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SHORT COMMUNICATION

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# Influences of Triton X-100 micelle on the microstructure of HSA

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**Abstract** The effects of Triton X-100 molecule and micelle on the microstructure and properties of HSA are studied by the some methods of UV spectrum, fluorescence spectrum, fluorescence polarization, circular dichroism, conductivity, and zeta potential. With the increase of Triton X-100 concentration, the UV absorbance, fluorescence intensity of HSA, and the system conductivity all first decrease and then increase. The zeta potential of HSA first goes up and then down. The percents of the β-sheet, random, turn structures, and the polarization of HSA increase but the percent of the α-Helix of HSA decreases. When Triton X-100 concentration is more than $1.0\times10^{-3}$ mol $l^{-1}$, the structure parameters change obviously especially for the percents of random and turn structures.

**Keywords** Influence · Micelle · Microstructure · HAS · Triton X-100

## Introduction

Human serum albumin (HSA) plays a special role in transporting metabolites and drug throughout the vascular system. It has a single peptide chain with 585 amino acids, possessing a single indole ring at tryptophan-214. It comprises three contiguous domains, each containing two subdomains (A and B) that possess common structural motifs by various intra- and inter-domain forces such as salt bridges, hydrophobic interactions [1–3]. More surfactants can interact with some proteins and affect the protein properties [4–9]. However, there is little report about the effects of surfactant on protein microstructure. In this paper, we study the effects of Triton X-100 molecule and micelle on the microstructures and properties of HSA by the methods of fluorescence spectrum, UV–Vis spectrum, circular dichroism, zeta potential and conductivity. These help to further probe into the interactive mechanism of surfactant with protein and the influences of surfactant colloids or other ligands on the interaction behaviors of surfactant with protein. The investigation may also provide some important theoretic information for life science, biologic technology, medicament application, and colloid science.

## Experiment

### Materials

Triton X-100 was bought from Aldrich (99+%, Milwaukee, WI, USA), human serum albumin from Shanghai Institute of Biology Produce (HSA, B.R.,). Doubly distilled and deionized water was used for the preparation of the solutions.

### Fluorescence spectrum determination

The fluorescence emission spectrum and the synchronous fluorescence spectrum of HSA all were determined by RF-5301 Fluorescence Spectrometer (Shimadzu, Japan) at

excitation wavelength, 298 nm. The slot widths of the excitation and the emission all were 3 nm.

### Fluorescence polarization determination

The fluorescence polarization $P$ of a protein deals with its microenvironment. The polarization can be calculated from the following equation [10]:

$$P = \frac{F_{//} - F_{\perp}}{F_{//} + F_{\perp}}, \quad (1)$$

where, $F_{\parallel}$ and $F_{\perp}$ are the fluorescence intensities of polarized components while parallel and mutually perpendicular between polarizer and checking polarimeter, respectively.

### UV–Vis spectrum determination

The UV–Vis absorption spectrum of HSA was determined by UV-2550PC UV–Vis Spectrum Spectrometer (Shimadzu, Japan) in different systems. The intrinsic absorbance of HSA is at 278 nm. The scope of the scanning wavelengh was from 650 to 190 nm.

### Circular dichroism measurement

Circular dichroism (CD) measurement was carried out with Jasco-810 Spectropolarimeter (Japan). The scanning rate was set at 100 nm/min. The helicities of protein have been frequently discussed on the basis of changes of $[\theta]_{222}$. The α-helicity, β-sheet, random and turn structures were estimated by the curve-fitting method of the CD spectrum.

### Zeta potential of protein determination

The protein electrophoresis can be taken on under an electric field. The zeta potential of HSA was measured from the electrophoresis by Js-94F Microelectrophoresis Meter (Shanghai Zhongcheng Instrument, China). The principle of the zeta potential measurement is similar to that of [11]. The measured voltage was 10~30 V, the stabilizing time 5 min, the measuring interval 50 s. The measured result obtained was the average of five replicates. The measured difference was less than ±0.2 mV.

### Conductivity determination

System conductivity was determined by DDS-11A Conductormeter (Shanghai No.2 Analytical Instrument, China). The calibration of the conductormeter had been made by 0.01 mol $l^{-1}$ KCl solution.

All of the above measurements were carried out at 25±0.1 °C. All the solutions with protein had to be placed at least 30 min for the interaction equilibrium.

## Results and discussions

### Effects of Triton X-100 on HSA fluorescence spectrum

The fluorescence of HSA can be quenched with Triton X-100 at low Triton X-100 concentration (Fig. 1). But when Triton X-100 concentration increases to $5\times10^{-6}$ mol $l^{-1}$, the fluorescence intensity of HSA also increases. Triton X-100 can associate with HSA or be adsorbed on the HSA chain by the strong interaction of hydrogen bonding and hydrophilic action. The association changes the microenvironment of the fluorescence groups in HSA molecule. The fluorescence groups may be shielded partially by Triton X-100 and causes the fluorescence intensity to decrease. On the other hand, with the association, the conformation of HSA changes also at the same time. The exposure of the amino acids enhances the fluorescence intensity. The increases of the system viscosity and the HSA rigidity all lead to the enhancement of fluorescence intensity.

With the increase of Triton X-100 concentration, the Triton X-100 molecule number adsorbed on the HSA chain increases. And then the Triton X-100 admicelle is formed [12] on the HSA chain. The HSA rigidity and viscosity further increase. The fluorescence intensity increases. When Triton X-100 concentration increases to about its first critical concentration ($3.0\times10^{-4}$) [13], the micelle is formed in the solution. The system viscosity, the HSA rigidity, and the fluorescence intensity increase continuously. When Triton X-100 concentration is more than the second critical concentration ($1.2\times10^{-3}$) [13], the fluorescence intensity increases rapidly. Here, the microstructure behaviors of HSA change markedly. HSA may begin to denature obviously.

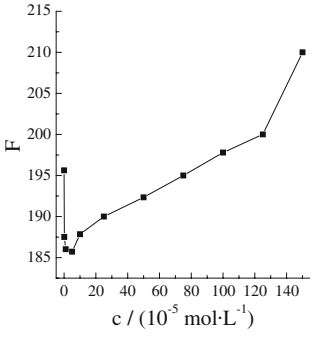



**Fig. 1** Influence of Triton X-100 on the intrinsic fluorescence intensity of HSA. HSA concentration is $5\times10^{-6}$ mol $l^{-1}$

Both the fluorescence intensity of Triton X-100 (excitation at 292 nm) and the fluorescence polarization of HSA increase with the increase of Triton X-100 concentration. In the synchronous fluorescence of HSA, the fluorescence spectrum at 373 nm for $\Delta\lambda$=40 nm increases slowly but that at 392 nm for $\Delta\lambda$=60 nm increases rapidly. This may be the reason that the number of tyrosine (Tyr) residue groups is high in the peptide chain of HSA (19 Tyrs).

## Effects of Triton X-100 on UV–vis spectrum of HSA

The intrinsic UV–vis absorption peak of HSA at 278 nm is mainly aroused by tryptophan and tyrosine in the peptides of HSA. With the increase of Triton X-100 concentration, the intrinsic peak enhances (Fig. 2). The increase of Triton X-100 molecular number adsorbed on the HSA chain makes the HSA chain unfold. The residuals of the amino acids in HSA may be exposed. When Triton X-100 concentration is more than $1.2\times10^{-3}$ mol $l^{-1}$, the intrinsic peak increases rapidly. This indicated that the microstructure behaviors of HSA changed markedly.

## Energy transferring efficiency between HSA and Triton X-100

Comparing the absorption spectrum of Triton X-100 with the fluorescence emission spectrum of HSA in low Triton X-100 concentration, it is obvious that the two spectra overlap mutually (Fig. 3). This indicates that the non-radiant energy transfer takes place in the quenching process of Triton X-100 to HSA.

According to the Föster theory about non-radiant energy transfer, the quenching extent of fluorescence substance lies on the distance between the fluorescence substance (energy deliverer) and the quencher (energy acceptor). If Triton X-100 associates with HSA in sequence, the association site and the distance can be calculated from the Föster theory [14, 15].

$$E = \frac{R_0^6}{R_0^6 + r^6} \tag{2}$$

$$E = 1 - F/F_0 \tag{3}$$

$$R_0^6 = 8.8 \times 10^{-25} JK^2 \varphi n^{-4} \tag{4}$$

$$J = \frac{\int_0^\infty F(\lambda)\varepsilon\lambda^4 d\lambda}{\int_0^\infty F(\lambda) d\lambda} \tag{5}$$

Here, $E$ is the energy transferring efficiency of non-radiancy. $R_0$ is the corresponding distance when $E$ is equal to 50%. The tropism factor is assumed to be the average value (2/3) of the isotropy for the deliverer (Trp) and the acceptor (Triton X-100). $\varphi$ is chosen as 0.14, which takes

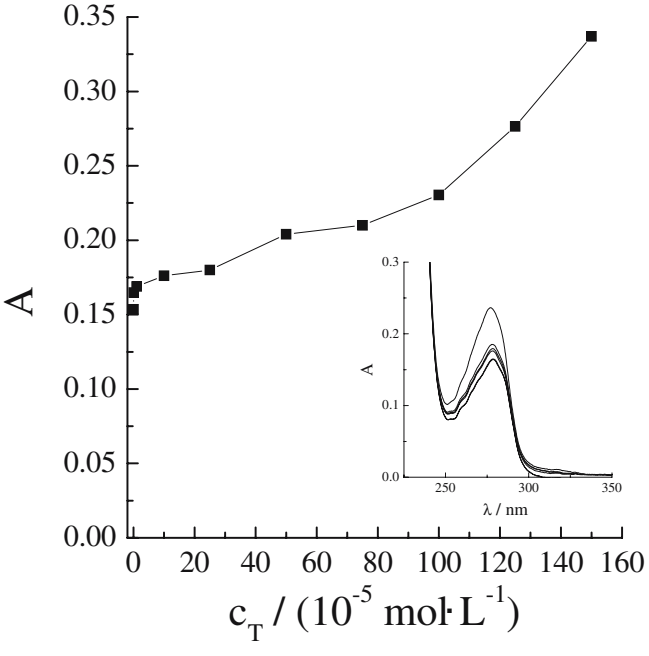



**Fig. 2** Effect of Triton X-100 concentration on the intrinsic UV–Vis absorption spectrum of HSA (at 278 nm). HSA concentration is $5\times10^{-6}$ mol $l^{-1}$

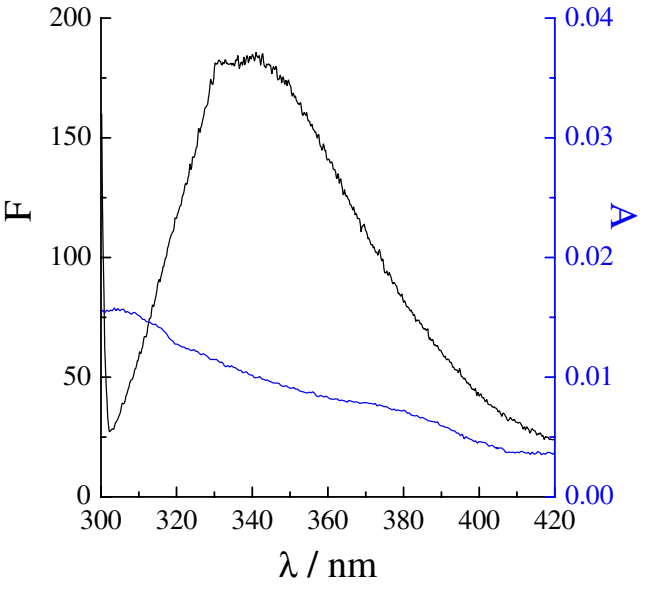



**Fig. 3** Absorption spectrum of Triton X-100 and fluorescence spectrum of HSA. HSA and Triton X-100 concentrations are $5\times10^{-6}$ mol $l^{-1}$, respectively

the tryptophan as standard substance. The refractive index is adopted as the average value ($n$=1.36) of water's and organic matter's refractive indexes. $J$ is the overlapping integral of the two spectra.

According to the overlapping part of the absorption spectrum of Triton X-100 with the fluorescence spectrum of HSA, Eq. 5 is applied to calculate the overlapping integral, $J_{HSA}=0.826\times10^{-15}$ cm$^6$ (mol l$^{-1}$). $R_0$=1.645 nm is from Eq. 4. The energy transferring efficiency is 0.067 from Eq. 3 while 214-tryptophan residue associates first with Triton X-100. So, the distance $r$ between the first associating site and 214-tryptophan residue is about 2.51 nm.

## Effects of Triton X-100 on microstructure parameters of HSA

Figure 4 shows effects of Triton X-100 concentration on the microstructure parameters of HSA. With the increase of Triton X-100 concentration, the percents of the β-sheet, random, turn structures, and the polarization of HSA increase, but the percent of the α-helix of HSA decreases (Fig. 4). The formation of the Triton X-100 sphere micelle further makes the α-helix content decrease, but the β-sheet content increases. The contents of random, turn structures do not change obviously. This indicates that Triton X-100 micelle can cause the α-helix to transform to β-sheet. When Triton X-100 concentration is more than the $1.0\times10^{-3}$ mol l$^{-1}$, the structure parameters change markedly especially for the percents of random and turn structures. This result makes known that the protein structure comes markedly forth to transform in high Triton X-100 concentration. The protein is evidently denatured.

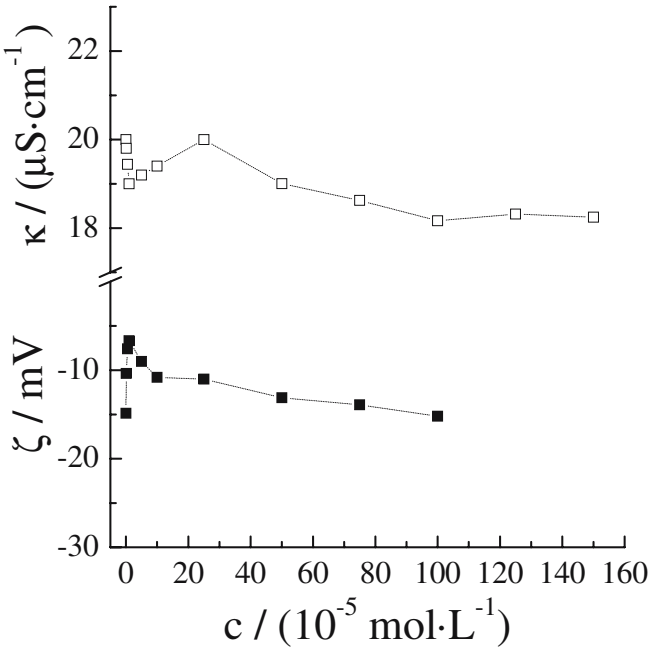
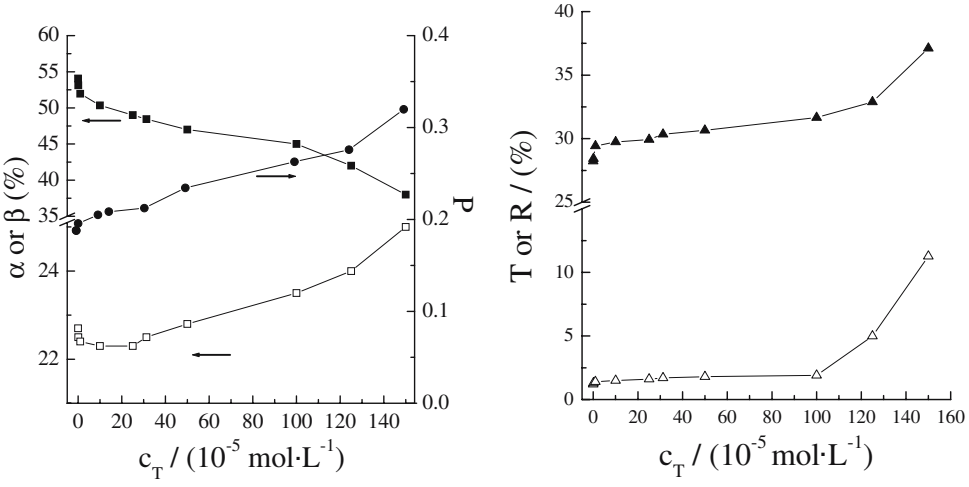



**Fig. 5** Zeta potential of HSA and system conductivity vary with Triton X-100 concentration. HSA concentration is $5\times10^{-6}$ mol l$^{-1}$. ■ Zeta potential of HSA; □ system conductivity

## Effects of Triton X-100 on electric property of HSA

The isoelectric point of HSA is 5.8 [16]. The HSA charge displays negative in neutral solution. With the increase of Triton X-100 concentration, the zeta potential of HSA first goes up and then down. The system conductivity first goes down and then up (Fig. 5). The association of HSA with Triton X-100 and the adsorption of Triton X-100 on the

**Fig. 4** Effects of Triton X-100 concentration on the structure parameters of HSA. HSA concentration is $5\times10^{-6}$ mol l$^{-1}$. ■ α-Helix; □ β-sheet; ● polarization; ▲ random; △ Turn



HSA chain all bring the apparent HSA volume and the system viscosity to enlarge. The surface charge of HSA is partially shielded by Triton X-100 molecule or micelle. So, the surface charge density of HSA reduces. The zeta potential goes up and the conductivity falls. On the other hand, the addition of Triton X-100 makes the system dielectric constant decrease. This also results in the system conductivity decrease. When Triton X-100 concentration is more than the second critical concentration ($1.3\times10^{-3}$ mol $l^{-1}$), HSA is markedly denatured. The image of HSA does not gain. So, the zeta potential does not appear in Fig. 4.

## Conclusion

Triton X-100 can obviously affect on the microstructure of HSA. The fluorescence of HSA can be quenched with Triton X-100 in low Triton X-100 concentration. The distance between the first associating site of Triton X-100 with 214-tryptophan residue in HSA is about 2.51 nm. The effects of Triton X-100 micelle on the HSA microstructure in high Triton X-100 concentration is more than those in low Triton X-100 concentration. The structure comes markedly forth to transform and indeed the protein is evidently denatured in high Triton X-100 concentration.

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