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Anionic Surfactant Binding to Lysozyme and Hydrophobic Interactions Effect to the Binding: A Novel Binding Model

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The interaction between anionic surfactants sodium dodecyl sulfate, sodium octyl sulfate and sodium decyl sulfate in various concentrations at pH 7.4 was investigated by the methods of fluorescence and circular dichroism techniques. Fluorescence data showed that the fluorescence quenching of lysozyme by anionic surfactants was the result of the formation of the surfactant-lysozyme complex. According to the Stern-Volmer equation, binding constant between anionic surfactants and lysozyme were obtained to be 21.08×10^3, 6.054×10^3 and 9.404×10^3 L mol^{-1}, respectively, indicating that the binding of sodium dodecyl sulfate to protein is more than others. Based on the results obtained, with increasing chain length of the surfactants, ΔG^o (H_2O) has become more positive. Therefore, hydrophobic interactions were the dominant intermolecular force in stabilizing the complex. The conformational investigation showed that the presence of sodium dodecyl sulfate decreased and in presence of sodium octyl sulfate, sodium decyl sulfate increased the α-helical content of lysozyme.

Key Words: Lysozyme, Anionic surfactants, Fluorescence quenching, Hydrophobic interaction, Stabilization.

INTRODUCTION

Lysozyme (1,4-β-N-acetylmuramidase; EC 3.2.1.17) is a lytic enzyme, which degrades a constituent of bacterial cell wall[1]. It splits the bond between N-acetyl-glucosamine and N-acetylmuramic acid in murein. This enzyme is found in tears, saliva, milk, respiratory and cervical secretions[2] but also in the small intestine where it is secreted by the planet cells[3,4]. Lysozyme is considered as part of the innate immune system. Lysozyme is a compact globular protein that takes part in the first barrier of defense. As an enzyme it is possible to study the functionality of lysozyme through its lytic activity[5]. Lysozyme, with low molecular weight (14.4 kDa)[6] with a pI[5] of 10.5 and high stability, owning six tryptophan and three tyrosine residues in its structure, is a photo-biological active protein[7]. Many proteins fold by two-state mechanism whereas other proteins populate one or more kinetic folding intermediates[8-11]. Hen lysozyme is often considered as a typical example of close adherence the equilibrium, two-state unfolding mechanism[12-16]. In the range of physiological pH values, the lysozyme does not show any detectable change in its structure up to

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77 °C and at the physiological temperature no detectable change in the structure was observed with a pH change from 1.2-11.3[17]. The stability lysozyme has been attributed to the four disulfide bonds[18,19] besides hydrogen bonds and hydrophobic interactions among the amino acid residues[20]. However, the inactivation of the enzyme is possible when solutions of surfactants are added to the lysozyme (are exothermic interactions which have been attributed to specific binding between the anionic sulphate head groups and cationic amino acid residues[21]) and numerous studies in this way have been reported. Surfactants are widely used in both consumer and industrial application: food processing, medicines and pharmaceuticals[22]. The interaction between ionic detergents and proteins has received much attention and it is generally accepted that the binding of such detergents to proteins occurs by a combination of electrostatic[23,24] and hydrophobic interactions[25]. The relative importance of these two types of interactions can be assessed by investigating the interaction of a typical globular protein like lysozyme which has been well characterized[13,26], with a series of detergents containing hydrocarbon moieties of varying length and different polar head groups. The binding of these anionic detergents by lysozyme induces conformational changes in the enzyme. Typical studies of surfactant-protein system have been developed keeping the protein concentration constant and varying the amount of surfactant. In the present paper, we describe the interaction of lysozyme with anionic detergents such as sodium dodecyl sulfate (SDS) and sodium decyl sulfate (SDeS), sodium octyl sulfate (SOS) at various detergent concentrations and pH 7.4 using fluorescence spectra and circular dichroism (CD) techniques. In this present work, by comparison the results of different kinds of anionic surfactant-induced complex states, we propose that the primary driving force for the formation of lysozyme-anionic surfactants complex is the reduction of electrostatic repulsion, although the hydrophobic effect does remains a factor. It is concluded that hydrophobic interactions play an important role in the detergent binding, whereas electrostatic interactions play only a minor role.

EXPERIMENTAL

Hen egg white lysozyme, purchased from sigma and used without further purification. Sodium dodecyl sulfate (SDS), sodium octyl sulfate (SOS) and sodium decyl sulfate (SDeS) were also obtained from sigma. All the pH values represent apparent pH meter readings. Tris (hydroxymethyl) aminomethane had a purity of not less than 99.5 %. All other chemicals were of analytical grade and used as received. Doubly distilled water was used throughout the experiment.

Fluorescence measurements were made on Hitachi 2500 spectrofluorometer at an excitation wavelength of 280 nm. All the spectra were an average of three scans and were corrected by subtraction of the blank spectrum. The temperature of the cell compartments was kept constant at 20 °C by water circulation. Circular dichroism (CD) measurements were performed on a Jasco-815 with protein concentrations 0.03 % mg/mL in a 1 mm path length quartz cuvette.

The protein solution, based on the molecular weights of 14400, was prepared by dissolving lysozyme in phosphate buffer solutions (0.05 mol L^{-1} phosphates, 0.2 mol L^{-1} NaCl, pH 7.4). The stock solution of surfactants were prepared by dissolving it in phosphate buffer solution with a final concentration of 5×10^{-3} mol L^{-1}.

In a typical fluorescence measurement, 2 mL lysozyme with the concentration of 10.03 % mg/mL was added to a quartz cell (1 cm × 1 cm). The surfactants solution was then gradually titrated to the cell using a trace syringes. The concentration of surfactants were 5×10^{-3} mol L^{-1}. An excitation wavelength of 280 nm was chosen and the emission wavelength was recorded from 300-600 nm. Circular dichroism (CD) spectra were recorded at 298 K under constant nitrogen flush over a wavelength range of 250-190 nm. The instrument was controlled by Jasco spectra manager software and the scanning speed was set at 200 nm min^{-1}. Each spectrum was the average of three successive scans and appropriate buffer solutions running under the same conditions were taken as blank and their contributions were subtracted from the experimental spectra.

RESULTS AND DISCUSSION

Fluorescence measurement: Fluorescence spectroscopy is one of the most powerful methods to study protein conformation. Taking advantage of the high spectroscopic sensitively and time-resolution power, this method provides valuable information on the conformational flexibility and other important entities closely related to the biological function of protein[27-29]. Hen egg-white lysozyme is an interesting subject to use examine the correlation between ligand induced conformational changes and enzymatic activities using the fluorescence spectroscopy, because lysozyme arranges Trp62 and Trp108 at the active site and Trp28 and Trp111 at the hydrophobic matrix box region where they participate in the substrate binding and maintaining the structural stability, respectively.

The fluorescence intensity of a compound can be decreased by a variety of molecular interactions including excited-state reaction, molecular rearrangement, energy transfer, forming ground state complex and collisional quenching, which is called fluorescence quenching. In this paper, we measured the fluorescence quenching spectra of lysozyme in the presence of different concentrations of surfactants to elucidate the quenching mechanism. Fig. 1 shows the fluorescence emission spectra of lysozyme in the presence of various concentrations of SDS. When different amount of SDS was titrated into a fixed concentration of lysozyme, the fluorescence intensity of lysozyme at around 340 nm decreased regularly with no shift of the emission wavelength (the excitation wavelength was 280 nm), suggesting that SDS could interact with lysozyme and quench its intrinsic fluorescence. The fluorescence quenching was usually analyzed using the well-known Stern-Volmer equation[30]:

$$F_0/F = 1 + K_{sv}[Q] = 1 + K_q \tau_0[Q] \quad (1)$$

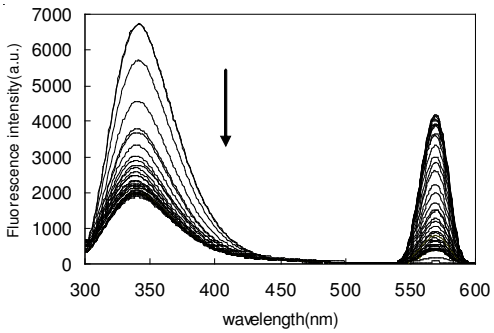

Fig. 1. Emission spectra of lysozyme in the presence of various concentrations of SDS at pH 7.4 and 24 °C. (λ_{ex} = 280 nm). C (Lys.) = 0.3 % mg/mL, C (SDS) = 5.0×10^{-3} mol L^{-1}, [SDS] from 0-0.93 mM

where F_0 and F denote the steady-state fluorescence intensities in the absence and in the presence of quencher, respectively. [Q] is the concentration of quencher, K_{sv} is the Stern-Volmer constant corresponding to the slope of the plot for F_0/F *versus* [Q], K_q the quenching rate constant for biomolecule and io the average fluorescence lifetime of biomolecule without quencher. In order to confirm the view, supposing the quenching process to be the dynamic model, K_q was calculated. It was easily obtained

$$K_{sv} = K_q \tau_0 \quad (2)$$

Because the fluorescence lifetime of biomolecule[16] was 10^{-8} s, the quenching constant K_q was calculated from the slope and listed in Table-1. The maximum of K_q for various quencher with biomolecule[17] is 2×10^{10} 1 mol^{-1} s^{-1}.

TABLE-1
STERN-VOLMER QUENCHING CONSTANTS FOR THE INTERACTION OF SDS, SdeS, SOS WITH LYSOZYME

pH	Surfactant	$K_{sv} \times 10^{-3}$	R^{2a}
7.4	SDS	21.088	0.9268
	SDeS	6.0541	0.9601
	SOS	9.4041	0.9443

a: R is the correlation coefficient. The results show that the Stern-Volmer quenching constant K_{sv} is not correlated with increasing chain length of the surfactants.

Hence, eqn. 1 was applied to determine K_{sv} by linear regression of a plot of F_0/F against [Q]. Fig. 2 shows the Stern-Volmer plots of F_0/F *versus* [Q] for the SDS-lysozyme complex. Fig. 3. shows the fluorescence emission spectra of lysozyme in the presence of various concentrations of sodium octyl sulfate. When different amount of sodium octyl sulfate was titrated into a fixed concentration of lysozyme, the fluorescence intensity of lysozyme at around 350 nm decreased regularly with no shift of the emission wavelength (the excitation wavelength was 280 nm), suggesting that sodium octyl sulfate could interact with lysozyme and quench its intrinsic

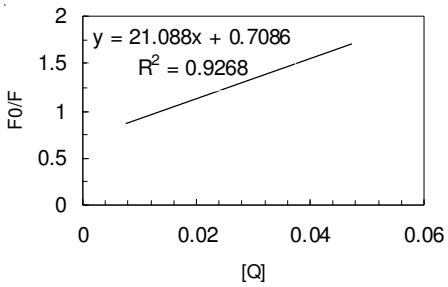

Fig. 2. Stern-Volmer plots for the SDS-Lys system at pH 7.4

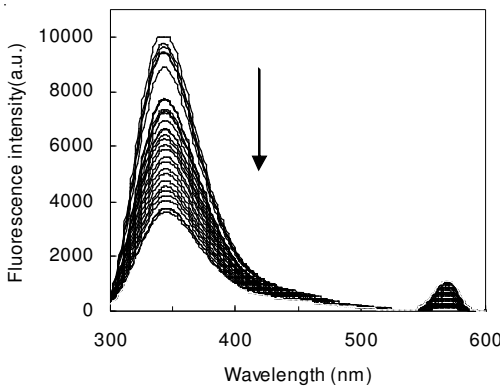

Fig. 3. Emission spectra of lysozyme in the presence of various concentrations of SOS at pH 7.4 and 24 °C. (λ_{ex} = 280 nm). C (Lys) = 0.3 % mg/mL, C (SOS) = 5×10^{-3} mol L^{-1}, [SOS] from 0-0.68 mM

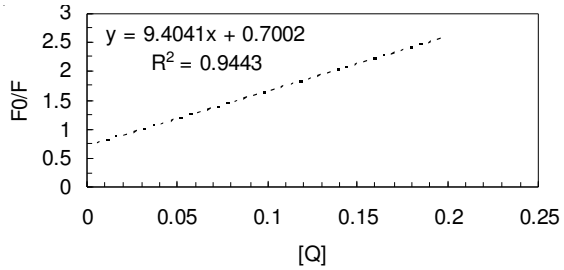

Fig. 4. Stern-Volmer plots for the SOS-Lys system at pH 7.4

fluorescence. Fig. 4. shows the Stern-Volmer plots of F_0/F *versus* [Q] for the SOS-lysozyme complex. Fig. 5. shows the fluorescence emission spectra of lysozyme in the presence of various concentrations of SDeS. When different amount of SDeS was titrated into a fixed concentration of lysozyme, the fluorescence intensity of lysozyme at around 350 nm decreased regularly with no shift of the emission wavelength (the excitation wavelength was 280 nm), suggesting that SDeS could interact with lysozyme and quench its intrinsic fluorescence. Fig. 6. shows the Stern-Volmer plots of F_0/F *versus* [Q] for the SDeS-lysozyme complex.

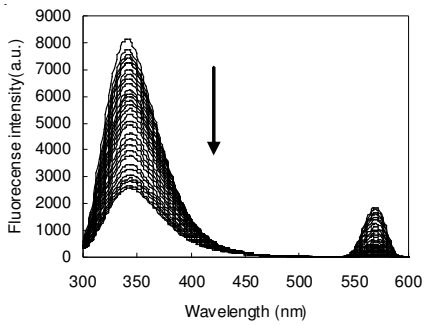

Fig. 5. Emission spectra of lysozyme in the presence of various concentrations of SDeS at pH 7.4 and 24 °C. (λ_{ex} = 280 nm). C (Lys) = 0.3 % mg/mL, C (SDeS) = 5×10^{-3} mol L^{-1}, [SDeS] from 0-0.14 mM

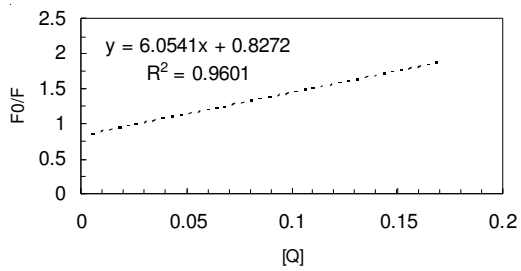

Fig. 6. Stern-Volmer plots for the SDeS-Lys system at pH 7.4

The acting forces between biomolecule and a surfactant may include hydrophobic effect, hydrogen bond, van der Waals force and electrostatic attraction and so on. According to the Gibb's free energy changes, ΔG^o, as follows[31]:

$$\Delta G^o_D = -RT \ln K = -RT \ln (A_{obs} - A_N)/(A_D - A_{obs}) \quad (3)$$

where R is the gas constant, T is the absolute temperature, A_N, A_D and A_{obs} are the physical parameters of extinction coefficient, molar ellipticity and fluorescence intensity of N, D and any observed states, respectively.

Fig. 7. shows the plot of ΔG^o against three surfactants concentrations (*i.e.*, SDS, SOS and SDeS). The free energies of complex formation in the absence of surfactants, ΔG^o (H_2O), were calculated from the following equation:

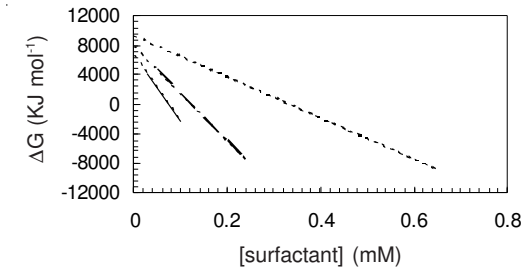

Fig. 7. Plot of ΔG^o against free surfactant concentrations

$$\Delta G^{o}_{D} = \Delta G^{o}_{D} (H_2O) - m[\text{anionic surfactants}] \quad (4)$$

where m is the slope of linear curve reflecting the coopratívity and also hydrophobicity of the transition state. The m-value correlates very strongly with the amount of protein surface exposed to the solvent upon unfolding. Table-2 shows the ΔG^{o}_{D} (H_2O) and m-values for the denatured state of lysozyme upon the addition of anionic surfactants such as SDS, SDeS and SOS at pH 7.4. It is apparent from Table-1 that as the chain lengths of anionic surfactants increase, the values of the midpoint concentration (C_m) and m-values decrease and increase, respectively. Table-1 also indicates the increase in ΔG^{o}_{D} (H_2O) and m-values that corresponds to the length of the hydrophobic chains. Therefore, hydrophobic forces play a dominant role in system. On the other hand, m-value is assign of coopratívity; therefore lysozyme-surfactant system induced by SDS is more cooperative than SDeS and SOS.

TABLE-2
ΔG^{o}_{D} (H_2O), m-VALUES AND INFLECTION TRANSITION POINTS FOR THE LYSOZYME-SURFACTANTS COMPLEX AT pH 7.4

Anionic surfactants	ΔG^{o}_{D} (H_2O)[a] (kJ mol^{-1})	m[b] (kJ mol^{-1} M^{-1})	Cm[c] (mM)
SDS	10.87×10^3	37.65×10^3	0.0082
SDeS	10.80×10^3	11.90×10^3	0.0330
SOS	8.67×10^3	8.89×10^3	0.1400

a: ΔG^{o}_{D} (H_2O) was calculated from eqn. 4. b: A parameter reflecting the hydrophobicity of the transition state. c: The midpoint concentration of transition.

It has been ascertained that it is the binding of surfactants to lysozyme caused the fluorescence quenching affects the conformation. Two negative bands at 208 and 222 nm indicated that the peptide chain adopted an α-helical structure in physiological conditions[32]. For further investigation, the methods of CD spectroscopy were studied. To prove the possible influence of surfactants binding on the secondary structure of lysozyme, the CD spectroscopy of lysozyme in the absence and presence of anionic surfactants were measured. The CD spectra of lysozyme exhibit two negative bands at 208 and 226 nm which is the characteristic of α-helix in the advanced structure of protein. The CD spectra of lysozyme with various concentration of SDS at pH 7.4 at room temperature are shown in Fig. 8, respectively. A decreasing tendency of the α-helices content and an increasing tendency of turn and unordered structure contents were observed with the increasing concentration of SDS but a increasing tendency of the α-helices content and turn content were observed with the increasing concentration of SDeS and SOS. As known, the secondary structure contents are related close to the biological activity of protein, thus a decrease in α-helical content from 30.3-26.7 % meant the loss of the biological activity of lysozyme upon interaction with high concentration of SDS. While an increasing in α-helical content from 27.6-30.7 % and from 29.6-32.6 % were observed with high concentration of SDeS and SOS, respectively. The secondary structural changes

here meant that surfactant bound with the amino acid residues of the main polypeptide chain of protein and destroyed their hydrogen bonding networks, making the lysozyme adopt a more incompact conformation state and its exposure to the hydrophobic cavities.

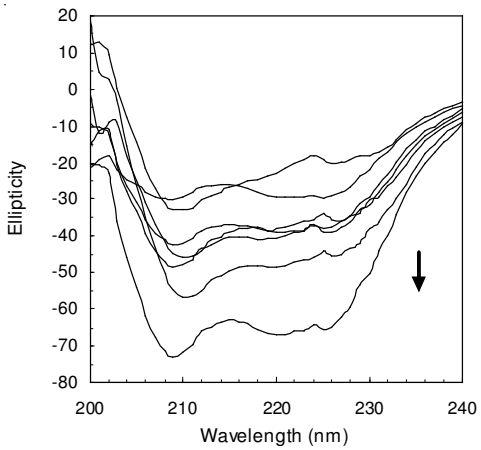

Fig. 8. CD spectra of SDS-lysozyme system obtained in phosphate-buffer at room temperature. C (Lys) = 0.3 % mg/mL; C (SDS) = 0.005 mol L^{-1}; from 0-0.83 mM

Conclusion

Interaction between anionic surfactants and lysozyme was investigated at different concentrations (pH 7.4) by fluorescence spectroscopy and CD spectroscopy. The fluorescence data showed that the fluorescence quenching of lysozyme was resulted mainly from static mechanism and Stern-Volmer quenching constant K_{sv} is not correlated with increasing chain length of the surfactants. ΔG^{o}_{D} (H_2O), indicating that hydrophobic forces play a dominant role in protein system. The spectral data revealed the conformational changes of lysozyme upon interaction with anionic surfactants.

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