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# BOVINE SERUM ALBUMIN DENATURATION IN THE PRESENCE OF HOECHST 33258 AND METHYLENE BLUE

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The interaction of Hoechst 33258 (H33258) and methylene blue (MB) compounds with bovine serum albumin (BSA) has been studied using the method of thermal denaturation. The obtained data showed that both ligands form complexes with BSA, moreover, MB binds to BSA stronger than H33258. Furthermore, H33258 destabilizes, while MB stabilizes the native structure of protein, leading to the decrease and increase of the denaturation temperature of BSA respectively.

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***Keywords*:** bovine serum albumin, methylene blue, Hoechst 33258, interaction, denaturation temperature.

**Introduction.** Serum albumins are known blood plasma components, released in liver and dissolves in water. It is known that serum albumins are the most abundant proteins in the circulatory system, the principle function of which is the transport of fatty acids as well as numerous endogenous and exogenous compounds [1–5]. The study of binding mechanisms of various drugs to serum albumins has an important value in many aspects of clinical medicine, biotechnology, pharmacology etc. [6, 7]. The bovine serum albumin (BSA) is a polypeptide chain, consisted of 583 amino-acids and contains two tryptophan residues [8]. The studies of BSA interaction with ligands, especially DNA-specific ligands, including methylene blue (MB), Hoechst 33258 (H33258), are of great interest, since they reach to nucleic acids, being transported via HSA.

MB is a coplanar polycyclic aromatic basic dye that belongs to the thiazine class (Fig. 1,a). It is important as a biological stain and diagnostic agent for many diseases, such as carcinoma [1, 9].

H33258 is known as a specific DNA-binding ligand that possesses high affinity to DNA, moderate cytotoxicity and good permeability through cellular membranes (fig. 1b) [10, 11]. These properties make H33258 a perspective material to design new drug preparations.

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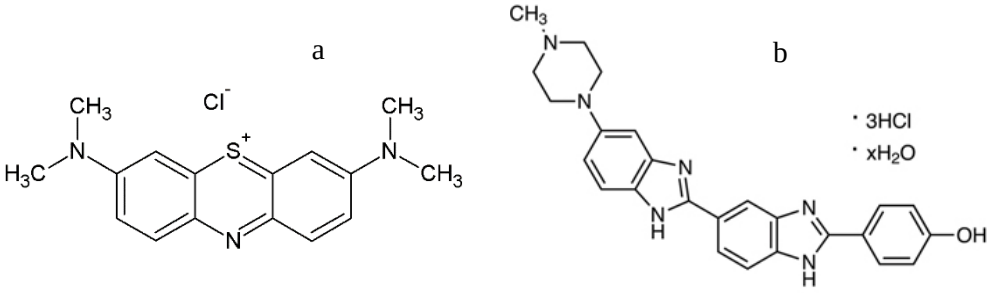


Fig. 1. The chemical structure of MB (a) and H33258 (b).

The present study is aimed at studying of BSA interaction with MB and H33258.

**Materials and Methods.** Bovine serum albumin 1% solution ("Sigma", USA), methylene blue, Hoechst 33258 ("Sigma", USA), physiological solution were used in experiments. Concentrations of MB and H33258 were determined spectrophotometrically, using the following coefficients of extinction: $\varepsilon_{664}$=76000 $M^{-1}cm^{-1}$ and $\varepsilon_{343}$=42000 $M^{-1}cm^{-1}$ respectively. Preparations were used without further purification. Protein denaturation was carried out on UV-VIS Unicam-SP-8-100 spectrophotometer (England). Preparations were heated in quartz cuvettes with hermetically closing Teflon caps, 1 *cm* optic pathway length, 3 *mL* volume. At melting the heating was realized with $0.5^0$C/*min* rate via Temperature Programme Controller SPX 876 equipment. At sufficiently temperature increasing a conformational transition of protein (denaturation) occurs and for melting curve construction denaturation degree ($1-\vartheta$) is used which is determined by the following equation:

$$1-\vartheta = \frac{A_t - A_{nat.}}{A_{denat.} - A_{nat.}},$$

where $A_t$ is protein absorption at given temperature, $A_{nat.}$ – native protein absorption, $A_{denat.}$ – denatured protein absorption. The concentration ratio ligand/albumin was equal to 1/5.

**Results and Discussion.** Earlier we have studied the denaturation of HSA in non-irradiated and irradiated forms, when the irradiation was carried out by millimeter range electromagnetic waves [12]. The thermal-denaturation curves of solutions of BSA, complexes BSA-H33258 and BSA-MB have been obtained. The denaturation curves are presented in Fig. 2. It was shown from the Fig. 2 that compared to BSA denaturation curve, the denaturation curve of the complexes BSA–H33258 is shifted to the lower temperature region, while for the complexes BSA–MB the curve is shifted to the higher temperature regions. It is also obvious from Fig. 2 that MB binds to BSA stronger, than H33258, and stabilizes the native structure of the protein, although H33258 destabilizes that structure. From the denaturation curves the values of denaturation temperatures ($T_m$) were determined and presented in Table.

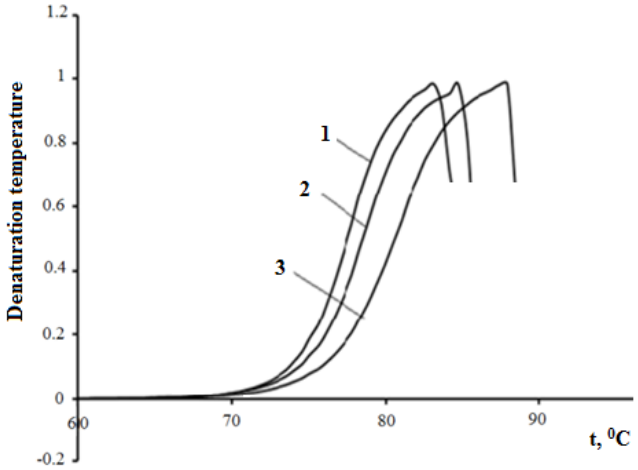


Fig. 2. Denaturation curves of BSA-H33258 (1) and the complexes of BSA (2) and BSA–MB (3).

From the denaturation experiments one can conclude that there occurs a complex-formation between BSA and mentioned ligands, i.e., there takes place a binding, not collision. As it is obvious from the data presented in Table, the highest temperature is obtained in the case of MB interaction with BSA, in contrast to H33258 that causes the decreasing of denaturation temperature of the protein.

*Values of denaturation temperatures of BSA, BSA–H33258 and BSA–MB complexes*

| Denaturation temperature | BSA | BSA–H33258 | BSA–MB |
|---|---|---|---|
| $T_m$, $^0$C | 78.5±0.2 | 76.6±0.1 | 82.3±0.2 |

**Conclusion.** Thus, based on the obtained data one can conclude that BSA interacts with MB and H33258 and forms complexes with them, that is, influences the values of $T_m$ of BSA–ligand complexes. Therefore, the denaturation curve of BSA with MB is shifted to higher temperature regions, whereas in case of H33258 there is a shifting of the denaturation curve of the complexes to the lower temperature regions, compared to that of the pure BSA. Thus, influence of MB stabilizes the native structure of BSA, meanwhile the H33258 destabilizes it. It is due to the fact that the extended molecule of H33258, most apparently, is localized in hydrophobic hole of heart-shaped molecule of BSA incompletely and changes the hydrophilic-hydrophobic medium in micro range of protein, as compared to MB, having more compact structure and localizing in hydrophobic hole of heart-shaped molecule of protein [12].

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## ՅՈՒԼԻ ԾԻՃՈՒԿԱՅԻՆ ԱԼԲՈՒՄԻՆԻ ԴԵՆԱՏՈՒՐԱՑԻԱՆ HOECHST 33258-Ի ԵՎ ՄԵԹԻԼԵՆԱՅԻՆ ԿԱՊՈՒՅՏԻ ԱՌԿԱՅՈՒԹՅԱՄԲ

Ջերմային դենատուրացիայի մեթոդով ուսումնասիրվել է Hoechst 33258-ի (H33258) և մեթիլենային կապույտի (ՄԿ)-ի փոխազդեցությունը ցուլի շիճուկային ալբումինի հետ (ՑՇԱ): Ստացված տվյալները ցույց են

տալիս, որ երկու լիգանդներն էլ ՑՇԱ-ի հետ կոմպլեքսներ են առաջացնում, ավելին ՄԿ-ն ՑՇԱ-ի հետ ավելի ուժեղ է կապվում, քան H33258-ը: Սակայն H33258-ը ապակայունացնում, իսկ ՄԿ-ն կայունացնում է սպիտակուցի նատիվ կառուցվածքը՝ հանգեցնելով ՑՇԱ-ի դենատուրացիայի աստիճանի նվազմանը և բարձրացմանը համապատասխանաբար:

П. О. ВАРДЕВАНЯН, М. С. МИКАЕЛЯН, Н. Р. ПЕТРОСЯН

## ДЕНАТУРАЦИЯ БЫЧЬЕГО СЫВОРОТОЧНОГО АЛЬБУМИНА В ПРИСУТСТВИИ HOECHST 33258 И МЕТИЛЕНОВОГО СИНЕГО

Исследовано взаимодействие Hoechst 33258 (H33258) и метиленового синего (МС) с бычьим сывороточным альбумином (БСА) с использованием метода терической денатурации. Полученные данные показывают, что оба лиганда формируют комплексы с БСА, более того, МС связывается с БСА сильнее, чем H33258. Однако H33258 дестабилизирует, в то время как МС стабилизирует нативную структуру белка, что приводит к уменьшению и увеличению температуры денатурации БСА соответственно.