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STUDY OF ABSORPTION SPECTRA OF THE COMPLEXES OF BOVINE SERUM ALBUMIN WITH HOECHST 33258 AND METHYLENE BLUE

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The study on the interaction of DNA-specific low-molecular compounds – groove binding material Hoechst 33258 and intercalating ligand methylene blue (MB) with serum albumin has been carried out. The absorption and differential absorption spectra of complexes of the mentioned ligands with protein were obtained. Changes of the absorption and differential absorption spectra indicate the binding of two ligands with albumin. The obtained results indicate that at the interaction with both ligands, the conformational state of the protein alters, though these changes are not similar, since in the case of MB a compactization of the protein folding occurs, while in the case of Hoechst 33258, most apparently, an unfolding of the compact structure takes place as a result of partial loss of helicity of α-structures.

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***Keywords*:** Bovine serum albumin, DNA-specific ligand, absorption spectra, differential absorption spectra.

Introduction. Proteins play an important role in living organisms, having various biological functions. From this point of view, the role of serum albumin is high, which is used as a model for research of inter-protein interaction in vitro. Many bioactive molecules and drug preparations reversibly bind to albumin and other components of blood serum and act as carriers [1]. Binding ability of albumin may significantly affect the distribution, the non-binding concentration and the metabolism of drug preparation in blood circulatory system.

Proteins, being targets for singlet, non-radical oxygen ($^1O_2$), possess a strong reaction ability in solved state due to the fact that $^1O_2$ shows a preferable reactivity to several amino-acidic residues [2–9]. Remarkably, singlet oxygen can be easily produced via ultraviolet (UV) or visible light in the presence of different photo-sensitive compounds, including methylene blue (MB) that receive a wide application in photodynamic therapy (PDT) [10–12]. It was revealed that a change in protein oxidation mechanisms occurs at the insertion of this ligand into its structure.

Another ligand, possessing an affinity toward proteins, is bisbenzimidazole compound – Hoechst 33258 (H33258), which is an anti-tumorous material. Bisbenzimidazole compounds are revealed to increase the irradiation cytotoxicity in some lines of tumors and, thus, can improve a radiotherapy effect. On the other hand,

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these compounds own radioprotector properties, since they decrease dangerous effect of irradiation on normal cells [13].

Serum albumin is a multi-functional protein and act as transporter of many endogenous and exogenous ligands [14, 15]. Albumin is consisted of three homologous α-helical subdomains – (DI, DII and DIII), each of which is divided into subdomains (A and B). Native conformation of albumin contains two principally important sites of binding for low-molecular compounds, including ligands – I and II, distributed in subdomains IIA and IIIA respectively [2]. That is why the knowledge on interaction mechanisms of ligands with proteins of plasma has crucial value for understanding of pharmacodynamics and pharmacokinetics of drug preparations. It should be mentioned that the data on interaction of MB and H33258 with albumin are few, besides hitherto the binding molecular mechanism of these ligands with albumin is not completely established [2]. Taking this fact into account the present work is aimed at the spectroscopic study of the binding of the mentioned ligands with bovine serum albumin (BSA).

Materials and Methods. Bovine serum albumin (BSA), MB, H33258 ("Sigma", USA), physiological solution, bi-distilled water were used in experiments. Concentrations of ligands and BSA were determined spectrophotometrically, using the following values of extinction coefficients: ε_{664}=76000 $M^{-1}cm^{-1}$ for MB, ε_{343}=42000 $M^{-1}cm^{-1}$ for H33258, ε_{280}=43824 $M^{-1}cm^{-1}$ for BSA [16]. Interaction of ligands with BSA by the absorption spectroscopy method was studied on the spectrophotometer PerkinElmer UV/VIS Lambda 365. Data on absorption were analyzed using program software Microsoft Excel 2010 and the absorption and differential absorption spectra in interval $500 \le \lambda \le 750$ *nm* for MB and $300 \le \lambda \le 450$ *nm* for H33258 were obtained. Experimental data error does not exceed 5%.

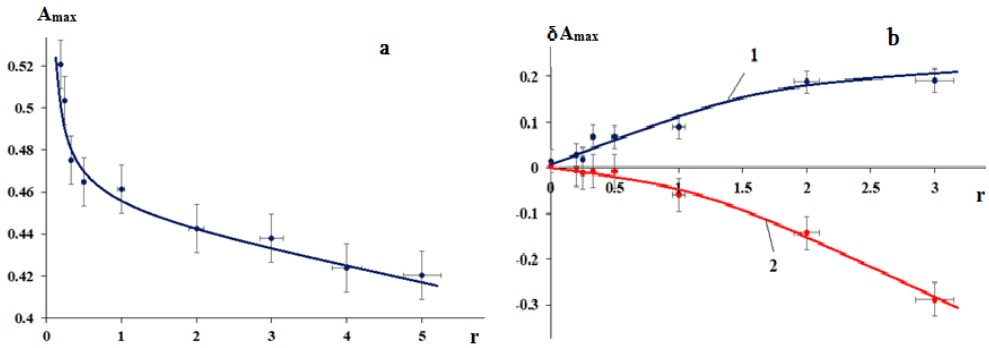

Fig. 1. Dependence curves of the absorption spectra maxima (A_{max}) on r for the complexes of H33258 with BSA (a); dependence curves of the maxima of the differential absorption curves (δA_{max}) on r for the complexes of H33258 with BSA (b).

Results and Discussion. Studies on interaction of ligands with macromolecules in UV/VIS region were carried out via absorption spectroscopy, which is a simple method, applied for investigation of structural changes and determination of complicated structures [17, 18]. Taking this fact into consideration we carried out a study of interaction of MB and H33258 with BSA by the method of absorption spectroscopy and the absorption as well as differential absorption spectra were

obtained at the interaction of the mentioned ligands with albumin. From these spectra the values of maxima were determined and the curves of their dependencies on the ratio *r* (*r*=ligand/albumin) were constructed. The dependence curves of absorption spectra maxima (A_{max}) of the complexes of H33258 with BSA were presented in Fig. 1a and the dependence curves of maxima of differential absorption spectra (δA_{max}) on *r* in Fig. 1b.

It is obvious from Fig. 1a, the maxima of the absorption spectra at the interaction of H33258 with BSA decrease along with increasing of *r*. This effect indicates that DNA-specific ligand H33258 binds to albumin, as a result of which a hypochromic effect is appeared. Meanwhile, the hypochromic degree decreases depending on increasing of r, which indicates that the binding sites of this ligand on albumin are limited and along with their saturation the interaction is stopped. It is also obvious in Fig. 1a that the dependence curve (A_{max}) has a hyperbolic form. If conditionally we divide this curve into two linear regions, it becomes obvious that the linear region, corresponding to low values of *r*, has big slope in the interval of change $0<r<1.0$. It can be the result of the fact that stoichiometry of H33258 binding to albumin tends to the ratio albumin: H33258 ≈ 1 : 1.

The maxima of the differential absorption complexes of H33258 with albumin are presented in Fig. 1b. To obtain differential absorption spectra (δA on λ), the absorption of the complexes of ligands with BSA was measured against absorption of the solution of free ligand. Though, in two solutions the ligand concentration was similar. It should be mentioned that the differential absorption spectra at the interaction of H33258 with albumin consist of two peaks: positive – in the interval of change $300\leq\lambda\leq350$ *nm* and negative – in the interval of change $350\leq\lambda\leq450$ *nm*. Nonetheless, the maxima of positive peaks correspond to $\lambda\approx335$ *nm*; those of negative peaks – $\lambda\approx380$ *nm*. It is obvious from Fig. 1b that the dependence curve δA_{max} (curve 1), corresponding to positive peaks of the differential absorption spectra (short-wave peaks), increases along with increasing of *r*, while the curve 2 (corresponding to negative peaks of differential absorption) decreases (long-wave peaks). Hyperchromic change at relatively short and hypochromic change at relatively long wavelengths in the differential absorption spectra of H33258 complexes with albumin, most apparently, are the result of the fact that a conformation of the protein changes due to the binding to H33258. Taking into account that hypochromic effect occurs at relatively long wavelengths, we assume that the molecules of this ligand form hydrogen bonds with amino-acidic residues of α-helices of the protein, as a result of which a decrease of parallel dipole-dipole interactions between peptide groups takes place. It is not also excluded that hypochromic effect is conditioned by stacking distribution of aromatic benzimidazole groups of bound molecules of H33258 [19–21]. Taking into account that denaturation temperature of albumin in the complex with H33258 decreases [22], we assume that the aforementioned hyper- and hypochromic effects are resulted from the partial losses of α-helical polypeptide chains of albumin, induced by the binding of H33258 with these regions.

We also carried out the studies on the interaction of BSA with MB. The dependence curves of the maxima of the absorption spectra (A_{max}) for the complexes

of MB with BSA (a) and those of the differential absorption spectra (δA_{max}) for the complexes of MB with BSA (b) on r are presented in Fig. 2.

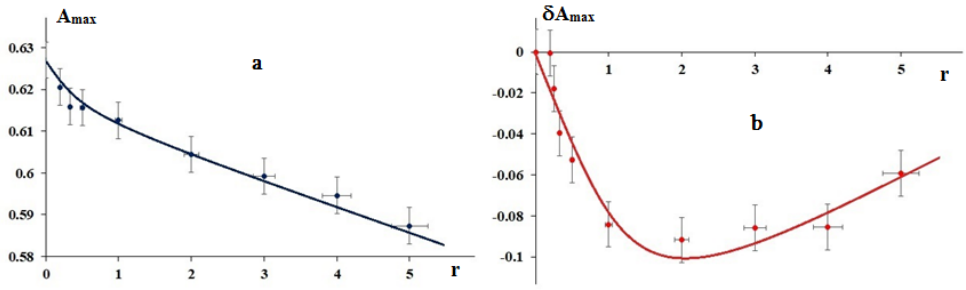

Fig. 2. Dependence curves of the maxima of the absorption spectra (A_{max}) on r for the complexes of MB with BSA (a); dependence curves of the maxima of the differential absorption spectra (δA_{max}) on r for the complexes of MB with BSA (b).

It is seen from the Fig. 2 that the maxima of the absorption spectra of the complexes MB-BSA monotonously decrease along with increasing of r, which indicates that MB, as H33258, binds to albumin. Though, the shape of the absorption spectra of the complexes MB-BSA sufficiently differs from the analogous curve of the complexes H33258-BSA. The differential absorption spectra are also obtained for the complexes MB-BSA (Fig. 2b). As it is obvious from the Fig. 2, the absolute values of the differential absorption curves decrease depending on r and passing through the minimum, start increasing. This effect is reflected on the binding of MB with BSA up to ratios ~1 : 1.5, as a result of which the differential absorption spectra change negatively, after which they start increasing, which, most apparently, indicates the fact that beginning from the values $r \approx 2$, the binding of MB to albumin is impeded and more molecules of the ligand are in free state. This result indicates that the binding sites of MB with BSA are more limited, than the adsorption centers for H33258. Most apparently, molecules of MB that have smaller sizes, than extended molecules of H33258, can be localized in inner cavity into site II, which is in subunit IIIA. This site has less hydrophobicity, than the site I, which is in subunit IIA. However, the site II is the main center of adsorption for MB [2]. Though, probably, BSA is subjected to such conformational changes that the adsorption centers for MB become more limited, than for H33258.

Conclusion. Thus, the obtained results indicate that H33258 and MB interact with blood serum albumin. These ligands are DNA-specific; meanwhile, they bind to DNA by different mechanisms: H33258 is localized in DNA minor groove and specifically binds to AT-sequences, MB binds by intercalation mechanism. As in the case of DNA, these ligands bind to albumin by various mechanisms: H33258 mainly binds to α-helices via hydrogen bonds, partially decreasing the helicity degree of these polypeptide structures; MB is localized in inner cavity of the protein, invoking some conformational reconstructions. We assume that in the case of MB some increase of the protein folding takes place, resulting in limitation of the binding sites for this ligand. In the case of H33258, probably, the reverse phenomenon occurs – some unfolding, as a result of which the protein denaturation temperature decreases, as it is revealed in [22]. Actually, both ligands, binding to albumin, induce

conformational reconstructions in the protein molecule, but these changes are not similar, which is reflected on the structural stability of macromolecule: in the case of H33258 some destabilization is revealed [22], while in the case of MB, vice versa, a stabilization of the protein dimensional structure occurs [23].

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Ն. Հ. ՊԵՏՐՈՍՅԱՆ

HOECHST 33258-Ի ԵՎ ՄԵԹԻԼԵՆԱՅԻՆ ԿԱՊՈՒՅՏԻ ՀԵՏ ՑՈՒԼԻ ՇԻՃՈՒԿԱՅԻՆ ԱԼԲՈՒՄԻՆԻ ՀԱՄԱԼԻՐՆԵՐԻ ԿԼԱՆՄԱՆ ՍՊԵԿՏՐՆԵՐԻ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆԸ

Իրականացվել է ցուլի շիճուկային ալբումինի հետ ԴՆԹ-սպեցիֆիկ լիգանդների[a] փոքր ակոսում կապվող Hoechst 33258-ի և ինտերկալացվող լիգանդ մեթիլենային կապույտի (ՄԿ) փոխազդեցության ուսումնասիրություն: Ստացվել են սպիտակուցի հետ նշված լիգանդների կոմպլեքսների կլանման և դիֆերենցիալ կլանման սպեկտրները: Կլանման և դիֆերենցիալ կլանման սպեկտրների փոփոխությունները վկայում են այն մասին, որ այս լիգանդները կապվում են ալբումինի հետ: Ստացված արդյունքները վկայում են այն մասին, որ երկու լիգանդների հետ էլ փոխազդեցության դեպքում սպիտակուցի կոնֆորմացիոն վիճակը փոխվում է, սակայն այն փոփոխությունները միանման չեն, քանի որ ՄԿ-ի դեպքում տեղի է ունենում սպիտակուցի խիտ փաթեթավորում, մինչդեռ Hoechst 33258-ի դեպքում, հավանաբար, կոմպակտ կառուցվածքը ապափաթեթավորվում է α-կառուցվածքների պարուրվածության մասնակի կորստի արդյունքում:

Н. Г. ПЕТРОСЯН

ИССЛЕДОВАНИЕ СПЕКТРОВ ПОГЛОЩЕНИЯ КОМПЛЕКСОВ БЫЧЬЕГО СЫВОРОТОЧНОГО АЛЬБУМИНА С HOECHST 33258 И МЕТИЛЕНОВЫМ СИНИМ

Проведено исследование по взаимодействию ДНК-специфических низкомолекулярных веществ – желобково-связывающегося соединения Hoechst 33258 и интеркалирующего лиганда метиленового синего (МС) с сывороточным альбумином. Получены спектры поглощения и разностного поглощения комплексов указанных лигандов с протеином. Изменения спектров поглощения и разностного поглощения указывают на связывание обоих лигандов с альбумином. Полученные результаты указывают на то, что при взаимодействии с обоими лигандами конформационное состояние протеина изменяется, однако эти изменения не одинаковые, поскольку в случае МС имеет место компактизация фолдинга белка, в то время как в случае Hoechst 33258, скорее всего, происходит анфолдинг компактной структуры в результате частичной потери спиральности α-структур.