

FLUORESCENCE STUDIES ON THE BLOOD PLASMA ALBUMIN INTERACTION WITH 4-HYDROXY-2-METHYLQUINOLINE

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Blood plasma albumin (bovine serum albumin, BSA) interaction with a biologically active reagent 4-hydroxy-2-methylquinoline (4H2MQ) has been studied using fluorescence spectroscopy methods (steady state, synchronous, excitation/emission matrix, 3D spectra). With the addition of 4H2MQ, no changes in the spectral characteristics of BSA (fluorescence emission intensity and λ_{\max} position) are observed; only the symmetry of the BSA emission spectrum is changed and the full width at half maximum is decreased. The analysis of synchronous and 3D fluorescence spectra showed the existence of three emitting species in this system, which could be a result of changes in the fluorescence and photochemical properties of 4H2MQ.

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Keywords: 4-hydroxy-2-methylquinoline, bovine serum albumin, fluorescence spectroscopy, synchronous fluorescence, excitation /emission matrix.

Introduction. Quinoline containing pharmacophores are widely used in drug formulations exhibiting antifungal, antibacterial, antiprotozoal, antiasthmatic, as well as antineoplastic properties [1–3]. Derivatives of quinolines, such as hydroxyquinolines, also demonstrate biological activity as alkenyl side chain of hydroxyquinolines/hydroxyquinolones can affect the antimicrobial properties due to hydrophobic interactions with transport proteins [4]. Chloroquine (a quinoline-based drug) is widely used for the prevention and treatment of malaria. Recent studies have provided evidence that this drug may also enhance the efficacy of chemotherapy, particularly the efficacy of cisplatin [5].

Human serum albumin (HSA) and bovine serum albumin (BSA) are commonly used as model proteins in biopharmaceutical studies. HSA and BSA display approximately similar structure and sequence of amino acid residues in three domains [6]. From a spectroscopic point of view, one of the main differences between the two proteins is that HSA has only one tryptophan (Trp) residue (Trp-214), whereas BSA has two Trp residues (Trp-134 and Trp-212), which are located in the subdomains IB and IA, respectively. Trp-134 is more exposed to

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solvent, and is very sensitive to any change in microenvironment polarity whereas Trp-212 is buried in a hydrophobic pocket of the protein. BSA possesses absorption (UV/Vis) and emission (fluorescence) properties due to the existence of aromatic amino acids phenylalanine (Phe), tyrosine (Tyr) and Trp residues [7]. By monitoring the intrinsic fluorescence properties of protein, binding affinities (binding mechanism, binding mode, binding constants, binding sites, intermolecular distance, etc.) of small molecules can be determined. In the present work, we attempted to understand the structural changes of BSA caused by the interaction with 4-hydroxy-2-methylquinoline (4H2MQ) using fluorescence spectroscopy methods. The structures of BSA and 4H2MQ are presented in Fig. 1.

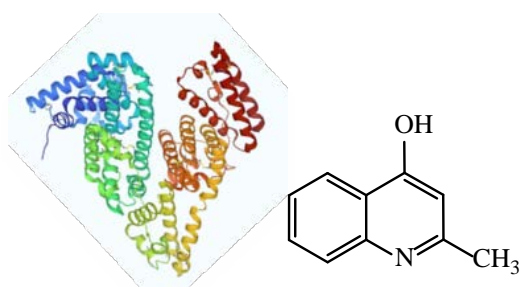


Fig. 1. Structures of BSA (from RCSB Protein Data Bank ID: 4F5S) and 4H2MQ.

Materials and Methods. BSA (fatty acid free < 0.05 %) was purchased from “Sigma” Chemical Co. (USA) and used without further purification, other reagents were of analytical grade. The concentration of 4H2MQ was varied in the range of $0\div 1\cdot 10^{-4} \text{ mol}\cdot\text{L}^{-1}$. BSA concentration (0.4 mg/mL) was determined at 280 nm with the molar extinction coefficient $4.3890\cdot 10^4 \text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$ [8]. BSA stock solution was prepared in saline solution. The fluorescence spectra were recorded on a Varian Cary Eclipse spectrofluorometer (Australia), equipped with a temperature-controlled accessory. The measurements were carried out at 293 K in the range of $\lambda=300\text{--}500 \text{ nm}$ upon the excitation wavelength $\lambda=280 \text{ nm}$, 1 cm quartz cells were used. The synchronous fluorescence spectra were registered with scanning ranges $\Delta\lambda=15$ and 60 nm in the absence and presence of 4H2MQ. The three-dimensional fluorescence spectra (3D spectra) were recorded under the following conditions: excitation range ($\Delta\lambda_{\text{ex}}$) $200\text{--}450 \text{ nm}$, emission range ($\Delta\lambda_{\text{em}}$) $250\text{--}450 \text{ nm}$, $\Delta\lambda_{\text{incr}}=10 \text{ nm}$. The number of scans was 21. The excitation and emission slits were set to 5 nm . The graphs and diagrams were constructed and analyzed, using ORIGIN 8.5 software.

Results and Discussion.

Steady State Fluorescence Spectra Analysis of BSA in the Presence of 4H2MQ. The interaction of a compound with a fluorophore can affect the fluorescence characteristics of the fluorophore due to ground-state complex formation, excited-state reactions, fluorescence resonance energy transfer (Förster’s theory) or molecular rearrangements [9–12]. Fluorescence spectra of BSA in the presence of 4H2MQ are presented in Fig. 2.

Fluorescence spectrum of BSA is characterized by a structureless band at 350 nm with an 864 a.u. emission intensity and full width at half maximum about 60 nm . Dashed line spectrum belongs to 4H2MQ, which indicates a little fluorescence in $300\text{--}500 \text{ nm}$ range. With the addition of 4H2MQ, no changes in the spectral

characteristics of BSA (fluorescence emission intensity and λ_{\max} position) are observed, only the symmetry of the emission spectra are changed.

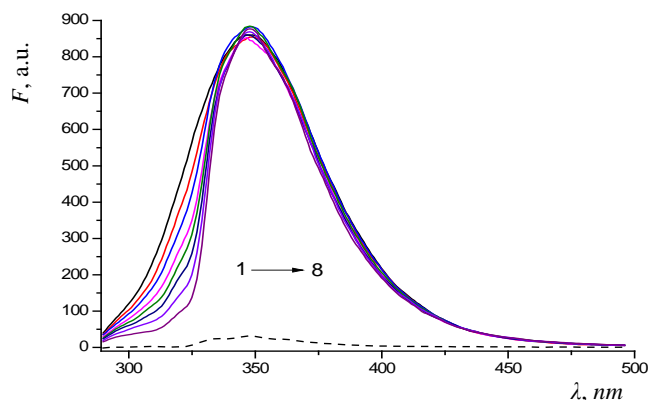


Fig. 2. Fluorescence spectra of BSA in the presence of 4H2MQ: $[BSA]=6 \cdot 10^{-6} \text{ mol} \cdot \text{L}^{-1}$; $[4H2MQ]=0 \div 1 \cdot 10^{-4} \text{ mol} \cdot \text{L}^{-1}$; $T=293 \text{ K}$. Dash curve shows the emission spectrum of 4H2MQ: $[4H2MQ]=1.0 \cdot 10^{-5} \text{ mol} \cdot \text{L}^{-1}$.

According to the model of discrete states of Burstain et al., there are five most probable forms of fluorescence 2D spectra of Trp residues (A, S, I, II and III) characterizing the indole fluorophore in proteins. Spectral form III ($\Delta\lambda_{\max}$ at about 350 nm , $\Delta\lambda=60 \text{ nm}$) corresponds to the emission of the indole chromophore located on the protein surface in contact with free water molecules [13]. In the unfolded state of BSA, fluorescence emission could be attributed to Trp-134 residue as Trp-212 is buried in a hydrophobic pocket, while in the folded state of protein, the emission band could be attributed to both residues. BSA emission characteristics (F and λ_{\max}) practically stay unchanged in the presence of 4H2MQ, only the full width at half maximum of the spectrum decreases from 62 to 48 nm . This could be a result of Trp-134 residue rotation caused by the interactions with the surrounding amino acids. Fluorescence spectra (excitation and emission) characterize the global state-structure of the fluorophore, whereas lifetimes characterize the observed substructures and their interactions with the surrounding environment. One Trp residue emission could be described with three fluorescence lifetimes. In BSA structure, it is not possible to assign a specific lifetime to a specific Trp residue. The two lifetimes around $0.3\text{--}0.4$ and $3\text{--}3.5 \text{ ns}$ seem to be independent of any structure around the Trp and simply characterize an internal property or/and organization of the Trp structure independently of its environment. The third lifetime could be attributed to the interaction between the Trp residue and the surrounding amino acids [14]. These results, obtained from fluorescence lifetime measurements, could be confirmed by synchronous fluorescence studies.

Synchronous Fluorescence Spectra Analysis of BSA in the Presence of 4H2MQ. This method involves measuring the fluorescence spectrum simultaneously changing the exciting and detecting radiation wavelengths. We used the synchronous fluorescence method for scanning ranges $\Delta\lambda=60$ and 15 nm in the absence and presence of 4H2MQ. When $\Delta\lambda=60 \text{ nm}$, the synchronous fluorescence emission characterizes the Trp residues, while when scanning range was 15 nm we also

observed fluorescence of the Tyr residues [15]. Fig. 3 shows the synchronous fluorescence spectra of BSA in the presence of 4H2MQ for $\Delta\lambda=15$ (a) and $\Delta\lambda=60$ nm (b).

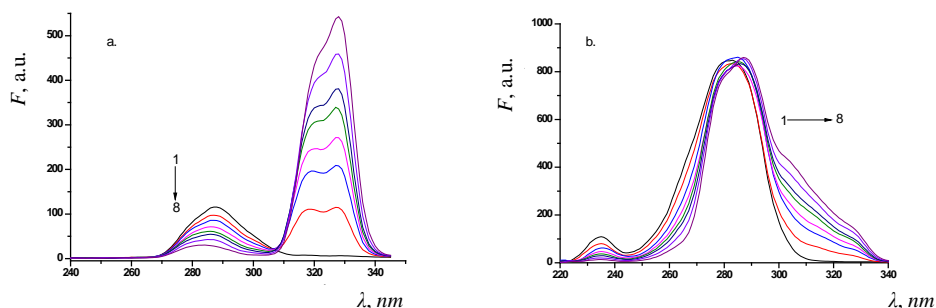


Fig. 3. Synchronous fluorescence spectra of BSA in the presence of 4H2MQ: $[BSA]=6 \cdot 10^{-6} \text{ mol} \cdot L^{-1}$; $[4H2MQ]=0 \div 1 \cdot 10^{-4} \text{ mol} \cdot L^{-1}$; $T=293 \text{ K}$; a) $\Delta\lambda=15 \text{ nm}$; b) $\Delta\lambda=60 \text{ nm}$.

Synchronous fluorescence spectrum of BSA, when $\Delta\lambda=15 \text{ nm}$, is characterized by a structureless band at $\lambda_{\text{max}}=288 \text{ nm}$ with a fluorescence intensity of 75.1 a.u., which shifts to shorter wavelengths ($\approx 4.5 \text{ nm}$) denoting on increasing hydrophobicity around the Trp residue. With the addition of 4H2MQ, a new emission band, splitted into two parts, appears at $\lambda_{\text{max}}=318 \text{ nm}$ (112 a.u.) and $\lambda_{\text{max}}=328 \text{ nm}$ (116 a.u.) reflecting the equilibrium between the two emitting species of 4H2MQ. With an increase in the 4H2MQ concentration, the fluorescence intensity of the band at 318 nm increases and it shifts to longer wavelengths (on about 9 nm). This indicates the prevalence of more polar emitting form of 4H2MQ in the solution. Synchronous fluorescence spectrum of BSA, when $\Delta\lambda=60 \text{ nm}$, is characterized by an intensive band (844.5 a.u.) at $\lambda_{\text{max}}=281 \text{ nm}$, which in the presence of 4H2MQ shifts to longer wavelengths ($\approx 5.1 \text{ nm}$) denoting on increasing polar interactions. In the presence of 4H2MQ, the symmetry of the band is disrupted, a shoulder appears approximately at 310 nm, which becomes more pronounced at the higher concentrations of 4H2MQ. Meanwhile, a less intense band is mentioned at $\lambda_{\text{max}}=234 \text{ nm}$ with a fluorescence intensity of 108 a.u., which decreases with the increase of 4H2MQ concentration. The nature of this band could be ascertained by the fluorescence excitation/emission matrix (3D spectra) method.

Fluorescence Excitation/Emission Matrix Studies. Fluorescence excitation/emission matrix method is widely used to analyze the systems of multiple fluorophores with overlapping spectra. The maximum emission wavelength and the fluorescence intensity of Tyr and Trp residues have a close relation to the polarity of their microenvironment [16, 17]. The contour maps of 3D spectra of BSA and BSA–4H2MQ are presented in Fig. 4, and the corresponding fluorescence characteristic parameters are listed in Table. 3D spectra of BSA are characterized by two bands with peak 1 at approximately $\lambda_{\text{ex}}/\lambda_{\text{em}}=230/350 \text{ nm/nm}$ and peak 2 at $\lambda_{\text{ex}}/\lambda_{\text{em}}=280/350 \text{ nm/nm}$. Peak 1 is often attributed to protein “backbone fluorescence”. As it was shown in [18], this peak originates from the fluorescence of aromatic residues and it is simply due to the excitation of the second excited state of the aromatic residues, which then emit from their lowest excited state. Peptide backbone does not contribute to protein fluorescence. Peak 2 corresponds to the emission of the aromatic amino acids Trp and Tyr residues.

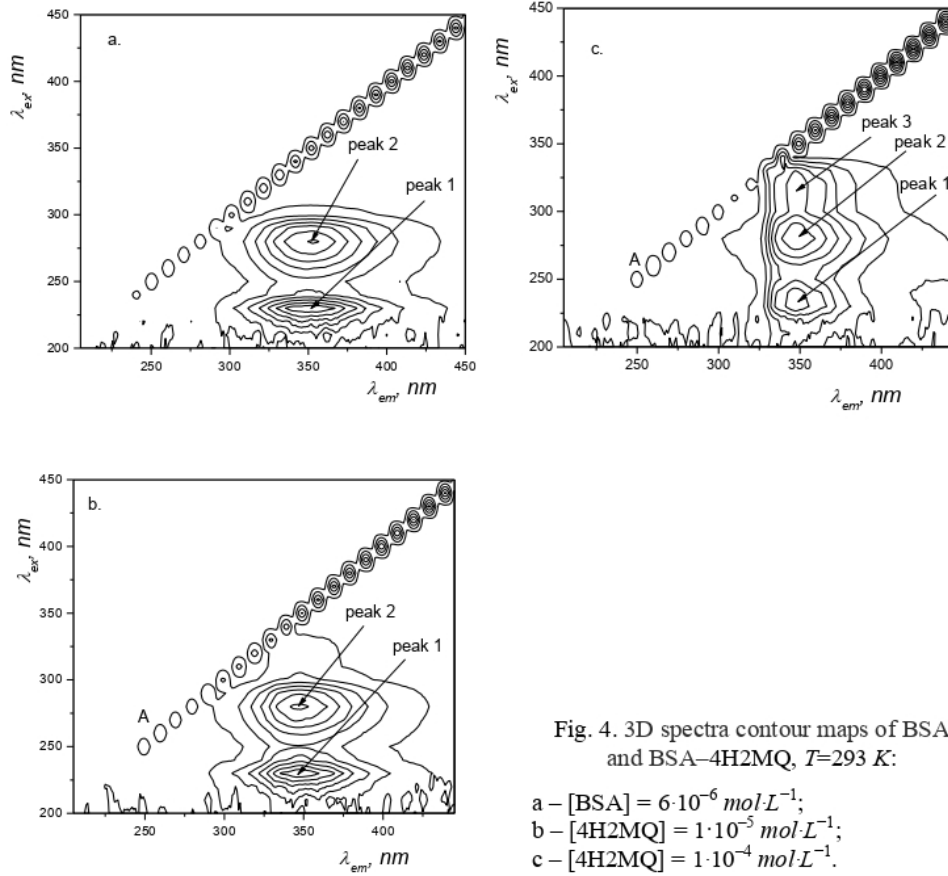


Fig. 4. 3D spectra contour maps of BSA and BSA-4H2MQ, $T=293\text{ K}$:

- a – $[\text{BSA}] = 6 \cdot 10^{-6} \text{ mol}\cdot\text{L}^{-1}$;
 b – $[\text{4H2MQ}] = 1 \cdot 10^{-5} \text{ mol}\cdot\text{L}^{-1}$;
 c – $[\text{4H2MQ}] = 1 \cdot 10^{-4} \text{ mol}\cdot\text{L}^{-1}$.

Fluorescence characteristic parameters of 3D spectra contour maps of BSA and BSA-4H2MQ

System	[4H2MQ], $\text{mol}\cdot\text{L}^{-1}$	$\lambda_{\text{ex}}/\lambda_{\text{em}}$, nm/nm	F , a.u.	Rayleigh scattering	
				$\lambda_{\text{ex}}/\lambda_{\text{em}}$, nm/nm	F , a.u.
BSA	0	230/350	165	240/240→440/440	15→86
		280/350	148		
BSA-4H2MQ	$1 \cdot 10^{-5}$	230/347	161	250/250→440/440	26→101
		280/347	144		
BSA-4H2MQ	$2 \cdot 10^{-5}$	230/347	162	250/250→440/440	27→107
		280/347	150		
BSA-4H2MQ	$3 \cdot 10^{-5}$	230/347	143	250/250→440/440	22→97
		280/347	144		
BSA-4H2MQ	$4 \cdot 10^{-5}$	230/347	145	250/250→440/440	21→108
		280/347	143		
BSA-4H2MQ	$5 \cdot 10^{-5}$	230/347	141	250/250→440/440	20→110
		280/347	145		
BSA-4H2MQ	$7 \cdot 10^{-5}$	230/347	134	250/250→440/440	18→114
		280/347	148		
		310/347	92		
BSA-4H2MQ	$1 \cdot 10^{-4}$	230/347	129	250/250→440/440	15→114
		280/347	150		
		310/347	109		

Peak A corresponds to Rayleigh scattering ($\lambda_{\text{ex}}=\lambda_{\text{em}}$). In the presence of 4H2MQ the intensities of both peaks decrease and a new peak (peak 3) appears at $\lambda_{\text{ex}}/\lambda_{\text{em}}=310/347 \text{ nm/nm}$ at higher concentrations of 4H2MQ ($7 \cdot 10^{-5} \text{ mol} \cdot \text{L}^{-1}$), which denotes the formation of new emitting species of BSA. This could be explained by the changes of the fluorescence and photochemical properties of 4H2MQ due to the equilibrium shift between keto (*K*) and enol (*E*) tautomeric and also protonated and deprotonated forms in solution. As it was shown in [19], in neutral solutions the *K* form is the major tautomeric structure for the ground, excited singlet and triplet states of 4-hydroxyquinolines, and the appearance of peak 3 could be a result of the triplet state of 4H2MQ. Changes in Rayleigh scattering are mentioned as well. Its intensity increases from 15→86 a.u. to 15→114 a.u., denoting the existence of more structured particles in the solution.

Conclusion. In this paper, the interaction of BSA with 4H2MQ was studied using fluorescence spectroscopy methods. It has been shown, that the fluorescence intensity of BSA is not change in the presence of 4H2MQ and no direct interaction between these molecules is observed. The Trp-134 residue of BSA demonstrates only a rotation caused by the interactions with the surrounding amino acids. Fluorescence 3D spectra show the existence of three emitting species in this system, which could be a result of changes in the fluorescence and photochemical properties of 4H2MQ.

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ԱՐՅԱՆ ՊԼԱԶՄԱՅԻ ԱԼԲՈՒՄԻՆԻ 4-ՀԻԴՐՕՔՍԻ-2-ՄԵԹԻԼՔԻՆՈԼԻՆԻ
ՀԵՏ ՓՈԽԱԶԳԵՑՈՒԹՅԱՆ ՖԼՅՈՒՐԵՍԵՆՏՏԱՅԻՆ
ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆԸ

Արյան պլազմայի ալբումինի (ցուլի շինուկային ալբումին՝ ՑՃԱ) փոխազդեցությունը կենսաբանորեն ակտիվ ռեագենտ 4-հիդրօքսի-2-մեթիլքինոլինի

(4H2MQ) հետ ուսումնասիրվել է ֆլուորեսցենտային սպեկտրաչափության տարրեր եղանակներով (ստացիոնար, սինքրոն, գրգռման/առաքման մատրիցայի, 3D սպեկտրներ): 4H2MQ-ն ավելացնելիս ՅՇԱ-ի ֆլուորեսցենտային առաքման ազդանշանի սպեկտրային բնութագրերի փոփոխություն (ֆլուորեսցենտային առաքման ինտենսիվության և λ_{\max} -ի տեղաշարժ) չի դիտվել, միայն փոխվել է ազդանշանի սիմետրիան և փոքրացել կիսաբարձրության լայնությունը: Այս փոփոխությունների պատճառը կարող է լինել Trp-134 ամինոթթվային մնացորդի պտույտը՝ հարևանությամբ տեղակայված ամինոթթվային մնացորդների հետ փոխազդեցությունների հետևանքով: Մինքրոն և 3D ֆլուորեսցենտային սպեկտրների վերլուծությունը ցույց է տվել, որ համակարգում առկա են երեք տիպի ֆլուորեսցենցող մասնիկներ, ինչը կարող է լինել 4H2MQ-ի ֆլուորեսցենտային և ֆոտոքիմիական հատկությունների փոփոխության արդյունք:

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ФЛУОРЕСЦЕНТНОЕ ИССЛЕДОВАНИЕ ВЗАИМОДЕЙСТВИЯ АЛЬБУМИНА ПЛАЗМЫ КРОВИ С 4-ГИДРОКСИ-2-МЕТИЛХИНОЛИНОМ

Исследовано взаимодействие альбумина плазмы крови (бычий сывороточный альбумин, БСА) с биологически активным реагентом 4-гидрокси-2-метилхинолином (4H2MQ) методами флуоресцентной спектроскопии (стационарное, синхронное, матрица возбуждения/эмиссии, 3D-спектры). При добавлении 4H2MQ изменений спектральных характеристик флуоресценции БСА (интенсивность флуоресценции и смещение λ_{\max}) не наблюдалось; изменилась только симметрия и уменьшилась полная ширина на половине высоты сигнала. Эти изменения могут быть связаны с вращением аминокислотного остатка Trp-134 БСА вследствие взаимодействия с соседними аминокислотными остатками. Анализ синхронных и 3D-флуоресцентных спектров показал, что в системе присутствуют три типа флуоресцирующих частиц, что может быть результатом изменения флуоресцентных и фотохимических свойств 4H2MQ.