A Thermodynamic Study on Lysozyme with Sodium Dodecyl Sulfate in Different Temperatures and PHs

GHOLAMREZA REZAEI BEHBEHENI
Chemistry Department, Imam Khomeini International University, Qazvin, Iran.
grb402003@yahoo.com

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Summary: The interaction of Sodium Dodecyl Sulfate (SDS) with hen egg lysozyme have been investigated at 298, 303 and 308 K in phosphate buffer at two different pH values (5 and 7), by isothermal titration calorimetry. The calorimetric data analysis allows the measurement of the complete set of thermodynamic parameters. The negative SDS ion binds to positive residues, neutralizes the protein surface charges and leads to precipitation and turbidity of the solution. At low concentrations of SDS, the binding is mainly electrostatic, with some simultaneous interaction of the hydrophobic tail with nearby hydrophobic patches on the lysozyme. The enthalpies of denaturation at pH 7 are 180.47, 198.51 and 216.56 $_{kJmol^{-1}}$ for 298, 303 and 308 K respectively.

Keywords: Sodium Dodecyl Sulfate, Lysozyme, Unfolding, Isothermal titration calorimetry.

Introduction

It is well known that sodium *n*-dodecyl sulfate (SDS), is an amphipathic anionic surfactant, employed widely in protein biochemistry for its powerful dissociation and solubilization properties. The study of surfactant+ protein interaction creates much interest for many physicochemical as well as conformational phenomena. From a technological perspective, studies of surfactant+ protein interactions are intriguing because they modulate the functional properties of proteins. Such interaction has been widely studied for many years because of its applications in industry, chemical, biological, pharmaceutical and cosmetic laboratories [1-3].

Surfactants can bind to proteins both as monomers and as micelles depending on the nature of the interaction and the surfactant concentration [2]. Surfactants have been divided into those, which bind and induce protein unfolding and those, which slightly interact without protein denaturation. Non-ionic or 'soft' surfactants are usually used for solubilization of membrane proteins. In principle all proteins retain their structures and activities in the presence of non-ionic detergents. In contrast ionic surfactants can bind to proteins and alter the conformation substantially that often leads to denaturation. Among these surfactants, SDS is most commonly used. The forces involved between protein and ionic detergents are both electrostatic and hydrophobic in nature [4-6].

Denaturation studies are capable of yielding information about the native state of a protein in terms of its cooperativity, intrinsic stability, and the nature of forces required to maintain its three dimensional structure [1-8]. 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. Many proteins possess specific binding sites for the surfactants [3-5].

Ionic surfactants, such as SDS, are unique in the way that they denature proteins at milli molar concentrations in marked contrast to other denaturants, such as guanidinum chloride or urea, which are effective only at molar concentrations [2-4]. The mechanism of the surfactant-induced unfolding of protein will improve our understanding of protein folding. One of the important applications of surfactants is the breakdown of protein structure (denaturation). Denaturation for providing additional information on the structure, properties and function of a protein can be brought about in many ways. 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 SDS, CH_3-$(CH_2)_{10}$-CH_2-$OSO_3^-Na^+$, which has found wide application in biology [3-7]. Then, in present study, we have investigated the interaction between SDS and hen lysozyme at different temperatures (298, 303 and 308 K) and pHs (5 and 7) by the extended solvation model.

Results and Discussion

We have shown previously that the heats of the macromolecules and ligands interactions can be reproduced by Eq. 1 in the aqueous solvent systems [5-14].

$$\Delta H=\Delta H_{max}x'_B-\delta^{\theta}_A(x'_AL_A+x'_BL_B)-(\delta^{\theta}_B-\delta^{\theta}_A)(x'_AL_A+x'_BL_B)x'_B+f_D\Delta H_D \quad (1)$$

The parameters δ^{θ}_A and δ^{θ}_B reflect to the net effect of SDS on the macromolecule conformational changes in the low and high SDS concentrations respectively. The positive values for δ^{θ}_A or δ^{θ}_B indicate that SDS stabilize the lysozyme structure and vice versa. x'_B can be expressed as follows:

$$x'_B=\frac{px_B}{x_A+px_B} \quad (2)$$

where x'_B is the fraction of bound SDS and $x'_A=1-x'_B$ is the fraction of unbound SDS. If the ligand binds at each site independently, the binding is non-cooperative and p is equal to 1. $p > 1$ or $p<1$ indicate positive or negative cooperativity of macromolecule for binding with ligand respectively. x_B is the total SDS concentrations divided by the maximum concentration of the SDS upon saturation of all lysozyme as follows:

$$x_B=\frac{[SDS]_t}{[SDS]_{\max}} \quad x_A=1-x_B \quad (3)$$

$[SDS]_t$ is the total concentration of surfactant and $[SDS]_{\max}$ is the maximum concentration of the surfactant upon saturation of all lysozyme. L_A and L_B are the relative contributions of unbound and bound SDS to the heats of dilution with the exclusion of lysozyme and can be calculated from the heats of dilution of SDS in buffer, ΔH_{dilut}, as follows:

$$L_A=\Delta H_{dilut}+x_B\left(\frac{\partial\Delta H_{dilut}}{\partial x_B}\right) L_B=\Delta H_{dilut}-x_A\left(\frac{\partial\Delta H_{dilut}}{\partial x_B}\right) \quad (4)$$

f_D is the fraction of lysozyme molecules undergoing denaturation which can be expressed as follows:

$$f_D=\frac{(\Delta H_N-\Delta H)}{(\Delta H_N-\Delta H_D)} \quad (5)$$

ΔH_N and ΔH_D are the heats of lysozyme and SDS interactions in the native and denatured state of lysozyme, respectively. $\Delta H_{\max}$ is the maximum heat of interaction upon saturation of all lysozyme.

The heats of lysozyme and SDS interactions were fitted to Eq. 1 over the entire surfactant concentration at different temperatures (298, 303 and 308 K) and pHs (5 and 7) and listed in Tables of 1 and 2. In the procedure the only adjustable parameter (p) was changed until the best agreement between the experimental and calculated data was approached over the whole range of solvent composition (Fig. 1 and 2 at pH 5 and pH 7, respectively). δ^{θ}_A and δ^{θ}_B parameters are recovered from the coefficients of the second and third terms of Eq. 1. Binding parameters for the interaction between SDS and lysozyme at different temperatures and pH recovered from Eq. 1 are listed in Table-3 and 4. The agreement between the calculated and experimental results (Fig. 1) is excellent, and gives significant support to the use of Eq. 1. Analysis of the ITC data using the extended solvation model gave the evidence for the existence of intermediate components during the cited interaction. Results also indicated a connection between turbidity of the protein solution upon interaction with SDS and distribution of the intermediates. δ^{θ}_A and δ^{θ}_B values for lysozyme and SDS interaction at all temperatures and pHs are positive, indicating that in the high concentrations of the SDS, the partially unfolded lysozyme structures have been stabilized.

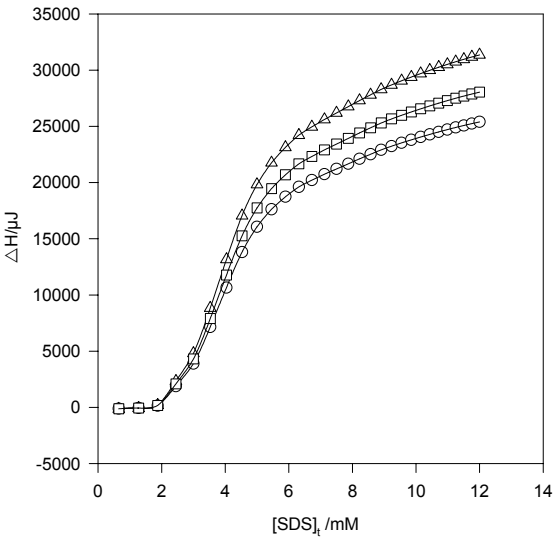

Fig. 1: Comparison between the experimental enthalpies for lysozyme+SDS interactions at 298 (o), 303 (□) and 308 K(Δ) at pH 5 and calculated data (lines) via Eq. 1.

Table-1: Enthalpies of SDS+lysozyme interactions, ΔH, at different temperatures of 298 (○), 303 (□) and 308 K (Δ) at pH 5. ΔH_{dilut} are the enthalpies of dilution of SDS with water. Precision is ±0.1 μJ or better.

$[SDS]_t$/mM	ΔH/μJ(○)	ΔH_{dilut}/μJ(○)	ΔH/μJ(□)	ΔH_{dilut}/μJ(□)	ΔH/μJ(Δ)	ΔH_{dilut}/μJ(Δ)
0.652	-117	-672	-130	-733	-145	-764
1.276	-61	-1250	-67	-1363	-75	-1420
1.875	152	-1723	168	-1879	187	-1958
2.448	1887	-2126	2084	-2318	2330	-2416
3.00	3892	-2470	4298	-2694	4805	-2807
3.529	7152	-2751	7898	-3000	8831	-3126
4.038	10654	-2996	11765	-3268	13155	-3405
4.528	13812	-3211	15253	-3502	17054	-3649
5.00	16061	-3388	17737	-3695	19832	-3850
5.454	17618	-3532	19456	-3852	21754	-4014
5.893	18731	-3664	20686	-3996	23129	-4164
6.316	19611	-3781	21657	-4123	24215	-4297
6.724	20210	-3887	22319	-4238	24955	-4417
7.118	20738	-3973	22902	-4332	25607	-4515
7.500	21209	-4051	23423	-4417	26189	-4604
7.869	21667	-4119	23928	-4491	26754	-4682
8.225	22108	-4183	24414	-4561	27298	-4755
8.571	22519	-4241	24869	-4625	27806	-4821
8.906	22907	-4294	25297	-4683	28285	-4881
9.231	23232	-4341	25657	-4735	28687	-4935
9.545	23528	-4382	25983	-4779	29052	-4981
9.851	23796	-4418	26279	-4819	29383	-5022
10.147	24048	-4451	26557	-4855	29694	-5060
10.435	24283	-4482	26817	-4889	29985	-5095
10.714	24504	-4511	27061	-4920	30257	-5128
10.985	24710	-4535	27288	-4946	30512	-5156
11.250	24901	-4557	27499	-4970	30747	-5181
11.507	25078	-4577	27695	-4992	30966	-5204
11.757	25243	-4594	27877	-5010	31169	-5223
12.000	25393	-4608	28043	-5026	31355	-5239

Table-2: Enthalpeis of SDS+lysozyme interactions, ΔH, at 298 (○), 303 (□), and 308 (Δ). ΔH_{dilut} are the enthalpies of dilution of SDS with water at pH 7. Precision is ±0.1 μJ or better.

$[SDS]_t$/mM	ΔH/μJ(○)	ΔH_{dilut}/μJ(○)	ΔH/μJ(□)	ΔH_{dilut}/μJ(□)	ΔH/μJ(Δ)	ΔH_{dilut}/μJ(Δ)
0.652	-102	-611	-112	-660	-122	-703
1.276	-53	-1136	-58	-1227	-64	-1307
1.875	132	-1566	145	-1691	158	-1802
2.448	1641	-1932	1805	-2086	1969	-2223
3.00	3384	-2245	3722	-2424	4061	-2583
3.529	6219	-2500	6841	-2699	7463	-2876
4.038	9264	-2723	10190	-2940	11117	-3132
4.528	12010	-2918	13211	-3151	14412	-3356
5.00	13966	-3079	15363	-3325	16759	-3541
5.454	15320	-3210	16852	-3467	18384	-3692
5.893	16288	-3330	17917	-3597	19546	-3830
6.316	17053	-3436	18758	-3711	20464	-3952
6.724	17574	-3532	19331	-3815	21089	-4062
7.118	18033	-3610	19836	-3899	21640	-4152
7.500	18443	-3681	20287	-3976	22132	-4234
7.869	18841	-3743	20725	-4043	22609	-4305
8.225	19224	-3801	21146	-4106	23069	-4372
8.571	19582	-3854	21540	-4163	23498	-4433
8.906	19919	-3902	21911	-4215	23903	-4488
9.231	20202	-3945	22222	-4261	24242	-4538
9.545	20459	-3982	22505	-4301	24551	-4581
9.851	20692	-4015	22761	-4337	24830	-4619
10.147	20911	-4045	23002	-4369	25093	-4654
10.435	21116	-4073	23228	-4399	25339	-4686
10.714	21308	-4099	23439	-4427	25570	-4716
10.985	21487	-4121	23636	-4451	25784	-4741
11.250	21653	-4141	23818	-4473	25984	-4764
11.507	21807	-4159	23988	-4492	26168	-4785
11.757	21950	-4174	24145	-4508	26340	-4802
12.000	22081	-4187	24289	-4522	26497	-4817

At low SDS concentrations, ITC isotherms feature an exothermic region that corresponds to specific electrostatic binding of SDS to positively charged amino acid residues on the lysozyme surface. This leads to charge neutralization of the complex and precipitation. At high SDS concentrations, hydrophobic interaction dominates the binding process. The positive value for δ_B^θ indicated that the extent to which lysozyme enhances the aqueous structure is increased by adding SDS.

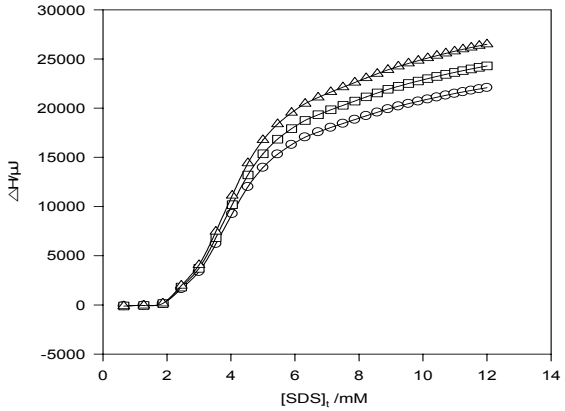

Fig. 2: Comparison between the experimental enthalpies for lysozyme+SDS interactions at 298 (o), 303 (□) and 308 K(Δ) at *p*H 7 and calculated data(lines) via Eq. 1

Table-3: Thermodynamic parameters for lysozyme+SDS interaction in 30 mM SDS solution with water via Eq. 1 at different temperatures of 298, 303 and 308 K, pH=5.

T/K	*p*	δ_A^θ	δ_B^θ	$\Delta H_D / kJmol^{-1}$
298	**1**	**0.088**	**0.304**	**207.516**
303	**1**	**0.0916**	**0.313**	**229.167**
308	**1**	**0.10**	**0.342**	**256.209**

Table-4: Thermodynamic parameters for lysozyme+SDS interaction in 30 mM SDS solution with water via Eq. 1 at different temperatures of 298, 303 and 308 K, pH=7.

T/K	*p*	δ_A^θ	δ_B^θ	$\Delta H_D / kJmol^{-1}$
298	**1**	**0.082**	**0.282**	**180.47**
303	**1**	**0.085**	**0.290**	**198.51**
308	**1**	**0.086**	**0.296**	**216.56**

The corresponding van't Hoff enthalpy was determined from difference spectral measurements at 301 nm as a function of temperature and found to be 29 kcal per mole [14]. There is no good agreement between the calorimetric and van't Hoff enthalpies, which indicate the presence of appreciably populated intermediate states between native and denatured forms of the protein. Proteins are generally susceptible to SDS denaturation during their partial and global unfolding transition. Several SDS+protein interactions from ITC enthalpograms have been recently characterized using cutinase, lysozyme and BSA [3-7]. In a very low SDS concentration range, SDS makes the globular protein loose through a specific binding in the structure, and then surface adsorption of SDS around the protein occurs. When the SDS concentration is larger than 1.0×10 molL^{-1}, the electrostatic repulsion between the head groups of SDS could initiate the partial unfolding. When the SDS concentration reaches the critical aggregation concentration (4.4×10 mM), the SDS binding and resulting protein denaturation are largely enhanced. Above the critical miceller concentration, the repulsion between the charged micelle-like clusters and the increased hydrophobic interactions of hydrophobic SDS chains with the hydrophobic backbone of BSA results in the complete unfolding of the protein.

Fig. 3 shows the heat capacities of lysozyme as a function of SDS concentration at two different pH of 5 and 7. Fig. 3 shows a three-step lysozyme phase transition in the presence of SDS. The two first transitions implies that HSA structure becomes more stabilized and compact through these transitions, presumably as a result of the interaction of negatively charged SDS heads with several positively charged residues on lsozyme surface. In contrast, during the third transition heat capacities of lysozyme is decreased, suggesting that lysozyme+SDS interaction in the high concentrations of SDS results in the denaturation (unfolding) of lysozyme, as a result of the hydrophobic interaction.

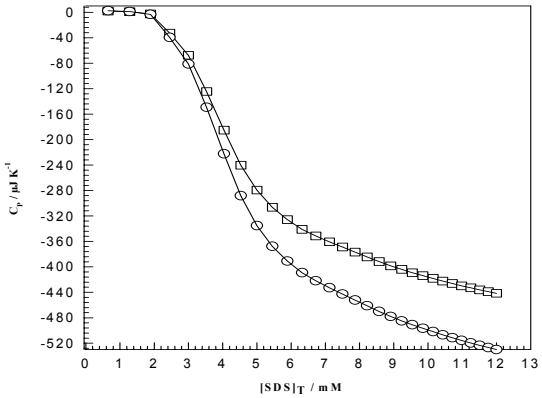

Fig. 3: The lysozyme heat capacities obtained for each studied concentration of SDS from the plot of enthalpy changes vs. temperature at pH 7 (□) and pH 5 (O).

Experimental

Hen egg-white lysozyme and were obtained from Sigma and SDS was purchased from Merck. Protein concentrations were determined from absorbance measurements at 277 nm in 1 cm quartz cuvettes. The molar extinction coefficient of lysozyme was 7690 M^{-1} cm^{-1}. All other materials and reagents were of analytical grade, and solutions were made in 50 mM buffer phosphate using double-distilled water. The isothermal titration calorimetric experiments were performed with the four channel commercial calorimetric system, Thermal Activity Monitor 2277, Thermometric, Sweden. The titration vessel was made from stainless steel. A solution of SDS (30 mM) was injected by use of a Hamilton syringe into the calorimetric titration vessel, which contained 1.8 mL lysozyme (68 μ M). Thin (0.15mm inner diameter) stainless steel hypodermic needles, permanently fixed to the syringe, reached directly into the calorimetric vessel. Injection of SDS solution into the perfusion vessel was repeated 30 times, with 40 μ L per injection. The heat of each injection was calculated by the "Thermometric Digitam 3" software. The heat of dilution of the SDS solution was measured as described above except lysozyme was excluded. The calorimeter was frequently calibrated electrically during the course of the study. The molecular weight of lysozyme was taken to be 14.7 kDa.

Conclusion

The results indicate that there are a three-step lysozyme phase transition in the presence of SDS. The two first transitions implies that HSA structure becomes more stabilized and compact through these transitions, presumably as a result of the interaction of negatively charged SDS heads with several positively charged residues on lsozyme surface. During the third transition heat capacities of lysozyme is decreased, suggesting that lysozyme+SDS interaction in the high concentrations of SDS results in the denaturation of lysozyme, as a result of the hydrophobic interaction.

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