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Chemistry

FLUORESCENCE STUDY OF BSA INTERACTION WITH (R)-5-(3-(4-(AZEPAN-1-YLMETHYL)-1H-1,2,3-TRIAZOL-1-YL)PROP-1--EN-2-YL)-2-METHYLCYCLOHEX-2-ENONE

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Interaction between bovine serum albumin (BSA) and (R)-5-(3-(4-(azepan-1--ylmethyl)-1H-1,2,3-triazol-1-yl)prop-1-en-2-yl)-2-methylcyclohex-2-enone (Triazole-A) was studied at 298 *K* and 308 *K* using fluorescence spectroscopy method. It was shown that the quenching mechanism of BSA by Triazole-A was initiated by a dynamic collision. Synchronous fluorescence measurements indicate that binding between Triazole-A and the protein does not lead to a change in the polarity of the microenvironment of the tryptophan and tyrosine residues.

Keywords: bovine serum albumin, fluorescence spectroscopy, synchronous fluorescence, Triazole-A.

Introduction. Serum albumins are the most abundant proteins in the circulatory system of a wide variety of organisms, being the major macromolecules contributing to the osmotic blood pressure. The most important physiological role of albumins is to transport numerous drugs, endogenous and exogenous compounds (ligands) in the bloodstream and deliver to target molecules. For many drugs, binding to serum albumin is critical for their distribution and pharmacokinetics [1–3]. Bovine serum albumin (BSA) is one of the most extensively studied proteins, particularly because of its structural homology with human serum albumin [4]. Triazole-A ((R)-(5-(3-(4-(azepan-1--ylmethyl)-1H-1,2,3-triazol-1-yl)prop-1-en-2-yl)--2-methylcyclohex-2-enone) is newly synthesized compound, which demonstrates an antibacterial activity (Fig. 1).

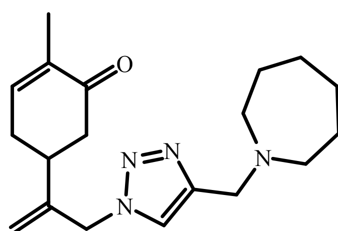

Fig. 1. Chemical structure of Triazole-A.

Spectral methods are a powerful tool to reveal the binding of drugs with albumin at low concentrations. Binding affinities of proteins can be determined by fluorescence quenching. The fluorescence quenching technique is used to monitor the molecular interactions, because of its high sensitivity, reproducibility, and relatively ease of use.

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Materials and Methods. We used Bovine Serum Albumin (BSA) from "Sigma Aldrich" (USA), a 0.9% solution of sodium chloride. Triazole-A was synthesized by the researchers of the Chair of Organic Chemistry of YSU. The concentration of BSA was 0.4 *mg*/*mL* and the concentration of Triazole-A was changed in the range $4\cdot10^{-5}$ to $2\cdot10^{-4}$ *M*. The fluorescence studies were carried out on a Cary Eclipse spectrofluorimeter ("Varian") at two temperatures (298.15 and 308.15 *K*) in the range from 300 to 500 *nm*. The excitation wavelength was chosen to be 280 *nm*. The temperature of the samples was regulated by the Lauda 100 water circulating thermostat. For synchronous fluorescence measurements, the excitation range was 260–360 *nm*, and $\Delta\lambda$ was set at 15 and 60 *nm*. A quartz cell of 1.0 *cm* length was used. Excitation and emission slit width was fixed to 5 *nm*. The ORIGIN 8.0 program was used to plot and analysis the graphs.

Results and Discussion.

Fluorescence Quenching Studies. The intrinsic fluorescence of BSA is obtained at 347 *nm* being excited at 280 *nm* [5]. The fluorescence quenching spectra of BSA with increasing concentrations of Triazole-A are shown in Fig. 2. The fluorescence intensity of BSA decreases, which indicates that Triazole-A interact with BSA.

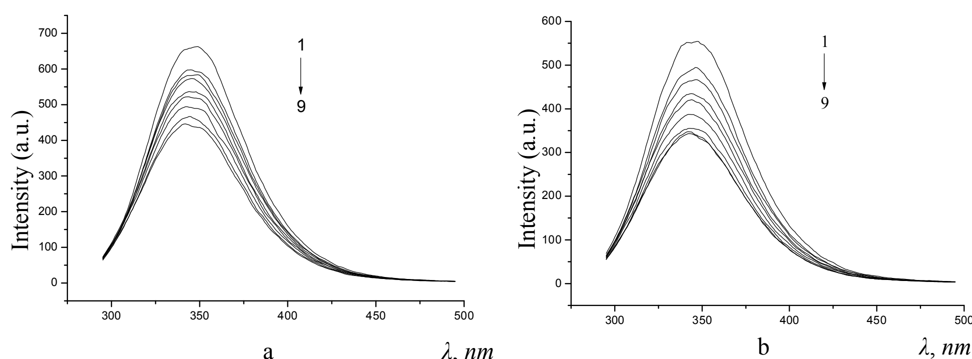

Fig. 2. The fluorescence quenching spectra of BSA in the presence of Triazole-A at T=298.15 *K* (a) and 308.15 *K* (b); [BSA]=0.4 *mg/mL*; [Triazole-A]: 1 – 0; 2 – $4\cdot10^{-5}$; 3 – $6\cdot10^{-5}$; 4 – $8\cdot10^{-5}$; 5 – $1\cdot10^{-4}$; 6 – $1.2\cdot10^{-4}$; 7 – $1.4\cdot10^{-4}$; 8 – $1.6\cdot10^{-4}$; 9 – $2\cdot10^{-4}$ (*M*).

Fluorescence quenching can occur by different mechanisms, which are usually classified as either dynamic quenching or static quenching. Dynamic and static quenching can be distinguished by their different dependence on temperature and viscosity. To further elucidate the quenching mechanism of BSA induced by Triazole-A, the fluorescence quenching data at different temperatures (298.15 and 308.15 *K*) are analyzed with the Stern–Volmer equation

$$F_0 / F = 1 + K_{SV}[Q] = 1 + k_q \tau_0 [Q],$$

where F_0 and F are the relative fluorescence intensities in the absence and presence of quencher respectively, $[Q]$ is the concentration of quencher, K_{SV} is the Stern–Volmer quenching constant, k_q is the bimolecular quenching rate constant and τ_0 is the average lifetime of the fluorophore in the excited state (usually for a biomacromolecule is $6\cdot10^{-8}$ *s*) [6].

Fig. 3 shows the plot of F_0/F versus [Triazole-A] for BSA at 298.15 and 308.15 *K*.

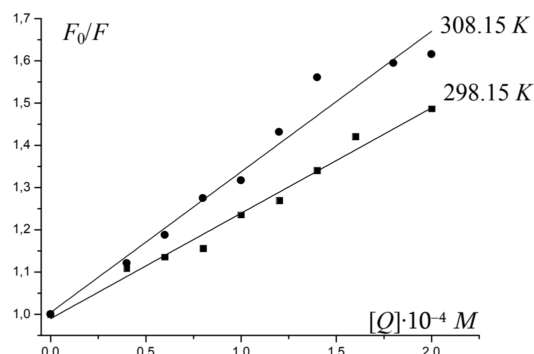

Fig. 3. Stern–Volmer plots of BSA quenched by Triazole-A at different temperatures.

K_{SV} and k_q are obtained from the linear fittings of the experimental data, which are presented in Table.

Stern–Volmer quenching constants and bimolecular quenching rate constants for the interaction of BSA with Triazole-A at various temperatures

T, K	K_{SV}, M^{-1}	k_q, $M^{-1}s^{-1}$	R
298.15	$2.5\cdot10^3$	$4.17\cdot10^{10}$	0.01181
308.15	$3.3\cdot10^3$	$5.50\cdot10^{10}$	0.02333

The Stern–Volmer quenching constant decreases with increasing temperature for the static quenching, and for the dynamic quenching the opposite effect is observed. The results show that the K_{SV} values increase with increasing temperature, which indicates that the quenching mechanism of BSA by Triazole-A is initiated by a dynamic collision.

Synchronous Fluorescence Spectra. Conformational changes in a protein molecule can be assessed by measuring the synchronous fluorescence intensity for the amino acid residues. This method involves measuring the fluorescence spectrum, while simultaneously changing the wavelengths of the exciting and detected radiation [7].

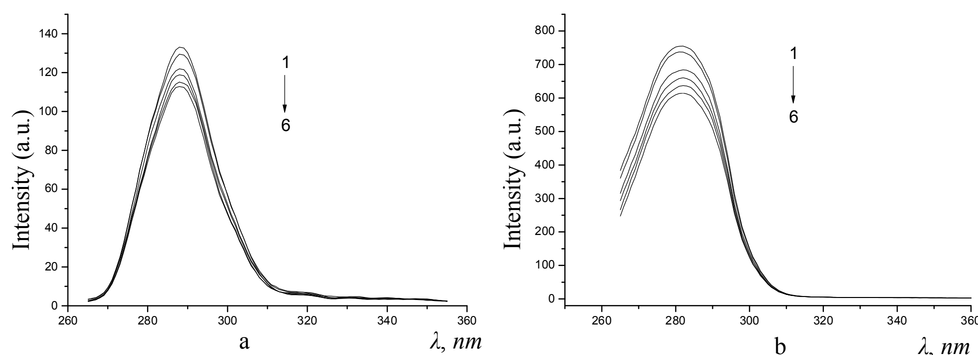

Fig. 4. Synchronous fluorescence spectra of BSA in the presence of Triazole-A at $\Delta\lambda$=15 *nm* (a) and 60 *nm* (b); [BSA]=0.4 *mg*/*mL*; [Triazole-A]: 1 – 0; 2 – $4\cdot10^{-5}$; 3 – $8\cdot10^{-5}$; 4 – $1.2\cdot10^{-4}$; 5 – $1.6\cdot10^{-4}$; 6 – $2\cdot10^{-4}$ (*M*).

We used the synchronous fluorescence method for scanning ranges $\Delta\lambda$=60 and 15 *nm* in the absence and presence of Triazole-A ($4\cdot10^{-5}$–$2\cdot10^{-4}$ *M*). For $\Delta\lambda$=60 *nm* the synchronous fluorescence signal for BSA belongs to the tryptophan residues, while for $\Delta\lambda$=15 *nm* we also observe fluorescence of the tyrosine residues. Fig. 4 shows the synchronous fluorescence spectra of BSA in the presence of Triazole-A additives for $\Delta\lambda$=60 and 15 *nm*.

In the presence of Triazole-A the intensity of the fluorescence of tryptophan and tyrosine decreases and no shift is observed in the signals. This indicates that binding between Triazole-A and the protein does not lead to a change in the polarity of the microenvironment of the tryptophan and tyrosine residues, but the internal packing of the protein changes.

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