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Thermodynamic investigation of effect of salt concentrations on denatured α-Amylase adsorbed onto a moderately hydrophobic surface

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Abstract The displacement adsorption enthalpies (ΔH) of denatured α-Amylase (by 1.8 mol L^{-1} GuHCl) adsorbed onto a moderately hydrophobic surface (PEG-600, the end-group of polyethylene glycol) from solutions (x mol L^{-1} $(NH_4)_2SO_4$, 0.05 mol L^{-1} KH_2PO_4, pH 7.0) at 298 K are determined by microcalorimeter. Further, entropies (ΔS), Gibbs free energies (ΔG) and the fractions of ΔH, ΔS, and ΔG for net adsorption of protein and net desorption of water are calculated in combination with adsorption isotherms of α-Amylase based on the stoichiometric displacement theory for adsorption (SDT-A) and its thermodynamics. It is found that the displacement adsorptions of denatured α-Amylase onto PEG-600 surface are exothermic and enthalpy driven processes, and the processes of protein adsorption are accompanied with the hydration by which hydrogen bond form between the adsorbed protein molecules favor formation of β-sheet and β-turn structures. The Fourier transformation infrared spectroscopy (FTIR) analysis shows that the contents of ordered secondary structures of adsorbed α-Amylase increase with surface coverages and salt concentrations increment.

Keywords Adsorption · Calorimetry · Thermodynamics · α-Amylase · Protein folding · Hydrophobic surface · Secondary structure

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Introduction

With the development of genetic engineering, protein folding is increasing concerned. The behavior of protein adsorption with refolding on a moderately hydrophobic surface is very complex, but of great significance for protein renaturing and purification, process of metabolism, pathological changes, and life process, etc. There are many factors affecting adsorption processes, such as pH [1, 2], temperature [2–5], salt concentration [1, 2, 6–8], and so on. The hydrophobic interactions involve in many biological processes and attract increasing attention. The salt in solution can provide a hydrophobic environment, which is in favor of protein adsorption onto hydrophobic solid surface. A lot of studies [7–10] showed that the affinities and adsorbed amounts of protein onto various solid surfaces increased with salt concentrations increment. The study on conformational changes of glycinin reported by Kim et al. [1] showed that in the absence of salt (NaCl), glycinin was most stable at pH 4.5, while with increasing of salt concentrations, glycinin was substantially stabilized even in acidic (pH 3.0) and alkaline (pH 11.5) conditions. The hydrophobic interaction chromatography (HIC) investigation [6] on dependence for salt concentrations of retention time for protein found that the retention time decreased with salt concentrations increment then grew after a minimum.

Although many investigations have been done to elucidate the effect of salt sort and concentration on protein adsorption processes, they seldom discussed the adsorption processes at the sub-molecular level in combination with thermodynamic functions and conformational changes. In our previous study [9, 10] on Lysozyme adsorption, the exploration of four subprocesses related to protein adsorption suggested that the possible two of them

contributed to some one (net adsorption or desorption thermodynamic fraction), making explanation of conformational change. In this study, based on the stoichiometric displacement theory for adsorption (SDT-A) the thermodynamic fractions of denatured α-Amylase adsorption in various salt concentrations and surface coverages were calculated and the secondary structures were analyzed by Fourier transformation infrared spectroscopy (FTIR), so as to deeply recognize the mechanism on effect of salt concentrations on adsorption and folding of denatured α-Amylase onto PEG-600 packings.

Calculating foundation [9, 10]

According to the SDT-A [11], the linear equation for description of the adsorption isotherms in dilute solutions in a liquid/solid system can be expressed as:

$$\ln P_a = \beta_a - \frac{q}{z}\ln C \tag{1}$$

and

$$z = n + q \tag{2}$$

where P_a represents partition coefficient of solute in the two phases, which can be calculated by adsorption isotherm [4, 8]. C represents equilibrium concentration of protein solution. β_a and q/z are constants and represent parameters for net adsorption of protein and net desorption of solvent in SDT-A, respectively, which can be obtained by linear plot of $\ln P_a$ vs. $\ln C$. n and q the water molecules of solvent released from the adsorbent and solvated solute, respectively.

The Gibbs free energy ΔG can be written as:

$$\Delta G = -RT\ \ln P_a \tag{3}$$

where R is gas constant and T the absolute temperature. Because $\ln P_a$ in Eq. 1 contains two independent terms, β_a and q/z, ΔG in Eq. 3 may be expressed as the sum of the two independent fractions: the (net) adsorption energy, ΔG_A, of the solute to the adsorbent, and the (net) desorption energy, ΔG_D, of the solvent molecules from the adsorbent, i.e.,

$$\Delta G = \Delta G_A + \Delta G_D \tag{4}$$

$$\Delta G_A = -RT\beta_a \tag{5}$$

and

$$\Delta G_D = RT\left(\frac{q}{z}\right)\ \ln C \tag{6}$$

Thermodynamic analysis of denatured protein folded on solid surface depends on the calculated fractions of thermodynamic functions and conformational changes associated with four subprocesses of displacement adsorption of protein. The calculating foundation of fractions of thermodynamic functions for protein adsorbed onto hydrophobic surface was expressed as in previous study [9]:

$$\Delta H = \Delta H_A + \Delta H_D = \Delta H_A + m\ln C \tag{7}$$

$$\Delta S = \Delta S_A + \Delta S_D \tag{8}$$

$$\Delta S_A = R\beta_a + \frac{\Delta H_A}{T} \tag{9}$$

and

$$\Delta S_D = \frac{\Delta H_D}{T} - \frac{q}{z}R\ln C \tag{10}$$

where ΔH_A and ΔS_A are all constants and, respectively, stand for (net) adsorption enthalpy and entropy of protein to the adsorbent, while ΔH_D and ΔS_D for (net) desorption enthalpy and entropy of solvent from the adsorbent in thermodynamics of SDT-A for a general displacement adsorption [12, 13]. Because m in Eq. 7 is a constant, ΔH_A can be obtained by linear plot of ΔH vs. $\ln C$ in Eq. 7.

The displacement adsorption of denatured α-Amylase on a moderately hydrophobic surface includes four subprocesses, i.e., (i) protein affinity to surface; (ii) following molecular conformational gain; (iii) dehydration between protein molecules and surface, and (iv) dehydration inside the hydrated protein molecules during formation of ordered structure, same as that for denatured lysozyme [10].

Experimental

Materials

α-Amylase (α-Amy, EC 3.2.1.1) from *Bacillus subtilis* was purchased from Fluka Co.(Germany), PEG-600 made of a silica base-HPHIC packings (particle size, 6.5 μm; pore diameter, 30 nm; the end-group of polyethylene glycol) were obtained from the Institute of Modern Separation Science, Northwest University, China. Guanidine hydrochloride (GuHCl) bought from Shanghai State-medicine Group Chemical Reagent Ltd. Co., ammonium sulfate ($(NH_4)_2SO_4$) from Tianjin Nankai Chemical Reagent Co., and potassium phosphate monobasic (KH_2PO_4) from Tianjin Dengfeng Chemical Reagent Co. Other chemicals are all analytic grade. The deionized water was produced by Milli-Q Academic (Millipore Co. Ltd, USA).

Adsorbed amounts determination

Put the mixtures of PEG-600 and the α-Amylase solutions as same as that in the calorimetric mixing batch vessel (see later) into isothermal vibrator and keep them shaking for

3 h at 298 K, so as to determine the adsorbed amounts of α-Amylase on the surface of PEG-600 packings corresponding to the calorimetric processes. After that use the UV–Vis spectrophotometer (SHIMADZU UV-2450) to determine the absorbency (280 nm) of the supernatants obtained by centrifuging. The concentrations of α-Amylase in supernatants can be determined and adsorbed amounts can also be calculated.

Microcalorimetric procedure

The calorimetric measurements were carried out by a Micro DSC-III (Setaram, Calurie, France) [14]. Transfer 0.500 mL α-Amylase solution with a syringe into the lower chamber of "measurement" mixing vessel and "reference" mixing vessel, respectively. The solutions with (0.4, 0.7, and 1.0 mg mL^{-1}) α-Amylase, 0.05 mol L^{-1} KH_2PO_4 (pH 7.0) and various concentrations of $(NH_4)_2SO_4$ (0, 1.0, 1.2, 1.5, 1.8, 2.1 mol L^{-1}) were denatured by 1.8 mol L^{-1} GuHCl for 24 h at 298 ± 0.001 K. Put 20 ± 0.01 mg PEG-600 packings in the upper chamber of the "measurement" vessel while the corresponding "reference" one being empty. The calorimetric operation is the same as that in the previous study [8]. The procedure to measure the blank heats, Q_{blank}, was the same except α-Amylase absent. The calorimetric data analysis was described in previous studies [8, 9].

FTIR spectroscopy

Infrared spectra were obtained using a FT-IR 5700 Spectrometer (America, Nicolet). The adsorbed α-Amylase was kept dry at 298 K. The spectra were collected in a single beam mode with 4 cm^{-1} resolution and the residual water vapor signals, if any, in the spectrum of protein were removed by subtracting the spectrum of gaseous water. Second-derivative spectra were obtained with a 7-point Savitsky–Golay derivative function by the Omnic software. Deconvolved spectra and second derivative [3, 15, 16] were employed to determine half width and positions of individual components. Curve-fitting procedures were carried out to amide I band of deconvolved spectra. Repetitious fitting make the coefficient of determination to be more than 0.99. Thus, the overlapping component bands (α-helix, β-sheet, β-turn, and random coil) would be distinguished. The areas of individual components were used to estimate the percentage of the relative secondary structure.

DSC measurement

The samples detected by differential scanning calorimetry (DSC) measurement were same as that in FTIR. Take the sample about 5 mg into the sample cell, while the reference cell was empty. Keep the sample at 293 ± 0.01 K for 10 min to baseline smooth prior to the initiation of the scanning experiment over a temperature range of 298 ± 0.01 K to 393 ± 0.01 K. The heating rate was 1.0 K/min for all experiments. The pure PEG-600 packings were measured under the same condition and no obvious transition peak was seen. Thus, the thermal stability of adsorbed α-Amylase can be tested.

Results and discussion

FTIR analysis of adsorbed α-Amylase

FTIR is an effective approach of the secondary structures of protein and a complementary exploration to calorimetry. FTIR can provide directly structural information about the protein at a sub-molecular level by analyzing the amide I band. The amide I band is composed of α-helix, β-sheet, β-turn, and randomly coiled conformation. Different kinds of secondary structure elements are related to the C=O stretching of the peptide bonds influenced by their different environment [17]. According to the areas of the component bands, the contents of the secondary structure elements in the present protein are expressed in percentage of the total amide I band area. The amide I band components of denatured α-Amylase can be assigned to α-helix (1,680–1,688 cm^{-1}), β-sheet (1,640–1,670 cm^{-1}), β-turn (1,690–1,730 cm^{-1}), and random coil (1,670–1,680 cm^{-1}), respectively, which may shift to higher frequency about 30 cm^{-1} than that in aqueous solution [18]. The curve-fitted results of adsorbed α-Amylase denatured by 1.8 mol L^{-1} GuHCl in various ammonium sulfate concentrations are listed in Table 1.

In Table 1, the percentage of β-sheet first decrease and then increase, while α-helix first increase and then decrease with salt concentrations increment at a given initial concentration of α-Amylase. Because in adsorption-induced rearrangements β-sheet is less sensitive than α-helix [19], some residual β-sheet structures are retained at lower salt concentrations, and intermolecular β-sheet formed at higher salt concentrations (1.8 and 2.1 mol L^{-1} $(NH_4)_2SO_4$) [20]. Since adsorbed amounts increase at higher salt concentrations (Fig. 2), the structures of α-helix may be influenced by the interactions inside or between protein molecules, and some α-helix structures maybe transform into β-sheet. Simultaneously, the sum of β-sheet and α-helix structures increase with salt concentrations increment, indicating that the adsorbed α-Amylase molecules gain more ordered secondary structures. The random coil structures decrease with salt concentrations increment, showing that in the processes of adsorption the random

Table 1 The percentage of the secondary structure elements for adsorbed α-Amylase in various salt concentrations

$C_{(NH_4)_2SO_4}$/mol L^{-1}	β-sheet %	α-helix %	(β-sheet + α-helix) %	β-turn %	Random coil %
$C_{\alpha\text{-Amy}} = 0.4$ mg mL^{-1}					
1.0	28.43	19.32	47.75	28.49	23.31
1.2	28.70	19.94	48.64	30.53	20.83
1.5	26.38	22.35	48.73	31.47	19.80
1.8	29.94	20.82	50.76	29.69	19.55
$C_{\alpha\text{-Amy}} = 0.7$ mg mL^{-1}					
1.0	29.16	19.96	49.12	28.18	22.70
1.2	28.78	21.49	50.27	28.91	20.82
1.5	28.75	22.58	51.33	29.95	19.26
1.8	30.73	21.59	52.32	28.70	18.98
2.1	39.83	15.12	54.95	37.81	7.25
$C_{\alpha\text{-Amy}} = 1.0$ mg mL^{-1}					
1.0	30.06	20.19	50.26	28.86	20.89
1.2	29.30	21.71	51.01	29.01	19.98
1.5	28.73	22.91	51.64	29.46	18.90
1.8	32.33	22.57	54.90	26.45	18.65
2.1	40.11	16.65	56.76	36.36	6.83

structures transform into half-ordered (β-turn) or ordered structures (β-sheet). This transformation can be detected obviously at 2.1 mol L^{-1} $(NH_4)_2SO_4$. At a given salt concentration, the contents of α-helix and β-sheet structures increase and randomly coiled conformation decrease with increasing concentrations of α-Amylase, showing that adsorbed α-Amylase gain more ordered secondary structures in the higher surface coverage. This is accordant with many reports [19, 21–23] that adsorbed protein molecules kept more secondary structures at higher surface coverage than that at lower coverage.

Thermal stability of adsorbed α-Amylase

DSC profiles can provide overall structural changes of protein at the molecular level via thermal stability [19], while FTIR profiles reflect only the secondary structural information on sub-molecular level. Therefore, DSC is an important complement to FTIR analysis for investigation of protein conformation. Figure 1 illustrates DSC profiles of adsorbed α-Amylase (denatured by 1.8 mol L^{-1} GuHCl) in various salt concentrations and surface coverages. The DSC profile for each sample was performed at least three times, and the changeable tendency was not affected by the slight differences (about ±0.20 °C). It is seen that the transition temperatures of endothermic peaks of adsorbed denatured α-Amylase increase with salt concentrations and surface coverages increment, indicating that the thermal stability of adsorbed denatured α-Amylase enhance, and their tertiary structures are more perfect. The result is

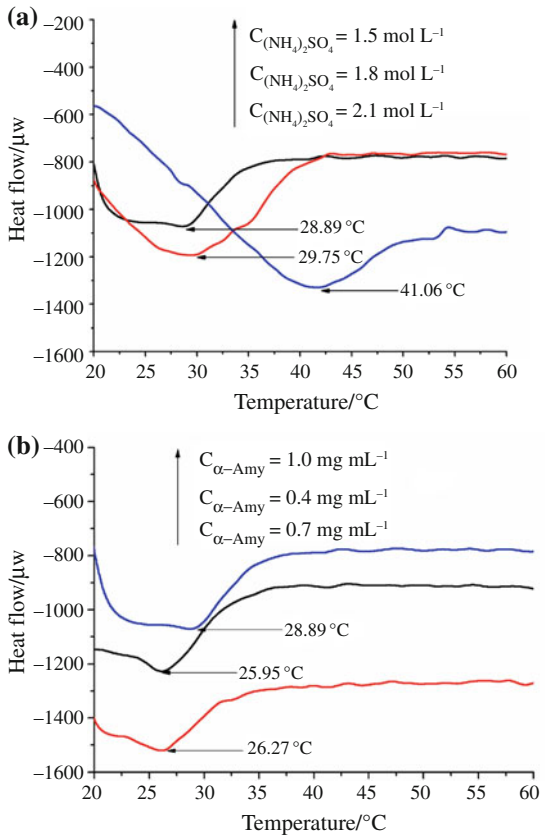

Fig. 1 DSC profiles of denatured (by 1.8 mol L^{-1} GuHCl) α-Amylase adsorbed onto PEG-600 surface from solutions **a** (1.0 mg mL^{-1} α-Amylase, 0.05 mol L^{-1} KH_2PO_4, pH 7.0) with different $(NH_4)_2SO_4$ concentrations, **b** (1.5 mol L^{-1} $(NH_4)_2SO_4$, 0.05 mol L^{-1} KH_2PO_4, pH 7.0) with different surface coverages

consistent with the FTIR analysis that adsorbed α-Amylase gains more ordered secondary structure with salt concentrations and surface coverages increment.

Linear parameters β_a and q/z in SDT-A

According to the data of adsorption isotherms illustrated in Fig. 2 for denatured (by 1.8 mol L^{-1} GuHCl) α-Amylase adsorbed onto hydrophobic PEG-600 surface at various concentrations of $(NH_4)_2SO_4$, the linear correlation coefficients r, adsorption parameter β_a, and desorption parameter q/z obtained by plot of $\ln P_a$ vs. $\ln C$ in Eq. 1 are listed in Table 2. As a basis to calculate the fractions of thermodynamic functions (see later), the obtained β_a and q/z are very reliable due to the satisfactory linear relationships. Because the adsorbed amounts are very little or even negative (in fact, apparent) at lower $(NH_4)_2SO_4$ concentrations (<1.0 mol L^{-1}), only the ones at higher salt concentrations (1.0–2.1 mol L^{-1} $(NH_4)_2SO_4$) are adopted to guarantee reliability of the calculated parameters. The corresponding partition coefficients P_a can be calculated by the method described in our previous study [4, 8].

All the negative values of q/z in Table 2 suggest that protein adsorption onto hydrophobic PEG-600 surface is accompanied with the hydration of adsorbed protein molecules, and the water molecules on hydrated protein are more than the released water molecules from protein based on the SDT-A. And, the hydration ($-q/z$) decrease with concentrations of $(NH_4)_2SO_4$ increment. The net adsorption parameters β_a increase with the concentrations of $(NH_4)_2SO_4$ increment indicating that conformational gain and protein affinity to surface increase with salt concentrations increment.

Displacement adsorption enthalpies

In order to obtain the displacement adsorption enthalpies or displacement adsorption heats, Q_i, the heats, Q_{blank}, of corresponding blank sample (α-Amylase absent) should be subtracted from the heats directly observed, Q_{obs}, during contacting the denatured α-Amylase solution with PEG-600 packings in a mixing batch vessel, i.e., $Q_i = Q_{obs} - Q_{blank}$. Every calorimetric test was performed at least three times.

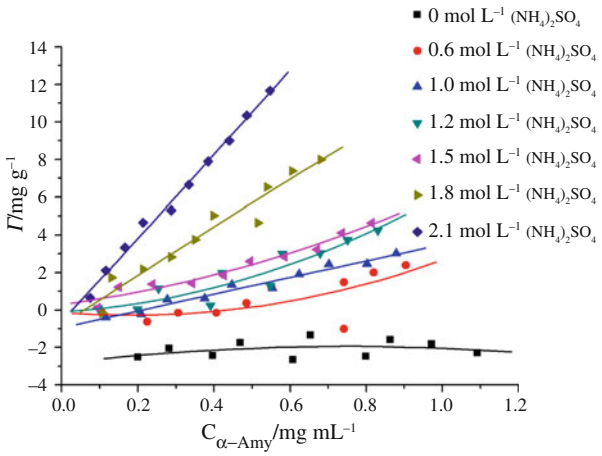

Fig. 2 The adsorption isotherms of denatured (by 1.8 mol L^{-1} GuHCl) α-Amylase adsorbed onto PEG-600 surface at various salt concentrations

Table 2 The linear parameters, β_a and q/z, and correlation coefficients r

$C_{(NH_4)_2SO_4}$/mol L^{-1}	$\ln P_a$ vs. $\ln C$		
	r	q/z	β_a
1.0	0.9867	−0.8373	4.0875
1.2	0.9997	−0.7868	4.4232
1.5	0.9939	−0.6866	4.7665
1.8	0.9964	−0.3298	6.0138
2.1	0.9976	−0.2271	6.4322

The individually calorimetric results in various protein concentrations (0, 0.4, 0.7 and 1.0 mg mL^{-1}) of 0.5 mL denatured α-Amylase solutions (1.8 mol L^{-1} GuHCl, x mol L^{-1} $(NH_4)_2SO_4$, 0.05 mol L^{-1} KH_2PO_4, pH 7.0) with mixing 20 mg PEG-600 packings at 298 K are listed in Tables 3, 4, and 5. The displacement adsorption enthalpies, ΔH, corresponding to foregoing conditions can be obtained by Q_i per molar protein. The values of adsorption enthalpies ΔH in Tables 3, 4, and 5 are all negative except that salt absence in Table 5, showing that the denatured α-Amylase adsorption onto PEG-600 is an exothermic process.

Table 3 Displacement adsorption enthalpies of denatured 0.4 mg mL^{-1} α-Amylase adsorbed onto PEG-600 surface from solutions (1.8 mol L^{-1}GuHCl, x mol L^{-1} $(NH_4)_2SO_4$, 0.05 mol L^{-1} KH_2PO_4, pH 7.0) at 298 ± 0.001 K

$C_{(NH_4)_2SO_4}$/mol L^{-1}	Q_{blank}/mJ	Q_{obs}/mJ	Q_i/mJ	ΔH/kJ mol^{-1}
0	−47.0 ± 0.4	−55.2 ± 1.1	−8.2 ± 1.5	−2,255 ± 413
1.0	−78.6 ± 1.6	−93.6 ± 0.7	−15.0 ± 2.3	−4,125 ± 633
1.2	−88.6 ± 1.2	−105.1 ± 1.5	−16.5 ± 2.7	−4,538 ± 743
1.5	−94.4 ± 1.2	−114.3 ± 1.6	−19.9 ± 2.8	−5,473 ± 770
1.8	−106.7 ± 1.2	−123.2 ± 1.4	−16.5 ± 2.6	−4,533 ± 715

Table 4 Displacement adsorption enthalpies of denatured 0.7 mg mL^{-1} α-Amylase adsorbed onto PEG-600 surface from solutions (1.8 mol L^{-1}GuHCl, *x* mol L^{-1} $(NH_4)_2SO_4$, 0.05 mol L^{-1} KH_2PO_4, pH 7.0) at 298 ± 0.001 K

$C_{(NH_4)_2SO_4}$/mol L^{-1}	Q_{blank}/mJ	Q_{obs}/mJ	Q_i/mJ	ΔH/kJ mol^{-1}
0	−47.0 ± 0.4	−51.4 ± 0.9	−4.4 ± 1.3	−691.4 ± 204
1.0	−78.6 ± 1.6	−93.2 ± 1.7	−14.6 ± 3.3	−2,294 ± 519
1.2	−88.6 ± 1.2	−105.6 ± 1.7	−17.0 ± 2.9	−2,671 ± 456
1.5	−94.4 ± 1.2	−112.2 ± 0.7	−17.8 ± 1.9	−2,797 ± 299
1.8	−106.7 ± 1.2	−117.9 ± 1.2	−11.2 ± 2.4	−1,760 ± 377

Table 5 Displacement adsorption enthalpies of denatured 1.0 mg mL^{-1} α-Amylase adsorbed onto PEG-600 surface from solutions (1.8 mol L^{-1}GuHCl, *x* mol L^{-1} $(NH_4)_2SO_4$, 0.05 mol L^{-1} KH_2PO_4, pH 7.0) at 298 ± 0.001 K

$C_{(NH_4)_2SO_4}$/mol L^{-1}	Q_{blank}/mJ	Q_{obs}/mJ	Q_i/mJ	ΔH/kJ mol^{-1}
0	−47.0 ± 0.4	−44.6 ± 1.4	2.4 ± 1.8	264.0 ± 198
1.0	−78.6 ± 1.6	−91.0 ± 1.5	−12.4 ± 3.1	−1,364 ± 341
1.2	−88.6 ± 1.2	−105.4 ± 2.0	−16.8 ± 3.2	−1,848 ± 352
1.5	−94.4 ± 1.2	−112.8 ± 0.2	−18.4 ± 1.4	−2,024 ± 154
1.8	−106.7 ± 1.2	−117.2 ± 1.2	−10.5 ± 2.4	−1,155 ± 264

Thermodynamic fractions of adsorption

According to our recent study [10], the net adsorption thermodynamic fractions (ΔH_A, ΔS_A, and ΔG_A) of denatured α-Amylase are attributed to contributions of both process (a) α-Amylase affinity to surface and process (b) following conformational gain of α-Amylase, while the net desorption ones (ΔH_D, ΔS_D and ΔG_D) of water molecules are cooperative results of process (c) dehydration between α-Amylase molecules and surface and of process (d) dehydration (squeezing water) inside the hydrated protein molecules during formation of ordered structure. These can be expressed as

$$\Delta H_A = \Delta H_a + \Delta H_{mo} \quad (11)$$

$$\Delta H_D = \Delta H_d + \Delta H_{md} \quad (12)$$

$$\Delta S_A = \Delta S_a + \Delta S_{mo} \quad (13)$$

$$\Delta S_D = \Delta S_d + \Delta S_{md} \quad (14)$$

$$\Delta G_A = \Delta G_a + \Delta G_{mo} \quad (15)$$

and

$$\Delta G_D = \Delta G_d + \Delta G_{md} \quad (16)$$

where the subscripts "a", "mo", "d" and "md" represent the subprocesses (a), (b), (c), and (d) of displacement adsorption of denatured protein, respectively. Based on Eqs. 11–16, conformational changes (Table 1) and adsorption isotherms (Fig. 2) of denatured protein, dominant subprocess of denatured protein refolding can be deduced.

According to Eqs. 4–10, the fractions of enthalpies, entropies, and free energies calculated in the present systems are listed in Tables 6 and 7. It is obvious in Tables 6 and 7 that the general thermodynamic functions for total displacement adsorption, ΔH, ΔG, and ΔS are all negative, suggesting that the adsorption of denatured α-Amylase on PEG-600 surface at 298 K is an exothermic and enthalpy-driven process, as same as that of lysozyme at the same conditions [10].

In Table 6, the measured ΔH and net adsorption enthalpies ΔH_A are all negative, while net desorption enthalpies ΔH_D are positive. According to Eqs. 7, 11, and 12, this indicates that the sum of subprocesses (a) (ΔH_a, exothermic) and (b) (ΔH_{mo}, exothermic) is predominant over the sum of subprocesses (c) (ΔH_d, endothermic) and (d) (ΔH_{md}, endothermic). The values of $-\Delta H$, $-\Delta H_A$, and ΔH_D first increase (maximum at 1.5 mol L^{-1}) and then decrease with salt concentrations increment. Because with increasing salt concentrations ($C_{(NH_4)_2SO_4} < 1.8$ mol L^{-1}), the adsorbed protein molecules gain more conformation (as shown in FTIR analysis), $-\Delta H_{mo}$ and ΔH_{md} increase. And, at the higher salt concentrations, the adsorbed amounts (in Fig. 2) and $-\Delta H_{mo}$ obviously increase, but the corresponding $-\Delta H_A$ decrease. Because the adsorption of protein may be not monolayer and hydrogen bond forms between the adsorbed protein molecules by hydration (exothermic), which leads to affinity of protein to surface (subprocess(a)) and dehydration between protein and surface (subprocess(c)) weaken, i.e., the decrease of $-\Delta H_a$ and ΔH_d.

With salt concentrations increment, $-\Delta G_A$ values, i.e., the sum of $-\Delta G_a$ (subprocess (a)) and $-\Delta G_{mo}$ (subprocess (b)) according to Eq. 15, increase, while $-\Delta G_D$ values, the sum of $-\Delta G_d$ (subprocess (c)) and $-\Delta G_{md}$ (subprocess

Table 6 Enthalpies and their fractions of denatured α-Amylase adsorbed onto PEG-600 surface from solutions of various $(NH_4)_2SO_4$ concentrations at 298 ± 0.001 K

C_0^a/mg mL^{-1}	ln$C$/μmol L^{-1}	ΔH/kJ mol^{-1}	ΔH_A/kJ mol^{-1}	ΔH_D/kJ mol^{-1}	r^b
1.0 mol L^{-1} $(NH_4)_2SO_4$					
0.4	1.9215	−4,125	−10,370	6,245	
0.7	2.4283	−2,294	−10,370	8,076	0.9971
1.0	2.7716	−1,364	−10,370	9,006	
1.2 mol L^{-1} $(NH_4)_2SO_4$					
0.4	1.9604	−4,538	−11,418	6,880	
0.7	2.3541	−2,671	−11,418	8,747	0.9810
1.0	2.7146	−1,848	−11,418	9,570	
1.5 mol L^{-1} $(NH_4)_2SO_4$					
0.4	1.8298	−5,473	−12,812	7,339	
0.7	2.3663	−2,791	−12,812	10,021	0.9852
1.0	2.6951	−2,024	−12,812	10,788	
1.8 mol L^{-1} $(NH_4)_2SO_4$					
0.4	1.6532	−4,538	−11,136	6,598	
0.7	2.2364	−1,760	−11,136	9,376	0.9882
1.0	2.5153	−1,155	−11,136	9,981	

[a] C_0: initial concentration of α-Amylase in solution

[b] r: linear correlation coefficient

Table 7 Thermodynamic fractions of denatured α-Amylase adsorbed onto PEG-600 surface from solutions of various $(NH_4)_2SO_4$ concentrations at 298 ± 0.001 K

C_0/mg mL^{-1}	ΔG/kJ mol^{-1}	ΔG_A/kJ mol^{-1}	ΔG_D/kJ mol^{-1}	ΔS/kJ mol^{-1} K^{-1}	ΔS_A/kJ mol^{-1} K^{-1}	ΔS_D/kJ mol^{-1} K^{-1}
1.0 mol L^{-1} $(NH_4)_2SO_4$						
0.4	−14.18	−10.13	−4.05	−13.79	−34.75	20.96
0.7	−15.76	−10.13	−5.63	−7.64	−34.75	27.11
1.0	−16.05	−10.13	−5.92	−4.52	−34.75	30.23
1.2 mol L^{-1} $(NH_4)_2SO_4$						
0.4	−14.27	−10.96	−3.31	−15.17	−38.26	23.09
0.7	−15.83	−10.96	−4.87	−8.91	−38.26	29.35
1.0	−16.19	−10.96	−5.23	−6.14	−38.26	32.12
1.5 mol L^{-1} $(NH_4)_2SO_4$						
0.4	−15.47	−11.82	−3.65	−18.30	−42.93	24.63
0.7	−15.85	−11.82	−4.03	−9.31	−42.93	33.62
1.0	−16.24	−11.82	−4.42	−6.73	−42.93	36.20
1.8 mol L^{-1} $(NH_4)_2SO_4$						
0.4	−16.24	−14.91	−1.33	−15.17	−37.30	22.13
0.7	−16.77	−14.91	−1.86	−5.85	−37.30	31.45
1.0	−16.99	−14.91	−2.08	−3.82	−37.30	33.48

(d)) in Eq. 16 decrease. This accords with both the analysis of enthalpy fractions in the subprocesses and the deduced conclusion that the ordered secondary structure increases with salt concentrations increment in FTIR analysis. The result that $-\Delta G_A$ values are always greater than the corresponding $-\Delta G_D$ confirms the inference that the sum of subprocesses (a) and (b) is predominant over the sum of subprocesses (c) and (d) for the foregoing enthalpy fraction analysis. Since the values of ΔG_D are all negative, which were different from that for lysozyme in our previous study [10], bovine serum albumin (BSA) and RNase A in our recent study, this indicates that the contribution of dehydration entropies, ΔS_D, to ΔG_D is greater than that of dehydration enthalpies, ΔH_D, and ΔG_D promotes adsorption

process of denatured α-Amylase. Whether the negative values of ΔG_D correlate with hydration or not should be further explored.

The values of ΔS, and ΔS_A are all negative, while ΔS_D values are all positive. This implies that the subprocesses (a) (ΔS_a) and (b) (ΔS_{mo}) is predominant over the cooperative subprocesses (c) (ΔS_d) and (d) (ΔS_{md}). The results are also accordant with the foregoing analysis for enthalpies and free energies, i.e., the subprocesses (a) and (b) play a major role in comparison with the cooperative subprocesses (c) and (d). With salt concentrations increment, the values of $-\Delta S$, $-\Delta S_A$ and ΔS_D first increase (maximum at 1.5 mol L^{-1} $(NH_4)_2SO_4$) and then decrease, same as the changed trend of corresponding $-\Delta H$, $-\Delta H_A$ and ΔH_D. Therefore, the same explanation and deduction can be drawn as follows. With salt concentrations increment, the adsorbed amounts (in Fig. 2) increase, i.e., $-\Delta S_a$ and ΔS_d increase, and the adsorbed protein molecules gain more conformation, i.e., $-\Delta S_{mo}$ and ΔS_{md} increase, which lead to $-\Delta S_A$ and ΔS_D increase according to Eqs. (13) and (14). However, at the higher salt concentration (1.8 mol L^{-1} $(NH_4)_2SO_4$) the adsorption of protein may be not monolayer and hydrogen bond forms between the adsorbed protein molecules by hydration, which leads to the decrease of $-\Delta S_a$ and ΔS_d, so $-\Delta S_A$ and ΔS_D decrease according to Eqs. (13) and (14).

The FTIR and DSC investigation show that adsorbed α-Amylase at higher surface coverage keep more ordered secondary structures and perfect tertiary structure. Thus, it can be deduced that with protein surface coverages increment, refolding of denatured α-Amylase or conformational gain (subprocess (b)) increases, while the affinity of α-Amylase to surface (subprocess (a)) decreases. Because at a given concentration of $(NH_4)_2SO_4$, ΔH_A, ΔS_A, and ΔG_A are all constants with α-Amylase coverages increment, the subprocesses (a) and (b) compensate each other: the former decreases, while the latter increases. With surface coverages increment, the protein gains more ordered structures, which leads to the increase of ΔH_{md} and ΔS_{md}. And, the increase of ΔH_D and ΔS_D can be attributed to the increase of ΔH_{md} and ΔS_{md}.

Conclusions

The adsorption process of denatured α-Amylase was investigated in combination with SDT, FTIR, DSC, and calorimetric measurements. Based on the thermodynamics of SDT, the thermodynamic fractions which related to four subprocesses of denatured α-Amylase refolding on the surface were calculated. Thermodynamic fraction analysis showed that in a displacement adsorption process, there also existed hydration of adsorbed protein besides dehydration, by which hydrogen bond formed and was in favor of the denatured α-Amylase adsorption and folding onto a moderately hydrophobic surface. The displacement adsorption of denatured α-Amylase onto PEG-600 were exothermic and enthalpy driven processes. FTIR analysis indicated that under the present conditions the adsorbed α-Amylase gained more ordered secondary structures with surface coverages and salt concentrations increment. At higher salt concentrations, the adsorption of protein may not be monolayer and hydrogen bond formed between the adsorbed protein molecules by hydration, which favored to β-sheet and β-turn formation.

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