Investigation on the Effect of Protein on the Properties of Bis(2-ethylhexyl) Sulfosuccinate/Isooctane Reverse Micelles

Mingtan Hai*,† and Feng Kong‡

School of Material Science and Engineering, University of Science and Technology Beijing, Beijing 100083, PRC, and Grikin Advanced Materials Co., Ltd., Beijing, PRC

The diameter of water-in-oil reverse micelles formed by sodium bis(2-ethylhexyl) sulfosuccinate (AOT) with water and with protein Bovine serum alumin (BSA) was investigated by dynamic light scattering (DLS) measurement of AOT/isooctane solution at 298.15 K. The diameter and viscosity of AOT reverse micelle increases with increasing ω_0 (mole ratio of water to surfactant: $\omega_0 = n_w/n_s$). The viscosity of AOT/isooctane with water or BSA inside did not change with shear rate, exhibiting Newtonian behavior. For $\omega_0 = 8.3$, the diameter of AOT reverse micelles increases with increasing BSA concentration from 2 mg of BSA·mL^{-1} of H_2O to 145 mg of BSA·mL^{-1} of H_2O. The diameter of AOT reverse micelles increases only slightly with BSA inside compared with water inside at the same ω_0. Incorporation of protein into the micelles does not affect their mean diameter significantly. The hydrodynamic diameter of BSA from 10 mg to 100 mg of BSA·mL^{-1} of H_2O in 0.1 mol·L^{-1} AOT reverse micelles increased almost linearly with ω_0 which confirmed that the diffusion coefficient was independent of ω_0, indicating a spherical structure for the AOT reverse micelles. The localization of BSA is in the center part of the water pool without perturbation of the reverse micelles. For highly concentrated BSA from 50 mg to 145 mg·mL^{-1} of water, only when ω_0 is higher than 5.6, the 0.1 mol·L^{-1} AOT reverse micelles can solubilize it.

Introduction

Reverse micelles are spherical aggregates made of hydrophilic molecules that self-organize with hydrocarbon chains facing the organic solvent and polar head groups pointing inward.[1] Reverse micelles have the ability to solubilize a relatively large amount of water or water-soluble solutes in the polar core to form a nanometer-sized waterpool. The system composed by the anionic surfactant sodium bis(2-ethylhexyl) sulfosuccinate (AOT) and the solvent isooctane (2,2,4-trimethylpentane) is the most commonly used due to its high capacity to solubilize water.[2] The diameter of the reverse micelle is proportional to the molar ratio of water-to-surfactant generally defined as ω_0 ($\omega_0 = n_w/n_s$). Reverse micelle solutions have been studied extensively for potential application to protein separation, enzyme reaction, and nanoparticle preparation. Reversed micelle size is a very important parameter to determine protein selectivity, enzyme activity, and particle size of synthesized nanomaterials.

Several studies addressing the mechanism of protein solubilization in reverse micelles have been reported. Three essential questions were raised by Luisi et al.:[1] (i) what are the driving forces for the protein solubilization; (ii) what is the localization of proteins in the reverse micelle; and (iii) what size or shape perturbation is induced in the reverse micellar droplets by the protein solubilization.

Since the pioneering work of Bonner et al.,[3] where the protein molecule was immersed in the water pool surrounded by a water-shell (water-shell model), these issues have been addressed. Experimental data from Levashov et al.[4] indicated no significant effect on the size of the reverse micelles upon solubilization of the proteins α-chymotrypsin, lysozyme, trypsin, egg albumin, horse liver alcohol dehydrogenase, and γ-globulin (fixed size model) except when the inner cavity of the micelle was smaller than the effective protein size (induced fit model). This contradicts the data obtained by Zampieri et al.,[5] where an increase of the dimensions of the micelles upon solubilization of α-chymotrypsin, lysozyme, and myelin basic protein had been determined. Geometrical considerations and experimental data have led to envisage two situations:[6,3] α-chymotrypsin is mainly in the water pool causing an equivalent increase in the water pool volume (increased radius), and cytochrome c is mainly located at the surfactant interface causing a decrease in the water pool radius. The localization of protein and size perturbation in reverse micelles is very important for protein and enzyme delivery from reverse micelles to water-in-oil emulsion.

The purpose of this work is to present size measurements by dynamic light scattering (DLS) to have insight on the localization of BSA (Bovine serum alumin) (model protein) encapsulation and size perturbation in AOT reverse micelles. The effect of BSA concentration, ω_0, and AOT concentration on the size and solubilization of AOT reverse micelles is emphasized and discussed. The viscosity and interfacial tension of AOT/isooctane reverse micelles with water or protein have been measured and discussed.

Experimental

Materials. Sodium bis(2-ethylhexyl) sulfosuccinate (AOT, solid) and BSA (Bovine serum alumin) were purchased from Sigma. 2,2,4-Trimethylpentane (isooctane) (99 %, spectroscopic grade) was purchased from Fluka. All reagents were used as received. The water used was highly purified dispersant water.

Solution Preparation. An appropriate volume of dispersant water or x mg of BSA·mL^{-1} of water (from 2 mg of BSA·mL^{-1} of water to 200 mg of BSA·mL^{-1} of water) was

* Corresponding author. E-mail: mingtanhai@mater.ustb.edu.cn.
† University of Science and Technology Beijing.
‡ Grikin Advanced Materials Co., Ltd.

10.1021/je700625m CCC: $40.75
Published on Web 02/09/2008

Table 1. Viscosity (η/mPa·s) of BSA in 0.1 mol·L^{-1} AOT/Isooctane Reverse Micelle Solutions at 298 K

	η/mPa·s			
ω_0	10 mg of BSA·mL^{-1} of H_2O	20 mg of BSA·mL^{-1} of H_2O	50 mg of BSA·mL^{-1} of H_2O	100 mg of BSA·mL^{-1} of H_2O
2.8	0.54		opaque	opaque
5.6				0.55
6.9				0.56
8.3	0.58	0.57	0.57	0.57
11.1				0.57
13.9	0.61	0.62	0.59	0.68
22.2	0.64			0.61
27.7	0.67	0.69	0.65	0.63
38.9	0.72	0.75	0.71	sedimentation
50.0	0.78	0.81	0.75	
55.6	0.80		sedimentation	
61.1	0.83	0.87		
66.7	sedimentation	0.96		
72.2		sedimentation		

added to 0.1 mol·L^{-1} or 0.3 mol·L^{-1} AOT/isooctane solution to achieve the desired ω_0 (ω_0 = [H_2O]/[surfactant]). This solution was mixed until it was clear and colorless.

For DLS measurements, the reverse micelle solutions were filtered through a 0.2 μm hydrophobic Teflon syringe filter unit, using a 5 mL nonrubber septa and a latex-free plastic syringe, into a 4 mL glass cuvette.

Viscosity Measurement. The viscosity measurements of reverse micelle solutions were carried out at 25 °C, using an automatic viscometer (programmable Brookfield DV-II+ viscometer) with spindle S18 (shear rate from (0 to 132) s^{-1} (1.32 rpm) and viscosity from 0.1 mPa·s to 30 Pa·s). The viscosity measurement uncertainty is less than 0.01 mPa·s of the full scale range, and the repeatability of the viscosity measurement is above 99.9 %. The sample volume is 8.0 mL.

Interfacial Tension Measurement. The interfacial tension measurements of reverse micelle solutions were carried out at 25 °C, using FTÅ200. The interfacial tension measurement uncertainty is less than 0.5 mN·m^{-1}, and the repeatability of the interfacial tension measurement is above 99.1 %.

Dynamic Light Scattering (DLS) Measurement. The size distribution by volume of water or BSA in AOT reverse micelle solution was measured by the dynamic light scattering instrument (Malvern-High Performance Particle Sizer (HPPS)) at 25 °C. The dispersant viscosity is measured, for isooctane, and at 0.1 mol·L^{-1} AOT/isooctane, the measured viscosity is 0.54 mPa·s; the dispersant refractive index is 1.39; the solute (water) refractive index is 1.33; and the absorbance is 0.

The Malvern HPPS is a unique instrument capable of measuring the size of molecules in solution as well as the size of dispersions and emulsions and up to 20 % from subnanometer to a few microns [from (0.6 to 6000) nm] using dynamic light scattering. Measurements are made in conventional cuvettes, eliminating the possibility of sample cross-contamination.

The particle size of proteins and macromolecules in solution requires exceptional sensitivity. Using a standard helium−neon laser, the HPPS has the highest sensitivity of any dynamic light scattering system available. Concentrated samples traditionally difficult to dilute without affecting their size distribution can often be measured in their original state, or with minimal dilution. To cover this range of applications, the HPPS uses a patented optical system called Noninvasive Back-Scatter or NIBS.

For a monosized particle, the translational diffusion coefficient can be related to the particle diameter using Stokes' diffusion law and the Einstein equation for Brownian motion.

$$D = kT/6\pi\eta R_h \quad (1)$$

where R_h is hydrodynamic radius; k is Boltzmann's constant; η is solvent viscosity; and T is absolute temperature. R_h is generally very close to, but slight larger than, the geometrical radius of a sphere due to solvation of solvent and some interaction effects.

Table 2. Solubility, Viscosity (η/mPa·s) of Water in AOT Reverse Micelles and the Diameter (d/nm) of Water in 0.1 mol·L^{-1} M and 0.3 mol·L^{-1} AOT/Isooctane by DLS at 298.15 K

	0.1 mol·L^{-1} AOT/isooctane			0.3 mol·L^{-1} AOT/isooctane	
x μL of [H_2O]	ω_0	η/mPa·s	d/nm	ω_0	η/mPa·s	d/nm
0	0	0.54	1.92 ± 0.90	0	0.84	1.71 ± 0.61
1	0.6	0.54	1.98 ± 1.12			
2	1.1	0.54	3.86 ± 1.56	0.37	0.84	1.93 ± 0.52
4	2.2	0.55	4.04 ± 1.34			
5	2.8	0.56	4.90 ± 1.59	0.93	0.84	2.31 ± 0.63
10	5.6	0.57	6.45 ± 1.84	1.87	0.84	3.22 ± 0.89
15	8.3	0.59	7.00 ± 2.80	2.77	0.84	4.00 ± 1.21
25	13.9	0.62	7.95 ± 2.55	4.63	0.90	4.51 ± 1.45
30	16.7	0.63	10.01 ± 3.75			
40	22.2	0.66	10.19 ± 3.03	7.4	0.94	4.74 ± 1.48
50	27.7	0.69	10.46 ± 3.17	9.2	1.00	4.92 ± 1.34
60	33.3	0.72	10.89 ± 2.79	11.1	1.02	5.04 ± 1.53
70	38.9	0.80	11.48 ± 4.02	12.97	1.06	5.21 ± 1.65
80	44.4	0.87	12.27 ± 4.25	14.8	1.11	5.52 ± 1.59
90	50.0	0.96	13.58 ± 4.69	16.7	1.17	5.76 ± 1.62
100	55.6	sedimentation		18.5	1.25	5.83 ± 1.86
120	66.7			22.2	1.41	6.47 ± 3.38
140	77.7			25.9	1.50	6.79 ± 3.49
160	88.9			29.6	1.62	8.21 ± 3.25
180	100.0			33.3	1.86	9.56 ± 2.97
250	138.9			46.3	5.01	
300	166.9			55.6	sedimentation	

Results and Discussion

Viscosity of BSA in AOT/Isooctane Reverse Micelles. Tables 1 and 2 list the viscosity of [x mg of BSA·mL^{-1} of water] and water in 0.1 mol·L^{-1} AOT/isooctane at 298 K. The viscosity of AOT/isooctane reverse micelle solutions did not change with shear rate and exhibited Newtonian behavior. The viscosity of AOT/isooctane reverse micelles filled with water or protein inside increases with increasing ω_0. The viscosity of 0.3 mol·L^{-1} AOT/isooctane solution is much higher than that of isooctane or 0.1 mol·L^{-1} AOT/isooctane solution due to its higher surfactant concentration.

Solubility of Water and Particle Size of Water in AOT/Isooctane Reverse Micelles. Although the linear length of the AOT molecule is 1.2 nm,[7] the average reverse micelle was found to be 1.62 nm by considering an icosahedral packing of an AOT polar head.[8] For the size of 0.1 mol·L^{-1} and 0.3 mol·L^{-1} AOT/isooctane, reverse micelles at ω_0 = 0 obtained by DLS were found to be (1.92 and 1.71) nm. It is confirmed that our results agreed with the literature.[5,8]

Table 2 lists the solubility, viscosity of water in AOT reverse micelles, and hydrodynamic diameter ($2R_h$) of water in 0.1 mol·L^{-1} and 0.3 mol·L^{-1} AOT/isooctane by DLS at 298.15

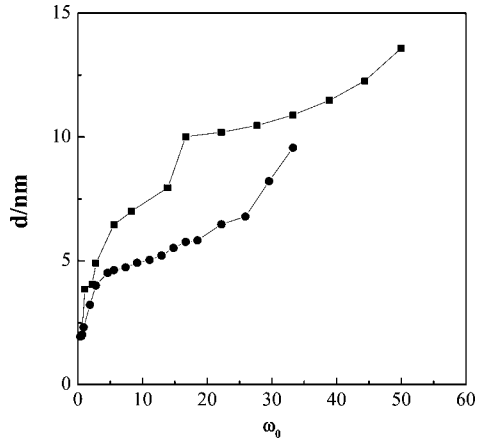

Figure 1. Relationship between the molar ratio of water-to-surfactant and the particle diameter of water in AOT/isooctane reverse micelles by DLS at 298.15 K: ■, x μL of [water]·mL^{-1} of [0.1 mol·L^{-1}] AOT/isooctane; •, x μL of [water]·mL^{-1} of [0.3 mol·L^{-1}] AOT/isooctane.

K. The dependence of mean diameter of water in 0.1 mol·L^{-1} AOT or 0.3 mol·L^{-1} AOT/isooctane reverse micelles (x μL·mL^{-1} of [0.1 mol·L^{-1} or 0.3 mol·L^{-1}] AOT/isooctane) on the ω_0 according to DLS is presented in Figure 1. The particle size increases with increasing ω_0 at different AOT/isooctane reverse micelles. For 0.1 mol·L^{-1} or 0.3 mol·L^{-1} AOT/isooctane, when ω_0 is higher than 50.0, the water in the reverse micelle solution is opaque with sedimentation, which means that the amount of water cannot be dissolved in the reverse micelles and the solubilization of water was the same for different AOT concentrations. The maximum solubility of water in 0.1 mol·L^{-1} and 0.3 mol·L^{-1} AOT/isooctane was found to be 90 μL·mL^{-1} [0.1 mol·L^{-1}] AOT/isooctane ($\omega_0 = 50.0$) and 260 μL·mL^{-1} [0.3 mol·L^{-1}] AOT/isooctane ($\omega_0 = 48.2$), respectively. As ω_0 was increased from 0 to 5.6, from 5.6 to 22.2, and from 22.2 to 50.0, the hydrodynamic diameter of water in 0.1 mol·L^{-1} AOT reverse micelles increased from (1.9 to 6.4) nm, from (6.4 to 10.2) nm, and from (10.2 to 13.6) nm and is almost linear with ω_0, confirming that the diffusion coefficient was independent of ω_0, indicating a spherical structure for the AOT reversed micelles. For 0.3 mol·L^{-1} AOT/isooctane reverse micelles, the maximum solubility of water was found to be 260 μL·mL^{-1} of [0.3 mol·L^{-1}] AOT/isooctane, which is much higher than that in 0.1 mol·L^{-1} AOT/isooctane reverse micelles. As ω_0 was increased from 0 to 0.9, from 0.9 to 25.9, and from 25.9 to 33.3, the hydrodynamic diameter of water in 0.3 mol·L^{-1} AOT reverse micelles increased from (1.7 to 2.3) nm, from (2.3 to 6.8) nm, and from (6.8 to 9.6) nm and is almost linear with ω_0, confirming that the diffusion coefficient was independent of ω_0, indicating a spherical structure for the AOT reversed micelles. At the same ω_0, the particle size of water in 0.3 mol·L^{-1} AOT reverse micelles is much smaller than that in 0.1 mol·L^{-1} AOT reverse micelles.

Effect of BSA Concentration on the Solubility and Particle Size of Reverse Micelles. Table 3 lists the solubility of BSA in 0.1 mol·L^{-1} AOT reverse micelles and the hydrodynamic diameter of BSA in 0.1 mol·L^{-1} AOT/isooctane by DLS at 298.15 K. The dependence of the mean diameter of BSA in 0.1 mol·L^{-1} AOT/isooctane reverse micelles (x μL of [x mg of BSA·mL^{-1} of H_2O]·mL^{-1} of [0.1 mol·L^{-1}] AOT/isooctane) on ω_0 according to DLS is presented in Figure 2. The hydrodynamic diameter increases with increasing ω_0 at different [x mg of BSA·mL^{-1} of H_2O] in 0.1 mol·L^{-1} AOT/isooctane reverse micelles from 10 mg of BSA·mL^{-1} of H_2O to 100 mg of BSA·mL^{-1} of H_2O. The hydrodynamic diameter of BSA in 0.1 mol·L^{-1} AOT/isooctane reverse micelles only changed slightly compared with that of water in the reverse micelles. The solubility of BSA in 0.1 mol·L^{-1} AOT/isooctane reverse micelles decreases with increasing BSA concentration. As ω_0 is below 5.6, the highly concentrated BSA from (100 to 145) mg·mL^{-1} of water is not dissolved in 0.1 mol·L^{-1} AOT reverse micelles. The maximum solubility of 100 mg, 50 mg, 20 mg, and 10 mg of BSA·mL^{-1} of H_2O in 0.1 mol·L^{-1} AOT/isooctane was found to be 50 μL, 90 μL, 120 μL, and 110 μL of [x mg of BSA·mL^{-1} of H_2O]·mL^{-1} of [0.1 mol·L^{-1}] AOT/isooctane. As ω_0 was increased from 2.8 to 66.7, the hydrodynamic radius of BSA from 10 mg to 100 mg of BSA·mL^{-1} H_2O in 0.1 mol·L^{-1} AOT reverse micelles increased almost linearly with ω_0 confirming that the diffusion coefficient was independent of ω_0, indicating a spherical structure for the AOT reverse micelles.

In a number of previously published works, it has been established that any change in the nature or in the ratio of the components forming the system of reverse micelles, as well as changes in surfactant hydration degree, resulted in the formation of surfactant micelles of different structural types. The evaluation of particulate size of AOT/isooctane reverse micelles containing water or protein BSA reveals that the entrapment of water or protein does not lead to a noticeable change in the mean diameter of the micelles at a given ω_0. This would mean at low hydration degrees small surfactant micelles contain a dimeric form of the protein, while larger micelles existing at higher ω_0 values solubilize several diametric molecules, maybe the BSA clusters as a whole, as refs 5, 6, and 8 showed.

Table 4 lists the particle size of 15 μL of [x mg of BSA·mL^{-1} of H_2O]·mL^{-1} of [0.1 mol·L^{-1}] AOT/isooctane reverse mi-

Table 3. Solubility of BSA in 0.1 mol·L^{-1} AOT Reversed Micelles and the Mean Diameter of BSA in 0.1 mol·L^{-1} AOT/Isooctane by DLS at 298.15 K

	d/nm			
ω_0	10 mg of BSA·mL^{-1} of H_2O	20 mg of BSA·mL^{-1} of H_2O	50 mg of BSA·mL^{-1} of H_2O	100 mg of BSA·mL^{-1} of H_2O
2.8	5.36 ± 1.92		opaque	4.33 ± 2.68 (opaque)
5.6				6.11 ± 2.82
6.9				8.81 ± 2.58
8.3	6.34 ± 3.08	7.09 ± 1.97	8.33 ± 2.15	9.53 ± 2.69
11.1				9.81 ± 3.14
13.9	7.49 ± 4.09	7.68 ± 2.11	9.92 ± 3.84	10.80 ± 2.93
22.2	9.83 ± 4.04			13.11 ± 4.85
27.7	11.26 ± 3.11	10.96 ± 3.63	11.64 ± 6.38	14.35 ± 4.30
38.9	15.04 ± 3.62	14.02 ± 4.43	13.40 ± 2.58	sedimentation
50.0	16.71 ± 4.83	15.66 ± 4.60	15.50 ± 7.41	
55.6	17.27 ± 8.33		sedimentation	
61.1	18.28 ± 8.40	17.32 ± 4.99		
66.7	sedimentation	18.48 ± 6.31		
72.2		sedimentation		

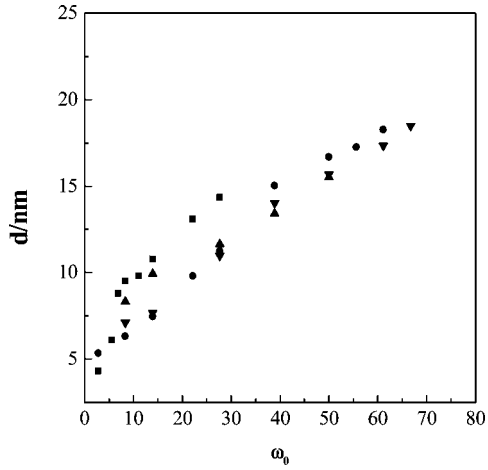

Figure 2. Effect of molar ratio of water-to-surfactant on the diameter of x μL of [x mg of BSA·mL^{-1} of water]·mL^{-1} of [0.1 mol·L] AOT/isooctane reverse micelles by DLS at 298.15 K: ■, 100 mg of BSA·mL^{-1} of water; ●, 10 mg of BSA·mL^{-1} of water; ▲, 50 mg of BSA·mL^{-1} of water; ▼, 20 mg of BSA·mL^{-1} of water.

Table 4. Mean Diameter (d/nm) of 15 μL of [x mg of BSA·mL^{-1} of H_2O]·mL^{-1} of [0.1 mol·L^{-1}] AOT/Isooctane in 0.1 mol·L^{-1} AOT Reverse Micelles by DLS at 298.15 K

[x mg of BSA·mL^{-1} of H_2O]·mL^{-1} of [0.1 mol·L^{-1}] AOT	d/nm
2	6.02 ± 2.49
10	6.34 ± 3.08
20	7.09 ± 1.97
50	8.33 ± 2.15
100	9.53 ± 2.69
145	11.0 ± 2.42
200	opaque

celles. Figure 3 shows the effect of BSA concentration on the hydrodynamic radius of BSA in 0.1 mol·L^{-1} AOT/isooctane reverse micelles at $\omega_0 = 8.3$ (15 μL of [x mg of BSA·mL^{-1} of H_2O]·mL^{-1} of [0.1 mol·L^{-1}] AOT/isooctane) by DLS at 298.15 K. The particle size increases linearly with increasing BSA concentration from 2 mg of BSA·mL^{-1} of H_2O to 145 mg of BSA·mL^{-1} of H_2O in 0.1 mol·L^{-1} AOT/isooctane reverse micelles. The maximum solubility of BSA in 0.1 mol·L^{-1} AOT/isooctane is 15 μL of 145 mg of BSA·mL^{-1} of H_2O. Though 0.3 mol·L^{-1} AOT reverse micelle has the higher solubility of water than 0.1 mol·L^{-1} AOT reverse micelles, the

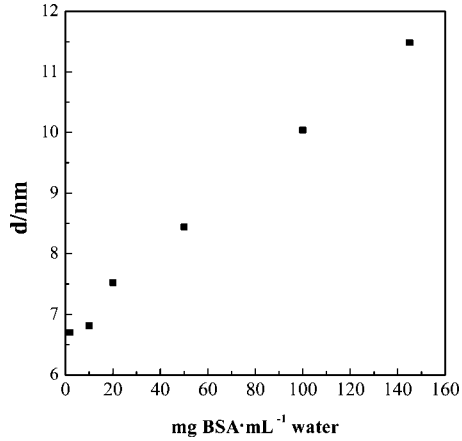

Figure 3. Effect of BSA concentration on the diameter of BSA in 0.1 mol·L^{-1} AOT/isooctane reverse micelles at $\omega_0 = 8.3$ by DLS at 298.15 K: ■, 15 μL of [x mg of BSA·mL^{-1} of water]·mL^{-1} of [0.1 mol·L^{-1}] AOT/isooctane.

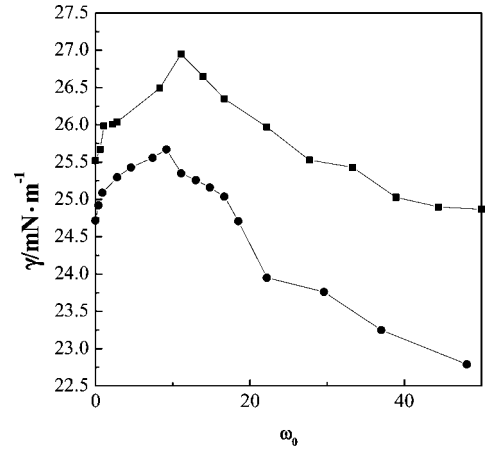

Figure 4. Relationship between the interfacial tension and molar ratio of water-to-surfactant in AOT/isooctane reverse micelle at 298 K: ■, x μL of [water]·mL^{-1} of [0.1 mol·L^{-1}] AOT/isooctane; •, x μL of [water]·mL^{-1} of [0.3 mol·L^{-1}] AOT/isooctane.

maximum solubility of BSA in 0.3 mol·L^{-1} AOT/isooctane is much lower than that in 0.1 mol·L^{-1} AOT reverse micelle, which is 15 μL of 100 mg of BSA·mL^{-1} of H_2O, maybe due to its smaller micelle diameter at the same ω_0 and the higher interaction between the AOT headgroups. 0.1 mol·L^{-1} AOT reverse micelles are much better for the protein delivery system than 0.3 mol·L^{-1} AOT/isooctane.

The localization of protein in the reverse micelles and the perturbation of the reverse micelles by protein uptake are important for understanding the role of the reverse micelles in protein solubilization. The water-shell model[3] as a typical example assumes that the protein adsorbs no surfactant molecule and is localized in the central part of the water pool of the reverse micelles. The size of the reverse micelle filled protein is different from that of empty one (filled water). For the same ω_0, from 8.3 to 50.0, the size of the reverse micelles filled protein with a higher concentration of BSA from 50 mg to 100 mg·mL^{-1} of water is a little larger than that of the empty one. But at ω_0 from 8.3 to 22.2, the size of the filled protein (10 mg of BSA·mL^{-1} of water, 20 mg of BSA·mL^{-1} of water) is a little smaller than that of the empty one. There is an increase of ω_0 for the filled micelles, attended by a decrease of ω_0 for the unfilled ones. The decrease of ω_0 for the unfilled micelles was so modest because of the small degree of occupancy of the micelles. There is an enlargement of the size of the micelles. The amount of water required in the inner cores is even larger than in the previous cases, which may be due to the larger size of the BSA molecule. When ω_0 is above 22.2, the size of the reverse micelle filled protein is larger than that of the empty one as the BSA concentration increases. The fact that the size of the reverse micelle filled protein changed, but not too much, confirmed that the incorporation of protein in the reverse micelles did not perturb the reverse micelles. The localization of BSA in the reverse micelles is in the center part of the water pool abiding the water-shell model.

Interfacial Tension of Water in AOT/Isooctane Reverse Micelles. The dependence of interfacial tension of water in 0.1 mol·L^{-1} AOT or 0.3 mol·L^{-1} AOT/isooctane reverse micelles (x μL of [H_2O]·mL^{-1} of [0.1 mol·L^{-1} or 0.3 mol·L^{-1}] AOT/isooctane) on ω_0 was presented in Figure 4. The interfacial tension first increased with increasing ω_0 at different AOT/isooctane reverse micelles, then decreased with increasing ω_0. The interfacial tension of water in 0.3 mol·L^{-1} AOT reverse micelles was only slightly smaller than that in 0.1 mol·L^{-1} AOT

reverse micelles. The interfacial tension changed slightly within a narrow range with ω_0, confirming that the addition of water did not perturb the reverse micelles. Incorporation of water in the reverse micelles did not perturb the reverse micelle, and the localization of water is in the center part of the water pool. Reverse micelles can solubilize and protect water-soluble solutes including drugs, genes, enzymes, and proteins. Protein and drug delivery from AOT reverse micelle to water-in-oil emulsions are underway. Great potentials exist in drug and enzyme delivery from reverse micelles to w/o emulsion.

Conclusion

That the diameter of AOT/isooctane reverse micelles with water or with protein inside increases with increasing ω_0 (molar ratio of water to surfactant) was investigated by dynamic light scattering (DLS) at 298.15 K. For $\omega_0 = 8.3$, the diameter of AOT reverse micelles increases with increasing BSA concentration from 2 mg of BSA·mL^{-1} of H_2O to 145 mg of BSA·mL^{-1} of H_2O. The diameter of AOT reverse micelles increases only slightly with BSA inside compared with water inside at the same ω_0. The hydrodynamic radius of BSA from (10 mg to 100 mg) of BSA·mL^{-1} of H_2O in 0.1 mol·L^{-1} AOT reverse micelles increased almost linearly with ω_0, confirming that the diffusion coefficient was independent of ω_0, indicating a spherical structure for the AOT reverse micelles. The solubility of water or protein increased with increasing AOT concentration. The localization of BSA in the reverse micelles is in the center part of the water pool without perturbing the reverse micelles much.

Literature Cited

(1) Luisi, P. L.; Giomini, M.; Pileni, M. P.; Robinson, B. H. Reverse micelles as hosts for proteins and small molecules. *Biochem. Biophys. Acta* **1988**, *947*, 209–246.

(2) Maitra, A. Determination of size parameters of water-AOT-oil reverse micelles from their nuclear magnetic resonance data. *J. Phys. Chem.* **1984**, *88*, 5122–5125.

(3) Bonner, F. J.; Woef, R.; Luisi, P. L. Micellar solubilization of biopolymers in hydrocarbon solvents. I. A structural model for protein containing reverse micelles. *J. Solid-Phase Biochem.* **1980**, *5*, 255–268.

(4) Levashov, A. V.; Khmelnitsky, Y. L.; Klyachko, N. L.; Chernyak, V. K.; Martinek, K. Enzymes entrapped into reversed micelles in organic solvents: Sedimentation analysis of the protein-aerosol OT-H_2O-Octane system. *J. Colloid Interface Sci.* **1982**, *88*, 444–457.

(5) Zampieri, G. G.; Jackle, H.; Luisi, P. L. Determination of the structural parameters of reverse micelles after uptake of proteins. *J. Phys. Chem.* **1986**, *90*, 1849–1853.

(6) Pileni, M. P.; Zemb, T.; Petit, C. Solubilization by reverse micelles: solute localization and structure perturbation. *Chem. Phys. Lett.* **1985**, *118*, 414–420.

(7) Zalauf, M.; Eicke, H. F. Inverted micelles and microemulsions in the ternary system water/AOT/isooctane as studied by photon correlation spectroscopy. *J. Phys. Chem.* **1979**, *83*, 480–486.

(8) Hirai, M.; Kawai-Hirai, R.; Yabuki, S.; et al. Aerosol-OT Reversed Micellar Formation at low water-surfactant ratio studied by synchrotron radiation Small-angle X-Ray Scattering. *J. Phys. Chem.* **1995**, *99*, 6652–6660.

Received for review October 29, 2007. Accepted January 14, 2008.

JE700625M