PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Chemistry and Biology 2013, № 1, p. 50–53

COMMUNICATIONS

Chemistry

INFLUENCE OF DIMETHYLSULFOXIDE ON THE FLUORESCENCE BEHAVIOR OF HUMAN SERUM ALBUMIN STUDIED BY EXCITATION-EMISSION MATRIX FLUORESCENCE SPECTROSCOPY METHOD

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Three-D excitation-emission matrix (EEM) fluorescence spectra of Human Serum Albumin (HSA) were measured at the presence of dimethylsulfoxide (DMSO) at t=25^0C during 270 *min* before and after adding DMSO in different concentrations. It has been shown that DMSO at low concentrations (5%) increase heat-resistance of HSA changing the solvent structure around the protein molecule, it causes conformational changes as well in protein. Drastic changes are observed in the protein struture at DMSO higher concentration (20%).

Keywords: human serum albumin, excitation-emission matrix, fluorescence spectroscopy, dimethylsulfoxide, structural changes.

Introduction Fluorescence spectroscopy has become a dominating technique in the areas of biochemistry and molecular genetics. In analytical sciences, its advantage is extremely high-sensitivity and selectivity, even single molecules can be detected. When the emission spectrum is obtained for all excitation wavelengths of a sample data matrix of fluorescence intensities are combined, which is called excitation emission matrix (EEM). It contains all steady state fluorescence properties of a substance. EEM has trilinear structure, there may be some linear deviations due to chemical interferences, Rayleigh or Raman scattering overlap fluorescence bands and instrumental noise [1].

Human Serum Albumin (HSA), the most prominent protein in plasma, plays an important role in the transportation and disposition of many endogenous and exogenous compounds, such as nonesterified fatty acids, heme, bilirubin, thyroxine, bile acids, as well as an extraordinarily broad range of drugs. The surface of the protein molecule in contact with aqueous solvent comprises hydrophilic and hydrophobic groups in almost equal number [2–4]. Dimethylsulfoxide (DMSO) highly affects on drug transportation and enhancement [5, 6].

In this paper we present the behavior of tryptophan residue in HSA in the presence of DMSO studied by EEM fluorescence spectroscopy method.

Materials and Methods. Human serum albumin fatty acids free (< 0.005%) and DMSO were purchased from “Sigma Chemical Co” (USA). Physiological solution (0.9% sodium chloride) was purchased from “Likvor Pharmaceutical

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Company" (Armenia). A series of solutions containing varying concentrations of DMSO (0 to 20%) and fixed concentration of HSA (0.4 *mg*/*ml*) were prepared using physiological solution.

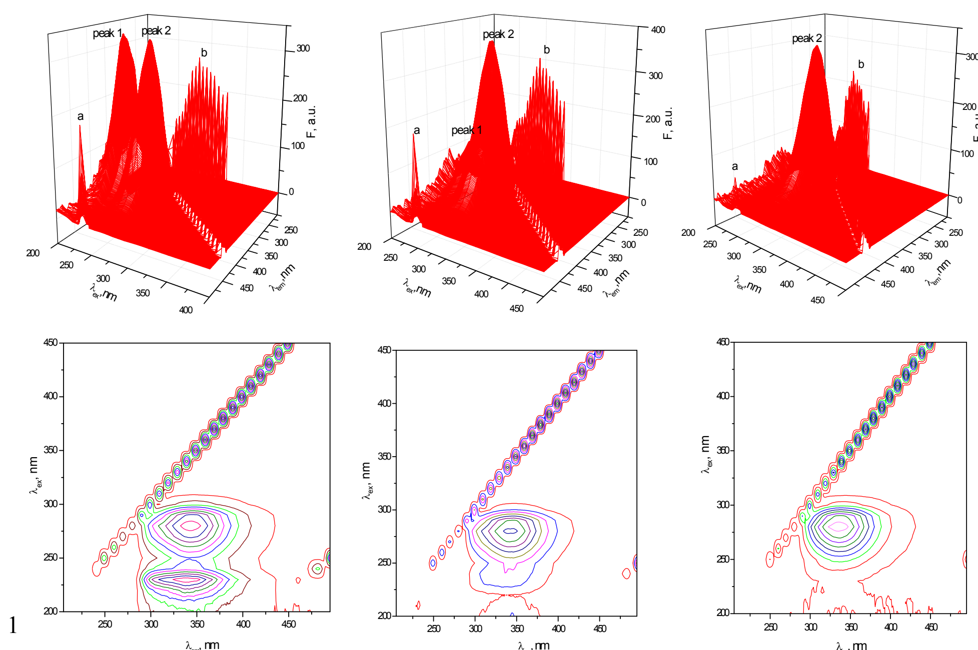
Fluorescence spectroscopy. Varian spectrofluorimeter (Australia) was used to measure the fluorescence spectra. The fluorescence 2D spectra scanning conditions were: $t=25^0C$, both the entrance and exit slits have a width of 5 *nm*. The excitation wavelength was adjusted at 295 *nm* and the emission spectra were recorded in the range of 300–500 *nm*. The 3D fluorescence spectra were performed under the following conditions: the emission wavelength was recorded between 200–500 *nm*, λ_{ex}=200 *nm* with increment of 10 *nm*, the number of scanning curves was 26. Each experiment was performed triply and the average data were used for analysis. To measure the time dependence of spectra we used Cary temperature controller. Spectra were measured after 5 *min* of incubation at corresponding temperature. To construct the graphs Origin 8.0 software was used.

Results and Discussion. HSA (a 585 amino acid residue monomer) contains three homologous α -helical domains (I–III) and a single residue (Trp-214), which is located in subdomain IIA. When 280 *nm excitation* wavelength is used, the fluorescence of HSA came from both Trp and Tyr residues whereas at 295 *nm* only the Trp residue is selectively excited [7].

Fluorescence is triparametric (excitation wavelength, emission wavelength, and emission intensity). Emission spectrum (2D spectrum) is the wavelength distribution of the emitted light measured at a single excitation wavelength. Excitation spectrum is defined by the wavelength distribution of exciting light, measured at a single emission wavelength. A full description requires excitation-emission data matrix. EEM is a 2D matrix, the elements of which I (λ_{ex}, λ_{em}) are the fluorescence intensity as a function of the excitation (λ_{ex}) and emission (λ_{em}) wavelengths. For a complex medium such as protein solution containing drug-complexes, EEM indicate the relative contributions of the component molecules. The advantage of 3D excitation/emission spectra over conventional 2D fluorescence spectra is that all changes in the fluorescence characteristics can be quantified that could be potentially missed in 2D spectra. The isometric projection of 3D spectra is most illustrative with the advantage that no fluorescence peaks are hidden. The higher the fluorescence intensities the darker the shading in the fluorescence plots. On the top of the matrix there is an area free of fluorescence. The diagonal area, where emission equals excitation wavelength, represents scattering of light [8].

Conventional 2D fluorescence spectra of HSA, HSA–DMSO (5 and 20%) systems were recorded at $t=25^0C$ during 270 *min* before and after adding DMSO. A blue-shift in 3 *nm* (from 348 *nm* to 345 *nm*) for 5% DMSO and 8 *nm* (from 348 *nm* to 340 *nm*) for 20% DMSO fluorescence emission was observed in the spectra. After 270 *min* the intensity of spectra was changed very little approximately in 5 a. u. for pure HSA and 5% DMSO, but for the 20% DMSO solution in 18 a. u. In our previous studies we mentioned that DMSO exhibits stabilizing (at low concentrations up to 12%) and destabilizing (at high concentrations up to 50%) effect on HSA structure [9]. The increasing of blue shift of $\lambda_{\max}$ can be explained by strengthening hydrophobic interactions between DMSO and protein molecules. Decrease in the intensity of fluorescence can be caused by the HSA denaturation. For more detailed studies of HSA structural changes in the presence of DMSO we measured 3D spectra (see Fig.). As shown in Figure, $\lambda_{ex} / \lambda_{em}$ two typical

fluorescence peaks (peak1 and peak 2) can be observed in 3D fluorescence spectrum: peak 1 ($\lambda_{ex}/\lambda_{em}$=230/338) and peak 2 ($\lambda_{ex}/\lambda_{em}$=280/344). Peak "a" is the Rayleigh scattering peak ($\lambda_{ex}=\lambda_{em}$), and peak "b" is the second-order scattering peak ($\lambda_{em}=2\lambda_{ex}$) [10]. Peak 1 reveals fluorescence spectral behavior of Trp and Tyr residue, and peak 2 may mainly represent the fluorescence characteristics of the polypeptide backbone structures.



3D Fluorescence spectra and excitation-emission contour plots of HSA–DMSO systems:
1 – HSA; 2 – HSA–DMSO 5%; 3 – HSA–DMSO 20%.

3D Fluorescence spectra characteristics of HSA, HSA–DMSO (5 and 20%) systems at t=25^{0}C during 270 min before and after adding DMSO

t, min			0	30	90	150	270
HSA	peak 1	$\lambda_{ex}/\lambda_{em}$=230 *nm* /338 *nm*					
		F, a.u.	336.7	330.3	329.0	329.0	329.0
	peak 2	$\lambda_{ex}/\lambda_{em}$=280/344					
		F, a.u.	326.0	330.1	328.2	328.2	328.2
HSA– –5% DMSO	peak 1	$\lambda_{ex}/\lambda_{em}$=230/338					
		F, a.u.	161.1	161.6	161.5	158.5	98.1
	peak 2	$\lambda_{ex}/\lambda_{em}$=280/344					
		F, a.u.	396.3	397.0	390.6	396.0	336.9
HSA– –20% DMSO	peak 1	$\lambda_{ex}/\lambda_{em}$=230/338					
		F, a.u.	–	–	–	–	–
	peak 2	$\lambda_{ex}/\lambda_{em}$=280/344					
		F, a.u.	371.3	368.1	368.7	374.9	308.0

The individual changes of peaks in these systems are shown in the Table. For pure HSA changes in the intensities for 2 peaks during 270 *min* are insignificant. In the presence of DMSO the intensity of peak 1 ($\lambda_{ex}/\lambda_{em}$=230/338)

decays and disappears when DMSO is 20%. After 270 *min* in the presence of DMSO 5% the intensity of peak 1 declines by 63.0 a. u. This demonstrates that the presence of DMSO affects not only on the polarity of the microenvironment of Trp and Tyr residues, but at higher concentrations it causes positional changes as well.

The intensity of peak 2 ($\lambda_{ex} / \lambda_{em}$=280/344) decreases by 59.4 a.u. at DMSO 5% and 63.3 a.u. at DMSO 20%. As it was mentioned above peak 2 represents the fluorescence characteristics of the protein polypeptide backbone structures. So, at DMSO higher concentrations we can observe structural changes in the polypeptide backbone.

From the analysis of 3D spectra of these systems we can conclude that DMSO low concentrations increase heat-resistance of HSA changing the solvent structure around the protein molecule. Drastic changes are observed at DMSO higher concentrations.

Received 24.01.2013

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