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CALORIMETRIC DETERMINATION OF ENTHALPIES OF LYSOZYME FOLDING AT A LIQUID-SOLID INTERFACE

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Calorimetric determination of the total enthalpy changes (ΔH_i) of guanidine-denatured lysozyme (Lys) during the adsorption with simultaneously refolding on the surface of hydrophobic interaction chromatography packings was carried out at 25±0.001°C. The measured ΔH_i in the circumstances should include the changes in the three fractions: adsorption, dehydration and molecular conformation. It was found that when the unfolded Lys molecules are adsorbed and refold on the surface, entropy-driving caused by the dehydration of Lys mainly dominates the foregoing process. The refolding enthalpies of Lys, $\Delta\Delta H_i$ were found to be 10~100 folds higher than that measured in usual solutions.

Keywords: *liquid-solid interface, lysozyme, micro-calorimetry, protein folding*

Introduction

The measuring free energy of protein folding with calorimetry is a very important and the most direct method to explore energetically the properties and mystery of protein folding, and also is one of prospective topic in molecular biology. Although the sensitivity of the calorimetry is not high enough, which is a disadvantage especially for some very expensive proteins, with the improvement of precisely calorimetric technology, the high accurate and reliable data about the energetic information of calorimetry of protein folding should be prior to others. The most current reports on this information are only restricted in homogeneous solutions. However, even though protein refolding is carried out in a solution in a container, the surface of the container inescapably contributes (either positive, or negative) to protein refolding. As it well known that the surface of the stationary phase of reversed phase liquid chromatography (RPLC) can make some native proteins denature, while hydrophobic interaction chromatography (HIC) can make some unfold proteins refold. Therefore, the effect of the character of a liquid-solid system on protein refolding should be investigated.

The bioactivity recovery of protein folding in solution is generally very low (about 5~20%). The transition enthalpies for lysozyme (Lys) sample in water and in glycerol for the native Lys and for that denatured after the thermal denaturation were measured by Brova *et al.* [1]. A characteristic single endothermic transition enthalpies for Lys between the native and unfolded states under various conditions were determined by Cueto *et al.* [2]. However, the report of micro-calorimetry on measuring free energy of protein folding at liquid-solid interface, in which the folding efficiency is very high, up to now, has seldom been seen. As pointed above that although the contribution of solid surface to protein folding is known to be far greater than that of solution, no any quantitative expression by calorimetry has been reported in literature. A straight way to express quantitatively the contribution of solid surface to protein folding is to use micro-calorimetric method to determine the enthalpy change of protein folding, $\Delta\Delta H$ and further to obtain its corresponding free energy change, $\Delta\Delta G$. In this study, selecting Lys as a model protein, guanidine hydrochloride (GuHCl) as denaturing agent, and an environment for the separation with simultaneous renaturation of Lys by high performance hydrophobic interaction chromatography (HPHIC), the total heat effects of Lys, ΔH_is, under various concentrations of GuHCl (C_{GuHCl}) were measured by using a Micro DSC-III calorimeter and the corresponding $\Delta\Delta H_i$s were calculated. The aim of this presentation is to establish a new calorimetric method to measure the heat effects and the free energy changes attained thereafter of protein folding on a liquid-solid interface. The contributions can be used to explore whether the energy barrier or the intermediate states of lowest energy ex-

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ist on the pathway of protein folding on the liquid-solid interface and so on, which have not been understood yet.

Experimental

Apparatus and materials

The calorimetric operations were carried out by a Micro DSC-III instrument (Setaram, Calurie, France), including a calorimeter, a controller, a computer and a printer-plotter. The calorimeter was equipped with a mixing 'batch' vessel, composing of a cylinder and a set of parts mounted on a rod provided for dividing the experimental volume into two distinct chambers, with a volume of 0.2 cm^3 for the upper chamber and 0.55 cm^3 for the lower chamber (Fig. 1).

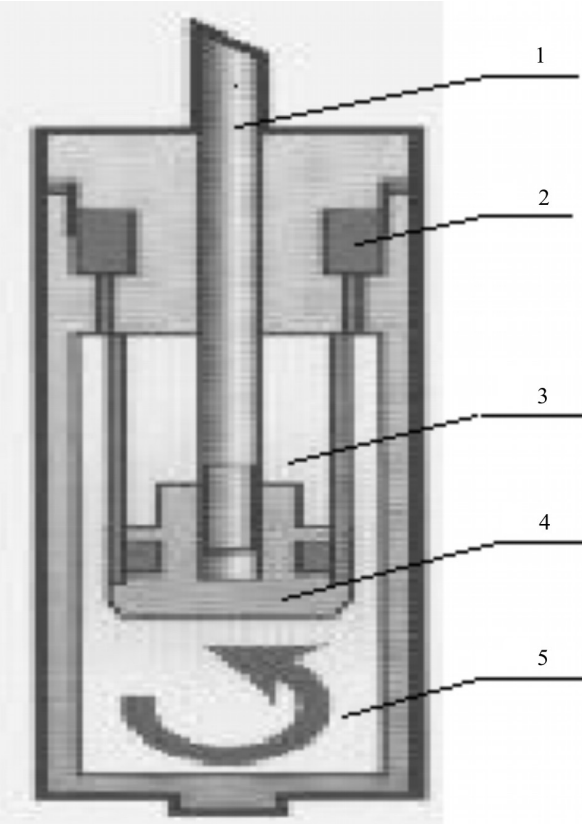

Fig. 1 The cutaway view of mixing vessel; 1 – the rod, 2 – ethylene propylene o-ring, 3 – upper chamber, 4 – the cover, 5 – lower chamber

An obturator is fixed at the lower end of the rod making the basis of the upper chamber via a hollow cylinder on the upper stopper. When the rod is up, both chambers are completely separated. When the rod is down, both chambers connect for the mixing. The rubbing due to the rod moving must be maintained at a minimum so as to avoid from generating heat. A nitrile o-ring on the obturator ensures the tightness of the upper chamber. The upper stopper provided with an o-ring (nitrile, ethylene propylene, viton) ensures the tightness of the vessel. The mixing vessel is able to stand a maximal internal pressure of 1 bar.

The concentrations of Lys solution were measured with a UV-Vis spectrophotometer (Model 8453, Agilent Co., USA). A Centrifugal (Type 800) for the separation of Lys solutions from PEG-600 packings and an isothermal vibrator (Type SHA-C/THZ-82) for adsorption of Lys on the PEG-600 packing surface were all made by Guohua Electromachine Co. (Changzhou, China).

PEG-600 made of a silica base-HPHIC packings (particle size, 6.5 μm; pore diameter, 30 nm; the end-group of polyethylene glycol) was obtained from the Institute of Modern Separation Science (Northwest University, China).

Chemicals

Lysozyme (Lys, chicken egg white) was purchased from Sigma Co. (St. Louis USA). Guanidine hydrochloride (GuHCl) bought from Beijing Chemical Reagent Co., ammonium sulphate ($(NH_4)_2SO_4$) obtained from Beijing Beihua Fine Chemical Co., potassium phosphate monobasic (KH_2PO_4) purchased from Tianjin Dengfeng Chemical Reagent Co. Other chemicals are all analytic-reagent grade. The deionized water was prepared with Milli-Q Academic (Millipore Co. Ltd, USA).

Preparations of denatured and calorimetric Lys solutions

A set of partially or/and fully denatured Lys solutions with 1.0 mg mL^{-1} Lys-0.05 mol L^{-1} KH_2PO_4 (pH 7.0) were prepared with different concentrations of GuHCl and standing for 24 h at 25°C. The Lys concentration in the reaction solution was 0.40 mg mL^{-1}. The C_{GuHCl} was 0.0, 0.40, 0.80, 1.30, 1.80, 2.20 and 2.60 mol L^{-1}. The concentrations of other chemical solutions (pH 7.0) were as: ammonium sulphate, 2.1 mol L^{-1} and potassium dihydrogen phosphate 0.05 mol L^{-1}.

Calorimetric procedure

Transfer 0.500 mL Lys solutions 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, corresponding the 'reference' one being empty. Close the vessels and keep them vertically until introduce into the calorimeter. Control the temperature of experiments at

25±0.001°C accurately. The signal of heat flow takes about 2 h to zero and maintain invariance, indicating the calorimetric system to approach to a stable equilibrium. After that, put down the rod of measurement mixing vessel to drop down the PEG-600 packings into the Lys solution and mix them with each other, and also start to adsorb. During the adsorption process, nitrogen gas should be blow to protect the semiconductor components around the mixing batch vessel. Record the heat flow–time curve until the heat flow approached to zero. The detection limit and calorimetric resolution of signal were 0.2 μW and 40 nW, respectively.

The procedures to measure the two disturbing heats were as same as the forgoing description except PEG-600 packings absent in the upper chamber for the rubbing heat of putting down operation rod, and except Lys absent in the liquid sample for the mix heat of PEG-600 packings with blank solution sample, respectively.

Adsorption amounts

In order to determine the adsorbed amounts of Lys at the surface of PEG-600 corresponding the calorimetric processes, the mixtures of PEG-600 packings and the Lys in various C_{GuHCl} solutions with the same ratios (W/V) as that in the mixing batch vessel employed were taken to shake for 5 h at 25±0.5°C in the SHA-C/THZ-82 isothermal vibrator. The obtained supernatants by centrifuging the mixtures at 2000 rpm were detected at 280 nm by 8453 UV-Vis Spectrophotometer to determine the concentrations of Lys. Based on the differences of equilibrium concentration between before and after adsorption, the adsorption amounts of Lys corresponding to the systems in mixing batch vessel were calculated.

Results and discussion

Total heat effects of displacement adsorption

Liquid-solid adsorption is practically a displacement adsorption including net adsorption of adsorbate to the surface and net desorption of solvent from both the solid surface and the solvated solute surface [3]. Thus, the heat of the process should be the total heat effects of displacement adsorption, Q_i or ΔH_i. The heats directly measured with the microcalorimeter during contacting the unfolded Lys solution sample with PEG-600 packings in the mixing batch vessel and denoted with Q_{obs} include not only above mentioned Q_i, but also both of the mixing (wetting) heat of PEG-600 packings with solution absent Lys (blank sample), Q_{mix}, and the rubbing heat produced due to the rod moving, Q_{rub}. Then, the following expression exists:

$$Q_i = Q_{obs} - Q_{mix} - Q_{rub} \quad (1)$$

The obtained calorimetric thermograms of the above mentioned three fractions shown on the right side in Eq. (1) are illustrated respectively in Fig. 2. The each area under the three curves represents the heat effect of the individual operation and the exact data of each area can be calculated with the multitask (simultaneous acquisition and processing) and multimodule software package offered by Setaram Micro DSC-III. Every calorimetric test in this presentation was performed more than four times. The individually measuring results of rubbing heats and mixing (wetting) heats are listed in Table 1. The mean values of measured Q_{rub} and Q_{mix} are –21.8±0.4 and –124.3±2.9 mJ, respectively, and the corresponding relative average errors of them are 1.83 and 2.25% respectively, indicating the measuring results to be quite satisfactory. Both the directly measured heats Q_{obs} and the total heats of displacement adsorption Q_i or the corresponding molar enthalpy change ΔH_i of denatured Lys corresponding to various C_{GuHCl} at the interface between PEG-600 packings and the solutions (2.1 mol L^{-1} $(NH_4)_2SO_4$, 0.05 mol L^{-1} KH_2PO_4 (pH 7.0) and x mol L^{-1} GuHCl) are listed in Table 2. It is shown

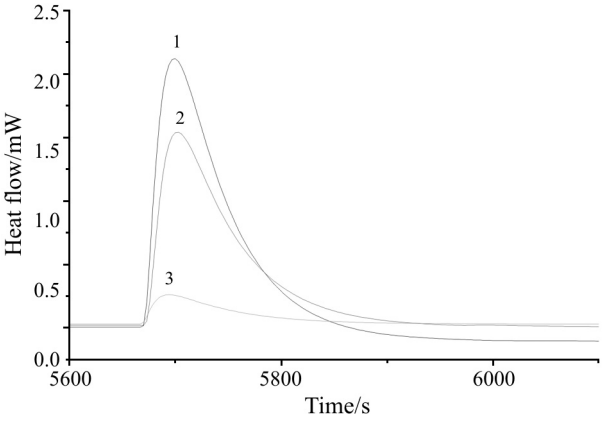

Fig. 2 Typical calorimetric curves of the involving fractions during mixing Lys solution with PEG-600 at 25±0.001°C; 1 – Q_{obs}(at 1.8 mol L^{-1} GuHCl), 2 – Q_{mix} (with Lys blank solution), 3 – Q_{rub}

Table 1 Rubbing heats and mixing heats at 25±0.001°C

No.	Q_{rub}/mJ	Q_{mix}/mJ
1	–20.1	–114.6
2	–22.0	–125.7
3	–23.1	–128.1
4	–21.1	–128.8
5	–22.8	
6	–21.9	
mean	–21.8±0.4	–124.3±2.9

Table 2 The $\Delta\Delta H_i$s of the Lys at interface of PEG-600/solution (2.1 mol L^{-1} $(NH_4)_2SO_4$, 0.05 mol L^{-1} KH_2PO_4 (pH 7.0) and x mol L^{-1} GuHCl) at 25±0.001°C

C_{GuHCl}/mol L^{-1}	m_{ads}/mg	Q_{obs}/mJ	Q_i/mJ	ΔH_i/kJ mol^{-1}	$\Delta\Delta H_i$/kJ mol^{-1}
0	0.1219	–54.9±2.6	91.2±3.9	10 777	0
0.4	0.1904	–63.1±2.7	83.0±3.9	6 277	4 500
0.8	0.1994	–120.8±7.3	25.4±7.8	1 831	8 946
1.3	0.1272	–124.0±7.1	22.1±7.7	2 505	8 272
1.8	0.0905	–155.4±0.3	–9.3±2.9	–1 477	12 254
2.2	0.0310	–121.1±0.6	25.1±2.9	11 641	–864
2.6	0.0290	–55.1±1.1	91.1±3.1	45 216	–34 439

m_{ads} – adsorbed amount of Lys on PEG-600; Q_{obs} – observed value; Q_i – total heat of displacement adsorption of Lys (offsetting the mixture heat of blank sample with the HPHIC packings, –124.3 mJ and the heat effect of putting down operation rod, –21.8 mJ)

that both Q_i and ΔH_i values are all endothermic except for that at 1.8 mol L^{-1} GuHCl solution. Moreover, with C_{GuHCl} decreasing, firstly the values of both Q_i and ΔH_i decrease until a minimum value (exothermic) corresponding to 1.8 mol L^{-1} GuHCl appears, and then they tend to increase. However, the forgoing results just make a sharp contrast to the changing tendency of the adsorbed amounts of denatured Lys, m_{ads}, listed in Table 2. The changeable tendency of m_{ads} exhibits firstly increasing up to a maximum value (0.1994 mg) corresponding to 0.8 mol L^{-1} GuHCl and then decreasing. It is shown that, at least, any simply corresponding correlation does not exist between Q_i or ΔH_i and m_{ads} listed in Table 2. In other words, during the process of refolding with simultaneous adsorption of Lys in variously denatured states on PEG-600 packing surface, the molecular conformations of the adsorbed Lys are different from each other due to the presence of GuHCl with various concentrations.

When the unfolded Lys molecules refold and are adsorbed on the PEG-600 packings with a moderate hydrophobicity, their total enthalpy changes include following three fractions: (*i*) dehydration enthalpy change ΔH_d. On the one hand, as the hydrated unfolded Lys molecules contact the hydrated PEG-600 packing surface, dehydration occurs at the contacted region. On the other hand, during the formation of the microdomains or intermediates of Lys, which is necessary for Lys refolding, a part of water molecules on the hydrated protein molecules will lose due to the hydrophobic interaction of amino acid residues in the polypeptide chain. Because dehydration must obtain energy from solid interface, where a chemical potential jump [4] exists, it is endothermic, i.e. dehydration enthalpy change ΔH_d is positive; (*ii*) adsorption affinity enthalpy change ΔH_a. When the unfolded Lys molecules are adsorbed by PEG-600 packing surface, because the orderly state of Lys molecules increases (entropy decreasing) and the adsorption should be a spontaneous process, thus, the adsorption affinity interaction is exothermic, resulting in its enthalpy change ΔH_a to be negative; (*iii*) the molecular conformation enthalpy change ΔH_m. When the unfolded protein molecules refold from its denatured state to native state or intermediate state, free energy change decreases. However, generally speaking, it is not sure that molecular conformation enthalpy change ΔH_m would be exothermic or endothermic, because ΔH_m is an apparent heat effect of two opposite interactions: formation of ordered domain of polypeptide amino acid residues (exothermic) and dehydration of them (endothermic).

The values of above three kinds of enthalpy changes in the different adsorbed states are various. Thus, it is easy to understand the changeable regulation of ΔH_i with C_{GuHCl}. When C_{GuHCl} is high, e.*g.* 2.6 mol L^{-1}, Lys molecules almost entirely exist in unfolded state and practically exist within the capillaries in solid phase rather than on solid surface, and the adsorbed amount is a little (0.0290 mg). It is dehydration between the hydrated unfolding protein molecules and the hydrated solid surface that plays a major role rather than adsorption affinity and molecular conformation, i.e. ΔH_d is predominant to ΔH_a and ΔH_m, resulting in ΔH_i being bigger endothermic. When C_{GuHCl} decreases to 1.8 mol L^{-1}, denaturation decreases. It is known from the data in HPHIC [5] that Lys had more retention on the stationary phase of HPHIC, and chemical equilibrium was favorable to make protein renature. Therefore, bonding to the ligands of the solid surface increases, and the adsorbed amount increases and ΔH_a and ΔH_m are relatively predominant to ΔH_d , resulting in ΔH_i being exothermic. However, with continuous decrease in the C_{GuHCl} from 1.8 mol L^{-1} to zero, reappearance of endothermic phenomenon and basically gradual rise of Q_i and ΔH_i indicates that when Lys refolds gradually into its native state, compared to the contribution from the endothermic action for dehydration and that from the exothermic action both for adsorption and for molecular conformation of ordered domain to native state of Lys, dehydration is still predominant, re-

sulting in forming more and more three-dimensional structure of Lys molecules.

From Table 2, the adsorbed amount of Lys firstly increases and then decreases with the decrease in the C_{GuHCl}. This is because that when the C_{GuHCl} is higher, Lys has at least two states, i.e. denatured and native states, and are simultaneously adsorbed on the adsorbent surface, while with C_{GuHCl} decreasing Lys in denatured state transforms gradually into its native state, and finally, completely becomes its native state. So, because of sterically hindered effect of the three-dimensional structure, the adsorption amounts of Lys decrease.

It is noted that the value of ΔH_i corresponding to 0.8 mol L^{-1} GuHCl (1831 kJ mol^{-1}) is lower than that at 1.3 mol L^{-1} GuHCl (2505 kJ mol^{-1}). This indicates that dehydration in former state is weaker than that in latter state and implies that a mutation (or tumult at least) of molecular conformation may occur. This phenomenon coincides with the fact that a lower energetic rise (as if it was an energy barrier but actually not) on the pathway at 0.8 mol L^{-1} GuHCl was found in the chromatography study [5] corresponding to the same experimental condition.

The values of ΔH_i listed in Table 2 are almost endothermic, indicating in the presence of the various C_{GuHCl} the spontaneous adsorption and refolding of unfolded Lys molecules on the PEG-600 surface to be an entropy-driving process. Obviously, this is an inevitable result due to the dehydration being predominant process. Both the dehydration occurring at the interface between the hydrated unfolded protein molecules and hydrated solid surface and that due to the hydrophobic interactions among polypeptide amino acid residues make water molecules out of the interior of Lys molecules, resulting in entropy increasing. In fact, the essence of the stoichiometric displacement theory for retention (SDT-R) of protein in HPHIC is that when the protein molecules are adsorbed by the stationary phase, a stoichiometric number of water molecules is squeezed out at the contact region between the protein and the stationary phase [6]. Perkin *et al.* [7] measured the amount of water both displaced from the contact region between protein molecules and solid surface and instantaneously lost from the hydrophobic region of a protein molecule when it was adsorbed by the surface. Thus, the conclusion may be drawn that entropy-driving dominates the unfolded Lys molecules to be adsorbed and refolded on the PEG-600 surface.

Refolding enthalpy change of Lys, $\Delta\Delta H_i$

Refolding enthalpy change of Lys, $\Delta\Delta H_i$, is defined as the difference between the total enthalpy change of native Lys ΔH at C_{GuHCl}=0 (end state) and that of denature Lys ΔH_i corresponding to various C_{GuHCl} (start state) under the same HPHIC condition. That can be expressed as

$$\Delta\Delta H_i = \Delta H(C_{GuHCl} = 0) - \Delta H_i \quad (2)$$

It is clear that those $\Delta\Delta H_i$s not only differ from the ΔH_i at the same states, but also differ among themselves corresponding to various states. This character can just right provide the kinetically important information for the gradually refolding of Lys on PEG-600 surface when the study on imitating the continuously removing GuHCl from sample solution in HPHIC is carried out.

It is seen from Table 2 that the absolute values of the $\Delta\Delta H_i$ at the denatured states cover the range from 864 to 34439 kJ mol^{-1} and are about 10~100 folds higher than that in solution. The energetical difference basically coincides with the reported results [8] that the refolding free energies of denatured proteins (Lys and α-amylase) on the stationary phase surface of HPHIC measured by HPHIC method were 10~100 folds higher than that in the usual solution. This is because under the condition of high concentration of ammonium sulphate (2.1 mol L^{-1}), the moderately hydrophobic PEG-600 surface provides high enough energy to a denatured or only partially folded protein molecule to make it dehydrate and refold to its native state, while only partially refolding existed in usual solution. Ammonium sulphate is one of the lyotropic salts, and a high concentration of ammonium sulphate provides a more favorable environment for both the protein and PEG-600 packing surface which facilitates adsorption in the surface. Energy provided by the surface of PEG-600 can propagate along the rigid peptide chain to the other (such as hydrophilic) amino acid residues of the unfolded protein molecules. Thus, the hydrated amino acid residues which originally do not contact the surface of PEG-600 are able to be dehydrated and to be promoted to refolding due to the interaction among those amino acid residues. This process may be rather complicated. A small number of hydrophobic region from protein molecules could possibly be adsorbed by PEG-600 surface due to their hydrophobicities and steric effect. The protein molecules firstly form microdomains on the solid surface and then grow up into their native or intermediate states. No matter $\Delta\Delta H_i$s whether exhibits to be exothermic corresponding to high C_{GuHCl} or to be endothermic corresponding to low C_{GuHCl}, the interactions between moderately hydrophobic solid surface and protein molecules during the process of protein refolding are rather strong. If in sight of imitating continuously removing the denaturing agent from sample solution in the HPHIC, the changeable tendency of $\Delta\Delta H_i$ from high C_{GuHCl} to low C_{GuHCl} shown in Fig. 3 is that the algebraic values of $\Delta\Delta H_i$ firstly increase until the maximum (12254 kJ mol^{-1}) at 1.8 mol L^{-1} GuHCl and then basi-

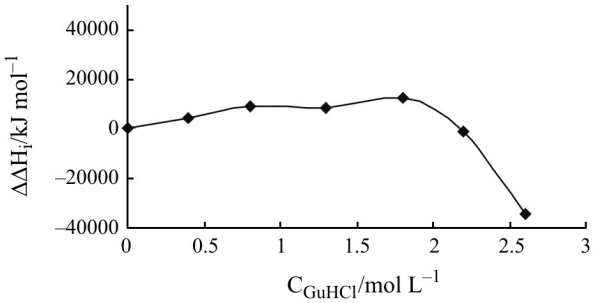

Fig. 3 $\Delta\Delta H_i$ varied with C_{GuHCl}

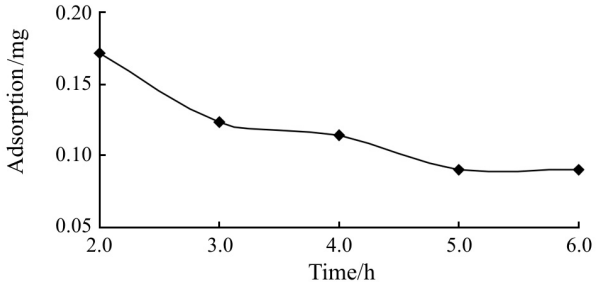

Fig. 4 Adsorption amount of Lys varied with adsorption time at 1.8 mol L^{-1} C_{GuHCl}

cally monotonous decrease. This substantially depends on the magnitudes of various ΔH_i discussed above in details. It is seen in Eq. (2) that the physical meaning of $\Delta\Delta H_i$ is a complementary enthalpy change needing to make a denatured protein corresponding to a given C_{GuHCl} and ΔH_i entirely refolding into the native protein corresponding to $\Delta H(C_{GuHCl}=0)$. In other words, $\Delta\Delta H_i$ and ΔH_i are complemented in enthalpy. Thus, the negative $\Delta\Delta H_i$ (exothermic) at high C_{GuHCl} reveals that more adsorption affinity and order of molecular conformation of the denatured protein on PEG-600 surface are needed to make it refold into the native state. In contrast, the positive $\Delta\Delta H_i$ (endothermic) at low C_{GuHCl} shows that more dehydrations of hydrated unfolding protein molecules, hydrated solid surface and hydrated amino acid residues are necessary for the entirely refolding of protein. These are in an accordance with the study on mechanism of refolding of protein by HPHIC [4].

It is worthy to be noted that the maximum refolding enthalpy change of Lys $\Delta\Delta H_i$ at 1.8 mol L^{-1} GuHCl suggested the existence of an intermediate state with relatively lower energy or called 'energy well' [5], although the literature [5] did not report the existence of 'energy well' for Lys. Burova *et al.* [1] reported that an intermediate state, the highly ordered molten globule in Lys's thermal unfolding-refolding dissolved in glycerol was discovered. Privalov *et al*. [9] considered that the intermediate states in Lys's unfolding-refolding were low-populated in aqueous solution and can not be detected in equilibrium experiments. These are strong support to the existence of intermediate states in Lys's unfolding-refolding. Again, the existence of the maximum $\Delta\Delta H_i$ at 1.8 mol L^{-1} GuHCl measured recently by us for the same studied system at 35°C may support the conjecture upon 'energy well'. For the exploring the mechanism of Lys adsorption and folding at C_{GuHCl}, 1.8 mol L^{-1}, the plotting of adsorption amounts of Lys m_{ads} *vs.* time was drawn in Fig. 4. The result indicates that with time increase, the adsorbed amounts m_{ads} decrease gradually down to a constant (0.0904 mg) corresponding to 5 h. It is shown that at the start denatured protein molecules may be adsorbed in the way of 'wrapped-up' on the PEG-600 surface, resulting in bigger adsorption amount. After that a part of the protein molecules may refolde on the surface and form a three-dimensional structure, while other molecules may be back to solution. This may show in other sight that the state at 1.8 mol L^{-1} C_{GuHCl} could be a relatively stable state. It correlates closely with exothermic ΔH_i (–1477 kJ mol^{-1}) and the maximum, $\Delta\Delta H_i$ (12254 kJ mol^{-1}).

Conclusions

A new micro-calorimetric method to measure directly the heat effects and the free energies attained thereafter of protein folding on liquid-solid interface was established.

It was pointed out that entropy-driving caused mainly by dehydration promotes the unfolded Lys molecules to be adsorbed and refolded on the PEG-600 surface with a moderate hydrophobicity. In the refolding pathway of Lys, the dehydration of both the hydrated unfolded Lys and hydrated solid surface exhibiting endothermic is predominant, and a lower energetic rise (as if it were an energy barrier but actually not) corresponding to 0.8 mol L^{-1} of the concentration of guanidine hydrochloride (C_{GuHCl}), while an intermediate state with relatively lower energy called 'energy well' at 1.8 mol L^{-1} C_{GuHCl} exist. The refolding enthalpies of Lys $\Delta\Delta H_i$ were found to be 10~100 folds higher than that in usual solutions, because the moderately hydrophobic PEG-600 surface can provide high enough energy to the hydrated and unfolded Lys molecules to make them dehydrate and refold into its native state, while only partially refolding existed in solution.

Acknowledgements

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References

1 T. V. Burova, N. V. Grinberg, V. Y. Grinberg, *et al*., Biochim. Biophys. Acta, 1478 (2000) 309.
2 M. Cueto, M. J. Dorta, O. Munguia, *et al*., Int. J. Pharm., 252 (2003) 159.
3 X. P. Geng, Thermochim. Acta, 308 (1998) 131.
4 X. D. Geng and Q. Bai, Sci. China, Ser.B, 45 (2002) 655.
5 J. Zhang, Master Thesis, Northwest University of China, 1999.
6 X. D. Geng, L. A. Guo and J. H. Chang, J. Chromatogr., 507 (1990) 1.
7 T. W. Perkins, D. S. Mark, T. W. Root, *et al.*, J. Chromatogr., A, 766 (1997) 1.
8 X. D. Geng, J. Zhang and Y. M. Wei, Chin. Sci. Bull., 45 (2000) 237.
9 G. Privalov, V. Kavina, E. Freire, *et al*., Anal. Biochem., 232 (1995) 79.

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