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# FRACTIONS OF THERMODYNAMIC FUNCTIONS FOR NATIVE LYSOZYME ADSORPTION ONTO MODERATELY HYDROPHOBIC SURFACE

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Calorimetric measurement of adsorption enthalpies of native lysozyme(Lyz) on a moderately hydrophobic surface at 25°C, pH 7.0 and various salt concentrations was performed. Based on the thermodynamics of stoichiometric displacement theory (SDT), we calculated the fractions of thermodynamic functions involving four subprocesses during a displacement adsorption process from the directly determined enthalpies in combination with adsorption isotherm measurements. The thermodynamic fractions reveal the relative degree of the four subprocesses for contributions to enthalpy, entropy and free energy. The results show that native Lyz adsorption on a moderately hydrophobic surface is an entropy driven process contributed mainly by conformational loss of adsorbed Lyz.

***Keywords:*** *adsorption, calorimetry, fractions of thermodynamic functions, hydrophobic surface, lysozyme*

## Introduction

Thermodynamic study by calorimetry of protein adsorbed at liquid/solid interface is a reliable method to explore the mechanism of protein adsorption. Directly measured adsorption heats combined with adsorption isotherm can be used to obtain the relative magnitudes of the subprocesses [1–3]. Adsorption of protein onto liquid/solid interface includes at least three subprocesses [4, 5]: (a) dehydration both between the hydrated protein molecules and the hydrated surface and between the hydrated residues of protein molecules during formation of microdomains or intermediates of protein; (b) adsorption affinity of protein molecules onto surface; (c) molecular conformation forming or losing ordered secondary structure. Stoichiometric displacement theory (SDT) [6–8] that subdivides the general thermodynamic functions of a displacement adsorption process into two thermodynamic fractions: (net) adsorption for adsorbate and (net) desorption for solvent has successfully been used for various adsorption systems [7–9] and can elucidate the subprocesses in protein adsorption.

Reports on protein adsorption onto hydrophobic and hydrophilic surfaces from solution reveal that conformational change of adsorbed protein occurred and more or less ordered structure was lost in many cases [10]. Haynes and Norde [11] found that adsorption onto hydrophobic polystyrene surface induced the exposure of hydrophobic fragments of lysozyme and α-lactalbumin. Approaches such as NR [12], FTIR [10], DSC [13–15] and HDX-MS [16] proved that proteins exhibited considerable structure perturbation when they were adsorbed onto solid surface. However, the relative degree of subprocesses of structure perturbation has not been fully understood. The aim of this study is to use the fractions of thermodynamic functions derived from directly measured displacement adsorption heats in combination with adsorption isotherms to describe the subprocesses including conformational loss for native lysozyme adsorption onto a hydrophobic surface.

## Calculating foundation [7–9]

The linear equation of SDT [17, 18] for description of the adsorption isotherms in dilute solutions in a liquid/solid system can be expressed as:

$$\ln P_a = \beta_a - \left(\frac{q}{z}\right)\ln a_{PDm} \quad (1)$$

and

$$z = n + q \quad (2)$$

where $P_a$ is the activity partition of the solute in the two phases, $\alpha_{PDm}$ the equilibrium activity of the solvated solute in bulk solution, $n$ and $q$ the moles of solvent released from the adsorbent and solvated solute, respectively, as one mole of solute is adsorbed by

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the solvated adsorbent and $\beta_a$ is the adsorption parameter. For a given adsorption system, $n$, $q$, $z$ and $\beta_a$ are all constants at a particular temperature. For convenience, $\alpha_{PDm}$ may be assumed equal to concentration, $C$ [6, 9, 18] and $P_a$ became into concentration partition of the solute [4–6, 9, 18], and Eq. (1) may be rewritten as

$$\ln P_a = \beta_a - \left(\frac{q}{z}\right)\ln C \tag{3}$$

Equation (3) is linear. The corresponding Gibbs free energy change, $\Delta G$, is

$$\Delta G = -RT\ln P_a \tag{4}$$

where $R$ is gas constant and $T$ the absolute temperature. Because $\ln P_a$ in Eq. (3) contains two independent terms, $\beta_a$, and the desorption parameter, $q/z$, of the solvent from the adsorbent [18], $\Delta G$ in Eq. (4) may be expressed as the sum of the two independent fractions: the (net) adsorption energy, $\Delta G_A$, of the solute to the adsorbent, and the (net) desorption energy, $\Delta G_D$, of the solvent molecules from the adsorbent, i.e.

$$\Delta G = \Delta G_A + \Delta G_D \tag{5}$$

Based on Eq. (3), the parameters $\beta_a$ and $q/z$ can be obtained from the linear plot of $\ln P_a$ *vs.* $\ln C$ with intercept and minus slope, respectively. Therefore, inserting Eq. (3) into Eq. (4) and comparing Eq. (5),

$$\Delta G_A = -RT\beta_a \tag{6}$$

and

$$\Delta G_D = RT(q/z)\ln C \tag{7}$$

$\Delta G$ in Eq. (4) may also be expressed as

$$\Delta G = -RT\ln P_a = \Delta H - T\Delta S$$

or

$$\ln P_a = -\Delta H/RT + \Delta S/R \tag{8}$$

$\Delta H$ and $\Delta S$ may be considered as constants as long as $\ln C$ is fixed and temperature range studied is not too wide. Therefore, Eq. (8) should be a linear equation for $\ln P_a$ *vs.* $1/T$.

So long as Eqs (6) and (7) hold, $\beta_a$ and $q/z$ should be similar to $P_a$ having a character of thermodynamic function and can also be expressed in the same form as that in Eq. (8) [6]. We then have

$$\beta_\alpha = \frac{\frac{-\Delta H_A}{T} + \Delta S_A}{R} \tag{9}$$

and

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

where $\Delta H_A$ and $\Delta H_D$ denote the adsorption enthalpy of solute onto the adsorbent and the desorption enthalpy of the solvent molecules from both the adsorbent and the solvated adsorbate molecules, respectively, while $\Delta S_A$ and $\Delta S_D$ are the entropy changes corresponding to $\Delta H_A$ and $\Delta H_D$, respectively. Similarly, we can elucidate that there are linear relationships between both $\beta_a$ and $q/z$ *vs.* $1/T$ in Eqs (9) and (10) under the same conditions as mentioned in Eq. (8). And moreover, at a given temperature, $\Delta H_A$ and $\Delta S_A$ are constants while $\Delta H_D$ and $\Delta S_D$ are variable with $\ln C$.

Similar to Eq. (5), we have

$$\Delta H = \Delta H_A + \Delta H_D \tag{11}$$

or

$$\Delta H = \Delta H_A + m\ln C \tag{12}$$

where $m$ is a constant at a given temperature, which is equal to $RT(q/z)$.

Equations (11) and (12) provide a possibility to study the contributions of two fractions of enthalpy change, if Eq. (12) is linear.

$\Delta H$ is the measured enthalpy, $\Delta H_A$ can be derived as intercept by plotting $\Delta H$ *vs.* $\ln C$. The term $m\ln C$ in Eq. (12) is equivalent to the net desorption enthalpy of solvent $\Delta H_D$, which relates to the enthalpy of water molecules loss and gain in protein molecules and can be calculated by the difference between $\Delta H$ and $\Delta H_A$.

Similarly, the entropy $\Delta S$ can be written as the algebraic sum of the two fractions:

$$\Delta S = \Delta S_A + \Delta S_D \tag{13}$$

where $\Delta S_A$ and $\Delta S_D$ have the same expanded meanings as the above $\Delta H_A$ and $\Delta H_D$, respectively. When $\Delta H_A$, $\beta_a$ and $\Delta H_D$, $q/z$ are inserted into Eqs (9) and (10), respectively, $\Delta S_A$ and $\Delta S_D$ can be obtained.

## Experimental

*Materials*

PEG-600 packings made of a silica base-HIC 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, lysozyme (Lyz, chicken egg white) from Sigma Co. (St. Louis USA), 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).

*Methods*

A set of concentrations of 0.4, 0.7 and 1.0 mg $mL^{-1}$ Lyz solutions with 0.05 mol $L^{-1}$ $KH_2PO_4$ (pH 7.0) and various concentrations of $(NH_4)_2SO_4$ (0, 0.3, 0.9, 1.5, 1.8, 2.1 mol $L^{-1}$) were separately prepared for calorimetric measurement and determination of adsorbed amounts.

The calorimetric measurements were carried out by a Micro DSC-III (Setaram, Calurie, France). Transfer 0.500 mL Lyz solution with a syringe into the lower chamber of 'measurement' mixing vessel and 'reference' mixing vessel, respectively. Put 20±0.01 mg PEG-600 packings in the upper chamber of the 'measurement' vessel, the corresponding 'reference' one being empty. The calorimetric operation is the same as that in the previous study [19]. The procedure to measure the blank heats, $Q_{blank}$, was the same except Lyz absent. The calorimetric data analysis is described in previous studies [4, 19].

To determine the adsorbed amounts of Lyz at the surface of PEG-600, which correspond to the calorimetric processes, the mixtures of PEG-600 packings and the Lyz in various $C_{(NH_4)_2SO_4}$ solutions with the same ratios (*W*/*V*) as that in the calorimetric mixing batch vessel employed were shaken for 5 h at 298 K. Supernatants obtained by centrifuging at 2000 rpm were analyzed at 280 nm by spectrophotometer (Shimadzu UV-2450) to determine the concentrations of Lyz.

## Results and discussion

*Adsorption enthalpies*

The original observed calorimetric data measured by mixing 20 mg PEG-600 packings with 0.5 mL solutions (*x* mg $mL^{-1}$ Lyz, *y* mol $L^{-1}$ $(NH_4)_2SO_4$, 0.05 mol $L^{-1}$ $KH_2PO_4$, pH 7.0) at 25°C are listed in Table 1. The data are average values with standard deviations. Every calorimetric test was performed at least four times. The corresponding displacement adsorption heat effects $Q_i$ were obtained by offsetting the blank heats $Q_{blank}$ ($C_{Lyz}$=0) due to both the identical salt and Lyz concentrations and the corresponding displacement adsorption enthalpies $\Delta H$ are shown in Table 2.

All the $\Delta H$ values in Table 2 are positive, different from that of partly denatured Lyz which exhibited exothermic at higher salt concentrations [4]. Our previous study [4] on denatured Lyz refolding pointed out that the $\Delta H$ includes three fractions, i.e. dehydration enthalpy $\Delta H_d$ (endothermic), adsorption affinity enthalpy $\Delta H_a$ (exothermic) and molecular conformation enthalpy $\Delta H_m$ (exothermic). For adsorption of native Lyz, however, the contribution of the endothermic effect due to losing part of ordered conformation in the adsorption can not be neglected. The molecular conformation enthalpy, herein, is a conformational loss enthalpy (to destroy the ordered structure) and endothermic. Therefore, the endothermic effect of $\Delta H$ should be attributed to both dehydration and conformation loss.

Molecular conformation changes in adsorption of protein are very complicate. They include two tendencies: ordered structure gain and loss, which associate with dehydration between hydrated protein molecular residues and with hydration of unfolded and exposed amino acid residues, respectively. For adsorption of denatured protein in previous works [4, 5], the first heat effect produced by ordered structure gain should be marked as $\Delta H_{mo}$ (exothermic), i.e. $\Delta H_m$ [4, 5], and the second one led by dehydration as $\Delta H_{md}$ (endothermic) which was included in $\Delta H_d$ [4, 5]. For adsorption of native protein in this paper, however, the heats which are opposite to $\Delta H_{mo}$ and $\Delta H_{md}$, are signed as $\Delta H_{ml}$ (endothermic) and $\Delta H_{mh}$ (exothermic), respectively. In short, if conformation gains more ordered structures, $\Delta H_{mo}<0$ and $\Delta H_{md}>0$; if conformation loses ordered structures, $\Delta H_{ml}>0$ and $\Delta H_{mh}<0$. Our recent FTIR research of adsorbed native Lyz on the PEG-600 surface showed that the characteristic adsorption band of α-helical structural elements was almost invisible, indicating loss of a part of domain. In this paper, therefore, the measured displacement adsorption enthalpies should include endothermic $\Delta H_d$ and $\Delta H_{ml}$ and exothermic $\Delta H_a$ and $\Delta H_{mh}$.

*Fractions of adsorption enthalpy*

To calculate the thermodynamic fractions we choose the data in 1.5 and 1.8 mol $L^{-1}$ ammonium sulfate, because the adsorption is stronger than in lower salt

**Table 1** The observed heats $Q_{obs}$ (mJ) of mixing 20 mg PEG-600 with 0.5 mL solutions of Lyz in $(NH_4)_2SO_4$ and 0.05 mol $L^{-1}$ $KH_2PO_4$ at pH 7.0 and 25°C

| $C_{Lyz}$/mg $mL^{-1}$ | $C_{(NH_4)_2SO_4}$/mol $L^{-1}$ | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 0.3 | 0.9 | 1.5 | 1.8 | 2.1 |
| 0.0 | –154.6±2.9 | –141.9±1.2 | –137.3±2.0 | –123.8±2.5 | –133.4±1.8 | –107.5±1.7 |
| 0.4 | –109.9±2.0 | –100.2±2.2 | –83.9±1.8 | –83.3±1.7 | –78.2±1.8 | –69.6±0.9 |
| 0.7 | –142.4±2.2 | –107.2±0.8 | –102.5±1.3 | –91.2±0.7 | –102.6±0.9 | |
| 1.0 | –143.0±0.7 | –119.7±0.8 | –112.1±0.6 | –100.4±0.8 | –114.9±2.1 | |

**Table 2** Adsorption enthalpies $\Delta H$ (kJ mol$^{-1}$) of Lyz adsorbed onto PEG-600 from solutions ($x$ mg mL$^{-1}$ Lyz, $y$ mol L$^{-1}$ $(NH_4)_2SO_4$, 0.05 mol L$^{-1}$ $KH_2PO_4$, pH 7.0) at 25°C

| $C_{Lyz}$/mg mL$^{-1}$ | $C_{(NH_4)_2SO_4}$/mol L$^{-1}$ | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 0.3 | 0.9 | 1.5 | 1.8 | 2.1 |
| 0.4 | 3218±353 | 3002±245 | 3845±274 | 2916±302 | 3974±259 | 2729±187 |
| 0.7 | 501.9±209.6 | 1428±82 | 1432±136 | 1341±131 | 1267±111 | |
| 1.0 | 334.1±104 | 639.4±57.6 | 725.8±74.9 | 673.9±95.0 | 532.8±112.3 | |

The adsorption heats $Q_i$=$Q_{obs}$–$Q_{blank}$. In the qualification of the $\Delta H$, only the initial concentrations of Lyz were used [4, 5].

concentrations and the calculated results are more reliable. The $\Delta H_A$ and $\Delta H_D$ obtained by plotting $\Delta H$ *vs*. logarithm of equilibrium concentration of Lyz ($C$) based on Eq. (12) are listed in Tables 3 and 4. The corresponding linear correlation coefficients ($r$) are quite satisfactory ($r$=0.9978 and 0.9941 in 1.5 and 1.8 mol L$^{-1}$ ammonium sulfate, respectively).

The endothermic $\Delta H$ in Table 2 indicate that the adsorption of Lyz is entropy driven which is in accordance with the investigation of Chen and his coworkers [20]. This can be attributed to greater positive values of $\Delta H_A$ which counteract the lower negative values of $\Delta H_D$ shown in Tables 3 and 4. In this study $\Delta H_A$ in SDT thermodynamics, which involve protein affinity attaching conformational loss, should consist of both $\Delta H_a$ (exothermic) and $\Delta H_{ml}$ (endothermic), as shown above, while $\Delta H_D$ in SDT thermodynamics, which involve water molecule gain (hydration of unfolded and exposed amino acid residues induced by conformational loss) and loss (dehydration), should be composed of both $\Delta H_d$ (endothermic) and $\Delta H_{mh}$ (exothermic).The relations between them can be expressed as

$$\Delta H_A = \Delta H_a + \Delta H_{ml} \quad (14)$$

and

$$\Delta H_D = \Delta H_d + \Delta H_{mh} \quad (15)$$

Tables 3 and 4 described practically the relative magnitude between the subprocesses with opposite heat effect, i.e. hydration of disordered (or unfolded) structure ($\Delta H_{mh}$<0) is stronger than dehydration of hydrated Lyz adsorption onto hydrated PEG-600 surface ($\Delta H_d$>0) due to $\Delta H_D$<0, and endothermic effect of ordered structure loss ($\Delta H_{ml}$>0) is greater than exothermic one of affinity adsorption of Lyz to surface ($\Delta H_a$<0) due to $\Delta H_A$>0. This reveals that $\Delta H_{ml}$ is predominant over others because the general enthalpy $\Delta H$ is endothermic. At fixed salt concentration, $\Delta H_A$ values are constant, while negative $\Delta H_D$ values increase with protein concentration increment, and the corresponding general enthalpies $\Delta H$ values decrease. This can be explained by decrease of adsorbed site number and equivalent enhancement of disordering (conformational loss) during the rise of adsorbed protein concentration. The result accords with the observation [21] that the extent of structural change decreased as the concentration of adsorbed protein decreased. With salt concentration increment, both $\Delta H_A$ values and negative $\Delta H_D$ values increase if the equilibrium concentrations of protein $C$ are identical. This means that stronger hydrophobicity promotes loss of more orderly secondary structure of Lyz.

*Fractions of adsorption entropy and free energy*

The linear correlation coefficients $r$, adsorption parameter $\beta_a$ and desorption parameter $q/z$ obtained by plot of ln$P_a$ *vs.* ln$C$ for native Lyz adsorbed onto hydrophobic PEG-600 surface from Lyz solutions ($x$ mol L$^{-1}$ $(NH_4)_2SO_4$, 0.05 mol L$^{-1}$ $KH_2PO_4$, pH 7.0) at 25°C are listed in Table 5. The linear relationships of them are very satisfactory, showing obtained $\beta_a$ and $q/z$ to be very reliable. The calculated fractions of entropy based on the Eqs (9) and (10) are also listed in Tables 3 and 4.

Like enthalpy fractions (Eqs (15) and (16)), $\Delta S_A$ and $\Delta S_D$ can also be regarded as the algebraic sum of both $\Delta S_a$ and $\Delta S_{ml}$ and that of both $\Delta S_d$ and $\Delta S_{mh}$, respectively, shown as follows:

$$\Delta S_A = \Delta S_a + \Delta S_{ml} \quad (16)$$

**Table 3** Thermodynamic fractions of native Lyz adsorbed onto PEG-600 in 1.5 mol L$^{-1}$ $(NH_4)_2SO_4$ solutions at 25°C

| $C_0$/ mg mL$^{-1}$ | $C$/ μmol L$^{-1}$ | $\Delta H_A$/ kJ mol$^{-1}$ | $\Delta H_D$/ kJ mol$^{-1}$ | $\Delta S_A$/ kJ mol$^{-1}$ K$^{-1}$ | $\Delta S_D$/ kJ mol$^{-1}$ K$^{-1}$ | $\Delta G_A$/ kJ mol$^{-1}$ | $\Delta G_D$/ kJ mol$^{-1}$ |
|---|---|---|---|---|---|---|---|
| 0.4 | 20.84 | 8827±302 | –5911±604 | 29.70±1.0 | –19.86±2.0 | –27.77 | 10.28 |
| 0.7 | 43.89 | 8827±131 | –7486±263 | 29.70±0.4 | –25.15±0.8 | –27.77 | 12.80 |
| 1.0 | 66.39 | 8827±95 | –8153±190 | 29.70±0.3 | –27.39±0.6 | –27.77 | 14.20 |

**Table 4** Thermodynamic fractions of native Lyz adsorbed onto PEG-600 in 1.8 mol $L^{-1}$ $(NH_4)_2SO_4$ solutions at 25°C

| $C_0$/ mg $mL^{-1}$ | $C$/ μmol $L^{-1}$ | $\Delta H_A$/ kJ $mol^{-1}$ | $\Delta H_D$/ kJ $mol^{-1}$ | $\Delta S_A$/ kJ $mol^{-1}$ $K^{-1}$ | $\Delta S_D$/ kJ $mol^{-1}$ $K^{-1}$ | $\Delta G_A$/ kJ $mol^{-1}$ | $\Delta G_D$/ kJ $mol^{-1}$ |
|---|---|---|---|---|---|---|---|
| 0.4 | 9.64 | 9426±259 | –5452±518 | 31.69±0.9 | –18.30±1.8 | –23.07 | 4.25 |
| 0.7 | 25.96 | 9426±111 | –8159±222 | 31.69±0.4 | –27.39±0.8 | –23.07 | 6.11 |
| 1.0 | 40.93 | 9426±112 | –8893±225 | 31.69±0.4 | –29.85±0.8 | –23.07 | 6.96 |

**Table 5** The adsorption affinity parameter $\beta_a$ and desorption parameter $q/z$

| $C_{(NH_4)_2SO_4}$/ mol $L^{-1}$ | ln$P_a$ vs. ln$C$ | | |
|---|---|---|---|
| | $r$ | $q/z$ | $\beta_a$ |
| 1.5 | 0.9996 | 1.3647 | 11.202 |
| 1.8 | 0.9997 | 0.7564 | 9.309 |

and

$$\Delta S_D = \Delta S_d + \Delta S_{mh} \quad (17)$$

The meanings of the subscripts are the same as that in enthalpy. The positive $\Delta S_A$ indicates that entropy gained by loss of ordered conformation ($\Delta S_{ml}>0$) is predominant over the entropy loss induced by adsorption affinity ($\Delta S_a<0$). Similarly, negative $\Delta S_D$ shows that entropy loss in hydration of polypeptide amino acid residues in disordered structure ($\Delta S_{mh}<0$) is greater than the entropy gain in dehydration of contacting of hydrated protein with hydrated ligand of surface ($\Delta S_d>0$). The changing trend of both $\Delta S_A$ and $\Delta S_D$ with both protein concentration and salt concentration increment can be also elucidated by the changes of four subprocesses as made in enthalpy. The general entropy $\Delta S$ values are positive, which can be explained that reduction of the protein's α-helical structure would cause an increased rotational mobility along the polypeptide chain, which in turn leads to larger conformational entropy of the protein [22]. This demonstrates again that entropy gain induced by loss of ordered conformation is the main driving force of adsorption of native Lyz onto PEG-600 surface.

The fractions of free energies in Tables 3 and 4 were calculated according to Eqs (6) and (7), to which the linear parameters $\beta_a$ and $q/z$ obtained by plots of ln$P_a$ *vs.* ln$C$ were inserted, respectively. Because, as shown in Table 5, the linear correlation coefficient of the plots of ln$P_a$ *vs.* ln$C$ are 0.9996 and 0.9997 at 1.5 and 1.8 mol $L^{-1}$ $(NH_4)_2SO_4$, respectively, the errors of $\beta_a$, $q/z$ and ln$C$ may be ignored, resulting in the transfer errors of calculation of $\Delta G_A$ and $\Delta G_D$ close to zero. The calculated fractions of free energies in Tables 3 and 4 show clearly that a spontaneous process ($\Delta G<0$) depends on greater negative $\Delta G_A$, which should be the original motive force and can be contributed mainly by entropy gain in conformational loss ($\Delta S_A>0$ in Tables 3 and 4) because $\Delta H_A>0$ (in Tables 3 and 4) and $T\Delta S_A>\Delta H_A$. The $\Delta G_D$ (>0) which is unfavorable to a spontaneous process substantially results from entropy loss of hydration ($\Delta S_{mh}<0$), because the entropy loss may be greater than an entropy gain of dehydration ($\Delta S_d>0$), resulting in $\Delta S_D<0$. $\Delta G_A$ values are all constant at a given salt concentration, and $-\Delta G_A$ decrease with salt concentration. $\Delta G_D$ values increase basically with surface coverage increment of Lyz and salt concentration decrement. These changes are determined by the corresponding ones of enthalpies and entropies. From the fractions of thermodynamics, we can see that the contributions to both entropy and enthalpy for orderly conformational loss of adsorbed native Lyz ($\Delta S_{ml}>0$, $\Delta H_{ml}>0$) predominate over that for adsorption affinity ($\Delta S_a<0$, $\Delta H_a<0$), resulting in $\Delta S_A>0$ and $\Delta H_A>0$. However, only entropy gain favors the displacement adsorption process.

## Conclusions

- The heat effects induced by molecular conformation changes in protein adsorption onto surface were specifically stipulated: if conformation gets more ordered structure, the enthalpy $\Delta H_{mo}<0$ and the accompanied dehydration enthalpy $\Delta H_{md}>0$; if conformation loses ordered structure, the enthalpy $\Delta H_{ml}>0$ and the accompanied hydration enthalpy $\Delta H_{mh}<0$.
- The relations between four subprocesses of protein adsorption and the fractions of thermodynamic functions calculated by SDT were first built up. For adsorption of native protein in this study, the (net) adsorption thermodynamic fractions in SDT correspond to contributions of protein affinity and conformational loss, while the (net) desorption thermodynamic fractions in SDT involve that of dehydration and hydration of unfolded and exposed amino acid residues induced by conformational loss.
- Native Lyz adsorption on hydrophobic PEG-600 surface is an entropy driven process contributed mainly by orderly conformational loss of adsorbed native Lyz ($\Delta S_{ml}>0$).

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