

Chemistry

FLUORESCENCE STUDY ON TRYPTOPHAN–POTASSIUM
IODIDE INTERACTION

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Interaction of tryptophan (Trp) with potassium iodide in aqueous and DMSO containing solutions at 293 and 303 K temperatures has been studied using fluorescence spectroscopy method. It has been shown that Trp fluorescence quenching by KI depends on Γ ion mobility, which is affected by the changes in temperature and viscosity of the environment. The physicochemical characteristics of binding process (K_b , n , ΔH , ΔS and ΔG) have been determined in H_2O and DMSO containing solutions.

Keywords: tryptophan, potassium iodide, fluorescence quenching, thermodynamic parameters.

Introduction. Tryptophan (Trp) is important for both protein synthesis and as a precursor of niacin, serotonin and other metabolites. Trp is an unusual amino acid, because of the complexity of its metabolism, variety and importance of its metabolites, number and diversity of the diseases it is involved in and because of its use as a pharmacological agent. The metabolism of Trp, its presence in the diet, the disorders associated with low Trp levels due to low dietary intake, malabsorption, or high rates of metabolism, the therapeutic effects of Trp and the side effects of Trp when it is used as a drug including eosinophilia myalgia syndrome are presented in [1].

DMSO is a medicinal product (Dimexidum, Demasorb, Dolocur, etc.), it smoothly penetrates via biological membranes, including cutaneous barriers, and intensifies the absorption of some drugs by skin [2]. Solutions containing DMSO affect the state of solvated ions and competing interactions of the particles. As it has been shown in [3], in these solvents cations (Li^+ , Na^+ , K^+) differ from anions (NO_3^- , Cl^- , ClO_4^-) by their solvated state.

Fluorescence spectroscopy and its multiple applications to the protein analysis have undergone rapid development during the past twenty years due to new theoretical and technical decisions. Fluorescence spectroscopy methods (steady-state and time-resolved fluorescence, synchronous and excitation emission matrix-EEM, two-dimensional) are widely used for monitoring bioprocesses, protein-ligand interactions, dynamics and kinetics of protein folding reactions [1–10].

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The studies on quenching mechanism of Trp by ions can help to find out the details of biological processes occurring with proteins in the presence of electrolytes. In this article we present the fluorescence study on Trp quenching by KI at 293 and 303 K in the presence of DMSO.

Materials and Methods. Chemically pure grade Trp was purchased from “Reanal” (Hungary); DMSO was purchased from “Sigma Chemical Co” (USA); KI (“chemically pure”) was purchased from “Reakhim” (Russia). Solutions of Trp and KI were prepared using double distilled water. Concentrations of Trp and DMSO were $4.3 \cdot 10^{-5} \text{ mol} \cdot \text{L}^{-1}$ and 25% (v/v) respectively. Fluorescence spectra were registered on a Varian Cary Eclipse spectrophotometer (Australia). Fluorescence spectra were recorded over the interval $\lambda=295\text{--}500 \text{ nm}$ at an excitation wavelength 290 nm. Quartz cells with $l=1 \text{ cm}$ were used for measurements. The reduced diagrams were plotted using the ORIGIN 8.0 software.

Results and Discussion. The effect of KI on the fluorescence intensity of Trp in H_2O at 293 K is presented in Fig. 1. Fluorescence of Trp is characterized by the emission maximum of $\lambda_{em}=356 \text{ nm}$.

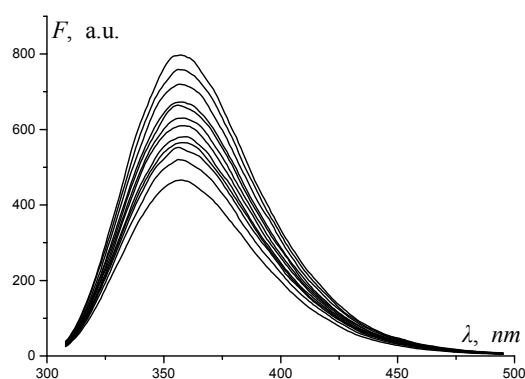


Fig. 1. Fluorescence quenching spectra of Trp by KI aqueous solution, $T=293 \text{ K}$:
 $[\text{Trp}] = 4.3 \cdot 10^{-5} \text{ mol} \cdot \text{L}^{-1}$,
 $[\text{KI}] = 0 - 31.5 \cdot 10^{-3} \text{ mol} \cdot \text{L}^{-1}$.

As shown in Fig. 1, Trp fluorescence intensity decreases considerably with the increase in KI concentration and λ_{max} shift is not observed. Trp fluorescence quenches in the presence of KI by collisional – dynamic quenching mechanism. Both static and dynamic quenchings require molecular contact between the fluorophore and quencher. In the case of collisional quenching, the quencher must diffuse to the fluorophore during the lifetime of the excited state [11]. Diffusion affects both the temperature and the viscosity. Collisional quenching of fluorescence is described by Stern–Volmer equation:

$$F_0 / F = 1 + k_q \tau_0 [Q] = 1 + K_D [Q], \quad (1)$$

where F_0 and F represent the fluorescence intensities of Trp in the absence and presence of quencher respectively, k_q is the bimolecular quenching rate constant, K_D is the dynamic quenching constant, τ_0 is the lifetime of fluorophore in the absence of quencher (the τ_0 for the biopolymer is often taken as 10^{-8} s [11]), $[Q]$ is the concentration of quencher. The Stern–Volmer quenching constant is given by $K_D = k_q \tau_0$. The Stern–Volmer plots, for Trp fluorescence quenching by KI in water and in the presence of DMSO at different temperatures, are shown in Figs. 2 and 3.

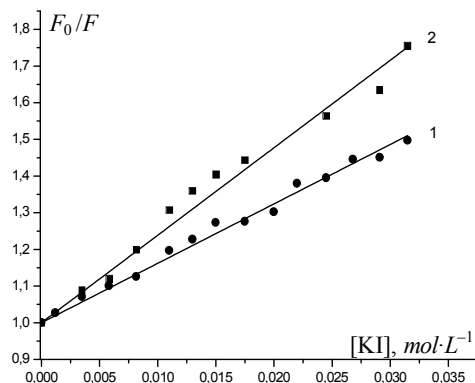


Fig. 2. Stern–Volmer plots of Trp fluorescence quenching by KI in H_2O at different temperatures, $[Trp] = 4.3 \cdot 10^{-5} \text{ mol} \cdot L^{-1}$.
1 – 293 K; 2 – 303 K.

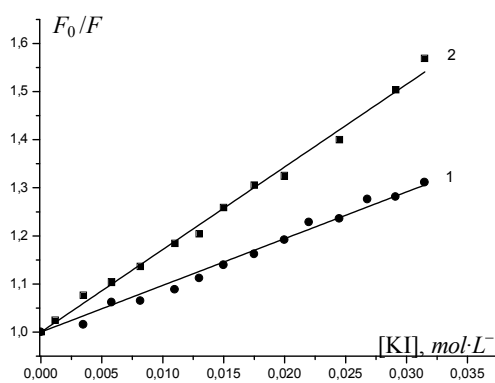


Fig. 3. Stern–Volmer plots of Trp fluorescence quenching by KI in H_2O –DMSO solution at different temperatures, $[Trp] = 4.3 \cdot 10^{-5} \text{ mol} \cdot L^{-1}$.
1 – 293 K; 2 – 303 K.

K_D and k_q have been determined from these dependencies. The results obtained are presented in Tab. 1.

Table 1

Dynamic quenching constants and bimolecular quenching rate constants for the interaction of KI with Trp in H_2O and H_2O –DMSO solution at different temperatures

System	Trp– H_2O		Trp– H_2O –DMSO (25 %, v/v)	
T, K	293	303	293	303
$K_D, L \cdot mol^{-1}$	16.50	23.86	10.29	19.17
$k_q \cdot 10^{-8}, L \cdot mol^{-1} \cdot s^{-1}$	16.50	23.86	10.29	19.17

It is clear that the increase of temperature leads to the increase of ions mobility and effects the diffusion. That is why the values of K_D and k_q are higher with a rise in temperature. As it was mentioned above, viscosity of the environment also effects ion diffusion. The viscosity of the aqueous solutions in the presence of DMSO (25% v/v) increases approximately twice [8]. The fluorescence intensities of Trp in the presence of KI in H_2O –DMSO solutions are presented in Tab. 2.

Table 2

Fluorescence intensities of Trp in the presence of KI in H_2O –DMSO solutions

Temperature	293 K	303 K
$[KI] \cdot 10^{-3}, M$	$F, \text{ a.u.}$	
0	973.61	586.82
1.20	953.51	561.68
8.17	889.59	471.06
15.00	836.74	465.88
17.50	814.02	426.43
29.10	745.21	415.52
31.50	709.77	374.12

The fluorescence intensity decreases drastically in the presence of DMSO and at 303 K it becomes more pronounced. K_D and k_q values are also smaller in the presence of DMSO (Tab. 1). The effect of temperature on ion mobility is observed in the presence of DMSO as well. The analysis of the results obtained shows, that the viscous environment reduces the migration of Γ^- ions (Trp quencher), which results negatively in K_D values.

Physicochemical Characteristics of Trp–KI Interaction. Physicochemical characteristics of Trp–KI interaction (K_b , n , ΔH , ΔS and ΔG) were determined at temperatures 293 and 303 K. The binding constant (K_b) and the number of binding sites (n) were determined graphically (Fig. 4), according to the equation

$$\lg \frac{F_0 - F}{F} = \lg K_b + n \lg [Q]. \quad (2)$$

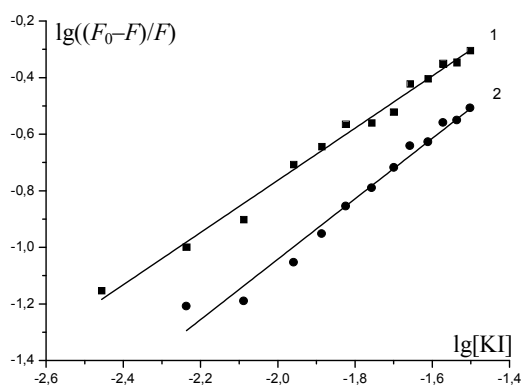


Fig. 4. Dependence of $\lg((F_0-F)/F)$ on $\lg[KI]$ for Trp–KI system. $T=293$ K. 1 – H_2O ; 2 – DMSO– H_2O (DMSO – 25% v/v).

ΔH (enthalpy change) and ΔS (entropy change) were determined according to the Van't-Hoff equation:

$$\ln K_b = -\frac{\Delta H}{RT} + \frac{\Delta S}{R}. \quad (3)$$

The change of Gibbs energy was determined according to the following equation:

$$\Delta G = \Delta H - T\Delta S. \quad (4)$$

The obtained results are summarized in Tab. 3.

Table 3

Binding constant, number of binding site and thermodynamic parameters of Trp–KI interactions at different temperatures

System	Trp– H_2O				
T, K	$K_b \cdot 10^2, L \cdot mol^{-1}$	n	$\Delta H, kJ \cdot mol^{-1}$	$\Delta S, J \cdot mol^{-1} \cdot K^{-1}$	$\Delta G, kJ \cdot mol^{-1}$
293	2.14	0.90	103.09	411.58	-16.69
303	7.41	0.90			-20.81
System	Trp– H_2O –DMSO, 25% v/v				
293	0.40	1.00	166.28	604.80	-10.93
303	4.30	0.92			-16.97

As one can see from Tab. 3, the Gibbs energy values are negative, indicating that KI interactions with Trp are spontaneous. ΔS is positive, which can be due to the increase in the number of particles in the system as KI dissociates. ΔH also is positive. Solvation of salts in water is usually accompanied by heat absorption. Solvation process consists of two stages: break down of crystal lattice structure and hydration of salt ions. The first stage is accompanied by heat absorption, and the second stage is heat release, and since the heat of the first stage is greater than the second one, heat is absorbed. In the presence of DMSO, the values of ΔH and ΔS differ from the values in aqueous solutions, which can be explained by the association and solvation processes occurring in the system. Temperature and the presence of DMSO do not affect the number of binding sites. The analysis of physicochemical parameters shows that the system is stabilized due to electrostatic and van der Waals interactions and the changes in temperature and viscosity of environment brings to the changes in quenching properties of Γ^- ions.

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