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Thermodynamics of Protein Denaturation by Sodium Dodecyl Sulfate

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The anionic surfactant sodium n-dodecyl sulfate (SDS) plays a variety of roles with regard to protein conformation, depending on its concentration. SDS at low concentrations mostly induces the compaction of protein (folding). Examples of this include: the molten globule state of acid-unfolded cytochrome c, associated with enhancement of the exothermic enthalpy values of isothermal titration calorimetry and a reversible profile by differential scanning calorimetry; the enzyme activation and compaction of Aspergillus niger catalase, and relationship of calorimetric enthalpy (ΔH_{cal}) to van't Hoff enthalpy (ΔH_{VH}), which proves the existence of intermolecular and intramolecular interaction during enzyme activation by SDS; the production of a new energetic domain for human apotransferrin and folded state for histone H_1 by SDS. SDS at moderate concentrations below the critical micelle concentration (cmc) is a potent denaturant for protein in solution. Protein denaturation is a key method in thermodynamics and binding site analysis and can be used to enhance our understanding of the protein structure-function relationship. The interaction between protein and surfactant, such as SDS, at the cmc level is a complicated interaction, thermodynamically, that should bring about enthalpy correction through micellar dissociation and micelle dilution.

Keywords: SDS, Proteins, Enthalpy, Molten globule, Folding, Unfolding

INTRODUCTION

The ionic surfactants consist of polar and non-polar portions on the same molecule. The dual nature of an anionic surfactant is typified by sodium n-dodecyl sulfate (SDS), CH_3-$(CH_2)_{10}$-CH_2-$OSO_3^-Na^+$, which has found wide application in biology [1,2]. The widespread application of surfactants, such as SDS, in the field of biochemistry has given impetus to fundamental studies of the nature of the interaction between protein and surface active agents in biological phenomena such as biological membranes [3] and protein solubilization [4]. It has also been suggested that surfactant-protein systems can be used as a model for biological membranes. Since phospholipid membranes help to stabilize the three-dimensional structure of membrane proteins, the use of detergents for the reconstitution of the proteins seems plausible. Further, there are similarities in the structures of some detergents and some of the phospholipids of cell membranes. It has been shown that both the head groups and the hydrophobic tails of surfactants are important for the stabilization of membrane proteins. The successful use of the detergent (*e.g.*, lauryl maltoside) for the solubilization/reconstitution of mitochondrial membranes has been demonstrated [5].

The effect of surfactants, such as SDS, on protein folding and unfolding depends on the concentrations of SDS and protein [6] as well as the nature of protein. Anionic surfactants, such as SDS, bind to protein in the monomeric state and in the micellar condition. The interaction between protein and micelle is a complicated situation that plays a role

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in the folding and unfolding of proteins [7-9]. Protein refolding and protein reactivation at high concentrations (cmc) using a detergent/phospholipid mixture has been reported. Proteins reactivated by mixed micelles are easy to purify since folding intermediates remain bound to the micelles and are released only after they have folded/reactivated. This is a successful method for preventing misfolding and/or aggregation, as well as promoting correct protein folding [7]. It has also been used in the regeneration of bacteriorhodopsin in mixed micelles [8] and reactivation of several enzymes by surfactant after treatment with guanidinium chloride [9]. It has also been reported that some aspects of the micelle model of alpha-crystallin can be related to its chaperone activity [10]. The use of a mixture of detergent and cyclodextrin can also act as an artificial chaperone, which promotes protein folding [11].

The binding of native cytochrome c to lipid micelles leads to a partially unfolded conformation of cytochrome c. This micelle-bound state has no stable tertiary structure, but remains as alpha-helical as the native cytochrome c in solution. In contrast, the binding of acid unfolded cytochrome c to lipid micelles induces folding of the polypeptide [12]. It has been reported that SDS refolds acid-denatured cytochrome c (Cyt) into a molten globule like state (MG-like state), which is obtained at low and high SDS concentrations (above its cmc), whereas aggregation and precipitation are observed at a medium SDS concentrations between these two states [13]. Formation of the MG-state with low SDS concentration is dependent on the [SDS]/[Cyt] ratio, as almost double the SDS concentration was needed to reach a MG-like state when the protein concentration was doubled [13].

The prevention of cytochrome c denaturation by urea using SDS at physiological pH suggests that the presence of SDS prevents the complete denaturation of cytochrome c by causing the formation of some stable partially folded forms of cytochrome c [14]. Another report implies that SDS induces the formation of a stabilized intermediate state for cytochrome c (at acidic and alkaline pH) [15] and for RNase A at low pH [16]. It has been reported that surfactants work as promoters for the refolding of protein (*e.g*., carbonic anhydrase II) through the formation of soluble folding intermediates and not by the dissolution of aggregates [17]. Another work suggests that SDS has a net stabilizing effect up to a molar ratio of 10:1 (ligand to protein) for bovine serum albumin and SDS, and at higher concentrations acts as a destabilizer and denaturant [18].

The mechanism of the surfactant-induced unfolding of protein will improve our understanding of protein folding. The SDS-unfolded cytochrome c structure is a unique state, which is structurally an intermediate between completely unfolded and native folded states. Hence, it allows the folding pattern to be studied starting from an intermediate step of cytochrome c unfolding of the protein by SDS, which occurs in a one-step cooperative process, and proceeds through an activation barrier. Another report states that the bisHis-ligated state captures a segment of the protein in a non-native orientation, thus folding from this state is a rate limiting step [19].

One of the important applications of surfactants is the breakdown of protein structure (denaturation). Ionic surfactants, such as SDS, are unique in the way that they denature proteins at millimolar concentrations in marked contrast to other denaturants, such as guanidinum chloride or urea, which are effective only at molar concentrations [20]. Denaturation studies are capable of yielding information about the native state of a protein in terms of its thermodynamic stability, cooperativity, and the nature of the forces required to maintain its tertiary structure [21,22]. Surfactant-protein interactions have many applications such as molecular characterization [23], determination of enzyme from membrane [24], dissociation of protein from DNA and tissue [25,26], protein dissociation [27], protein precipitation [28], and protein fragmentation [29,30].

In this paper, we attempt to show the different roles of SDS at various concentrations upon interaction with protein, particularly from the thermodynamic viewpoint.

Materials and Methods

Materials. Horse cytochrome c (type IV), human apotransferrin (hTF), horseradish peroxidase (HRP), Aspergillus niger catalase, and SDS were obtained from Sigma. Human adult hemoglobin (HbA) was prepared as previously reported [31]. All other reagents were of analytical grade. Solutions were prepared with double-distilled water.

Methods. The various instruments and methods used were from our previous reports as follows: isothermal titration calorimetry (ITC) (thermal activity monitor 2277, Thermo-

metric, Sweden) [32]; differential scanning calorimetry (DSC) (Scal-1 microcalorimeter, Russia) [33]; circular dichroism (CD) (JASCO J-715 spectropolarimeter, Japan); spectrophotometry (model Shimadzu-3100 spectrophotometer) and microviscometry (Haake D8 microviscometer Germany) [34]; densitometry (model DMA 58 densitometer, Austria) [34]; equilibrium dialysis [35].

RESULTS AND DISCUSSION

As Table 1 shows, SDS plays different roles at various concentrations with regard to protein conformation. Here, we classify the SDS concentrations as very low, low, medium and high. Each classification corresponds to a specific role affecting protein structure and function.

Figure 1 represents the formation of the MG-like state of cytochrome c induced by a very low concentration of SDS. The effect of salts (*e.g.*, $Na\text{-}OSO_3^-Na^+$) on the MG formation of cytochrome c and other proteins *via* the acid unfolded state through the folded state has been reported [36,37]. In addition, evidence for the stabilization of the MG state of cytochrome c by SDS as a salt with a hydrophobic chain ($CH_3\text{-}(CH_2)10\text{-}CH_2\text{-}OSO_3^-Na^+$) has been presented. Accordingly, SDS has been referred to as a hydrophobic salt [34,38]. The results show that SDS is much more effective than sodium sulfate for the formation of the MG state for cytochrome c (see Table 2 from Ref. [34]) due to the presence of its hydrophobic tail. It has been reported that the main driving force of the salt-induced MG state is the reduction of the electrostatic repulsion between the charged groups in the protein molecule [39].

In this paper, SDS is selected because of its dual electrostatic and hydrophobic interactions; the electrostatic contribution is more dominant than the hydrophobic moiety at very low concentrations [34]. Therefore, the hydrophobic chain increases the ability of SDS at a very low concentration to form the MG state of cytochrome c, mostly through electrostatic contribution. Figure 1c shows the calorimetric titration of the acid unfolded state of cytochrome c with the SDS generated exothermic process for forming the MG state. The effects of various n-alkyl sulfates on the acid unfolded state of cytochrome c indicate that the increased alkyl chain length has enhanced the exothermic values of calorimetric enthalpy, with a more compact structure [32] and more electron transference for the MG states [40].

Table 1. The Role of SDS at Different Concentrations upon Interaction with Proteins

[SDS][a]	Status
Very low	Hydrophobic salt, molten globule state, protein folding
Low	Protein folding, mild denaturation
Medium	Protein unfolding and denaturation
High	cmc, protein salvation, protein folding/unfolding and other applications

[a] The effective concentration range of SDS for each step in Table 1 is dependent upon the protein's condition and the chemical and physical conditions of the protein-SDS interaction in aqueous solution.

One of the advantages of the MG state induced by SDS for cytochrome c (and the heme proteins) is the conversion of thermal irreversibility to the reversible state. The differential scanning calorimetry-thermal denaturation of cytochrome c has always been calorimetrically irreversible, whereas the MG state induced by SDS at very low concentrations results in a reversible profile [41]. The spectroscopic properties indicate that the hydrophobic tail of SDS utilizes the hydrophobic contribution to stabilize the heme conformation at the MG state of cytochrome c. This would be the main reason for thermal profile reversibility of the MG state in cytochrome c. The reversibility of the DSC thermogram would allow its deconvolution and thermodynamic analysis of the energetic domains for this protein [41].

SDS exhibits different roles at low concentrations upon interaction with proteins. SDS probably induces the folding or partial unfolding for proteins (depending on the protein characterization).

Aspergillus niger catalase could be activated up to 180% by a low concentration of SDS at pH 6.4 because of the compaction (folding) of the catalase-SDS complexes under these circumstances [42]. The thermodynamic and hydrodynamic results show the compaction of catalase at low concentrations of SDS (≤ 2 mM) (see Table 2). The value of

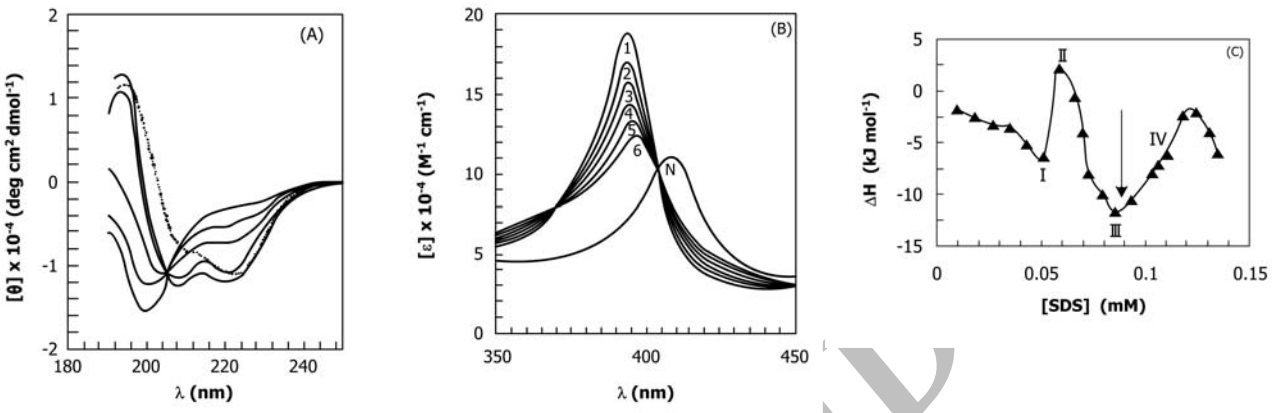

Fig. 1. (A) Far UV circular dichroism (CD) spectra of cytochrom c (ellipticity, [θ]) as a function of SDS concentrations. 1, 0 mM SDS (denatured state at pH = 2); 2, 0.01 mM SDS; 3, 0.037 mM SDS; 4, 0.06 mM SDS; 5, 0.08 mM SDS; dotted curve, the spectrum of the native state in 25 mM phosphate buffer at pH = 7. (B) Soret absorption spectra of cytochrome c (molar extinction coefficient, [ε]) as a function of SDS. 1, 0 mM SDS (denatured state at pH = 2); 2, 0.02, mM SDS; 3, 0.04 mM SDS; 4, 0.06 mM SDS; 5, 0.07 mM SDS; 6, 0.08 mM SDS; N, native curve at pH = 7, in 25 mM phosphate buffer. (A) and (B) are taken directly from Ref. [34]. (C) Calorimetric (ITC) enthalpy (ΔH) plotted against concentrations of SDS upon interaction with cytochrome c, obtained after subtracting the effect of SDS dilution. Arrow shows the MG state indication induced by various concentrations of SDS: I, 0.05 mM; II, 0.06 mM; III, 0.08 mM; IV, 0.11 mM. This curve is directly taken from Ref. [32].

$\Delta H_{VH}/\Delta H_{cal}$ at 2 mM SDS is very high and entropy is very low, which may indicate the strong intermolecular force between the subunits of the catalase-SDS complexes in this condition. Table 2 also shows the values of Stokes radius, partial specific volume and heat capacity, indicating the folding of the catalase during the optimum activation at 2 mM SDS [42].

The results also show that SDS at low concentrations (≤ 1 mM) induces the compaction of HbA (see Fig. 2). The DSC profile, melting point and partial specific volume (V) indicate the degree of folding of HbA upon addition of SDS at low concentrations [31].

Histone H_1 is also folded by SDS at low concentrations (< 0.5 mM), which was confirmed using various techniques including binding enthalpy curve, calorimetry, polyacrylamide gel electrophoresis, protein titration, and viscometry [27,43,44].

Sometimes SDS at low concentrations induces the partial unfolding of proteins (*e.g.*, hTF). Figure 3 shows the reversible DSC profiles for hTF in the absence and presence of 0.5 mM SDS. This figure depicts two distinct peaks for hTF, left shifted by the addition of 0.5 mM SDS, which means that the SDS has somewhat destabilized the hTF-SDS complexes by the reduction of the T_m of hTF, as seen in Fig. 3A. For the most part, the decrease in T_m corresponds to the unfolding or partial unfolding of the protein. Figure 3 shows the deconvoluted curve of excess heat capacity (Cp_{excess}) for hTF in the presence of SDS (0.5 mM) having four subpeaks, that is two subpeaks for the C domain and two other subpeaks for the N domains (Fig. 3B). The area under Cp_{excess} and the top of the curve of thermal unfolding (subpeaks) indicate the enthalpy of unfolding (ΔH_{unf}) and T_m of the protein domains, respectively. The amount of ΔH_{unf} for all subpeaks is generally diminished during the presence of SDS specially for N domains (for more information, see Refs. [33,45]).

Sodium dodecyl sulfate at medium concentration (below the critical micelle concentration, cmc) induced almost the denaturation for proteins while the hydrophobic moiety is predominant. Proteins denaturation by surfactant such as SDS provides extra information local site-specific properties of protein systems that lie at the heart of many biochemical regulatory phenomena and is the basic key to biological

Table 2. The Relationship of $\Delta H_{VH}/\Delta H_{cal}$ (van't Hoff Enthalpy and Calorimetric Enthalpy), Heat Capacity (ΔC_P), Viscosity (η), Partial Specific Volume (V) and Stokes Radius for Aspergillus Niger Catalase upon Interaction with SDS at Various Concentrations. These Results are Directly Taken from Ref. [42]

[SDS] (mM)	$\Delta H_{VH}/\Delta H_{Cal}$	ΔS_V (J mol^{-1} K^{-1})	$\eta \times 10^{-4}$ (M^{-1})	V (cm^3 g^{-1})	Stokes radius (Å)	ΔCp (kJ mol^{-1} K^{-1})
0.0	-	-	1.12	0.7696	149.96	1.98
1.0	-	-	1.15	-	122.32	-
2.0	7.33	-10	0.85	0.6576	110.60	1.70
3.0	4.54	5	A	-	-	1.73
4.0	2.66	10	-	0.8225	-	-
5.0	2.22	15	-	-	-	1.75
6.0	1.90	30	-	-	-	-
6.5	1.79	35	-	-	-	-
7.0	1.84	40	2.2	0.8236	151.85	-
8.0	2.08	-	-	-	-	1.80
9.0	2.75	-	-	-	-	-
10.0	-	-	-	0.8484	-	-

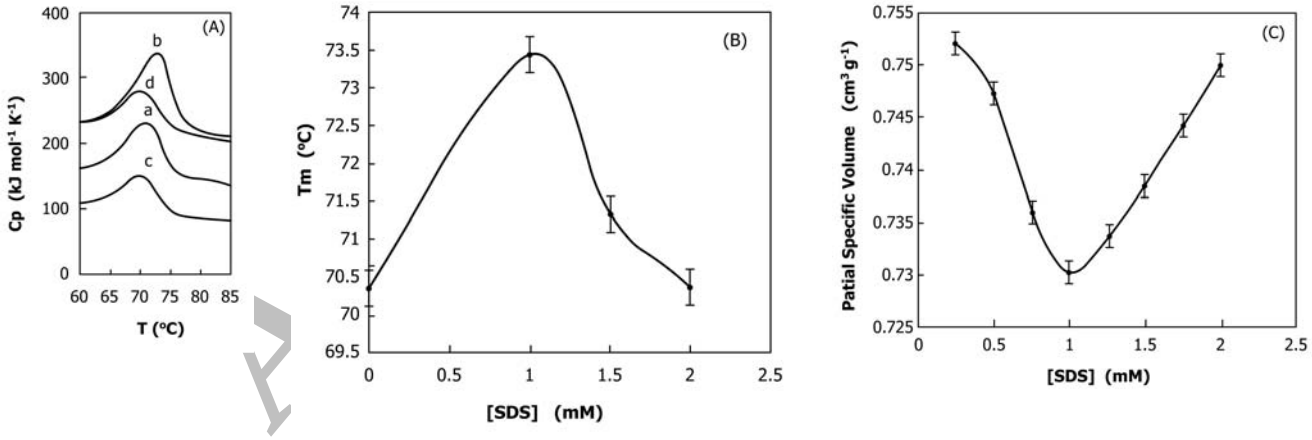

Fig. 2. (A) DSC profiles for hemoglobin (HbA) upon interaction of SDS at different concentrations. The top of DSC profile shows the T_m for HbA: a, 0 mM SDS; b, 1 mM SDS; c, 1.5 mM SDS; d, 2 mM SDS. (B) Plot of T_m versus SDS at corresponding concentrations. (C) Effect of corresponding SDS concentrations on partial specific volume changes for HbA. All results indicate the protein folding at a low concentration of SDS. These results are directly taken from Ref. [31].

control. The binding is the specific virtue of protein-surfactant complexes that directly occurs [46]. The availability of binding data opens the way to the extensive area of macromolecular ligand binding theories that provide a deep understanding of protein structure [47]. For example, the denaturation of HRP by SDS is explained from the perspective

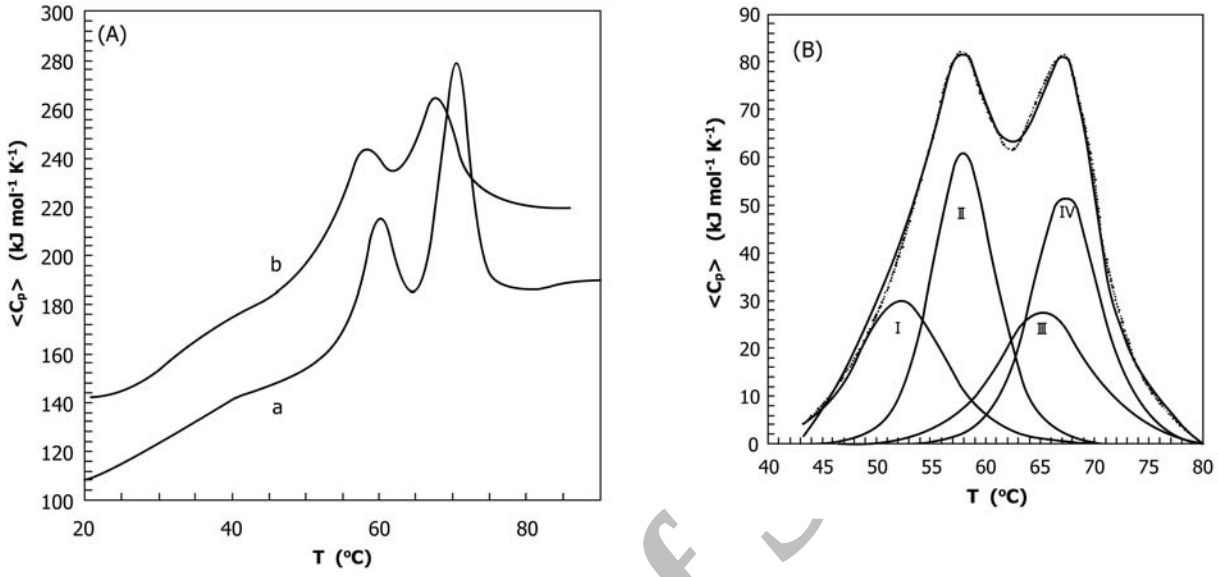

Fig. 3. (A) DSC profiles of hTF in the absence (a) and presence of 0.5 mM SDS (b). (B) Deconvoluted excess molar heat capacity of hTF in the presence of 0.5 mM SDS. Subpeaks of I, II and III, IV belong to C and N domains of hTF, respectively. These results are directly taken from Ref. [33].

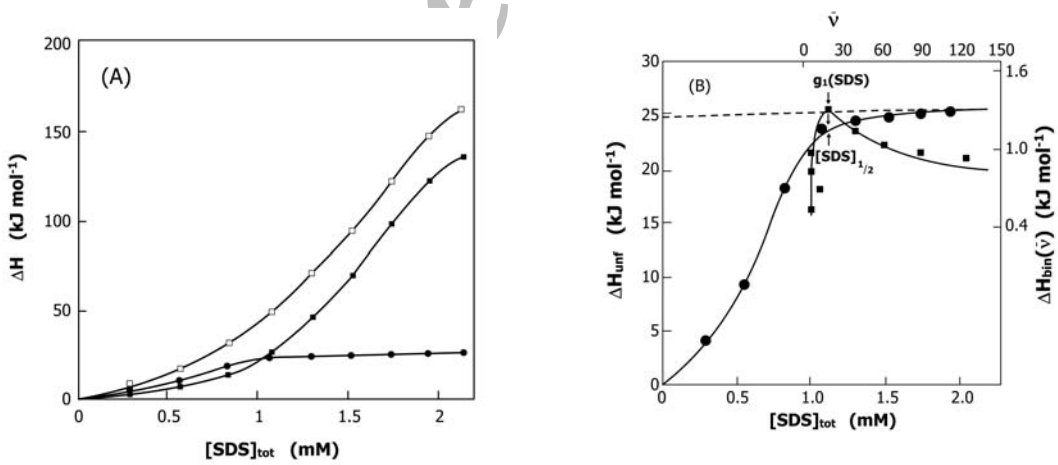

Fig. 4. (A) Enthalpy changes of the interaction of HRP with SDS at 27 °C: (□) enthalpy of calorimetry (ITC), (■) enthalpy of binding, (●) enthalpy of unfolding. (B) (●) Dependence of enthalpy of unfolding (ΔH_{unf}) for HRP upon interaction with various total concentrations of SDS and (■) plot of $\Delta H_{bin(\bar{\nu})}$ *vs.* $\bar{\nu}$ (SDS) at 27 °C. $\bar{\nu}$ is the number of moles SDS per number of moles HRP and g_1 is the maximum of $\bar{\nu}$ belonging to the first set of cited interactions. These results are directly taken from Ref. [35].

of enthalpy. Figure 4B shows the different enthalpies of denaturation for HRP: calorimetric enthalpy (ΔH_{cal}), binding enthalpy (ΔH_{bin}) and enthalpy of unfolding (ΔH_{unf}). Figure 4B shows the relationship of ΔH_{unf} to $\Delta H_{bin(\bar{\nu})}$ ($\bar{\nu}$ is the number of moles SDS per number of moles HRP). This figure shows that 95% of HRP unfolding coincides with g_1 (g_1 is the

number of electrostatic binding sites for the first set of bindings by SDS). This means that the degree of HRP unfolding (95%) occurred at the end of the ionic interaction and at the start of the hydrophobic contribution (for more information, see Ref. [35]).

Surfactants such as SDS induce the formation of micelles at high concentrations. The interaction between protein and surfactant at the cmc has not been studied extensively due to the thermodynamic complexity of the interaction. During protein-surfactant interaction at the cmc level, we note the absorption of heat (endothermic enthalpy) during demicellization (micellar dissociation) and the dilution of the micelles [48]. The cmc for anionic surfactants is highly dependent on ionic strength. The higher the ionic strength, the lower the concentration of surfactant needed to reach the cmc. We have previously studied the enthalpy of the interaction between SDS and Aspergillus niger catalase by micro-calorimetry and equilibrium dialysis over a range of ionic strengths [48]. The observed enthalpies of these interactions were corrected for micellar dissociation (see Fig. 1 from Ref. [48]).

The study of protein-surfactant interactions (especially protein-SDS interactions) can assist us in the structural elucidation of proteins, improvement of our understanding of protein binding site magnitudes, recognition of the thermodynamic forces involved, and recognition of protein cooperativity.

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REFERENCES

[1] F. W. Putnam, Adv. Protein Chem. 4 (1984) 79.

[2] D. Attwood, A.T. Florence, Surfactant Systems, Their Chemistry, Pharmacy and Biology, Chapman and Hall, London, 1983.

[3] M.N. Jones, Biological Interfaces, Elsevier, Amsterdam, 1975, Chap. 5.

[4] A. Helenius, K. Simons, Biochim. Biophys. Acta 415 (1975) 29.

[5] G. Zardeneta, P.M. Horowitz, Anal. Biochem. 223 (1994) 1.

[6] P. Jing, T. Kaneta, T. Imasaka, Anal. Sci. 21 (2005) 37.

[7] G. Zardeneta, P.M. Horowitz, Anal. Biochem. 218 (1994) 392.

[8] R. Renthal, C. Hannapel, A.S. Nguyen, P. Haas, Biochim. Biophys. Acta 1030 (1990) 176.

[9] S. Tandon, P.M. Horowitz, Biochim. Biophys. Acta 955 (1988) 19.

[10] T. Aerts, J. Clauwaert, P. Haezebrouck, E. Paeters, Eur. Biophys. J. 25 (1997) 445.

[11] D. Rozema, S.H. Gellman, J. Am. Chem. Soc. 117 (1995) 2373.

[12] N. Sanghera, T.J.T. Pinheiro, Protein Sci. 9 (2000) 1194.

[13] Q. Xu, T.A. Keiderling, Protein Sci. 13 (2004) 2949.

[14] K. Chattopadhyay, S. Mazumdar, Biochem. 42 (2003) 14606.

[15] A. Naeem, R. Hasan Khan, Int. J. Biol. Macromol. 36 (2004) 2281.

[16] A.A. Moosavi-Movahedi, M. Gharanfoli, K. Nazari, M. Shamsipur, J. Chamani, B. Hemmateenejad, M. Alavi, A. Shokrollahi, M. Habibi-Rezaei, C.M. Sorenson, N. Sheibani, Colloids Surf. B 43 (2005) 150.

[17] D.B. Wetlaufer, Y. Xie, Protein Sci. 4 (1995) 1535.

[18] C. Giancola, C. Sena, D. Fessas, G. Graziano, G. Barone, Int. J. Biol. Macromol. 20 (1997) 193.

[19] T.K. Das, S. Masumdar, S. Mitra, Eur. J. Biochem. 245 (1998) 662.

[20] A.A. Moosavi-Movahedi, G.A. Naderi, B. Farzami, Thermochim. Acta 239 (1994) 61.

[21] C. Tanford, Adv. Protein Chem. 24 (1970) 1.

[22] S. Lapanje, Physicochemical Aspects of Protein denaturation, John Wiley & Sons, New York, 1978.

[23] M.N. Jones, Chem. Soc. Rev. 21 (1992) 127.

[24] A.T. Andrews, Electrophoresis, Theory, Techniques and Biochemical and Clinical Application, 2nd ed., Clarendon Press, Oxford, 1990, pp. 117-147.

[25] C. Tanford, J.A. Reynolds, Biochim. Biophys. Acta 457 (1976) 133.

[26] A.L. Dounce, Arch. Biochem. Biophys. 111 (1965) 506.

[27] S.Z. Bathaie, A.A. Moosavi-Movahedi, B. Ranjbar,

A.A. Saboury, Colloids Surf. B 28 (2003) 17.
[28] A. Takeda, A. Hachimori, M. Murai, K. Sato, T. Samejima, J. Biochem. 78 (1975) 911.
[29] K.S. Birdi, Biochem. J. 135 (1973) 253.
[30] A. Weda, K. Takeda, J. Colloid Interface Sci. 138 (1990) 277.
[31] M.R. Dayer, A.A. Moosavi-Movahedi, P. Norouzi, H.Q. Ghourchian, S. Safarian, J. Biochem. Mol. Biol. 35 (2002) 364.
[32] J. Chamani, A.A. Moosavi-Movahedi, A.A. Saboury, M. Gharanfoli, G.H. Hakimelahi, J. Chem. Thermodyn. 35 (2003) 199.
[33] M. Rezaei-Tavirani, A.A. Moosavi-Movahedi, S.Z. Moosavi-Nejad, J. Chamani, D. Ajloo, Thermochim. Acta 408 (2003) 9.
[34] A.A. Moosavi-Movahedi, J. Chemical, Y. Goto, G.H. Hakimelahi, J. Biochem. 133 (2003) 93.
[35] K. Nazari, A.A. Saboury, A.A. Moosavi-Movahedi, Thermochim. Acta 302 (1997) 131.
[36] Y. Goto, S. Nishikori, J. Mol. Biol. 222 (1991) 679.
[37] Y. Goto, A.L. Fink, Biochemistry 28 (1989) 945.
[38] A.A. Moosavi-Movahedi, J. Chamani, in: J.E. Ladbury, M. Doyle (Eds.), Biocalorimetry II, John Wiley & Sons, Ltd., New York, 2004, Chap. 12.
[39] Y. Goto, N. Takahashi, A.L. Fink, Biochemistry 29 (1990) 3480.
[40] A.A. Moosavi-Movahedi, J. Chamani, H. Ghourchian, H. Shafiey, C.M. Sorenson, N. Sheibani, J. Protein Chem. 22 (2003) 23.
[41] A.A. Moosavi-Movahedi, J. Chamani, M. Gharanfoli, G.H. Hakimelahi, Thermochim. Acta 409 (2003) 137.
[42] A.A. Moosavi-Movahedi, Pure Appl. Chem. 66 (1994) 71.
[43] A.A. Moosavi-Movahedi, M. Goodarzi, M.R. Housaindokht, in: Z.H. Zaidi, A. Abbassi, J. Smith (Eds.), Protein Structure-Function TWEL Publishers, London, 1990.
[44] A.A. Moosavi-Movahedi, A. Rabbani, M. Goodarzi, B. Goliaei, Thermochim. Acta 154 (1989) 205.
[45] M. Rezaei-Tavirani, A.A. Moosavi-Movahedi, A.A. Saboury, G.H. Hakimelahi, B. Ranjbar, M.R. Housaindokht, Thermochim. Acta 383 (2002) 103.
[46] A.A. Moosavi-Movahedi, Encyclopedia of Surface and Colloid Science, Marcel Dekker, Inc., New York, 2002, pp. 5344-5354.
[47] M.N. Jones, Biochem. J. 151 (1975) 109.
[48] A.A. Moosavi-Movahedi, M.N. Jones, G. Pilcher, Int. J. Biol. Macromol. 11 (1989) 26.