Biology

COMPARISON OF METHYL VIOLET INTERACTION WITH BOVINE SERUM ALBUMIN AND HUMAN SERUM ALBUMIN BY UV-DENATURATION METHOD

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In the present work the interaction of methyl violet (MV) with human serum albumin (HSA) and bovine serum albumin (BSA) has been studied by the UV-denaturation method and the obtained data were compared. The denaturation parameters – denaturation temperature and denaturation interval width, were determined. It was shown that MV, binding to serum albumins, stabilizes their structure. At the same time, the stabilization degree is different. It was also shown that BSA is stabilized more, than HSA, while binding to MV.

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Keywords: methyl violet, serum albumins, complex-formation, denaturation temperature, denaturation interval width.

Introduction. In recent years the studies on the serum albumin interaction with various low-molecular compounds – ligands are of great interest, first of all, conditioned by the fact that albumins are the major drug-transporters in blood [1–5]. Serum albumins are known to carry different endogenous and exogenous ligands, interacting with them [6–8]. From this point of view, it is very interesting to find out how this interaction affects the protein structures and which conformational changes it can induce, taking into consideration that answers to these questions have a crucial value in clinical medicine, biotechnology, pharmacology, etc. [9–11]. For scientific researches human serum albumin (HSA) and bovine serum albumin (BSA) are being used due to their structural and functional similarity and difference. HSA is one of the most spread proteins in blood, synthetized in liver. HSA composes of almost 60% of all plasma proteins, having a concentration about 40 *mg*/*mL.* Its dimensional structure is determined by X-ray crystallographic measurements [12]. The globular protein consists of a single polypeptide chain, consisted of 585 amino-acids, with molecular width 66 *kDa* [13]. HSA is composed of 3 homologous domains I, II and III, each containing 2 sub-domains A and B, each, in turn, having 6 and 4 α-helices respectively [14]. The tertiary structure of HSA is stabilized by 17 disulfide bonds, giving it a heart-shaped form [13]. BSA is also a globular protein, which is consisted of 583 amino-acids and has 17 disulfide bonds, with a molecular mass 66.4 *kDa.* BSA has different pH-dependent conformational isomers. According to Forster in

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the range of pH 4.3–8.0, BSA has a form "N", with 3 homologous domains (I, II, III), each domain consists of 2 sub-domains A and B [15, 16]. In model studies as an example of ligand, methyl violet is chosen, due to its properties. Methyl violet (MV), also known as crystal violet, is triphenylmethane industrial dye, which is used for different purposes: as a biological stain, a fungicide in agriculture and exterior drug for skin diseases [2]. There are some works, devoted to the interaction peculiarities of MV with BSA [1, 2] and HSA [4, 17], indicating that MV binds to serum albumins and in both cases induces a quenching of protein own fluorescence intensity.

In the present work the interaction of MV with HSA and BSA is studied by thermal UV-denaturation method.

Materials and Methods. HSA (1%), BSA (1%), MV ("Sigma", USA), physiological solution were used in experiments. All preparations were used without additional purification. MV concentration was determined spectrophotometrically, using the following value of extinction coefficient for MV: $\varepsilon_{590} = 87000\ M^{-1} \cdot cm^{-1}$.

Thermal UV-denaturation of the albumins and their complexes with MV was carried out on spectrophotometer PerkinElmer Lambda 365 (USA). The temperature was raised by PerkinElmer Peltier Controller (USA) with rate 0.5°C/*min*. Values of temperature and corresponding absorption were displayed on PC and then elaborated in Excel MS software. After receiving the mentioned data group, the denaturation curves were constructed by the method described in [18]. Based on the denaturation curves the denaturation temperature and denaturation interval width were determined; the concentration ratio MV: albumin = 1:10. Each datum, presented here, is an average of 5 experiments; the error does not exceed 5%.

Results and Discussion. Denaturation curves of BSA and its complexes with MV are presented in Figure.

Denaturation curves of BSA (1) and BSA–MV (2) complexes.
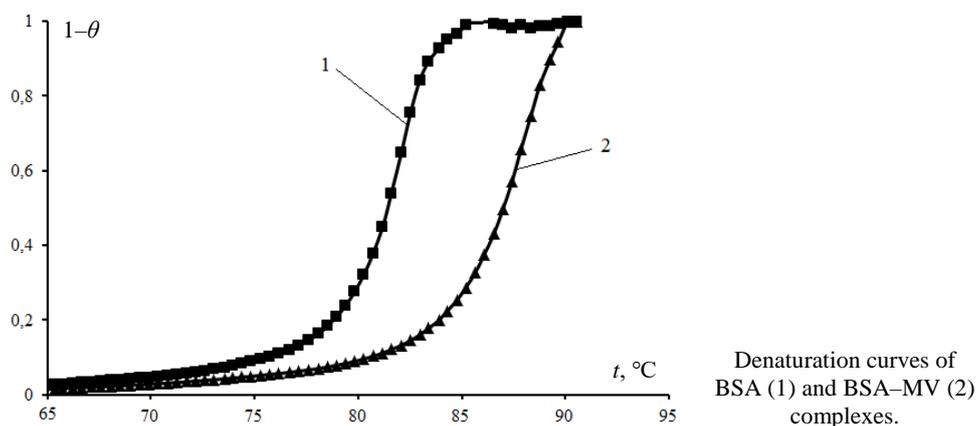
It is obvious from Figure, that the binding of MV to BSA results in stabilization of the protein, due to which the denaturation curve (curve 2) shifts to higher temperature region, as compared to the pure BSA denaturation curve (curve 1). The analogous curves were constructed for HSA and HAS–MV complexes, but curves are not presented, since they are similar in shape with the presented ones. Values of the denaturation parameters – denaturation temperature (T_m) and denaturation interval width (ΔT) are presented in Table.

Denaturation parameters of HSA and BSA and their complexes with MV

Complexes	T_m, °C	ΔT, °C
HSA	81.6±0.1	4.8±0.2
HAS–MV	84.8±0.3	9.9±0.1
BSA	81.3±0.2	5.1±0.1
BSA–MV	87.1±0.1	11.1±0.1

It is obvious from the Table that both BSA and HSA denature almost at the same temperatures; in reality the difference is negligible. At the same time, being bound to the proteins, MV, in both cases, induces some stabilization of the protein structure. It is remarkable that forming a complex with BSA, MV raises the denaturation temperature more, than with HSA. Probably it indicates that MV invokes such conformational changes in BSA, which make difficult to denature earlier. This assumption is justified from the obtained values of ΔT as well. The denaturation interval widths of pure BSA and HSA differ insignificantly, while for the complexes there appear differences. First of all, the denaturation interval widths of BSA and HSA grow after complex-formation with MV, which is connected to more stable conformations of complexed proteins. But for BSA–MV complexes the value of ΔT is a little higher, than that of HAS–MV complexes – by 1.2°C. Possibly, MV binds to BSA stronger, than to HAS, causing the compactization of BSA structure. Due to this binding the structure of BSA turns to be more compact, compared to HSA. This compactization, most probably, is due to the fact that BSA contains two tryptophan residues, while HSA contains only one of that. This fact, in all appearances, results in the differences in denaturation parameters of their complexes with MV.

Conclusion. Thus, generalizing the obtained data, one can conclude that MV, binding to serum albumins, stabilizes the conformations of the proteins, which is reflected in the denaturation curves, since the latters are shifted to higher temperature region. On the other hand, the complex of MV with BSA is stronger, than that with HSA. It is indicated by the denaturation data, showing that the denaturation temperature of the BSA–MV complexes is higher, than that of HAS–MV complexes. At the same time, the denaturation interval width of BSA–MV complexes is larger, than that of HAS–MV complexes, which means that the structure of the first complex is more compact, than that of HAS–MV complex.

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ՄԵԹԻԼԱՅԻՆ ՄԱՆՈՒՇԱԿԱԳՈՒՅՆԻ ՓՈԽԱԶԴԵՑՈՒԹՅԱՆ ՀԱՄԵՄԱՏՈՒԹՅՈՒՆԸ ՑՈՒԼԻ ՇԻՃՈՒԿԱՅԻՆ ԱԼԲՈՒՄԻՆԻ ԵՎ ՄԱՐԴՈՒ ՇԻՃՈՒԿԱՅԻՆ ԱԼԲՈՒՄԻՆԻ ՀԵՏ ՈՒՄ-ԴԵՆԱՏՈՒՐԱՑԻԱՅԻ ՄԵԹՈԴՈՎ

Աշխատանքում ուսումնասիրվել է մեթիլային մանուշակագույնի (ՄՄ) փոխազդեցությունը մարդու շիճուկային ալբումինի (ՄՇԱ) և ցուլի շիճուկային ալբումինի (ՑՇԱ) հետ ՈւՄ-դենատուրացիայի մեթոդով և ստացված տվյալները համեմատվել են: Որոշվել են դենատուրացիայի պարամետրերը՝ դենատու-րացիայի ջերմաստիճանը և դենատուրացիայի միջակայքի լայնությունը: Ցույց է տրվել, որ ՄՄ-ն, կապվելով շիճուկային ալբումինների հետ, կայունացնում է դրանց կառուցվածքը: Միևնույն ժամանակ կայունացման աստիճանը տար-բեր է: Ցույց է տրվել նաև, որ ՄՄ-ի հետ կապվելիս ՑՇԱ-ի կայունացումն ավելի մեծ է, քան ՄՇԱ-ի դեպքում:

М. А. ШАГИНЯН, М. С. МИКАЕЛЯН, М. А. ПАРСАДАНЯН, А. П. АНТОНЯН

СРАВНЕНИЕ ВЗАИМОДЕЙСТВИЯ МЕТИЛОВОГО ФИОЛЕТОВОГО С СЫВОРОТОЧНЫМИ АЛЬБУМИНАМИ БЫКА И ЧЕЛОВЕКА МЕТОДОМ УФ-ДЕНАТУРАЦИИ

В работе исследовано взаимодействие метилового фиолетового (МФ) с сывороточным альбумином человека (САЧ) и сывороточным альбумином быка (САБ) методом УФ-денатурации, полученные данные сравнивались. Определены параметры денатурации – температура денатурации и ширина интервала денатурации. Показано, что МФ, связываясь с сывороточными альбуминами, стабилизирует их структуру. В то же время степень стабилиза-ции разная. Показано также, что САБ стабилизируется больше, чем САЧ при связывании с МФ.