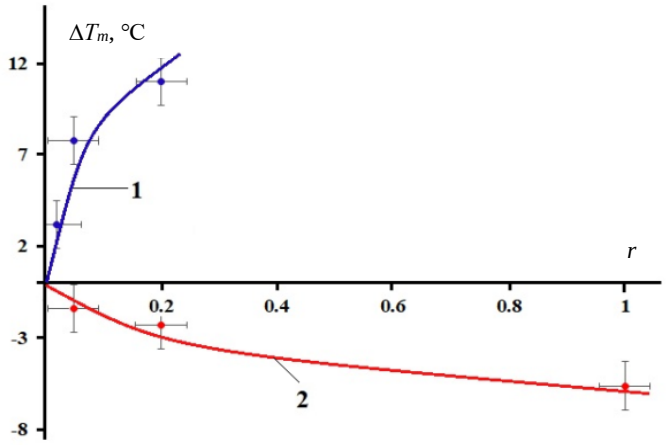


Fig. 2. Dependence curves of denaturation temperature changes ($\Delta T_m$) on concentration ratio $r$ = [H33258]/[biomacromolecule].

Thus, the obtained data indicate that the thermal denaturation is sufficiently informative in the study of structural transformations of proteins and nucleic acids. Moreover, this method permits also studying the interaction of various ligands with both DNA and proteins, as well as conformational transformations of their complexes. This method can light out molecular basis of binding mechanisms of ligands to biomacromolecules.

**Conclusion.** The obtained data allow to conclude that MB and H33258 interaction with DNA is realized both specifically (intercalation mechanism) and non-specifically (electrostatic mechanism) [11–13]. In the case of albumin there is no revealed a certain specific mechanism of binding by UV-denaturation method, however, it is not excluded that these ligands bind to proteins by hydrophobic and electrostatic interactions or formation of hydrogen bonds, which all together result in increasing of protein folding and stabilizing of their molecules in relation to temperature, as denaturing factor.

These results can light out the peculiarities of de-protonation and transportation of biologically active compounds, being specific to NA, toward targets (DNA and RNA). It, in turn, can have an important biological value, since it can lie at the basis of modulation of various cellular processes through biologically active compounds. It is also important to mention that biologically active compounds, including drug preparations, should be tested, if the possess and affinity to blood albumin toevaluate the effect of their effective concentrations on organism.

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## ԴՆԹ-Ի ԵՎ ՑՇԱ-Ի ՀԵՏ ՏԱՐԲԵՐ ԼԻԳԱՆԴՆԵՐԻ ԿՈՄՊԼԵՔՍՆԵՐԻ ԿԱՌՈՒՑՎԱԾՔԱՅԻՆ ԱՆՑՈՒՄՆԵՐԻ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆԸ

Ուսումնասիրվել են ԴՆԹ-ի և ցուլի շիճուկային ալբումինի (ՑՇԱ), ինչպես նաև մեթիլենային կապույտի (ՄԿ) և Hoechst 33258-ի (H33258) հետ դրանց կոմպլեքսների կառուցվածքային անցումները ՈՒՄ-դենատուրացիայի մեթոդով: Ստացված արդյունքները բացահայտում են, որ ՄԿ-ն կայունացնում է և սպիտակուցի տարածական կառուցվածքը և ԴՆԹ-ի երկշղթա կառուցվածքը, մինչդեռ H33258-ը կայունացնում է ԴՆԹ-ի երկշղթա կառուցվածքը և ապակայունացնում է սպիտակուցի կառուցվածքը: Ցույց է տրվել նաև, որ ՄԿ-ի կայունացնող ազդեցությունը ԴՆԹ-ի նատիվ կառուցվածքի վրա ավելի արտահայտված է լիգանդի փոքր կոնցենտրացիաների դեպքում[a] համեմատած սպիտակուցի հետ:

П. О. ВАРДЕВАНЯН, Н. Р. ПЕТРОСЯН

## ИЗУЧЕНИЕ СТРУКТУРНЫХ ПЕРЕХОДОВ В КОМПЛЕКСАХ РАЗЛИЧНЫХ ЛИГАНДОВ С ДНК И БСА

Изучены структурные переходы в ДНК и бычьем сывороточном альбумине (БСА) и в их комплексах с метиленовым синим (МС) и Hoechst 33258 (H33258) методом УФ-денатурации. Полученные результаты выявили, что МС стабилизирует как пространственную структуру белка, так и двухцепочечную структуру ДНК, в то время как H33258 стабилизирует двухцепочечную структуру ДНК и дестабилизирует структуру белка. Выявлено также, что стабилизирующий эффект МС на нативную структуру ДНК достигается при существенно более низких концентрациях лиганда, чем в случае белка.