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HEAT INDUCED DENATURATION OF BOVINE SERUM ALBUMIN IN DIMETHYLSULFOXIDE CONTAINING SOLUTIONS IN THE PRESENCE OF POTASSIUM IODIDE

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Heat induced denaturation of bovine serum albumin (BSA) in dimethylsulfoxide (DMSO) containing solutions in the presence of potassium iodide has been studied using UV/vis spectroscopy method. It has been shown that the thermal stability of protein depends on competitive interactions taking place in the solution. At low DMSO concentrations (5 vol. %) BSA is more stable than at higher concentrations (10–25 vol. %).

***Keywords*:** bovine serum albumin, UV/vis spectroscopy, dimethylsulfoxide, potassium iodide.

Introduction. Proteins are biomolecules of great importance in the biochemical processes such as medical, pharmaceutical and food fields, since they exhibit outstanding biological activities under mild conditions. However, most of proteins dissolved in an aqueous solution are immediately denatured and inactivated at high temperatures due to the disruption of weak interactions including ionic interactions, hydrogen bonds and hydrophobic interactions, which are prime determinants of protein tertiary structures [1].

Thermal denaturation of proteins is a serious problem not only in the separation and storage of proteins, but also in the processes of biotransformation, biosensing, drug production and food manufacturing. The addition of stabilizing agents is one of the most convenient methods for minimizing thermal denaturation. Inorganic salts, sugars, amino acids and water-miscible organic solvents play a major role in wide range of the physical properties and stability of proteins in solution [2]. Organic solvents, such as dimethyl sulfoxide (DMSO), have been used to precipitate, crystallize and denature proteins [3]. These solvents affect the state of solvated ions and competing interactions of the particles. As it has been shown in [4], cations (Li^+, Na^+, K^+) differ from anions by their solvated state. This also has an impact on protein stability [5].

In this paper we present the results of UV/vis studies on heat induced denaturation of bovine serum albumin (BSA) in DMSO containing solutions in the presence of KI.

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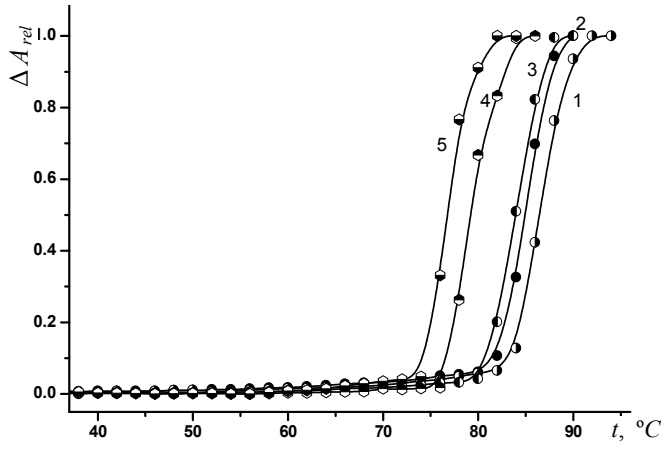
BSA, the most abundant protein in plasma, is a globular protein comprised of a single chain of 582 amino acids, cross linked with 17 cystine residues. Because of its structural similarity to that of human serum albumin (HSA), BSA is commonly used as a model protein for biophysical and physicochemical studies. Crystallographic analysis of BSA reveals that it contains three homologous α-helical domains (I–III), each of which is composed of two sub domains, A and B. It has two tryptophan (Trp) residues that possess intrinsic fluorescence: Trp-134, which is located on the surface of sub-domain I B, and Trp-212, locating within the hydrophobic binding pocket of sub-domain IIA [6].

Materials and Methods. BSA and DMSO were purchased from "Sigma" (USA). KI of chemically pure grade was purchased from "Reakhim" (Russia). A series of solutions containing varying concentrations of DMSO (0 to 25 vol.%) and fixed concentration of BSA (0.4 *mg*/*mL*) and KI (0.9 vol.%) were prepared using bidistilled water.

UV/vis Measurements. UV/vis spectra were registered on Specord PC 50 spectrophotometer (Germany). BSA thermal denaturation was studied in the range 36–94°*C*. The scanning rate was 1°*C*/*min*. For maintaining permanent temperature, a circulating Lauda A100 thermostat (Germany) was used. UV/vis profiles of BSA thermal denaturation were normalized (0 to 1) using the Origin 8.0. Melting temperatures were determined as midpoint of sigmoidal curves.

Results and Discussion. UV/vis profiles of BSA thermal denaturation in water–DMSO solutions in the presence of KI within a temperature range of 36–94 °*C* are shown in the Figure.



UV/vis profiles of BSA thermal denaturation in the temperature range 36–94 °*C*: C_{BSA}=0.4 *mg/mL*, C_{KI} = 0.9 vol. %, C_{DMSO} = 0 (2); 5 (1); 10 (3); 20 (4); 25 vol. % (5).

It is noteworthy that in the presence of DMSO, pH values were practically invariable, being within the range of 6.2–7.3. Hence, variations in pH values could not affect BSA thermal denaturation [5]. In the presence of 5 vol.% DMSO melting curves shift to the region of higher temperatures, but with increasing DMSO concentration 10–25 vol. % melting curves shift to the region of low temperatures. From these melting profiles melting temperatures (T_m) were determined, which are presented in the Table.

Denaturation temperatures of BSA in KI containing DMSO–water solutions

C_{DMSO}, vol. %	0	5	10	20	25
$T_m \pm 0.25$, °C	84.07	87.03	83.09	80.50	78.14

Apparently at low DMSO concentrations (5 vol. %) BSA is more thermally stable than at higher concentrations (10, 20, 25 vol. %). High thermal stability of BSA in solvents with low DMSO content can be explained by the fact that in the presence of DMSO lattice structure of solvent becomes more branched due to formation of hydrogen bonds between DMSO and water molecules.

The solvent structure branching effect of DMSO on protein stability is more pronounced than the medium structure breaking effect of I^- ions. The shift of melting curves to low temperature region at higher DMSO concentrations is complex. It can be caused not only by the break of water structure and breaking effect of I^- ions, but by the electrostatic interactions between low solvated I^- ions and protein positively charged groups as well.

It is known that the thermal unfolding of albumins is described by a multistep mechanism based on Eyring and Lamry approximation model [7], according to which albumin denaturation occurs via two steps, reversible and irreversible. One can hardly distinguish the areas of reversible and irreversible steps on the BSA melting curves observed in water–DMSO solutions in the presence of potassium iodide as the mechanism of denaturation is changed.

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