

MOLECULAR INTERACTIONS OF L-TYROSINE
IN DIMETHYL SULFOXIDE AQUEOUS SOLUTIONS.
PART 1: ELECTRONIC ABSORPTION AND FLUORESCENCE
SPECTROSCOPY STUDIES

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Molecular interactions of L-tyrosine in dimethyl sulfoxide (DMSO) aqueous solutions have been studied using electronic absorption and fluorescence spectroscopy methods. It has been shown that at low concentrations of DMSO in aqueous media hetero-associates such as 1DMSO:1H₂O or 1DMSO:2H₂O are formed, while at high concentrations, associates of a more complex structure (mixed clusters) are formed that interact with L-tyrosine to form complex compounds.

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Introduction. In the field of biomedical physical chemistry, the studies on protein interactions, stability, structural changes and complex formation with different ligands are of great interest as these studies can help elucidate the mechanisms of complex biological processes. In aqueous solutions, the structure of proteins is stabilized due to intermolecular and intramolecular hydrogen bonds formed by amino acids, which are the fundamental units of protein structure [1–3]. L-tyrosine is a nonessential amino acid with a considerable role in the bioactivity of the organism and is added to foods, pharmaceuticals and dietary products [4]. The fluorescent properties of aromatic amino acids (tryptophan – Trp, tyrosine – Tyr, and phenylalanine – Phe) are widely used in protein studies to trace and analyze molecular interactions and dynamics of conformational changes. These amino acids are very sensitive to polarity changes in the environment. The spectral characteristics of Trp and Tyr are very close, and the quantum yield of these amino acids is high enough (0.14 and 0.13, respectively) to give good fluorescence. Tyr exhibits substantial fluorescence and high sensitivity to medium polarity, making it a useful natural probe for studying protein structure and dynamics. In a hydrophobic environment (when buried within the core of the protein), Tyr and Trp exhibit high fluorescence intensity. In contrast, in a hydrophilic environment (exposed to solvent) the quantum yield of these amino acids decreases leading to a low fluorescence

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intensity [5, 6]. Changes in the polarity of the solvent environment can affect the structure and resonance energy transfer from Tyr to Trp amino acid residues. Tyr (molecular structure is shown in Fig. 1), due to its phenolic hydroxyl group, possesses unique reactivity [7].

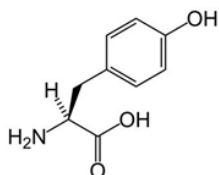


Fig. 1. Molecular structure of L-Tyrosine.

Dimethyl sulfoxide (DMSO) is a dipolar aprotic solvent with high relative permittivity ($\epsilon_r=48$). It is miscible with organic solvents as well as with water. DMSO dissolves both polar and non-polar compounds due to S=O and two hydrophobic methyl groups with the +I effect. It can alter the solubility and structural stability of DNA, phospholipid liposomes, proteins, peptides and amino acids [8–11]. The spectral properties of amino acids/proteins were studied extensively to get more characteristics for monitoring protein structure and dynamics [12–15]. As a model system for these studies, especially for UV/Vis and fluorescence studies, can serve aqueous solutions of aromatic amino acid (Trp, Tyr and Phe). The objective of the present work is to reveal the nature of molecular interactions of L-tyrosine in DMSO–H₂O mixed solvent.

Materials and Methods. Chemically pure grade L-Tyr was purchased from “Reanal” (Hungary) and DMSO was obtained from “Sigma Chemical Co” (USA). The Tyr solution was prepared in bidistilled water. Electronic absorption spectra of L-Tyr in DMSO solutions, containing a fixed concentration of L-Tyr ($10^{-4} \text{ mol}\cdot\text{L}^{-1}$) and various concentrations of DMSO (the mole fraction of DMSO was changed from 0 to 0.7), were registered on a Specord 50 spectrophotometer (Germany) in the wavelength range of 200–400 nm. Fluorescence spectra were registered on a Varian Cary Eclipse spectrophotometer (Australia) in the wavelength range of 280–500 nm at an excitation wavelength $\lambda_{ex}=275 \text{ nm}$. The widths of the excitation and emission slits were 5 nm. 3D fluorescence spectra were recorded in the following mode: excitation wavelength (λ_{ex}) range was 200–400 nm; emission wavelength (λ_{em}) range was 200–450 nm. The width of the excitation slit was 5, and that of the emission slit was 2.5 nm. $\Delta \lambda_{incr}$ was 10 nm, and the number of scans was 26. For the measurements, a quartz cell with $l=1 \text{ cm}$ was used.

Results and Discussion.

UV/Vis Spectra Analysis. Two broad bands in the UV region of the electromagnetic spectrum characterize proteins and amino acids in aqueous media. The peptide bond in proteins has a strong absorption at around 190 and a weak absorption between 210 and 220 nm. Absorption in the region of 255–280 nm is attributed to aromatic amino acids: Trp ($\lambda_{max}\approx 280 \text{ nm}$, $\epsilon=5600 \text{ M}^{-1}\text{cm}^{-1}$), Tyr ($\lambda_{max}\approx 275 \text{ nm}$, $\epsilon = 1490 \text{ M}^{-1}\text{cm}^{-1}$) and Phe ($\lambda_{max}\approx 257 \text{ nm}$, $\epsilon=0.200 \text{ M}^{-1}\text{cm}^{-1}$) [16]. UV/Vis spectrum of Tyr is characterized by two bands at $\lambda_{max}=223$ and 275 nm originating from $\pi\rightarrow\pi^*$ transitions to the singlet L_b and singlet L_a states.

The electronic absorption profile of Tyr in the presence of DMSO is presented in Fig. 2. Upon adding DMSO, the absorbance of the characteristic band of Tyr at 275 nm increases and slightly shifts to a longer wavelength ($\Delta\lambda=5.43$ nm). At $X_{\text{DMSO}}=0.7$, a decrease in the absorbance is observed, which can be explained by a change in ε values of the formed Tyr–DMSO–H₂O structures. The shift of the band is a result of the increasing polar interactions in the Tyr–DMSO–H₂O system due to the phenolic hydroxyl group, which can be involved in various forms of hydrogen bonding. At low concentrations of DMSO in aqueous media, DMSO molecules can form hetero-associates with water molecules, such as 1DMSO:1H₂O or 1DMSO:2H₂O, which can act as a ligand in complexes with L-tyrosine. In the case of high concentrations, DMSO molecules can form water-bridged mixed cluster structures or can directly interact with L-tyrosine.

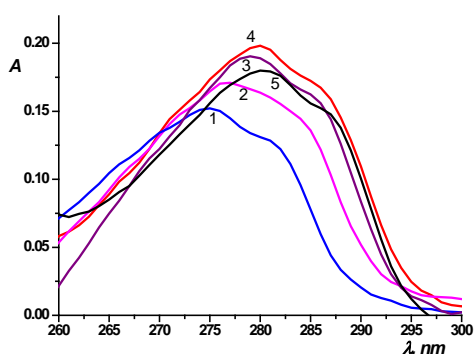


Fig. 2. Electronic absorption spectra of Tyr in the aqueous solution of DMSO.

[Tyr] = 10^{-4} mol·L⁻¹;
 X_{DMSO} = 0 (1), 0.2 (2), 0.4 (3), 0.6 (4), 0.7 (5).

Solubility studies of L-Tyr in aqueous solutions of methanol, ethanol, *n*-propanol and DMSO showed that the mole fraction of solubilized L-Tyr was higher in DMSO–water solution than in the other three alcoholic solutions. In the case of polar protic solvents (methanol, ethanol, *n*-propanol) L-Tyr was preferentially solvated by water molecules, while in the case of polar aprotic solvent, such as DMSO, L-Tyr was not preferentially solvated neither by water nor by DMSO molecules [14]. So, changes in the UV/Vis spectra can confirm the formation of such structures (complexes and mixed clusters of different size and polarity) in DMSO–H₂O mixed solvent.

Fluorescence Spectra Analysis. The fluorescence spectrum of Tyr in aqueous solutions (Fig. 3) is characterized by a single emission band at $\lambda_{\text{max}}=303$ nm at an excitation $\lambda_{\text{ex}}=275$ nm. In the presence of DMSO, fluorescence intensity of Tyr decreases upon adding DMSO and the emission band shifts to longer wavelength region.

Fluorescence of Tyr may be affected by solvent properties (polarity, viscosity, etc.), and by structural or conformational changes in nearby molecules in several ways: formation of a hydrogen bond with a proton acceptor or donor, cation- π or hydrophobic interactions of the aromatic ring [17]. The steady-state and time-resolved fluorescence studies of the photophysical properties of Tyr in pure organic and water-organic solvents also confirm these observations [18]. When an aromatic hydroxyl group/carboxyl group of Tyr interacts with a proton acceptor, a red-shifted emission of Tyr is observed. In the presence of DMSO, the red shift is about 6.00 nm, which indicates the existence of strong intermolecular interactions between Tyr and the components of the binary solvent.

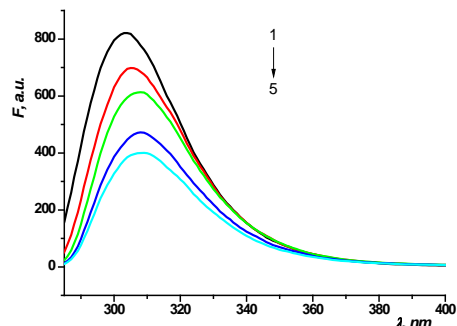


Fig. 3. Fluorescence emission profiles of Tyr in the aqueous solution of DMSO.

$[\text{Tyr}] = 10^{-4} \text{ mol}\cdot\text{L}^{-1}$;
 $X_{\text{DMSO}} = 0$ (1), 0.2 (2), 0.4 (3), 0.6 (4), 0.7 (5).

The 3D fluorescence spectra and contour maps (excitation/emission matrix) of Tyr in aqueous solutions (Fig. 4) are characterized by two excitation/emission peaks: peak 1, $\lambda_{ex}/\lambda_{em}=228/304$ (nm); peak 2, $\lambda_{ex}/\lambda_{em}=275/304$ (nm) and peak A – Rayleigh scattering peak ($\lambda_{ex}=\lambda_{em}$) [19, 20].

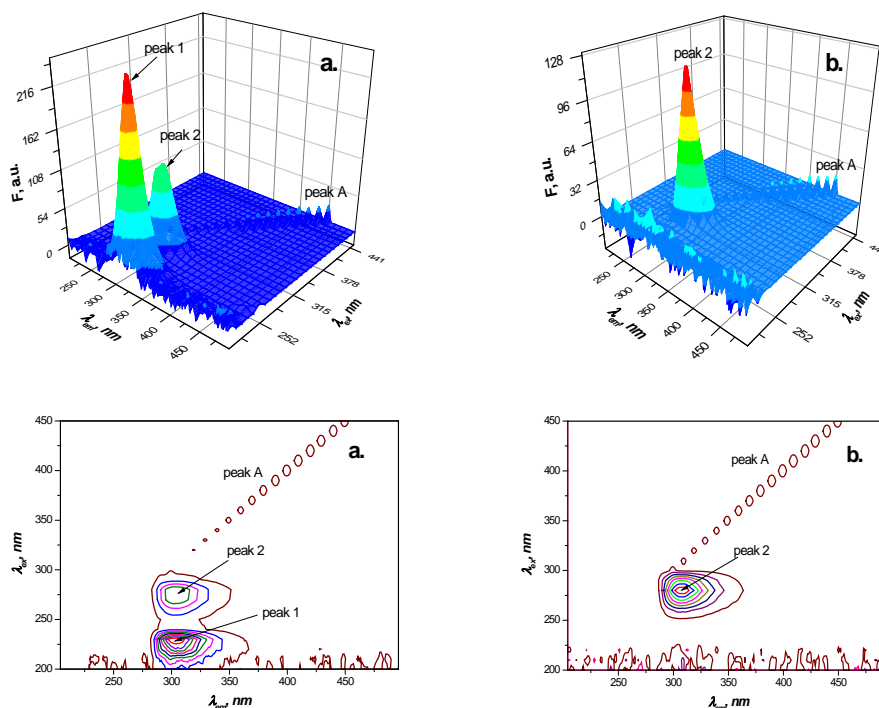


Fig. 4. Fluorescence 3D emission profiles of Tyr in the aqueous solution of DMSO. $\lambda_{ex}/\lambda_{em}=200\text{--}400/200\text{--}450$ nm/nm region. $[\text{Tyr}] = 10^{-4} \text{ mol}\cdot\text{L}^{-1}$, $X_{\text{DMSO}} = 0$ (a), 0.4 (b).

The intensity of fluorescence emission at $\lambda_{ex}/\lambda_{em}=228/303$ (nm) is stronger, than that of at $\lambda_{ex}/\lambda_{em}=275/303$ (nm) (2.1 : 1.0). Peak 1 is simply due to the excitation of the second excited state of the aromatic amino acid, which then emits from the lowest excited state [21]. Peak 2 corresponds to the emission of the aromatic amino acids. In the presence of DMSO, peak 1 is not observed in the registered region of the spectrum, and the intensity of the Rayleigh scattering peak increases. The results

of the analysis of 3D fluorescence spectra of the Tyr–DMSO–H₂O system are presented in Table. As it is mentioned in [5, 22], under some circumstances, Tyr can display complex spectral properties. The fluorescence of Tyr can be quenched because of resonance energy transfer, or Tyr can undergo excited-state ionization, resulting in the loss of a proton from the aromatic hydroxyl group.

Emission bands fluorescence characteristics of Tyr 3D spectra in the presence of DMSO

System	X_{DMSO}	Peak 1		Peak 2		Rayleigh scattering peak	
		$\lambda_{\text{ex}}/\lambda_{\text{em}}$, nm/nm	F, a.u.	$\lambda_{\text{ex}}/\lambda_{\text{em}}$, nm/nm	F, a.u.	$\lambda_{\text{ex}}/\lambda_{\text{em}}$, nm/nm	F, a.u.
Tyr–H ₂ O	0	228/303	242.9	275/303	113.5	350/350→450/450	14.5→24.9
Tyr–H ₂ O–DMSO	0.2	–	–	280/307	118.2	310/310→450/450	14.0→33.2
Tyr–H ₂ O–DMSO	0.4	–	–	280/310	120.1	360/360→450/450	12.7→16.6
Tyr–H ₂ O–DMSO	0.6	–	–	280/309	98.2	310/310→450/450	15.5→25.1
Tyr–H ₂ O–DMSO	0.7	–	–	280/309	81.5	330/330→450/450	10.5→19.5

As a result of strong intermolecular interactions between DMSO and water molecules, both water and DMSO molecules are preferentially involved in mixed clusters, depending on the concentration of DMSO, which brings to an increase or decrease in the Rayleigh scattering peak. From the analysis of 3D fluorescence spectra, we can conclude, that in the presence of DMSO, Tyr undergoes excited-state ionization, and/or resonance energy transfer can take place, by the involvement of the aromatic hydroxyl group in hydrogen bonding. Complexes of different compositions (1DMSO:1H₂O and 1DMSO:2H₂O) in a DMSO aqueous solution can be formed.

More detailed studies of the L-Tyr–DMSO–H₂O system based on FTIR spectral and theoretical quantum chemical calculations will be presented in further publications.

Conclusion. In this paper, molecular interactions of L-tyrosine in DMSO aqueous solutions have been studied using electronic absorption and fluorescence spectroscopy methods (intrinsic fluorescence, excitation/emission matrix). It has been shown that the photophysical properties of Tyr are highly sensitive to its local environment structure. At low concentrations of DMSO (up to $X_{\text{DMSO}} = 0.5$) hetero associates, such as 1DMSO:1H₂O and 1DMSO:2H₂O, are the dominant structures interacting with L-tyrosine, and at high concentrations (up to $X_{\text{DMSO}} = 0.7$), DMSO molecules can form water-bridged mixed cluster structures or can directly interact with L-tyrosine.

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Լ-ԹԻՐՈՉԻՆԻ ՄՈԼԵԿՈՒԼԱՅԻՆ ՓՈԽԱԶԴԵՑՈՒԹՅՈՒՆՆԵՐԸ
ԴԻՍԵԹԻԼՍՈՒԼՖՕՔՍԻԴԻ ՋՐԱՅԻՆ ԼՈՒԾՈՒՑԹՆԵՐՈՒՄ:
ՄԱՍ 1. ԷԼԵԿՏՐՈՆԱՅԻՆ ԿԼԱՆՄԱՆ ԵՎ ՖԼՈՒՐԵՍԿԵՆՏԱՅԻՆ
ՄՊԵԿՏՐՈՍԿՈՊԻԱԿԱՆ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆՆԵՐ

Ուսումնասիրվել են Լ-թիրոզինի մոլեկուլային փոխազդեցությունները դիմերիլսուլֆօքսիդի (ԴՄՍՕ) ջրային լուծույթներում էլեկտրոնային կլանման և ֆլուորեսցենտային առաքման սպեկտրոսկոպիայի մեթոդների կիրառմամբ: Ցույց է տրվել, որ ջրային միջավայրում ԴՄՍՕ-ի ցածր կոնցետրացիաների դեպքում գոյանում են հետերոասոցիատներ 1ԴՄՍՕ:1H₂O կամ 1ԴՄՍՕ:2H₂O բաղադրությամբ, իսկ բարձր կոնցետրացիաների դեպքում՝ ավելի բարդ կառուցվածքի ասոցիատներ (խառը կլաստերներ), որոնք փոխազդում են Լ-թիրոզինի հետ՝ առաջացնելով կոմպլեքս միացություններ:

К. Р. ГРИГОРЯН, А. А. ШИЛАДЖЯН, Р. Г. АРУТЮНЯН

МОЛЕКУЛЯРНЫЕ ВЗАИМОДЕЙСТВИЯ L- ТИРОЗИНА
В ВОДНЫХ РАСТВОРАХ ДИМЕТИЛСУЛЬФОКСИДА.
ЧАСТЬ 1: ЭЛЕКТРОННО-АБСОРБЦИОННЫЕ
И ФЛУОРЕСЦЕНТНЫЕ ИССЛЕДОВАНИЯ

Методами электронно-абсорбционной и флуоресцентной спектроскопии изучены молекулярные взаимодействия L-тирозина в водных растворах диметилсульфоксида (ДМСО). Показано, что при низких концентрациях ДМСО в водной среде образуются гетероассоциаты типа 1ДМСО:1H₂O или 1ДМСО:2H₂O, а в случае высоких концентраций – ассоциаты более сложных структур (смешанные кластеры), которые взаимодействуют с L-тирозином, образуя комплексные соединения.