Journal of Thermal Analysis and Calorimetry, Vol. 87 (2007) 2, 453–456

INTERACTION OF A HOMOLOGOUS SERIES OF *n*-ALKYL TRIMETHYL AMMONIUM BROMIDES WITH EGG WHITE LYSOZYME
Microcalorimetric and spectroscopic study

A. K. Bordbar[1*], *R. Hosseinzadeh*[1,2] *and M. H. Norozi*[1]

[1]Laboratory of Biophysical Chemistry, Department of Chemistry, University of Isfahan, Isfahan 81746-73441, Iran
[2]Food and Chemical Analysis Research Laboratory, Jahad-E-Daneshgahi, Urmia University, Urmia, Iran

The interaction of a series of *n*-alkyl trimethyl ammonium bromides (C_{12}, C_{14} and C_{16}) with egg white lysozyme have been studied using fluorescence and UV-Vis spectroscopies and isothermal titration calorimetry (ITC). The trend of variation of molar absorptivity at 281 nm, quantum yields (λ_{ex}=281 nm) and heat of interaction with respect to surfactant concentration, were measured. The spectrophotometric results show that the hydrophobic interactions have a major role in denaturation mechanism and it would be increased with increasing in hydrocarbon tail length of surfactant. The ITC results indicated the two-step mechanism for unfolding of lysozyme due to its interaction with surfactants.

Keywords: *absorption spectroscopy, cationic surfactants, fluorescence spectroscopy, ITC, lysozyme, unfolding*

Introduction

Lysozyme is one of the most thoroughly studied proteins containing 129 residues. This is a small two domain protein with high content of secondary structure, which has four disulfide bonds [1]. Denaturation of lysozyme by ionic surfactants, gives useful information about its conformational stability that is one of the main interesting issue in the field of biochemistry and biotechnology [2–5]. The binding studies of lysozyme with a number of surfactants have been carried out by several researchers using different techniques. Jones and Manely have carried out an extensive investigation on the interaction of lysozyme with *n*-alkyl sulfates by equilibrium dialysis method in order to study the effect of chain length of the surfactants on the Gibbs free energy of binding of surfactant anion, and the binding isotherms have been discussed in terms of Wyman binding potential concept [6]. Their studies indicate that the binding of surfactants to lysozyme initiate conformational changes in the protein. Imoto *et al.* [7] also reported such conformational changes in lysozyme as a result of its binding with sodium dodecyl sulphate using nuclear magnetic resonance, fluorescence and UV spectral methods. Hayashi *et al.* [8, 9] have studied the interaction of lysozyme with cationic surfactants such as dimethyl benzylmyristyl ammonium chloride (DBMA) in order to investigate the sites of interaction and the pH dependence of such interaction. Their studies reveal that the DBMA involves both hydrophobic and electrostatic interactions with lysozyme in the pH range 1.5 to 8.0. The binding of cetyl trimethyl ammonium bromide (CTAB) and dodecyl trimethyl ammonium bromide (DTAB) at various surfactants concentrations and pH using equilibrium dialysis, UV-Vis and circular dichroism techniques have been studied by Subramanian *et al.* [10]. They concluded that hydrophobic interactions play a major role, while electrostatic interactions play only a minor in the binding process. However, there is no comprehensive study on interaction of a homologous series of cationic surfactants with different hydrocarbon tail length with lysozyme using spectroscopic and isothermal titration calorimetric (ITC) techniques. Such study has been done in the present work. The heats of interactions and the conformational changes accompanying interactions are followed by ITC, fluorescence and UV-vis spectroscopies for better understanding of denaturation mechanism and the role of hydrocarbon tail length of surfactant on this process. This study can provide more valuable results with respect to the accuracy and validity of ITC as a direct thermodynamic tool [11–13].

Experimental

Materials

Chloroform, sodium dihydrogen phosphate, sodium hydrogen phosphate, sodium chloride, chloridric acid, sodium hydroxide, phosphoric acid and lysozyme enzyme from egg white powder was pur-

* Author for correspondence: bordbar@chem.ui.ac.ir

1388–6150/$20.00

Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

chased from Merck Co. It was used without further purification. Dodecyl trimethyl ammonium bromide (DTAB), tetradecyl trimethyl ammonium bromide (TTAB) and hexadecyl trimethyl ammonium bromide (HTAB) obtained from Sigma Chemical Co. A phosphate buffer (5 mM), pH 7.2, ionic strength 0.125 was used as buffer.

All of the experiments were carried out at 27°C. $A^{1\%}_{1cm,280nm}$=26.4 for lysozyme was used to calculate the concentration of lysozyme [14]. All of the protein solutions were freshly prepared and used. Double distillated deionized water was used for preparation of solutions.

Methods

Spectral absorption measurements were carried out on a Shimadzu-160 double beam spectrophotometer with a thermostat cell compartment at 27°C. A titration experiment was done by placing 2 mL of buffer solution in reference cell and 2 mL of protein solution (about 1 mg mL^{-1}) in sample cell. A certain amounts of surfactant stock solution were added to both cells and the difference spectra were recorded 5 min after addition of surfactant solution. The concentrations of surfactants were used to be below its critical micelle concentration (CMC) and appropriate to obtain molar ratios of surfactant to lysozyme as high as several thousand to one. The path length of spectrophotometer cell was 1 cm. The effect of the dilution of lysozyme solution by the added surfactant solution was corrected using Beer's law.

Fluorecence spectra between 300 and 380 nm were recorded on RF-5000 Shimadzu spectrofluorimeter with a thermostat cell compartment at 27°C. The following procedure was used for titration of lysosyme with cationic surfactants: 2 mL of protein solution with concentration about 0.3 mg mL^{-1} was placed in a fluorescence cell and small increments (about 10 μL) of the stock surfactant solution were injected in the cuvette with a micropipette. The lysozyme emission spectra was monitored using excitation at 281 nm and by selecting 5 nm for both excitation and emission band widths. The absorbance of the samples at excitation wavelength did not exceed 0.20, so the possibility of inner-filter effect was eliminated. In order to eliminate the dilution of lysozyme solution by the added surfactant solution, a blank containing lysozyme solution titrated with buffer was monitored as described above. The fluorescence intensity changes of the blank were subtracted from fluorescence intensity measurements of the surfactant/lysozyme complexes for every considered titration point. Conductometric measurements of surfactant solutions were carried out using ORION conductometer, model 180, with a thermostated cell compartment at 27°C.

The enthalpy of surfactant-lysozyme interaction was measured using a four-channel microcalorimetric system, Thermal Activity Monitor 2277 from Sweden Thermometric, interfaced with IBM Pentium (III) and DIGITAM-4.1 software. The surfactant stock solution was placed in 250 μL injection syringe and 2.5 mL of lysozyme solution (1 mg mL^{-1}) in a 5 mL titration cell. The interaction enthalpy was measured by sequential injection of specified amounts of surfactant solution to the titration cell. The enthalpy of dilution and demicellization of the surfactant solution was measured as described previously in the absence of lysozyme [15–19]. The enthalpy of dilution and demicellization for surfactant micelles was subtracted from the enthalpy of lysozyme-surfactant interaction. Heat of lysozyme dilution was negligible and system frequently calibrated electrically during experiments. The ITC measurements have been done twice and the reproducibility of the results has been certified within experimental error.

Results and discussion

The values of 13.20, 1.90 and 1.25 mM were obtained for CMC of DTAB, TTAB and HTAB at 27°C, 5 mM phosphate buffer pH=7.2, respectively, using conductometric technique. These values are comparable with literature values [20]. The concentration of surfactant in the titration experiments did not exceeded from its CMC value.

The relative effect of DTAB, TTAB and HTAB on the absorption spectrum of lysozyme represents a laminar increasing in all of the spectral regions by increasing of cationic surfactant concentration. However, there is no any significant change in the maximal absorption wavelength (281 nm). This can be due to existence of non-covalent interactions in protein-surfactant complexes. The variation of molar absorptivity at 281 nm, ε_{281}, of lysozyme solution *vs*. total concentration of surfactant was shown in Fig. 1.It represents an increase in ε_{281} with increasing of surfactant concentration. The rate of this increasing has been reduced by decreasing of hydrocarbon tail length of surfactant.Lysozyme has 6 tryptophan, 3 tyrosine and 3 phenyl alanine residues which are responsible for its absorption and emission spectrum. However, with respect to absorption spectrum of these amino acids, it can be said that tryptophan and tyrosine have considerable contribution in absorption spectrum of lysozyme. The change in chemical environment of this chromophore causes the change in protein spectrum.With respect to Fig. 1, curve for HTAB reaches to a flat region after surfactant concentration about

1 mM, while the corresponding curve for TTAB has a shoulder at concentration about 1.4 mM. This state was observed for DTAB at concentration about 10.3 mM. This behavior can be related to a significant change in folding state of protein at these surfactant concentrations. Figure 2 represents the change in fluorescence intensity of lysozyme at λ_{em}=339.2 for λ_{ex}=281 nm, *vs*. total concentration of surfactant. The

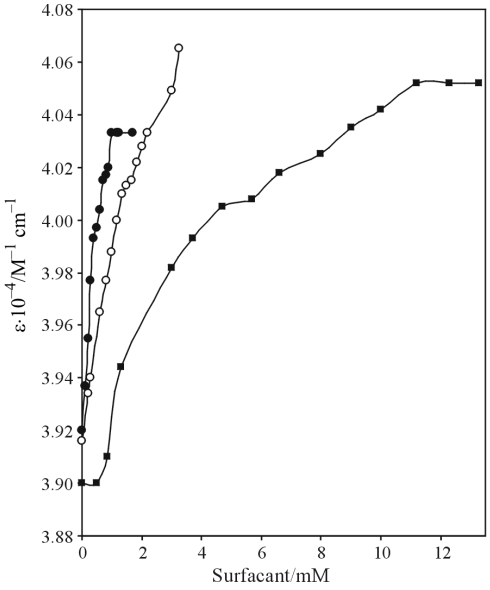

Fig. 1 Variation of molar absorption coefficient of lysozyme at 281 nm *vs*. total surfactant concentration at 5 mM phosphate buffer, pH 7.2 and 27°C. ■ – DTAB, ○ – TTAB and ● – HTAB

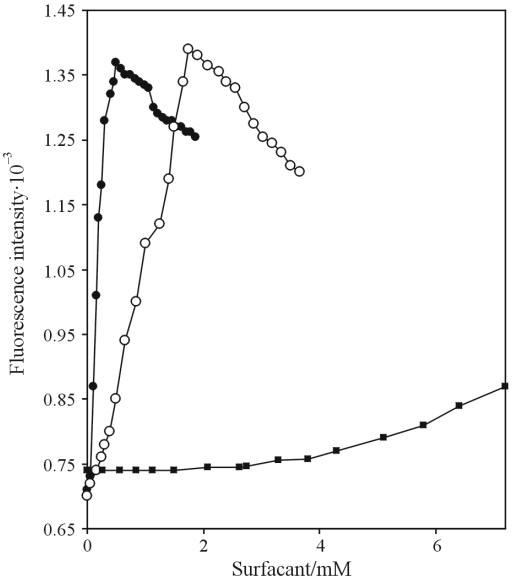

Fig. 2 Variation of fluorescence emission intensity of lysozyme at 339.2 nm (λ_{ex}=281 nm and band mass are 3 nm for both excitation and emission band slit), *vs.* total surfactant concentration at 5 mM phosphate buffer, pH 7.2 and 27°C. ■ – DTAB, ○ – TTAB and ● – HTAB

curves for HTAB and TTAB show a distinct maximum at 0.5 and 1.7 mM, respectively. This extreme point was not observed for DTAB. This represents the higher power of TTAB and HTAB for unfolding of lysozyme. It seems the denaturation power of surfactant does not have a linear relation with hydrocarbon tail length. This can be related to cooperative nature of protein folding process. However, this trend of variation in fluorescence intensity of lysozyme can be interpreted as follows.The initial binding of ionic surfactant to ionic charge sites at the lysozyme surface rigid the chemical environment of tryptophan residues so that the quantum efficiency increased. This initial interaction ultimately perturbs the protein structure and unfolded protein in a cooperative manner. The unfolding exposes the tryptophan residues and also increases the flexibility of these residues, consequently, the quenching effects favored and quantum yield of protein decreased. This behavior does not clearly observed for DTAB which also confirmed the UV-results about the relation between denaturation power of surfactant and its hydrocarbon tail length.

Figure 3 shows the enthalpograms for interactions of DTAB, TTAB and HTAB with lysozyme. These curves determined from calorimetric measurements and represent the values of total interaction heat of surfactant to lysozyme at any surfactant concentration. The corresponding curves for DTAB and TTAB show two distinct maximum which related to two endothermic processes, while the enthalpic curve for HTAB have a distinct maximum and a shoulder.

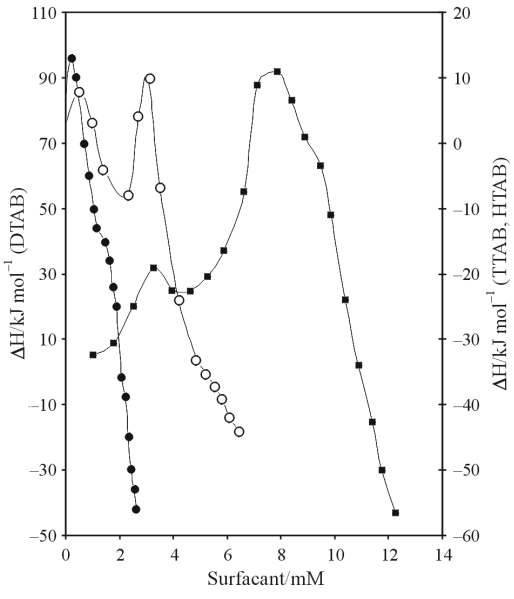

Fig. 3 The plot of calorimetric enthalpy (ΔH) for interaction of ■ – DTAB, ○ – TTAB and ● – HTAB with lysozyme, *vs.* total concentration of surfactant, at 5 mM phosphate buffer, pH 7.2 and 27°C

The exothermicity of process increased by increasing hydrocarbon tail length of surfactant, this behavior can be interpreted as follows.The initial ionic interactions induced a conformational change in native protein which changes the decreasing trend of enthalpy curve. The subsequent binding to the new folded state ultimately unfolded lysozyme which is an all-or-none and endothermic process. Hence, the following mechanism can be proposed for this process

$$F_1 \rightarrow F_2 \rightarrow U$$

where F_1 and F_2 are two different folded states of lysozyme which relate to first maximum and U is unfolded state of lysozyme and relates to second maximum in enthalpic curves. This process is endothermic. The clearity of these maximum must be corresponded to the relative amount of endothermic unfolding enthalpy to exothermic binding enthalpy. The results represent the increasing of exothermic binding enthalpy by increasing of hydrocarbon tail length. This causes that the second maximum for HTAB dose not appears clearly and converted to a shoulder in the enthalpic curve. Consequently, the second maximum in enthalpic curves is related to predominant unfolding of lysozyme and the first to different forms of folded state. The position of second maximum is related to denaturation power of surfactant and confirms the spectroscopic results about the nonlinear relation between this character and hydrocarbon tail length of surfactant. Moreover, this can be representing the special power of calorimetric method for monitoring all of the structural changes in protein which spectroscopic methods can not. A two-step mechanism on basis of ITC data was also reported for denaturation of jack bean urease with these surfactants, previously [21, 22]. Our interpretation is in agreement with these studies.

Acknowledgements

Financial supports of Center for Graduate Studies and Research Council of Isfahan University are gratefully acknowledged.

References

1 S. Chodankar and V. K. Aswal, Phys. Rev. E, 72 (2005) 041931.
2 G. D. Manetto, C. La Rosa, D. M. Grasso and D. Milardi, J. Therm. Anal. Cal., 80 (2005) 263.
3 A. Michnik, K. Michalik and Z. Drzazga, J. Therm. Anal. Cal., 80 (2005) 399.
4 C. N. Pace, Trends Biotechnol., 8 (1990) 93.
5 C. Vieille and J. G. Zeikus, Trends Biotechnol., 14 (1996) 183.
6 M. N. Jones and P. Manley, Surfactants Solution, Proc. Int. Symp., 4th (1984), Meeting Date 1982, pp. 2, 1403.
7 T. Imoto, S. Sami, M. Tsuru and K. Yagishita, Agric. Biol. Chem., 43 (1979) 1809.
8 K. Hayashi, M. Kugimiya, T. Imoto, M. Funatsu and C. C. Bigelow, Biochemistry, 7 (1968) 1461.
9 K. Hayashi, M. Kugimiya, T. Imoto, M. Funatsu and C. C. Bigelow, Biochemistry, 7 (1968) 1467.
10 M. Subramanian, B. S. Sheshadri and M. P. Venkatappa, J. Biochem., 95 (1984) 413.
11 A. A. Saboury, J. Therm. Anal. Cal., 72 (2003) 93.
12 L. J. Waters, S. A. Leharna and J. C. Mitchell, J. Therm. Anal. Cal., 80 (2005) 43.
13 M. R. de Rivera and F. Socorro, J. Therm. Anal. Cal., 80 (2005) 769.
14 A. J. Sophianopoulos, C. K. Khodes, D. N. Holcomb and K. E. Holde. J. Biol. Chem., 237 (1962) 1107.
15 G. C. Kresheck and W. A. Hargraves, J. Colloid Interface Sci., 48 (1974) 481.
16 G. C. Kresheck, J. Phys. Chem. B, 102 (1998) 6596.
17 G. Olofsson, J. Phys. Chem., 89 (1985) 1473.
18 I. Johnson, G. Olofsson and B. Jonsson, J. Chem. Soc. Faraday Trans., 183 (1987) 3331.
19 S. Paula, W. Sus, J. Tuchtenhagen and A. Blume, J. Phys. Chem., 99 (1995) 11742.
20 M. J. Rosen, 'Surfactants and Interfacial Phenomena' Surfactant Research Institute, Brooklyn College, 1989, Chapter 3, p. 122.
21 A. A. Saboury, A. K. Bordbar and A. A. Moosavi-Movahedi, J. Chem. Thermodyn., 28 (1996) 1077.
22 A. A. Saboury and A. A. Moosavi-Movahedi, Bull. Chem. Soc. Jpn., 69 (1996) 3031.

Received: January 10, 2005
Accepted: October 10, 2006

DOI: 10.1007/s10973-005-6906-2