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Surfactant Effects on Tautomeric and Microscopic Equilibria of Tryptophan: Experimental and Theoretical Studies

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Surfactant molecules are used as interesting tools to study the structure, function and stability of proteins. Protonation states of amino acids may be changed in the presence of surfactants. In this work, using experimental observations and molecular dynamic simulation, the effects of sodium dodecyl sulfate on the acid dissociation constants of tryptophan was examined. The acid-base equilibrium of tryptophan molecules was examined in the aqueous solution and in the presence of different concentrations of SDS. Different concentrations of SDS have diverse effects on the values of pKa_1. However, the effect of SDS on pKa_2 is similar in all concentration ranges. Furthermore, the microconstants related to the same equilibria (k_{11}, k_{22}, k_{12} and k_{21}) have different values in the presence and absence of SDS. These results show the different protonation states of tryptophan molecules in the presence and absence of surfactant. Molecular dynamic simulation showed that in the absence of SDS, tryptophan molecules form molecular aggregates similar to that of stacking. However, in the presence of SDS, stacking between tryptophan molecules is disrupted by hydrophobic and hydrogen bonding interactions of surfactant.

Keywords: Tryptophan, Surfactant, Microscopic constant, Simulation

INTRODUCTION

Study of the interactions between surfactants and proteins is of great scientific and technological importance [1]. Local charges of the enzymes such as lipases which function in the aqueous and non-aqueous environments may be altered in different media as a result of different protonation states of their amino acids [2,3]. Proteins which interact with lipid bilayers in cell membranes may undergo different ionic states in the presence of lipids or any hydrophobic molecules. Tryptophan is commonly used to study the protein structure. Thus, the description of acid dissociation constants of this molecule in different media is an important subject [4]. Since surfactant-protein interaction is interesting for pharmacists and researchers who study macromolecular structure (*e.g.*, proteins), effects of the anionic surfactant sodium dodecyl sulfate (SDS) on the properties of individual amino acids such as dissociation constants can be an indicator of the effects of surfactant on the macromolecular structure [5,6]. Accordingly, studying the interactions between amino acids and surfactants provides useful tool for analyzing the effects of surfactant on the overall structure of proteins [7].

In addition, determination of the microscopic constants and tautomeric ratios plays an important part in understanding the ionic composition of the proteins. The chemical and biological activity of proteins would be expected to vary with the degree of ionization [8,9]. Accurate knowledge of the ionization constants for zwitterions (*e.g.* amino acids) is a prerequisite for understanding the corresponding mechanism. On the other hand, the microscopic constants and tautomeric ratios describe the amount of various species as a function of pH [10,11]. These parameters are especially useful in the research areas relating to the amino acids in the living systems.

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Microscopic protonation constants and tautomeric ratios have been determined only for few amino acids. To the best of our knowledge, the physicochemical properties and behavior of tryptophan in the aqueous solution and in the presence of SDS has not been well understood to this time. Present work is an attempt to study the effect of anionic surfactant (SDS) on the structure of tryptophan and also to examine the effect of surfactant on the acid dissociation constants of tryptophan. To this end, the macroconstant values (K_{a1} and K_{a2}), tautomerization constant (k_z) and microconstant values (k_{11}, k_{12}, k_{21} and k22) have been determined. Molecular Dynamic (MD) simulation is a useful and reliable tool to study the bimolecular structures and dynamics. Bimolecular interactions and assemblies, which are difficult to study experimentally, can be investigated by this molecular modeling method. To justify the experimental results, intermolecular interaction between SDS and tryptophan molecules, that is the main cause of changes in acid dissociation constants, has been investigated by MD simulation.

MATERIALS AND METHODS

Materials

Tryptophan, sodium dodecyl sulfate (SDS), hydrochloric acid, sodium hydroxide, and methanol were purchased from Merck (Darmstadt, Germany) and used without further purification. All materials used were of analytical grade. Stock solutions of surfactant SDS were prepared by dissolving the appropriate amounts of this substance in deionized water. Deionized water was used throughout the entire work.

Methods

Determination of macroconstants K_{a1} and K_{a2} values. Macroconstants were determined using pH-metric titration method. Titrations were carried out using a glass electrode calibrated with standard buffer solutions (pH 4 and 7). All the measurements were carried out under nitrogen atmosphere at 25 ± 0.1 °C. A 5 ml portion of the tryptophan solution (~6×10^{-3} M), in the absence or the presence of different concentrations of SDS, below and above the critical micelle concentration (1, 2, 5 and 10 mM), was placed in the cell and titrated. To perform the titrations, HCl or NaOH solution (0.006 M, freshly prepared everyday) was added with continuous stirring and the pH recorded after each addition.

Determination of microconstant values. UV-Vis spectrophotometric technique has been used to determine the tautomeric (k_z) and microscopic constant (k_{11}, k_{12}, k_{21} and k_{22}) values. The technique is based on the spectral differences between the zwitterions form of the molecule (found mainly in aqueous solution) and the neutral form (found mainly in methanol). Stock solutions of pure tryptophan (0.006 M) and tryptophan in the presence of SDS (2 and 10 mM) in the mixed water/methanol (0 to 100%) media have been prepared. The absorption spectra of these solutions were recorded by a SHIMADZU UV-2550 spectrophotometer in the region of 240-300 nm. Each experiment has been repeated three times.

Simulation details. For SDS and tryptophan molecules force field parameters were derived from Gromos96 [12] force field. To avoid any dependencies on initial conditions, a random initial distribution of SDS and tryptophan molecules was applied. Three systems of SDS solution were constructed. System 1 contains 8 mM SDS, system 2 contains 6 mM tryptophan, and system 3 contains 8 mM SDS + 6 mM tryptophan. The well-tested SPC/E water model is used in the simulation [13]. All MD calculations were carried out using GROMACS 4.5.4 [14]. A steepest-descent algorithm was performed to minimize the energy of each system and to relax the solvent molecules. To maintain a constant temperature and pressure for various components during simulations, the Berendsen coupling algorithm was used [15]. PME algorithm was applied for each component of the systems to estimate the electrostatic interactions [16].

RESULTS AND DISCUSSION

Depending on pH values of the aqueous solutions, tryptophan can be found in four different species, namely cation (H_2R^+), zwitterion ($HR^{\pm}$), neutral (HR^0) and anion (R^-) forms. The equilibria between these species are shown in Fig. 1. In order to calculate microconstant values, knowledge about the macroconstant values (K_{a1} and K_{a2}) is necessary. These constants are shown in Eqs. (2) and (3) [11,17,18].

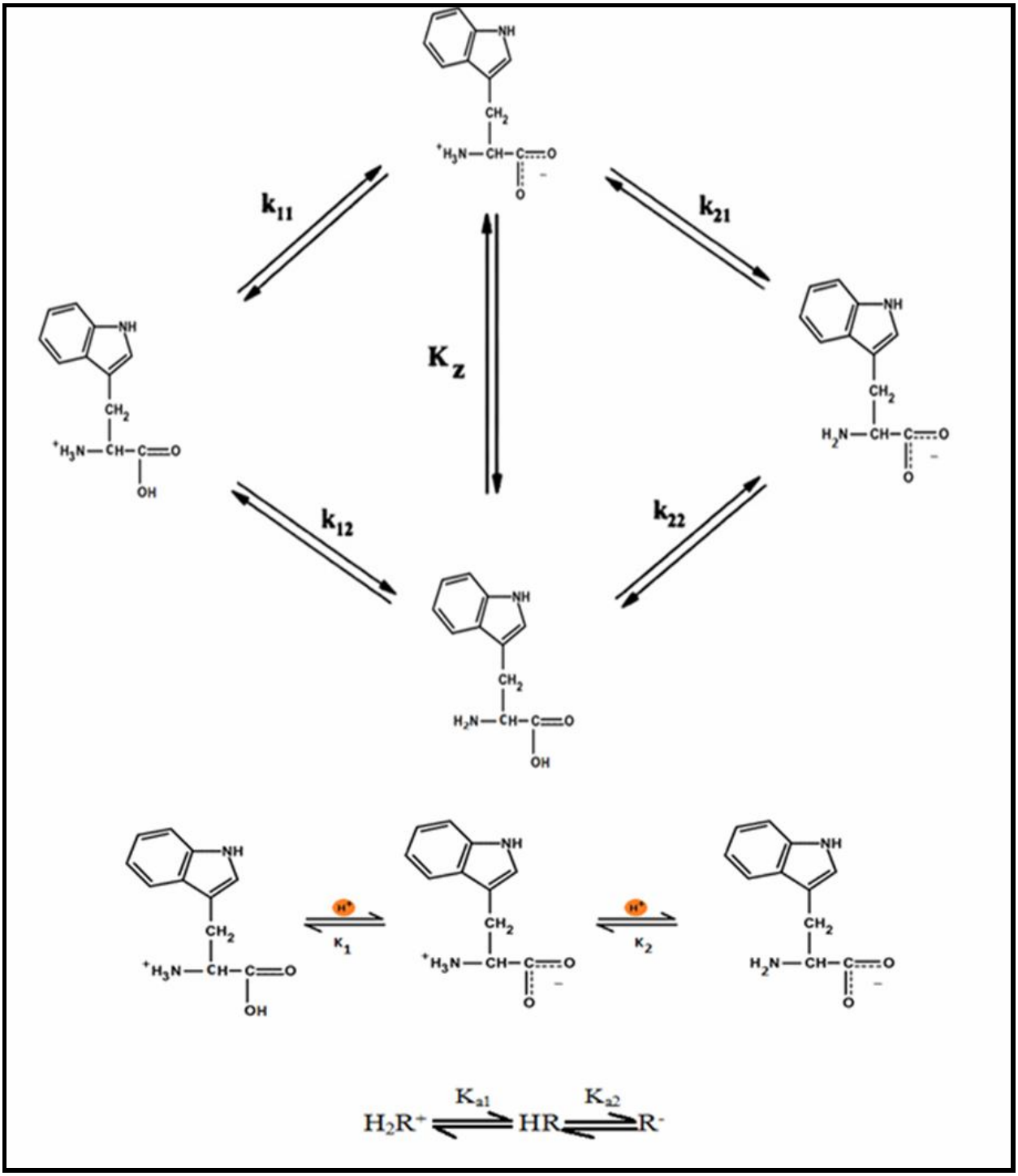

Fig. 1. Ionization equilibria of tryptophan.

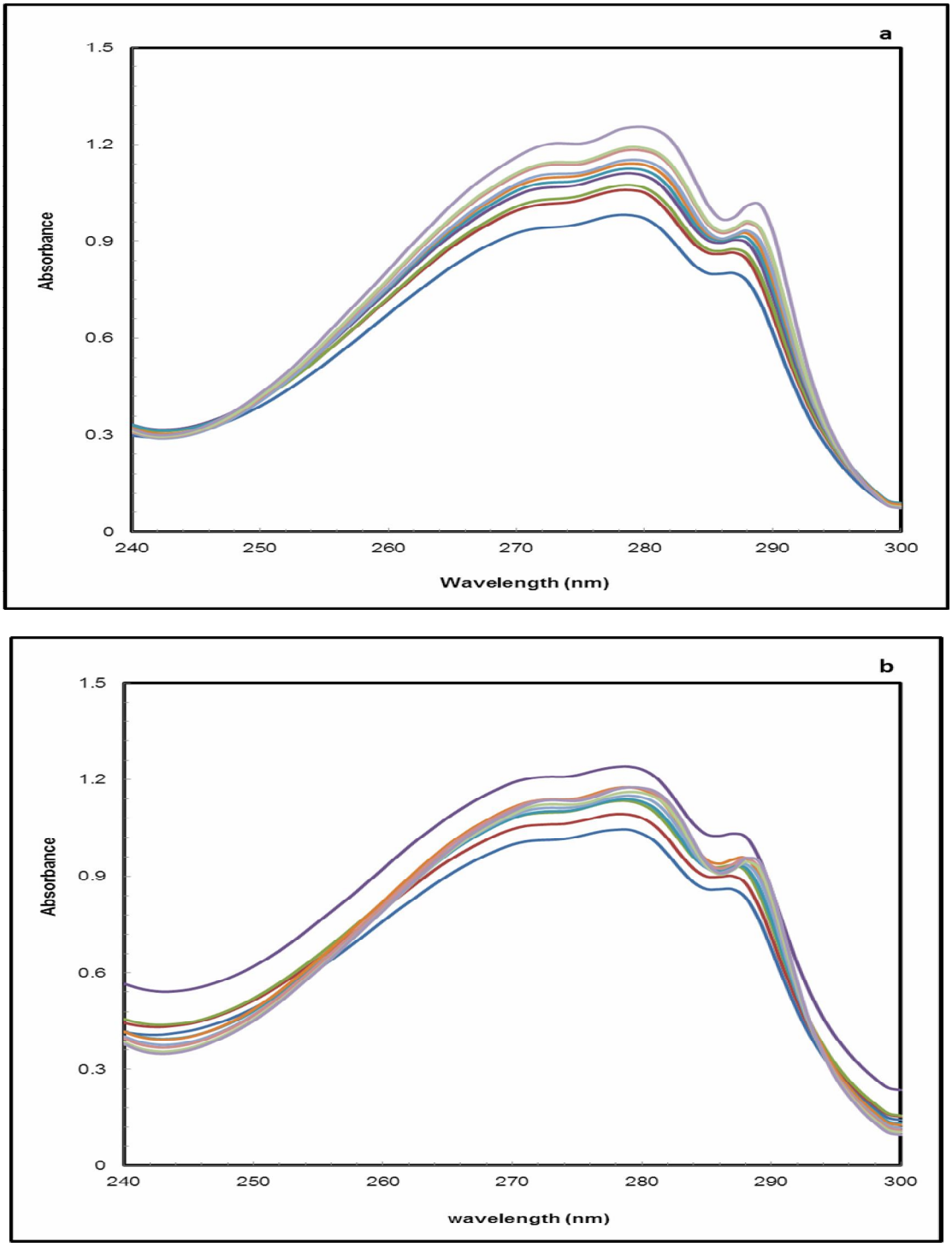

Fig. 2. Absorption spectra of solutions of tryptophan in the mixed water/methanol (0-100%) media (a) in the absence of SDS (b) in the presence of 2 mM SDS (c) in the presence of 10 Mm SDS. The methanol percentage of this solution changes from down to up (from 0 to 100%).

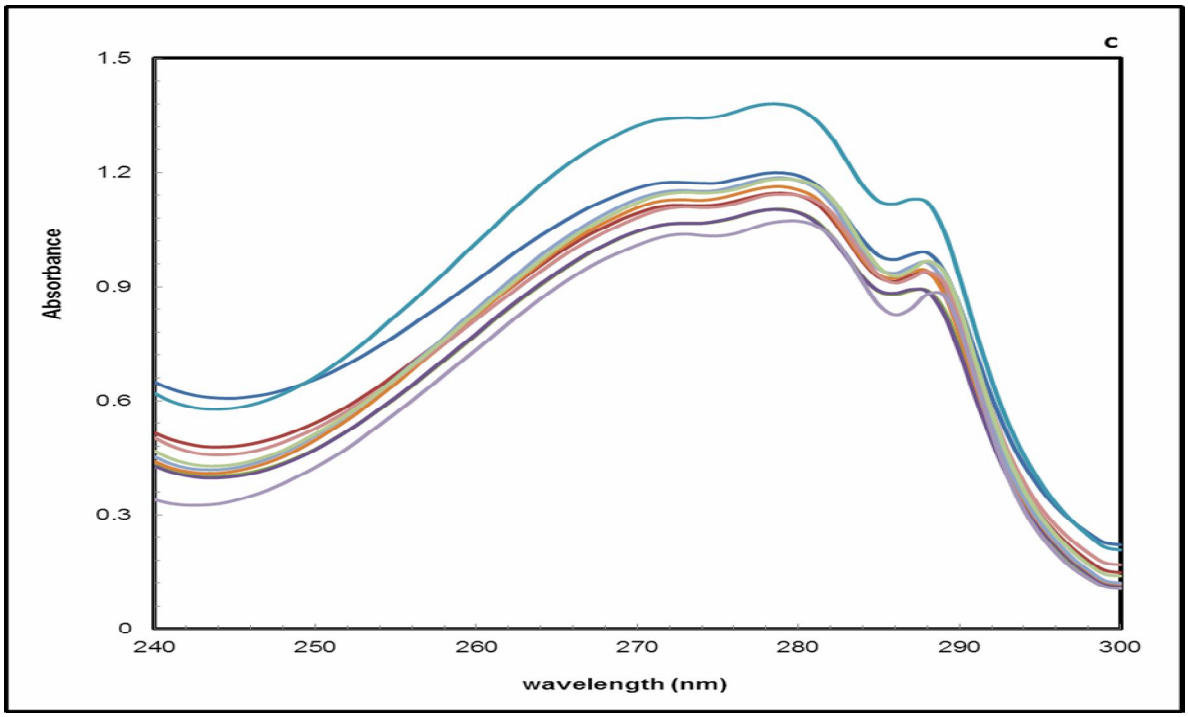

Fig. 2. Continued.

Table 1. Dissociation Constants of Tryptophan in Pure Water and at Different Concentrations of SDS at 250 °C

SDS (M)	pKa1	pKa2
0.000	2.93	9.02
0.001	2.89	8.89
0.002	2.86	8.80
0.005	2.99	8.62
0.010	3.16	7.85

$$[HR] = [HR^{\pm}] + [HR^{0}] \quad (1)$$

$$K_{a1} = \frac{([HR^{\pm}] + [HR^{0}])\{H^{+}\}}{[H_2R^{+}]} \quad (2)$$

$$K_{a2} = \frac{[R^{-}]\{H^{+}\}}{([HR^{\pm}] + [HR^{0}])} \quad (3)$$

where ([HR$^{\pm}$] + [HR^{0}]) is the total concentration of zwitterions and neutral forms, which are indiscernible by

cid-base titration, $[H_2R^+]$ and $[R^-]$ are the concentrations of cationic and anionic forms, respectively, and $\{H^+\}$ is the activity of proton. Titration of tryptophan solutions was carried out by HCl and NaOH solutions in the absence and presence of different concentrations of SDS (1, 2, 5 and 10 mM). At the half-titration point in the titration of tryptophan, the measured pH is equal to pK_a.

The pK_a values obtained by this technique for tryptophan in pure water and at different concentrations of SDS at 25 °C were shown in Table 1. The results show that the high and low concentrations of SDS have different effects on the pKa_1 values. It was shown that SDS would increase the acidity of the amino group at the low concentrations (2 mM), whereas it decreases the acidity of this group at high concentrations, at below (5 mM) and above CMC (10 mM). However, the effect of SDS on pKa_2 is similar at all concentration range, *i.e.* SDS increases the acidity of the amino group.

The relation of microconstants with microspecies concentrations can be written as follows [11,17]:

$$k_{11} = \frac{[HR^{\pm}][H^+]}{[H_2R^+]} \quad (4)$$

$$k_{12} = \frac{[HR^0][H^+]}{[H_2R^+]} \quad (5)$$

$$k_{21} = \frac{[R^-][H^+]}{[HR^{\pm}]} \quad (6)$$

$$k_{22} = \frac{[R^-][H^+]}{[HR^{\circ}]} \quad (7)$$

$$k_t = \frac{[HR^{\pm}]}{[HR^0]} \quad (8)$$

The relationships between the micro- and macroconstants have been expressed as [17-19]:

$$k_{a1} = k_{11} + k_{12} \quad (9)$$

$$\frac{1}{k_{a1}} = \frac{1}{k_{21}} + \frac{1}{k_{22}} \quad (10)$$

$$k_{a1}k_{a2} = k_{11}k_{21} = k_{12}k_{22} \quad (11)$$

$$k_1 = \frac{k_{11}}{k_{12}} = \frac{k_{22}}{k_{21}} \quad (12)$$

These equations can be solved when one of the microscopic constants is identified. Here, k_z can be found from the UV spectra of tryptophan. The spectra obtained at different methanol-water mixtures are shown in Fig. 2. Both ionizable groups show measurable shifts and the microconstants can be determined by measuring the tautomeric ratios spectrophotometrically from mixture of methanol and water. This method has been found and indicated by Takács-Novák and co-workers [20]. On this basis, the microconstant values of tryptophan were determined with the assumption that the spectrum of tryptophan in methanol is recognized as that of the neutral form HR^0 while the spectrum in aqueous solution is apportioned to that of the zwitterion ion $HR^{\pm}$.

The tautomerization microconstant can be calculated from the spectroscopic data using the relationship [17,21,22].

$$k_z(\%) = \frac{A_{HRO} - A_{\%}}{A_{\%} - A_{HR^{\pm}}} \quad (13)$$

where:

k_z (%): tautomerization constant in a given percent of solvent mixture;

$A_{\%}$: absorbance of the compound in the given percent of solvent mixture;

A_{HR0}: absorbance of the compound in the pure organic solvent;

$A_{HR\pm}$: absorbance of the compound in aqueous solution.

From the spectroscopic data k_z (%) values were calculated. The aqueous k_z value was obtained from the intercept of the following equations:

$$\log k_{z(\%)} = -1.3476\ R\ (\%) + 0.5089 \quad (14)$$

(tryptophan in the absence SDS)

$$\log k_{z(\%)} = -0.22\ R\ (\%) + 1.3 \quad (15)$$

(tryptophan in the presence of 2 mM SDS)

$$\log k_{z(\%)} = -15.51\ R\ (\%) + 1.49 \quad (16)$$

(tryptophan in the presence of 10 mM SDS)

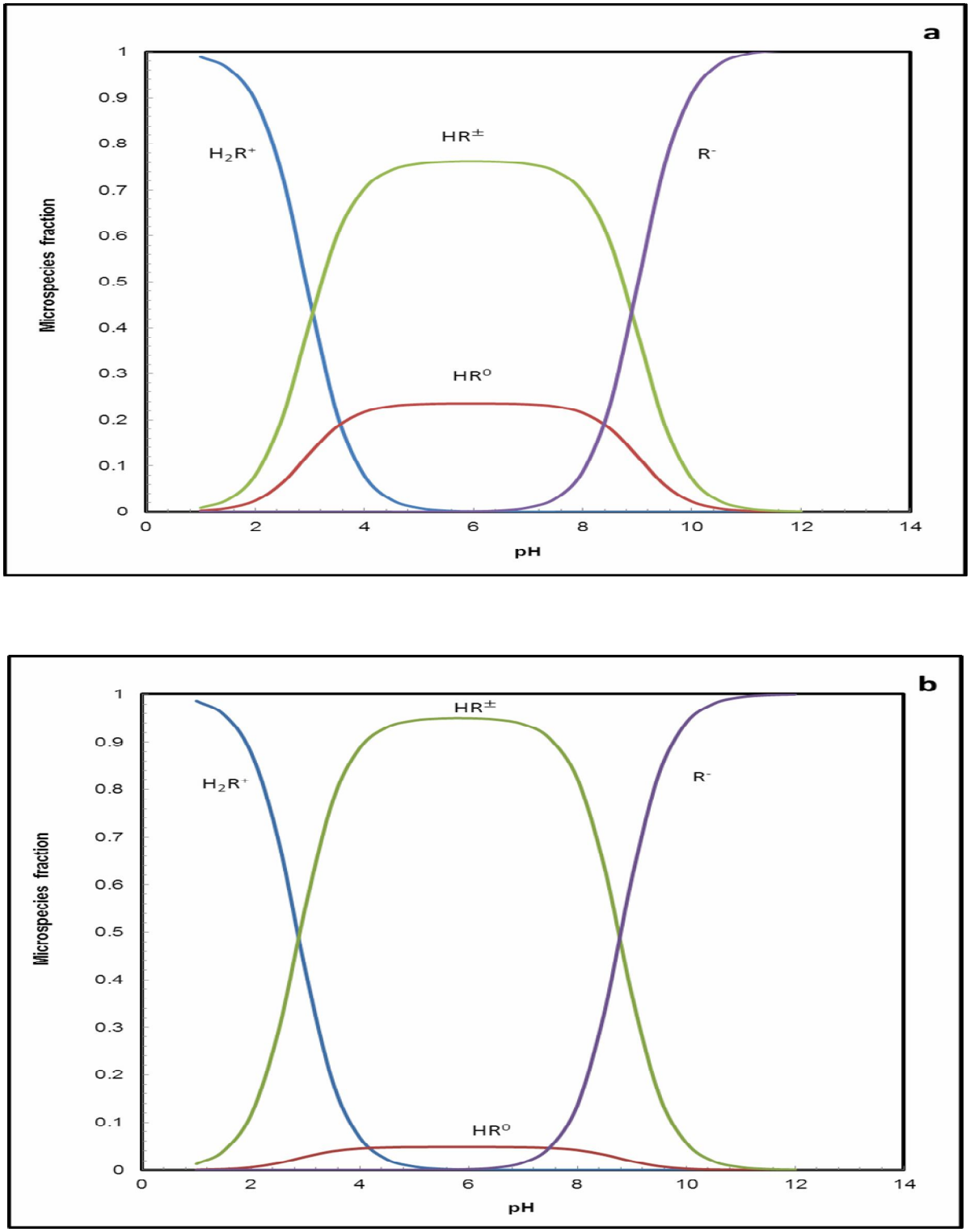

Fig. 3. Microspeciation diagram of tryptophan (a) in the absence of SDS (b) in the presence of 2 mM SDS (c) and in the presence of 10 mM SDS.

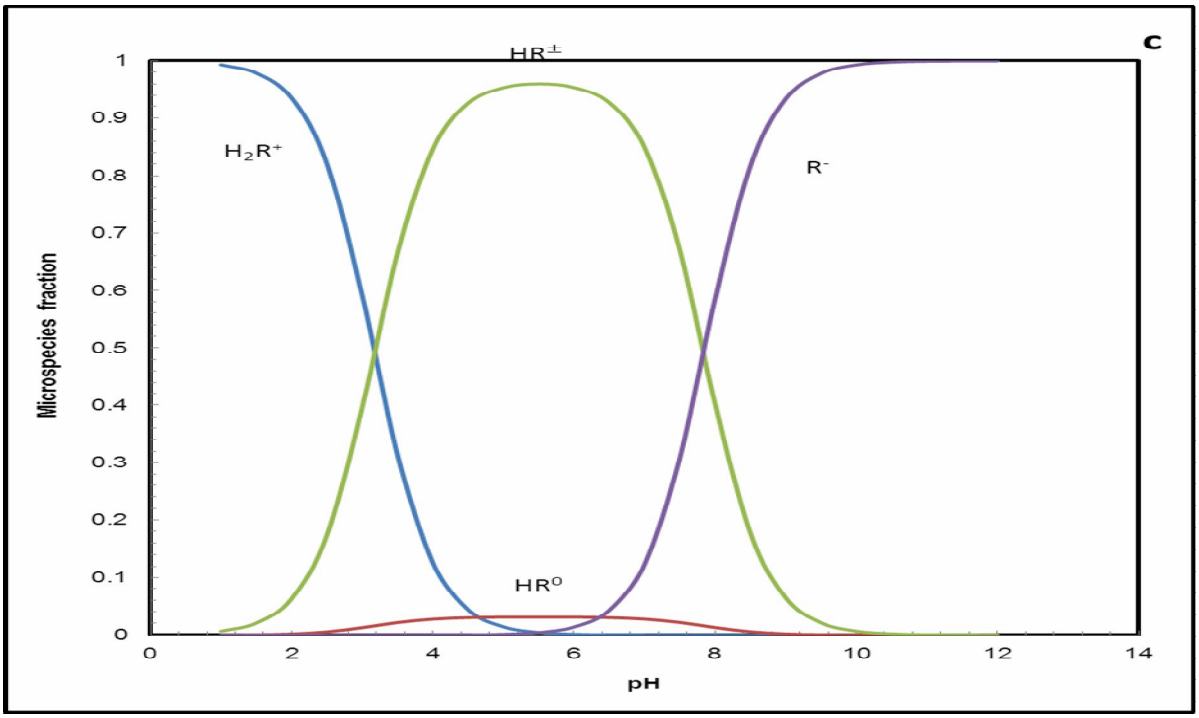

Fig. 3. Continued.

Table 2. Dissociation Macroconstants, Microconstants and Tautomeric Constants of Tryptophan in the Absence of SDS and in the Presence of 2 and 10 mM SDS

Parameter	Tryptophan	Tryptophan+SDS 2 mM	Tryptophan+SDS 10 mM
pKa1	2.93	2.86	3.16
pKa2	9.02	8.80	7.85
Kz	3.23	19.95	30.90
pk11	2.94	2.88	3.17
pk12	3.54	4.18	4.66
pk21	8.90	8.78	7.84
pk22	8.39	7.48	6.35

The obtained tautomerization k_z constants, macroconstants (K_{a1} and K_{a2}) and microconstants (k_{11}, k_{12}, k_{21} and k_{22}) values of tryptophan in the absence of SDS and in the presence of 2 and 10 mM SDS solutions were obtained that are summarized in Table 2. It can be seen that the constants related to the same equilibria (k_{11}, k_{22} or k_{12} and k_{21}) have different values. This means that the dissociation at one site causes a substantial decrease of the acidity of the other site

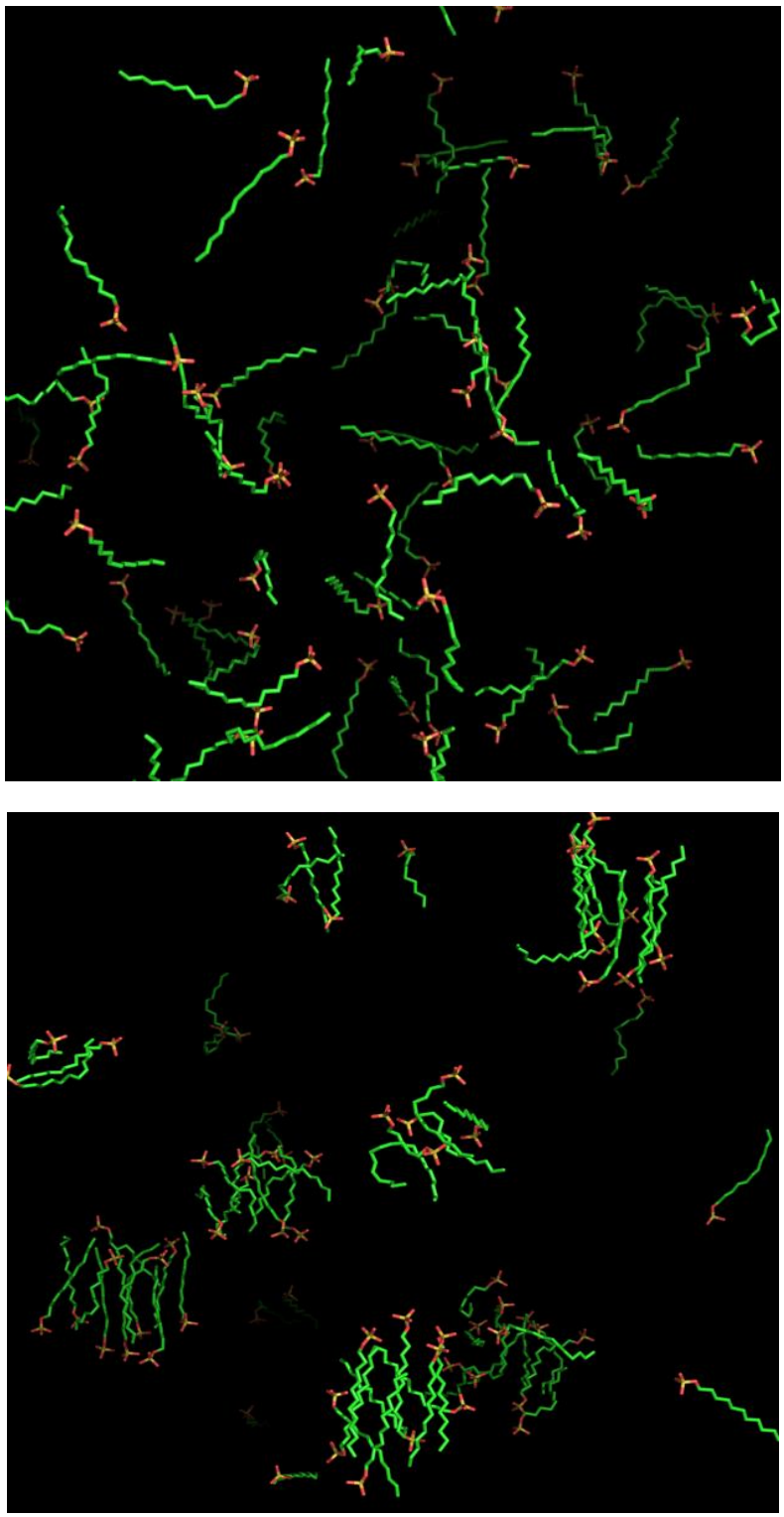

Fig. 4. Snapshots of MD simulation of SDS molecules (a) before simulation (b) after simulation.

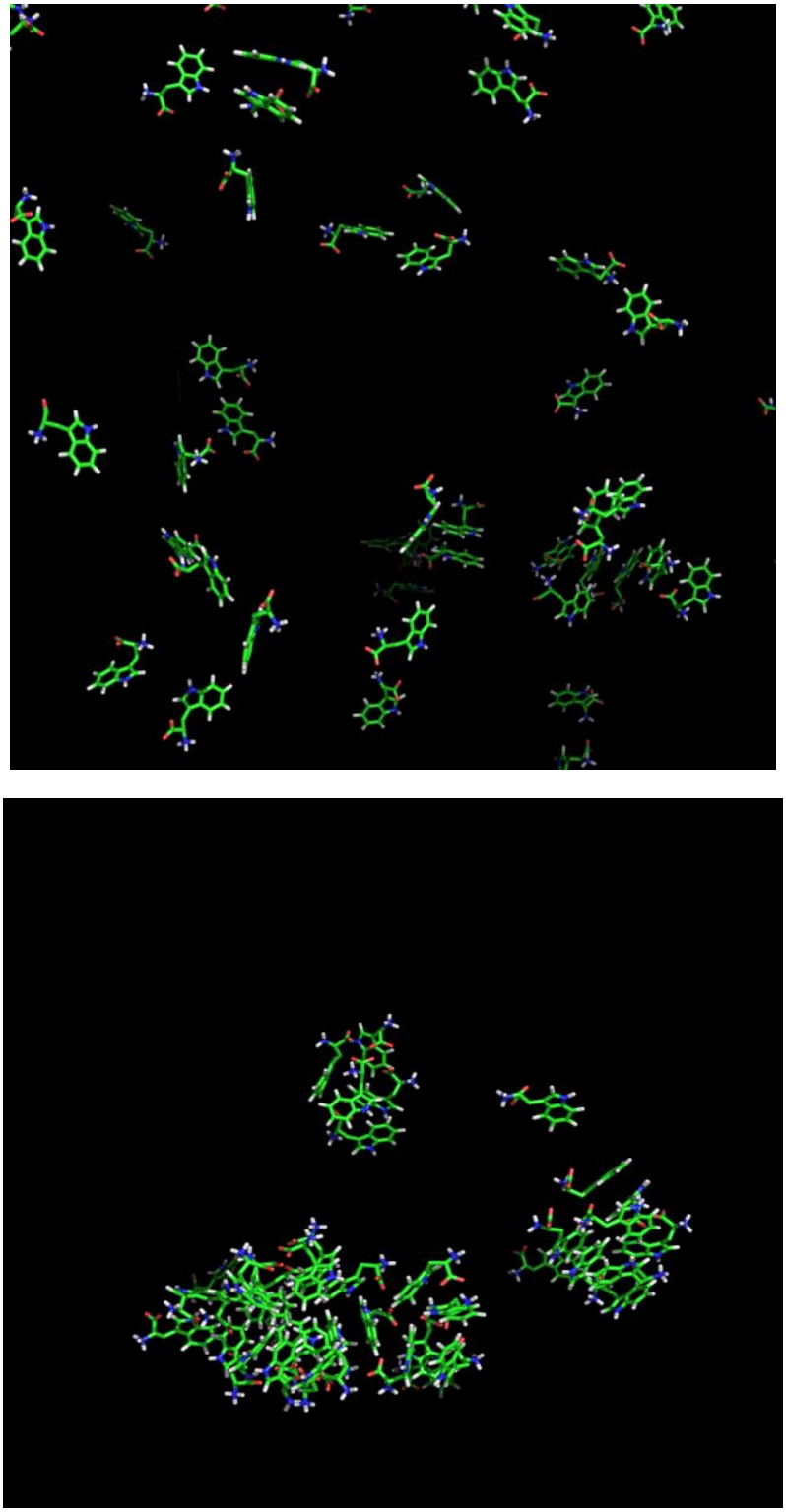

Fig. 5. Snapshots of MD simulation of tryptophan molecules (a) before simulation (b) after simulation.

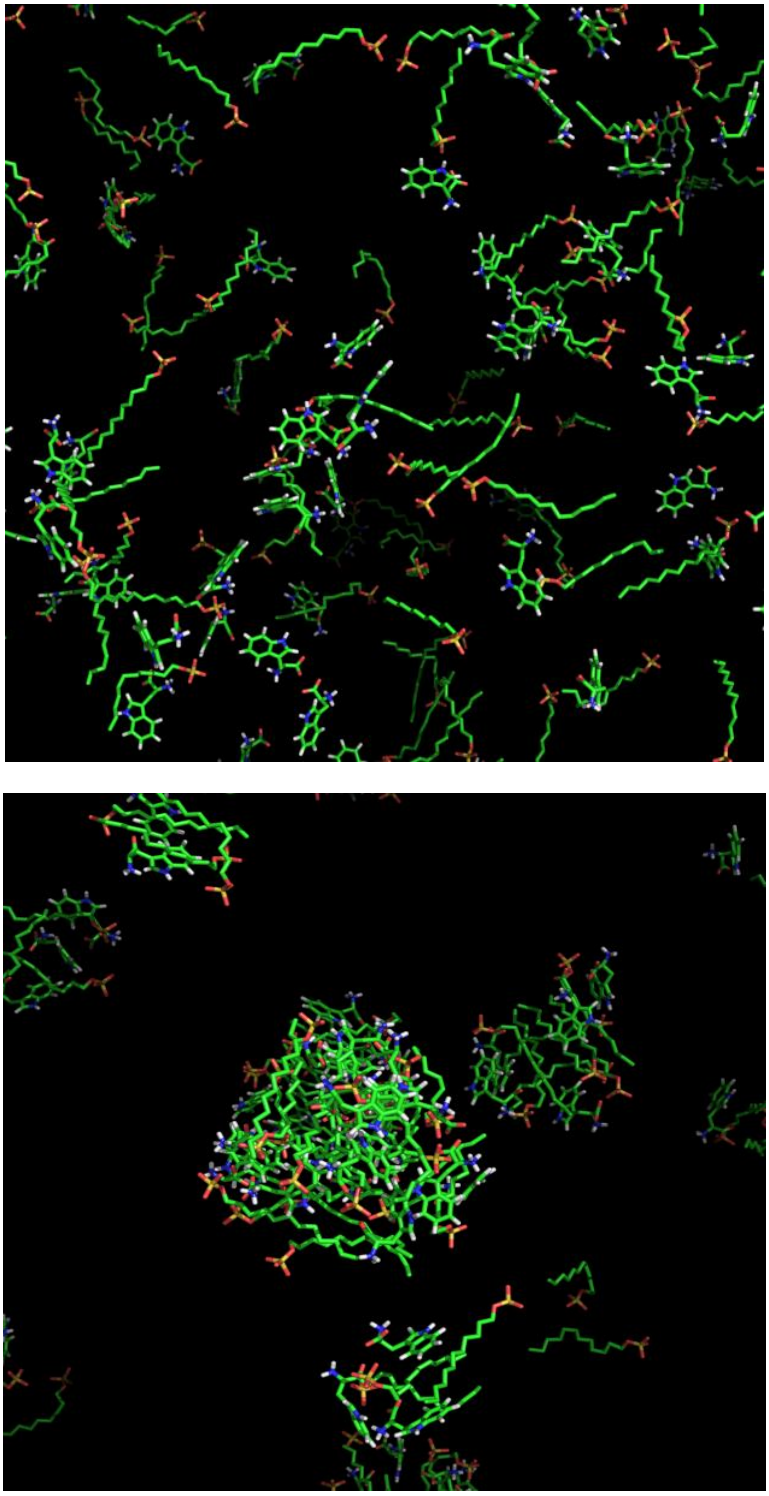

Fig. 6. Snapshots of MD simulation of the mixture of SDS and tryptophan molecules (a) before simulation (b) after simulation.

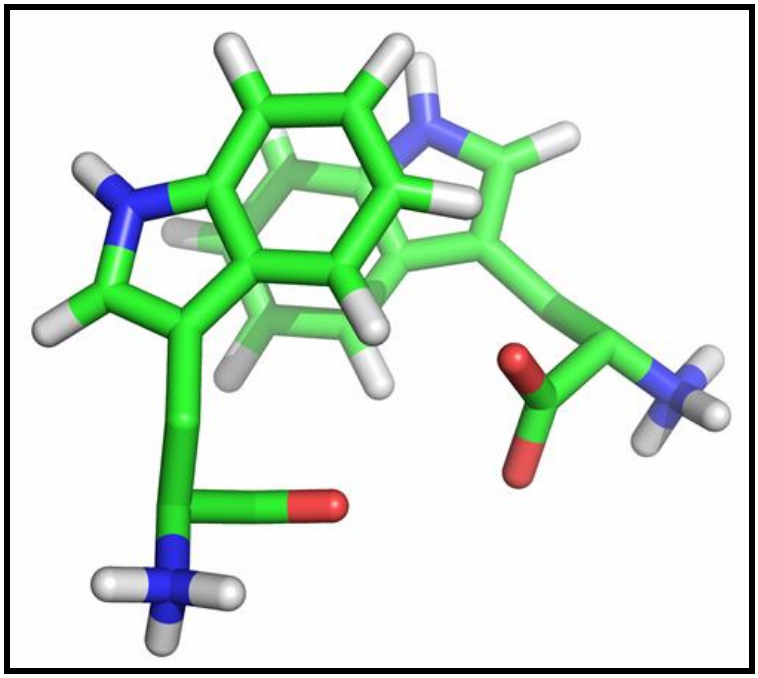

Fig. 7. Interaction between two tryptophan molecules after MD simulation.

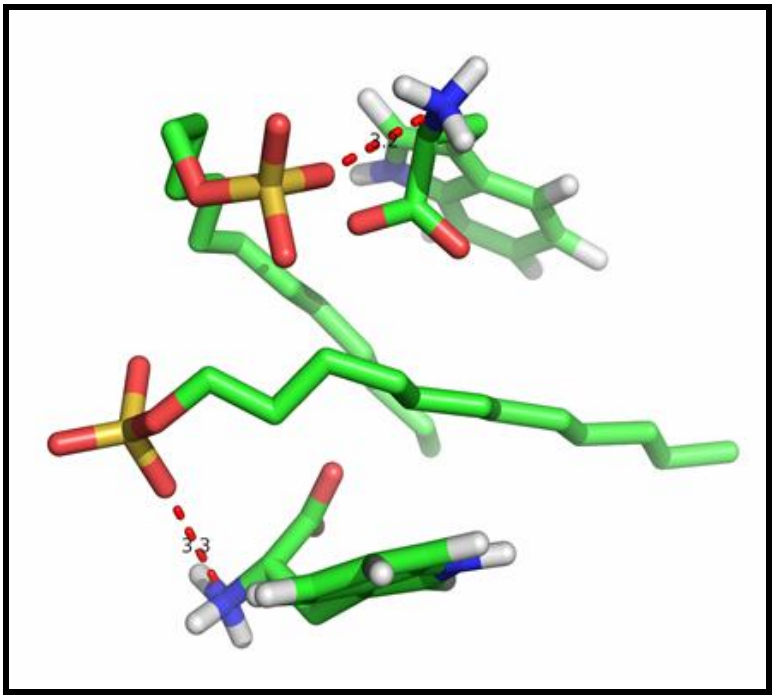

Fig. 8. Hydrophobic and hydrogen bonding interaction between SDS and tryptophan after MD simulation.

($pk_{11} < pk_{22}$; and $pk_{12} < pk_{21}$) [10,17].

The distribution diagrams for the four microspecies of tryptophan were also obtained based on the values of macro and microconstants. As show in Fig. 3, the cationic form (H_2R^+) dominates at the pH < 3.0. In the pH interlude of 4.0-8.0 the principal form is the zwitterions $HR^{\pm}$ that preponderates over the neutral species HR^0 and both reach a maximum at pH 6. At the pH higher than 6, the mole fractions of the species $HR^{\pm}$ and HR^0 decrease while the mole fraction of the negative species R^- increases and reaches to the value of 1 at pH ~11.

The changes in the pKa values of tryptophan in the presence and absence of SDS molecules can be related to the stabilization and destabilization effects of the surfactant. After adding SDS to the amino acid solution K_z is elevated (Table 2). The product of this equilibrium is a charged molecule and presence of SDS as charged molecule and also sodium ions can stabilize these molecules. pK_{11} remained unchanged in low concentration of SDS but is increased at high concentrations. This is due to the higher solubility of cation in comparison to zwitterionic form. Cationic form can interact strongly with SDS through electrostatic interactions in concentration above CMC in which micelles have negative charge. Increasing of pK_{12} also confirms that cationic form is stabilized by adding SDS. pK_{21} remained constant in low concentration of SDS, but this parameter is reduced at higher concentrations (Table 2). This shows that presence of sodium ions and also SDS molecules increases the ionic strength leading to stabilization and solubility of the charged forms. Reduction of pK_{22} also confirms the higher solubility of anion in comparison to neutral form.

Molecular modeling has been used for better understanding of molecular situation of these different systems. MD simulation is carried out in three different states: SDS solution, tryptophan solution, and SDS-tryptophan. The concentrations were selected based on the experimental conditions. The situations of the solution component before and after MD simulation are graphically shown in Fig. 4-6. It is shown that some aggregates are formed in SDS and tryptophan solutions during simulation, while micellar-like aggregates with larger size is obtained in the mixture of SDS and tryptophan. We found that tryptophan molecules predominately interact with each other through stacking of their rings. One example structure is shown in Fig. 7. SDS molecules disintegrate these stacks by hydrophobic interaction of their hydrocarbon chain and also by hydrogen bonding (Fig. 8). We can conclude that acid dissociation constants of the tryptophan molecules in the presence of SDS molecules change by three different mechanisms: 1) changes in the solubility and stability of ions by different ionic strength 2) hydrophobic interactions with SDS and 3) hydrogen bonding.

CONCLUSIONS

The present work investigates the effects of anionic surfactant SDS on the acid dissociation equilibria of the amino acid tryptophan. The macroconstant values (K_{a1} and K_{a2}), tautomerization constants (k_z) and microconstant values (k_{11}, k_{12}, k_{21} and k_{22}) of the tryptophan in the absence or the presence of different concentrations of SDS were determined. It was indicated that the potentiometric method is acceptable for determination of macroconstants values (K_{a1} and K_{a2}). Furthermore, the application of the UV-Vis analysis permitted to determine tautomerization constants (k_z) and micro constant values (k_{11}, k_{12}, k_{21} and k_{22}). In addition, the distribution diagrams for the four microspecies of tryptophan in aqueous solution and at different concentrations of SDS are plotted. Experimental observations showed that presence of SDS can alter the values of equilibrium constants. Molecular dynamic simulation was used to explain the effects of SDS on the equilibrium constants. Simulations showed that SDS can interact with tryptophan molecules through hydrogen bonding and hydrophobic interaction which leads to changes in equilibrium constants.

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