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## COMMUNICATION

*Biology*

# FLUORESCENCE CHANGE OF HUMAN SERUM ALBUMIN INDUCED BY METHYL VIOLET

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The interaction of methyl violet (MV) with human serum albumin (HSA) has been studied, using the fluorescence spectroscopy method. It was shown that MV chnages the own fluorescence of HSA. It was also shown that MV does not induce any conformational change in the structure of HSA, since there is no change of the wavelength of HSA fluorescence intensity maximum. MV binds to HSA, near to fluorescing tryptophan, which in the hydrophilic environment, and changes the own fluorescence of the protein.

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***Keywords*:** human serum albumin, methyl violet, interaction, fluorescence spectra.

**Introduction.** Human serum albumin (HSA) is one of known proteins in human organism, the main function of which is the transport of various endogenous and exogenous compounds, among which drugs are the important transporting material [1, 2]. From this point of view it is very important to study the interaction of HSA with biologically active compounds. In the presented work as a drug preparation methyl violet (MV) is chosen.

Methyl violet, which is also known as crystal violet, is a triphenylmethane industrial dye, which is widely used in various purposes: a biological stain, a fungicide in the aquaculture, an external drug for skin diseases (Fig. 1) [3].

Crystal violet is a cationic dye, which has been used for detection of metal ions. It was also used as a detector of nucleic acids, based on its interaction with DNA [4]. It is also known by its good sterilization, little toxicity and hormesis. Taking into consideration of important properties of crystal violet, it has a scientific value to study the interaction of methyl violet (MV) with known transporter in organism – serum albumin.

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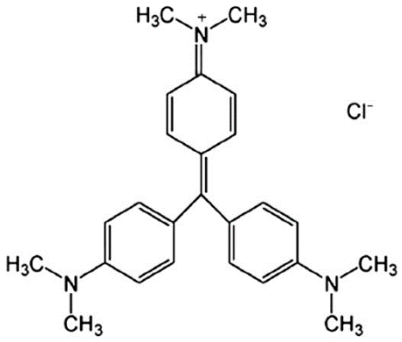


Fig. 1. The chemical structure of MV.

The present study is aimed at studying of HSA interaction with MV by fluorescence spectroscopy.

**Materials and Methods.** Human serum albumin 1% solution ("Sigma", USA), methyl violet ("Sigma", USA), physiological solution were used in experiments. Concentrations of MV was determined spectrophotometrically, using the following coefficient of extinction: $\varepsilon_{590} = 87000\ M^{-1}cm^{-1}$. Each datum in the experiments was averaged from 5 values.

Fluorescence spectra of HSA and MV were obtained using a spectro-fluorometer Cary Eclipse (Australia). Excitation of the samples was made at 280 *nm.* The fluorescence spectra were registered in the interval $300 \le \lambda \le 500$ *nm*. After obtaining of the pure HSA fluorescence spectrum, the titration of the protein solution was carried out by the solutions of MV in the following concentration ratio of ligand/albumin – from 1/2 up to 1/10. The measurement error does not exceed 5–10%.

**Results and Discussion.** The fluorescence spectra of HSA and HSA–MV complexes were presented in Fig. 2.

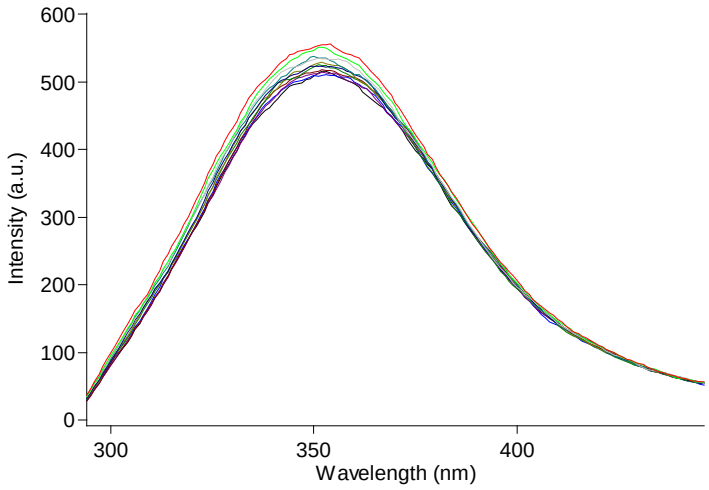


Fig. 2. Fluorescence spectra of HSA and the complexes of HSA-MV.

It is obvious from Fig. 2 that the fluorescence intensities of HSA increase with the increasing of MV concentration, which means that MV acts rising HSA own fluorescence. This conclusion differs from the literature data, which is connected to differences of structures of human and bovine serum albumins (consistence of 1 and 2 tryptophan residues respectively) [5]. On the other hand, it is seen from the Fig. 1 that the maximum of pure HSA fluorescence intensity is equal to 516 at 353 *nm* while after the addition of 500 *mL* MV, when the concentration ratio MV/HSA becomes equal to 1/2, the maximum fluorescence intensity is equal to 554 at 353 *nm*. This means that MV does not invoke any structural change in the structure of HAS, but leads to significant changing of hydrophobicity in the vicinity of a single tryptophan residue, responsible for protein fluorescence. However, the binding of MV with HSA results in changing of charge density of globular protein, which may invoke a little change of the fluorescence. According to the literature data [6], if the fluorescence intensity maximum is in the vicinity of 355 *nm*, the fluorescing tryptophan is in the polar surrounding.

**Conclusion.** Proceeding from the data presented in this work, one can conclude that MV interacts with HSA, forming a complex with it and increases the own fluorescence intensity of the latter. On the other hand, MV does not change HSA conformation, which is shown from the wavelength of the fluorescence intensity maximum – it does not change. Binding to the region of HSA, close to tryptophan, MV changes the fluorescence intensity of HSA.

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## ՄԱՐԴՈՒ ՇԻՃՈՒԿԱՅԻՆ ԱԼԲՈՒՄԻՆԻ ՖԼՈՒՈՐԵՍՑԵՆՑԻԱՅԻ ՓՈՓՈԽՈՒԹՅՈՒՆԸ՝ ՀՐԱՀՐՎԱԾ ՄԵԹԻԼ ՄԱՆՈՒՇԱԿԱԳՈՒՅՆՈՎ

Ուսումնասիրվել է մեթիլ մանուշակագույնի (ՄՄ) հետ մարդու շիճուկային ալբումինի (ՄՇԱ) փոխազդեցությունը ֆլուորեսցենտային սպեկտրոսկոպիայի մեթոդով: Ցույց է տրվել, որ ՄՄ-ն փոխում է ՄՇԱ-ի սեփական ֆլուորեսցենցիան: Ցույց է տրվել նաև, որ ՄՄ-ն որևէ կոնֆորմացիոն փոփոխություն չի հրահրում ՄՇԱ-ի կառուցվածքում, քանի որ չի փոխվում ՄՇԱ-ի ֆլուորեսցենտային ինտենսիվության մաքսիմումին համապատասխանող ալիքի երկարությունը: ՄՄ-ն կապվում է ՄՇԱ-ի հետ այն տեղամասին մոտ, որտեղ գտնվում է ֆլուորեսցենցող տրիպտոֆանը, որը գտնվում է հիդրոֆիլ միջավայրում, և փոփոխության է ենթարկում սպիտակուցի սեփական ֆլուորեսցենցիան:

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## ИЗМЕНЕНИЕ ФЛУОРЕСЦЕНЦИИ СЫВОРОТОЧНОГО АЛЬБУМИНА ЧЕЛОВЕКА ИНДУЦИРОВАННОГО МЕТИЛОВЫМ ФИАЛЕТОВЫМ

Исследовано взаимодействие метилового фиалетового (МФ) с сывороточным альбумином человека (САЧ) с использованием метода флуоресцентной спектроскопии. Показано, что МФ изменяет собственную флуоресценцию САЧ. Показано также, что МФ не индуцирует никаких конформационных изменений в структуре САЧ, поскольку не наблюдается изменение длины волны, соответствующей максимуму флуоресцентной интенсивности САЧ. МФ связывается с САЧ вблизи флуоресцирующего триптофана, находящегося в гидрофильном окружении, и меняет собственную флуоресценцию белка.