

Study of the interaction between bovine serum albumin and molecules of halogenated fluorescein derivatives in anionic reverse micelles

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The effect of spatial confinement in reverse micelles (RMs) on the spectral and fluorescent characteristics of eosin (E), erythrosin (ER), rose bengal (RB), bovine serum albumin (BSA), and their mixtures was studied. It was found that the size of the reverse micelles (their hydrodynamic radius R_h) affects the spectral and luminescent characteristics of both BSA and dye molecules (DM). The microenvironment of tyrosine residues in BSA does not undergo any significant changes with changing R_h , while a decrease in the micelle radius leads to an increase in the fluorescence intensity of tryptophan residues. For DMs, spatial confinement in aqueous pools of micelles affects the change in the dipole moment of the molecules upon their excitation. The dependences of the BSA fluorescence quenching coefficient (K_{SV}) on DM were experimentally obtained, and it was determined that for the studied dyes, this characteristic differs for micellar solutions compared to buffer solutions. The static quenching constants (K_S) for complexes in micelles in the studied region of their hydrodynamic radii are two orders of magnitude smaller than the Stern–Volmer constant (K_{SV}) for aqueous solutions. K_S values decrease in the $E \rightarrow ER \rightarrow BR$ series, which is due to conformational changes in the molecular structure in this series associated with the redistribution of charges on the molecular atoms. Dynamic fluorescence quenching, which is the main one, is caused by the transfer of electronic excitation energy from BSA to DM. The efficiency of electronic excitation transfer increases with decreasing R_h .

Keywords: reverse micelles, fluorescence, fluorescent dyes, serum albumin, halogenated fluorescein.

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Introduction

Being key building blocks and one of the main components of organisms, albumins play a critical role in live organisms, performing various biological functions. They interact with various substances, such as other proteins, DNA, RNA and drugs [1]. Many drugs and other bioactive small molecules bind with albumin, which in this case functions as their carrier. In their turn, the emerging interactions may also impact the stability and toxicity of a drug in chemotherapeutic processes. Study of such interactions will enable better understanding of biological processes at a molecular level. To study the mechanisms and the nature of such binding, as well as the surface-induced conformation changes in the protein interfaces, „albumin+dye molecules (DM)“ complexes are used [2,3]. The generally accepted methods for the study of binding of small ions or molecules with albumins are various spectral methods, and the main study tool of such processes is fluorescent spectroscopy due to its high sensitivity in the molecule glow detection [4–6].

Usually the interaction between DM and protein is studied in the dissolved buffer solutions. Therefore, it becomes necessary to study such interactions in the conditions close to the cells. For this purpose it is necessary to engage simple cell models, one of which is a reversed micelle, which is a

nanosized particle made of surfactant molecules that get together around a water nucleus, suspended in a nonpolar solvent [7]. The size of such „cell“ (micelle) may be changed by the ratio of water and surfactant concentrations in a solution.

Recently to study the interaction of protein–DM, derivatives of fluorescein (F) are used, which are produced by substitution of hydrogen atom in F with halogen atoms. These dyes are widely used in medicine as contrast dyes, in treatment of melanoma [8] and in antimicrobial photodynamic therapy [9–11].

This paper studied spectral-luminescent characteristics of bovine serum albumin (BSA) and DM of eosin (E), erythrosin (ER), Rose Bengal (RB) and their mixes in the conditions of spatial limitation of sodium 1,4-bis (2-ethylhexyl) sulfosuccinate (AOT) in reversed micelles.

Experiment

Water-micelle solutions of dyes and protein were prepared using the method described in [12]. To prepare the studied solutions, AOT, decane and dyes of fluorescein family (fluorescein and its halogen derivatives: bromated derivative — eosin Y, iodinated derivative — erythrosin, tetra-iodine- tetra-chlorine derivative — Rose Bengal) were used. Concentrations of the mentioned dyes were 10^{-5} ,

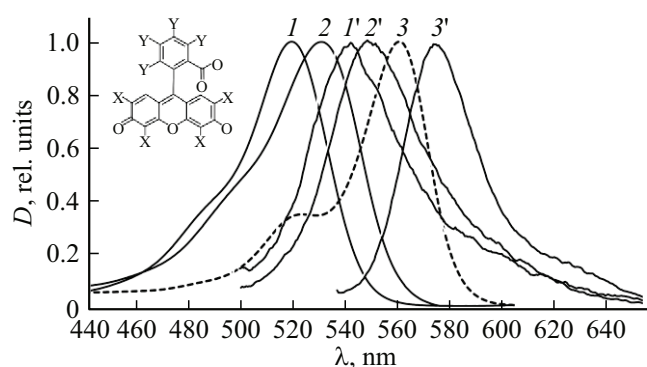


Figure 1. Spectra of absorption ($I-3$) and fluorescence ($I'-3'$) of dyes: E (I, I'), ER ($2, 2'$), RB ($3, 3'$) in water micelle solutions with $R_h = 11.0$ nm. In insert — structural formulae of DM: E ($X=\text{Br}$ and $Y=\text{H}$), ER ($X=\text{I}$ and $Y=\text{H}$), RB ($X=\text{I}$ and $Y=\text{Cl}$).

$1.5 \cdot 10^{-5}$, $2.5 \cdot 10^{-5}$ and $3 \cdot 10^{-5}$ mol/l. Concentration of BSA $C = 5 \cdot 10^{-5}$ mol/l. The pH value was 6.0, at this medium acidity the studied complexes create stable structures without denaturation effects from serum albumin.

The size of micelles was adjusted by changing the degree of hydration of $\omega = \frac{[\text{H}_2\text{O}]}{[\text{AOT}]}$ ($[\text{AOT}]$ and $[\text{H}_2\text{O}]$ — molar concentrations of water and surfactant in the solution) and was determined by the method of dynamic light scattering [13] in a Photocor Complex instrument. The hydrodynamic radius of micelles R_h was determined in the assumption of their spherical shape using Stokes–Einstein–Debye formula.

Water-micelle solutions of protein and a mix of protein and DM were prepared for the study for four values of ω 18.6, 37, 55 and 70, which corresponded to $R_h = 3.3$, 6.8, 11.0 and 15.0 nm.

The absorption spectra of the solutions were measured in a spectrophotometer SOLAR PB 2201 (Belarus), spectra of fluorescence and synchronous spectra — in a spectrofluorimeter SOLAR PB 2203 (Belarus).

The sample temperature in all measurements was 295 K.

The obtained spectra were processed with OriginPro 8.5 software. The statistical processing of data was carried out by calculation of the arithmetic mean (M), standard deviation (σ), arithmetic mean error (m). The sample was 5 repetitions for each experiment.

Results and discussion

When BSA and DM interact, their spectral characteristics change, and protein and DM fluorescence is quenched [14,15]. Therefore, at first we studied the spectra of fluorescence of water micelle solutions of proteins and mixture of BSA and DM for four values of $R_h = 3.3$, 6.8, 11.0 and 15.0 nm.

Fig. 1 presents as the example the spectra of absorption and fluorescence of the studied dyes for water-micelle solutions with $R_h = 11.0$ nm.

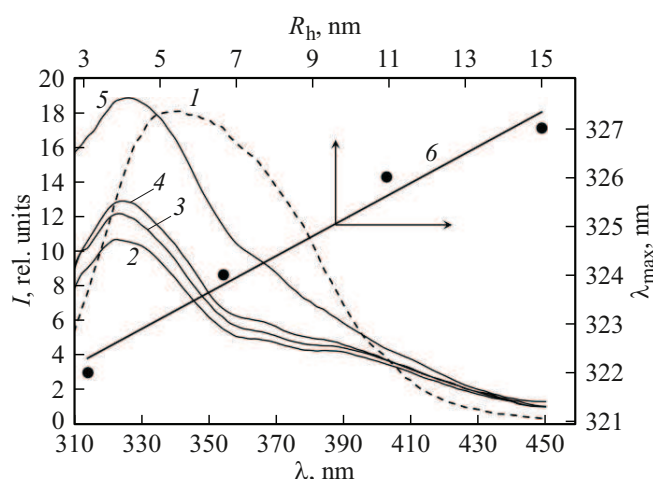


Figure 2. Spectra of fluorescence of BSA solutions with concentration of $5 \cdot 10^{-5}$ mol/l in water (I) and in reversed micelles (RM) with various R_h : 3.3 (2), 6.8 (3), 11 (4) and 15 nm (5). Dependence of the position of maximum intensity of fluorescence of BSA spectrum λ_{max} on R_h (6).

Fig. 2 presents spectra of fluorescence of BSA in micelles with various values of hydrodynamic radius and in water. Internal fluorescence of protein mainly depends on amino acid residues (tryptophane (Trp), phenylalanine (Phe) and tyrosine (Tyr)). Besides, Phe has a very low quantum yield of fluorescence, therefore its spectrum of radiation is practically not observed. BSA contains two tryptophane residues: Trp 213 is located in a pocket of hydrophobic binding in the second domain (Sudlow I), and Trp 134 — on the surface of BSA in the first domain. Tyr residues are distributed in all BSA domains.

When BSA is introduced in the reversed micelle, its spectral characteristics will be determined by its charge and hydrophilic or hydrophobic characteristics, and also a charge of a surfactant. AOT molecules are anionic. Solutions with pH 6 were studied. At such pH value the BSA charge is deemed negative [16]. When protein–reversed micelle interaction is considered, the protein size is important. In our experiments at pH 6 the hydrodynamic radius is $R_h = 3.4$ nm [17].

When dissolved in water, and also in water biological solutions the locally charged areas of the albumin macromolecule are redistributed, which changes its hydrophilic and hydrophobic properties. Hydrophobicity may cause compaction of albumin macromolecules, whereas predominance of hydrophilic interactions may promote conversion of compact domain structure into a quasi-linear structure. Seemingly, opening of the protein molecule changes fluorescence of amino acid residues of serum albumin.

You can see that introduction of the BSA molecule into RM causes a short-wavelength shift of the protein fluorescence spectrum compared to water solutions, the value of which depends on the RM size: largest for $R_h = 3.3$ nm and smallest for $R_h = 15.0$ nm. To describe

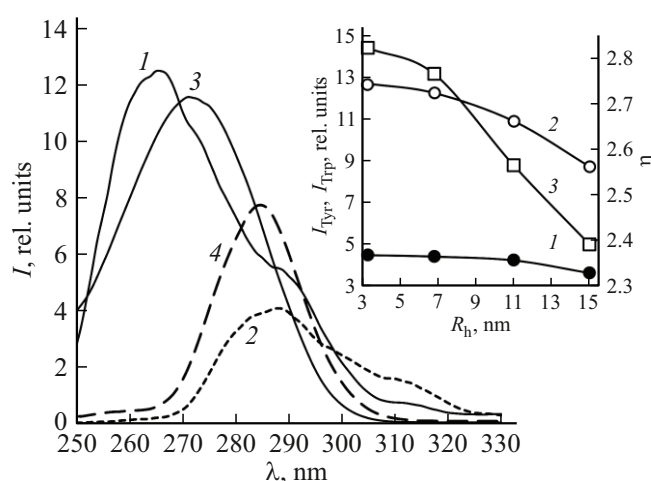


Figure 3. Synchronous BSA fluorescence spectra in reversed micelles with $R_h = 3.3$ nm (1, 2) and in water solutions (3, 4) for $\delta\lambda = 60$ (1, 3) and $\delta\lambda = 15$ nm (2, 4). BSA concentration is $5 \cdot 10^{-5}$ mol/l. Insert: dependences of intensity I (in relative units) in maxima of spectra I_{Tyr} (1), I_{Trp} (2) and ratios of intensities in maxima $\eta = \frac{I_{\text{Trp}}}{I_{\text{Tyr}}}$ (I_{Trp} — glow intensity at $\delta\lambda = 60$ nm, I_{Tyr} — at $\delta\lambda = 15$ nm) (3) on hydrodynamic radius R_h . BSA concentration is $5 \cdot 10^{-5}$ mol/l.

the BSA fluorescence spectra in reversed micelles, it is possible to attract several protein solubilization models [18]. The most realistic ones for the studied system are the following two: 1) contact with the organic solution and interaction with the micelle wall, 2) water pool and AOT shell. At $R_h = 3.3$ nm the water pool may not fully cover the BSA macromolecule, which causes direct contact of certain BSA fragments with the organic substance solution, as a result a significant shift is observed in the protein fluorescence spectrum (curve 2, Fig. 2). When the water pool of the reversed micelle increases (at $R_h = 6.8$ nm), the molecules are localized at the expense of the electrostatic interaction (BSA and AOT molecules carry negative charge) in the micelle center, and the BSA fluorescence spectrum shifts towards the long-wavelength area (curve 3, Fig. 2). With further increase of R_h the long-wavelength shift of the BSA fluorescence spectrum is observed (Fig. 2, curve 6), provided for by the increase in hydrophilic interactions between the protein and the intramicellar water due to decrease in the electrostatic interactions between the BSA molecules and charged AOT heads. Besides, the position of the BSA fluorescence spectrum maximum does not reach its position for the water solution.

To analyze the change in the microenvironment of Tyr and Trp residues, the synchronous fluorescence spectra of water micelle solutions of protein were measured and analyzed — fluorescence spectra obtained from scanning along wavelengths of excitation (λ_{exc}) and radiation (λ_{rad}) at constant difference $\delta\lambda = \lambda_{\text{rad}} - \lambda_{\text{exc}}$ (both monochromators of spectrofluorimeter are scanned simultaneously). The studies were carried out at two values of BSA fluorescence

registration and excitation wavelength differences $\delta\lambda$: 15 nm (glow spectrum Tyr) and 60 nm (glow spectrum Trp) [19]. Synchronous BSA fluorescence spectra were measured in micellar structures for all four R_h values and for water solutions of protein. The scanning interval for the systems studied in the paper was 250–350 nm. Fig. 3 presents as an example the synchronous spectra of micellar solutions with $R_h = 3.3$ nm (curves 1, 2) and water solutions (2, 4) of BSA for $\delta\lambda = 60$ (curves 1, 3) and $\delta\lambda = 15$ nm (curves 2, 4).

The insert to Fig. 3 shows the dependences of intensity I (in relative units) in maxima of Tyr (curve 1) and Trp (curve 2) spectra on hydrodynamic radius R_h . You can see that the glow intensity I_{Tyr} practically does not depend on R_h , then I_{Trp} with growth of R_h decreases. The same figure presents the dependence of the ratio of intensities in maxima of Trp (I_{Trp}) and Tyr (I_{Tyr}) — $\eta = \left(\frac{I_{\text{Trp}}}{I_{\text{Tyr}}}\right)$ on R_h (curve 3). As values R_h grow due to weakening of the spatial limitation inside the RM pool, the ratio η drops with the growth of R_h , and $\eta > 1$ (Fig. 1, c, curve 3). Therefore, the microenvironment of Tyr residues undergoes no clear changes, whereas decrease of R_h causes more intense fluorescence of Trp residues.

Introduction of halogen-substituting fluoroscein into AOT micelles, which contain BSA, causes a change in the DM fluorescence spectra due to their interaction with protein. The DM size in water is the following: for eosin and erythrosin the dimensions estimated as the distance (diameter) between most remote atoms, was 1.08 and 1.12 nm accordingly [20], for the Rose Bengal the diameter measured using the dynamic light scattering, 1.4 nm [21]. I.e. DMs are located in the water pool of the reversed micelle. To describe these changes, the Stokes shifts $\Delta\nu = \nu_g - \nu_e$ were defined, where ν_g , ν_e — frequencies of maxima of absorption and fluorescence spectra in the RM accordingly and change of the Stokes shift of dye spectra upon addition of BSA, i.e. the difference between the Stokes shift of

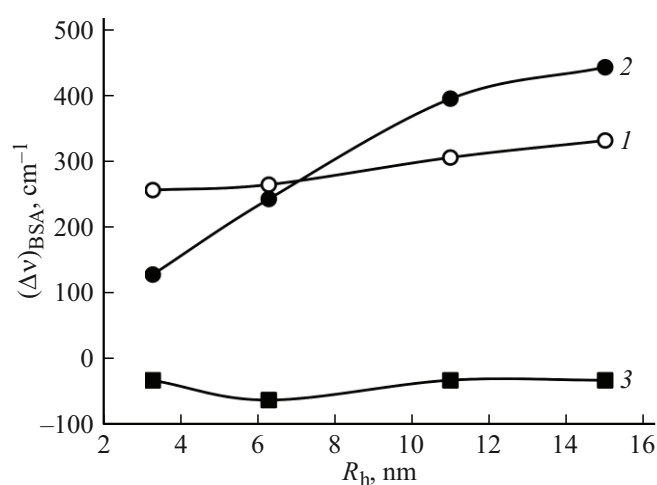


Figure 4. Dependences of change of Stokes shifts of dye spectra with concentration $C = 3 \cdot 10^{-5}$ mol/l in the mix with BSA on R_h for E (1), ER (2) and RB (3).

dye spectra in the micelle and Stokes shift of dye spectra in the „dye + BSA“ in the micelle ($(\Delta\nu)_{BSA} = \Delta\nu - \Delta\nu_{BSA}$, $\Delta\nu_{BSA}$ — Stokes shift of dye spectra in RM of protein and dye, $\Delta\nu$ — Stokes shift for DM spectra in RM) for various R_h .

In Fig. 4 (curves 1–3) the $\Delta\nu_{BSA}$ dependences are presented as an example on R_h for the studied dyes at dye concentration of $C = 3 \cdot 10^{-5}$ mol/l. The value $\Delta\nu_{BSA}$ characterizes the influence of „protein–DM“ on the Stokes shift of the dye spectra. For the entire area of the studied R_{exth} upon addition of BSA to micelles, the reduction is observed in the Stokes shift of the spectra. From Fig. 4 you can see that for the „BSA + RB“ system the value $(\Delta\nu)_{BSA}$ is not changing practically (curve 3). This indicates poor interaction of RB and BSA. For E and ER for all R_h the interaction in the „BSA + DM“ system causes a decrease in the Stokes shift DM (Fig. 4, curves 1, 2). At the same time as R_h increases, $(\Delta\nu)_{BSA}$ grows.

Influence of R_h micelles at $\Delta\nu$ DM may be due to the change of their dipole moments upon excitation: $\Delta\mu = \mu_e - \mu_g$, where μ_e and μ_g — dipole moments of fluorophore in the excited and main states. To determine such changes, let us use equation proposed by N.G. Bakhshiev [22,23] within the method taking into account its solvatochromism, which binds $\Delta\mu$ and $\Delta\nu = \nu_g - \nu_e$:

$$\Delta\mu = (\nu_g - \nu_e) \frac{hca^3}{2F}, \quad (1)$$

where

$$F = \frac{2n^2 + 1}{n^2 + 2} \left(\frac{\varepsilon - 1}{\varepsilon + 2} - \frac{n^2 - 1}{n^2 + 2} \right), \quad (2)$$

h — Planck's constant, c — light speed, n — refractive index, ε — dielectric constant of solvent, radius of solvent cavity (Onsager radius), which contains fluorophore [24],

$$a = \left[\frac{3M}{4\pi\rho N_A} \right]^{\frac{1}{3}}, \quad (3)$$

where N_A — Avogadro's number, ρ — density, M — molecular weight of DM.

Onsager's radii estimated using (3), for the studied dyes are: $a_E = 6.98 \text{ \AA}$, $a_R = 7.65 \text{ \AA}$, $a_{RB} = 5.53 \text{ \AA}$.

Fig. 5 presents dependences $\Delta\mu$ of studied dyes on R_h (curves 1–3), determined using formula (1) from the experimental spectral characteristics of DM. From Fig. 5 you can see that between $\Delta\mu$ and R_h the direct dependence is observed. Besides, changes $\Delta\mu$ are highest for E (curve 2) and minimum for RB (curve 3). Fig. 5 shows also dependences $\delta\mu = \Delta\mu - \Delta\mu_{BSA}$ ($\Delta\mu_{BSA}$ — difference of dipole moments of DM in the excited and main states in micellar solutions of protein and dye, $\Delta\mu$ — difference of dipole moments of DM in the excited and main states in micellar solutions) on R_h (curves 4–6). Value $\delta\mu$ specifies the influence of „BSA + DM“ interaction at the change of dipole moments in the excited and main states of DM. From Fig. 5 you can see that in the „BSA+DM“

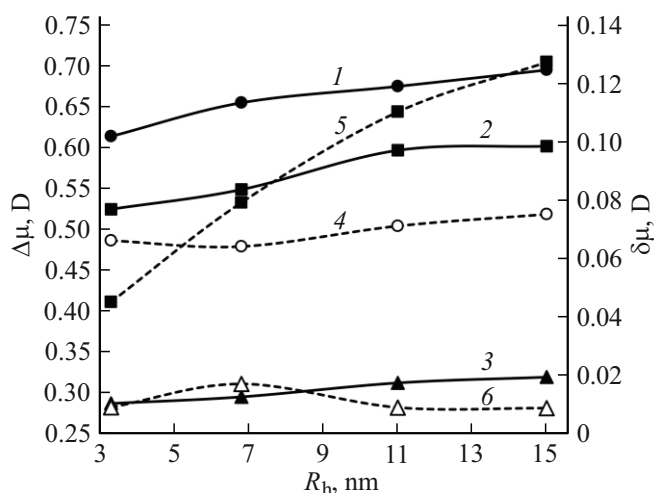


Figure 5. Dependences $\Delta\mu$ (1–3) of dyes with $C = 3 \cdot 10^{-5}$ mol/l and change of their dipole moment $\delta\mu$ (4–6) in a mix with BSA on R_h for E 1, 4), ER (2, 5) and RB (3, 6).

system the difference $\Delta\mu$ decreases for all studied dyes and dimensions of micelles R_h . The highest „dimensional“ effect is observed for ER: upon increase of R_h from 3.3 to 15 nm $(\Delta\mu)_{BSA} \approx 0.08$ changes (Fig. 5, curve 5). Besides, for E this change is ≈ 0.01 (Fig. 5, curve 4), and for RB the influence of „protein–DM“ interaction at the difference of dipole moments μ_e and μ_g is practically absent (Fig. 5, curve 6).

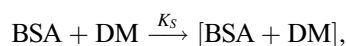
As noted above, the growth of DM concentration causes quenching of BSA fluorescence. Fig. 6 shows as examples the BSA fluorescence spectra in a mixture with ER of various concentration for RM with $R_h = 3.3$ nm.

Processes of intermolecular quenching of fluorescence may be both dynamic and static [25,26].

Static quenching may happen, when fluorophore and quenching agent form non-fluorescent complexes, it does not depend on diffusion or collisions of molecules [26]. In case of static quenching of fluorescence there is also a linear dependence between the reduction of fluorescence intensity and quenching agent concentration.

Formation of non-luminescent complexes (associates) affects the absorption spectra — addition of DM to BSA solutions improves absorption.

Let us assess the efficiency of this process. The equilibrium to form a complex between BSA and DM is described as follows:



where K_S — constant of complex binding that is equal to

$$K_S = \frac{\alpha}{(1 - \alpha)C_{DM}}, \quad (4)$$

where α — degree of association.

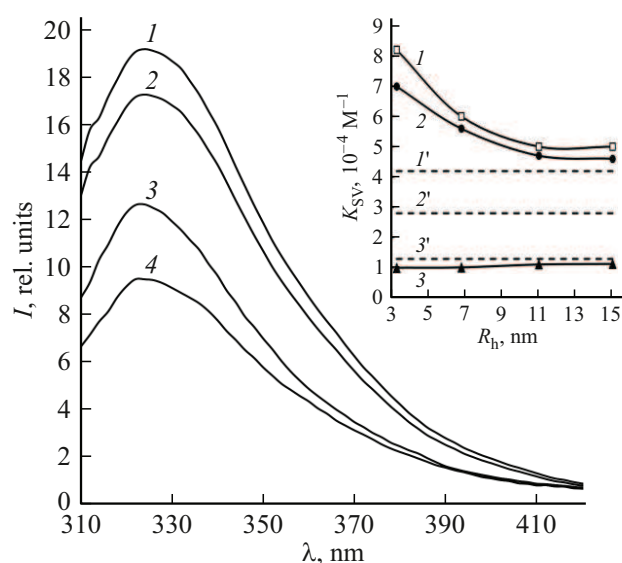


Figure 6. BSA fluorescence spectra in a mix with ER with dye concentration 10^{-5} (1), $1.5 \cdot 10^{-5}$ (2), $2.5 \cdot 10^{-5}$ (3) and $3 \cdot 10^{-5}$ (4) mol/l. The hydrodynamic radius of micelle is equal to 3.3 nm. Insert: K_{SV} dependences on the radii of reversed micelles R_h for solutions of BSA with E (1), ER (2) and RB (3). I' – $3'$ — K_{SV} values for buffer solutions of dyes: I' — E, $2'$ — ER, $3'$ — RB.

The constant of the complex binding K_S was calculated using Benesi–Hildebrand method [27]:

$$\frac{1}{D - D_0} = \frac{1}{D_c - D_0} + \frac{1}{K_S(D_c - D_0)C_{MK}}, \quad (5)$$

where D — optical density of BSA solutions that contain DM of various concentration in the maximum of absorption spectra, D_0 — optical density of BSA solutions, D_c — optical density of the complex „BSA+MK“.

From (5) it follows that dependence $\frac{1}{D - D_0}$ on $\frac{1}{C_{MK}}$ is linear, and $\frac{1}{K_S(D_c - D_0)}$ — the straight line inclination coefficient, and $\frac{1}{D_c - D_0}$ — the point of the straight line crossing with the axis of coordinates. From the experimentally obtained spectral characteristics of solutions, using (5), the least square method was used to define K_S values. The results are presented in Table 1.

From Table 1 you can see that first of all, K_S of complexes in the micelles with hydrodynamic radii from 3.3 to 15.0 nm is lower than in water solutions. Second, K_S values decrease in $E \rightarrow ER \rightarrow RB$ row, which is due to conformation changes of the molecule structure in this row, bound with redistribution of charges in atoms of molecules [20].

Using values K_S , with the help of formula (4) you can determine the degrees of association for any DM concentrations in the solutions. Thus, for the study area of DM concentrations ($C_{MK} = 10^{-5} - 3 \cdot 10^{-5}$ mol/l) and for the highest value $K_S = 526 \pm 75$ (E in micelle with $R_h = 15$ nm) value α is 0.015. I.e. the quantity of the complexes of E and DM molecules that form complexes in

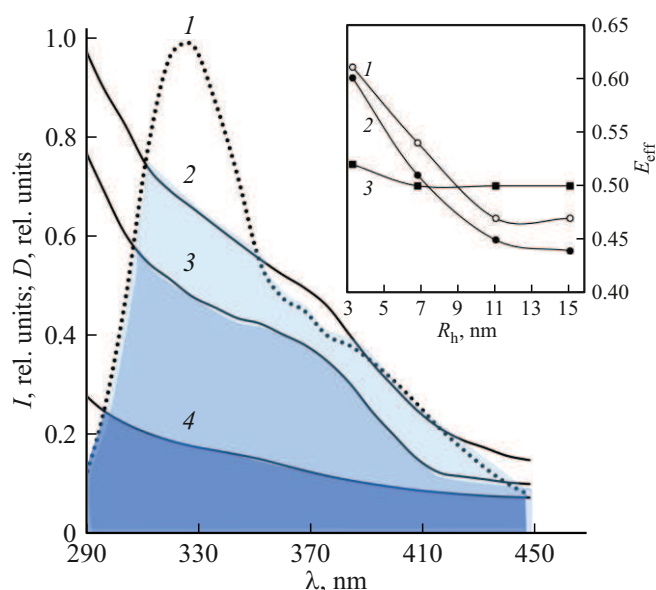


Figure 7. Normalized spectra of BSA fluorescence (I) and DM absorption: E (2), ER (3) and RB (4). Insert: dependences of efficiency of electron excitation energy transfer from BSA to E (1), ER (2) and RB (3) from the hydrodynamic radius of the micelle.

the main state is low. Therefore, the influence of formation of such complexes at BSA fluorescence quenching is very small, and one may assume that the main mechanism of fluorescence quenching is dynamic.

According to the guidelines of the International Union of Theoretical and Applied Chemistry [28], dynamic quenching is a process that prevents release of fluorophore from the excited state after the excited state has formed. According to this definition, the dynamic quenching of fluorescence is caused by both deactivation of fluorophore in the excited state in a contact or a collision with another molecule (extinguisher) in a solution, but also by energy transfer, electron transfer etc.

To establish the efficiency of such quenching of BSA fluorescence, values $\frac{I_{BSA}}{I_{BSA}^{MK}}$ were estimated for BSA at different concentrations of dyes C_{MK} . Here I_{BSA} , I_{BSA}^{MK} — intensities of fluorescence in micellar solutions and solutions of mixes of BSA and DM accordingly. Dependences of $\frac{I_{BSA}}{I_{BSA}^{MK}}$ on CD_M described by Stern–Volmer relationship were built [25]:

$$\frac{I_{BSA}}{I_{BSA}^{MK}} = 1 + K_{SV}C_{MK}, \quad (6)$$

where K_{SV} — Stern–Volmer constant.

From the experimentally obtained values of solution fluorescence intensity, using (6), the least square method was used to define K_{SV} values. The insert to Fig. 6 presents dependences K_{SV} (R_h) for the studied dyes in solutions with proteins and DM (curves 1–3). The same figure shows K_{SV} values for buffer solutions of these dyes (curves $1'$ – $3'$). You can see that for E and ER the DM BSA fluorescence

Table 1. Constant values K_S of „BSA + DM“ complexed for various dyes in reversed micelles for various R_h and water solutions

R_h, nm	K_S, M^{-1}				
	3.3	6.8	11.0	15.0	Water solution
E	350 ± 52	357 ± 54	434 ± 65	526 ± 75	1111 ± 130
ER	351 ± 52	357 ± 53	384 ± 58	416 ± 62	1000 ± 120
RB	165 ± 25	169 ± 25	17 ± 33	384 ± 58	588 ± 70

Table 2. Value of BSA and DM fluorescence spectra overlapping integral

$J(\lambda) \times 10^{-16}, \text{M}^{-1} \text{cm}^{-1} \text{nm}^4$		
BSA–E	BSA–ER	BSA–RB
15.4	11.7	5.44

quenching constant is higher than in the buffer solutions. As R_h increases for these dyes, K_{SV} decreases (Fig. 6, curves 1, 2). For BR the K_{SV} quenching constant for all R_h is lower than for E and ER, and at the same time it practically does not depend on R_h (insert to Fig. 6, curve 3).

From Fig. 6 you can see that K_{SV} is higher by two orders of magnitude than K_S (Table 1). It is related to the fact that BSA and DM molecules are charged negatively and are located at the distances that are not sufficient to form a complex. As a result at low DM concentrations ($C_{MK} = 10^{-5} - 3 \cdot 10^{-5} \text{ mol/l}$) the probability of complex formation in the main state is low.

Therefore, the main mechanism of fluorescence quenching is dynamic, which is caused by electron excitation energy transfer (EEET), since collisions of the BSA and DM molecules charged identically are not likely in the studied system. For EEET it is necessary that the distance between the interacting molecules was around 5–7 nm and significant overlapping of the fluorescence spectra of the donor (BSA) and absorption of acceptor (DM). These two conditions are met for the studied system: the corresponding distance is provided by local concentration of molecules in a micelle, the BSA fluorescence and DM absorption spectra overlapping is observed. Fig. 7 presents the normalized BSA fluorescence spectra (curve 1) and DM absorption spectra (curves 2–4). From Fig. 7 you can see that the BSA fluorescence and DM absorption spectra overlap, besides, the value of such overlapping decreases in the row E→ER→RB.

Let us determine the BSA fluorescence and DM absorption spectra overlapping using formula [25]

$$J(\lambda) = \int_0^{\infty} f_D(\lambda) \varepsilon_A \lambda^4 d\lambda, \quad (7)$$

where $f_D(\lambda)$ — fluorescence spectrum of donor (BSA), $\varepsilon_A(\lambda)$ — absorption spectrum of acceptor (DM), λ — wavelength.

Table 2 presents values J for the studied systems. From Table 2 you can see that the highest integral of spectra overlapping is observed in E, and the lowest one — in RB. This indicates that the efficiency of electron excitation energy transfer decreases in E→ER→RB row.

To account for this effect, let us define for various DM concentrations the relative efficiency index E_{eff} of electron excitation energy transfer between BSA and DM equal to [25]

$$E_{\text{eff}} = 1 - \frac{I_{\text{BSA}}^{\text{MK}}}{I_{\text{BSA}}}. \quad (8)$$

The obtained dependences of index E_{eff} for the studied systems are shown in the insert to Fig. 7.

As it follows from Fig. 7, index E_{eff} decreases as R_h increases for all dyes. At the same time the least change of E_{eff} related to the increase of R_h is observed for RB.

Conclusion

It was shown experimentally that coefficients K_{SV} of dynamic quenching of DM BSA fluorescence in micelles and buffer solutions were different. For micelles with hydrodynamic radius from 3.3 to 15.0 nm the highest value of K_{SV} is recorded for E and ER. As R_h increases, K_{SV} values for E and ER decrease, and at the same time they substantially differ from the similar coefficients in the buffer solutions. Constants K_{SV} of protein fluorescence quenching with RB molecules practically do not depend on the size of micelles (in the range of R_h from 3.3 to 15.0 nm) and are lower than constants of quenching in the buffer solutions.

For DM the spatial limitation in the water pools of micelles affects the change of the DM dipole moment upon their excitation. It was established that as the micelle size decreases (upon decrease of R_h), the difference of dipole moments decreases in the excited and main states of $\Delta\mu$. Besides, the changes of this value $\Delta\mu$ are highest for E and minimum for RB ($\Delta\mu(R_h)$ practically does not change). Since the presented row of fluorophore dyes is characterized by the subsequent substitution of hydrogen atoms with halogen atoms (bromine, iodine and chlorine), changes of $\Delta\mu$ parameter are related to the „effect of the internal heavy atom“. Indeed, for the studied dyes the constants of

intercombination conversion are equal to the following: for E — $0.042 \cdot 10^{10} \text{ s}^{-1}$, for ER — $0.85 \cdot 10^{10} \text{ s}^{-1}$, for RB — $1.0 \cdot 10^{10} \text{ s}^{-1}$ [29].

Dependences of the dynamic quenching coefficient K_{SV} of DM BSA fluorescence obtained from the experimental data differ in the micelle compared to the buffer solutions. For E and ER K_{SV} is higher for the entire area of their studied dimensions. If R_h increases, for E and ER the decrease of K_{SV} is observed, at the same time the values of constants of BSA fluorescence quenching coefficients do not achieve their value in the buffer solutions. If there is RB in micelles, K_{SV} of protein in the solutions practically does not change depending on their size and is lower than the value of the quenching constants in the buffer solutions.

The constant of static quenching K_S for the complexes in micelles is lower by two orders of magnitude than K_{SV} in water solutions. K_S values decrease in E→ER→RB row.

Therefore, the main mechanism of fluorescence quenching is dynamic supported by energy transfer of electron excitation from BSA to DM. Efficiency of electron excitation transfer increases, if R_h decreases. Besides, if R_h decreases, the increase in the energy transfer efficiency for electron excitation is different for various DMs: the lowest for RB, the highest for E.

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Conflict of interest

The authors declare no conflict of interest.

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