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THE INTERPRETATION OF PROTEIN STRUCTURE THROUGH RELATIONSHIP OF MELTING POINT (TM) AND ENTHALPY OF UNFOLDING (ΔHU)

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ABSTRACT: Melting point (Tm) and enthalpy of unfolding (ΔHU) for protein denaturation are used as characterization of protein stability. Protein stability is one of the main property of protein considering drug – protein interaction. However, it is reported that these two parameters (ΔHU and Tm) correspond to stability of proteins; there are evidences that indicate Tm and ΔHU change during protein denaturation irregularly. Here it is presented a novel glance about relationship between ΔHU and Tm for protein denaturation based on structural aspects of proteins. The finding indicated that there are nine conditions related to Tm and enthalpy changes. These conditions are described and documented by correspond proteins. The clear description for Tm parameter as reflector of local intramolecular interactions and enthalpy of unfolding as protein compactness index are provided. Based on this hypothesis new description of Tm and ΔHU can lead to better understanding of structural aspect of protein molecules

Keywords: Melting Point, Enthalpy of Unfolding, Protein Stability, Protein Compactness, Protein Frame Lock.

INTRODUCTION

Biothermodynamics is an important field in Biophysics and Biochemistry [1]. One of the well known branch of Biothermodynamics is study of protein thermal denaturation [2]. Thermal denaturation has provided the most useful information about tertiary structure and activity of globular proteins [3]. Two important parameters such as melting point (Tm) and enthalpy of unfolding (ΔHU) can be obtained via protein denaturation [4]. In fact the exposure of non-polar groups of protein to the water leads to the significant reduction of □HU, which specifies the extent of the compactness and flexibility of proteins [5]. However it is reported that there are some flexible proteins with more stability [6]. ΔHU is defined as the amount of the heat required for converting the native state of protein to unfolded state [7]. Tm is the temperature when the free energy change of protein unfolding (ΔGU) is equal to zero [8] or the heat capacity function is maximum [9]. Increase in Tm value is consisted with enhancement of the protein thermostability [11 – 12]. Based on molecular population, Tm is a temperature that corresponds to existence 50% of molecules in native or denatured state [13]. However, it is reported that these two parameters (ΔHU and Tm) correspond to stability of proteins; there are evidences that indicate Tm and ΔHU change during protein denaturation irregularly [14]. Here it is presented a novel glance about relationship between ΔHU and Tm for protein denaturation based on structural aspects of proteins [15]. Advantage of this description can lead to new interpretation of structural details of protein [16]. Changes of proteins Tm in various conditions is independent of changes in ΔHU, we have proved this topic by presentation of 9 situation. When Tm increases for a protein molecule, this is not due to increase in energy component of molecule. In other words, increment of temperature is no means increasing energy. In fact, increased temperature is a reason for creation of new mode for protein which is thermally stabilized without considering changes of energy component of molecule. Indeed, increment Tm is indicated formation lock in structure of protein. When Tm is increased, this

does not mean that the structure is complexes but in part of the structure is created a lock (e.g. a loop in structure connected to other cites). Better understanding of this thermodynamic parameter gives us the ability for saving protein in unfavorable conditions. Goal of this article is presents of sufficient documentations to confirm this hypothesis.

RESULTS

All possible combinational conditions (including 9 situations) of Tm and ΔHU for 11 proteins in two different conditions are considered. In order to define the relationship between Tm and ΔHU and better elucidation, it is attempted to give various examples (see Table 1). Finally desired relationship is interpreted by considering structural aspect of proteins.

Table 1: Relationship between Melting Temperatures (Tm) and Enthalpy Change (ΔHU) of 11 proteins under two different conditions is showed. Nine situations consisted: (1) Increased Tm and ΔHU, (2) increased Tm and decreased ΔHU, (3) increased Tm and constant ΔHU, (4) decreased Tm and increased ΔHU, (5) decreased Tm and decreased ΔHU, (6) decreased Tm and constant ΔHU, (7) constant Tm and increased ΔHU, (8) constant Tm and decreased ΔHU, (9) constant Tm and constant ΔHU. (↑, ↓, ↔ correspond to increased, decreased and constant respectively)

1	Ribonuclease T1	Tm ΔHU	42.7 51.1	91 94	↑ ↑	7
2	CryIIIA	Tm ΔHU	47 840	70 670	↑ ↓	10
3	calmodulin	Tm ΔHU	48.3 84	74.6 86	↑ ↔	11
4	RNase HI	Tm ΔHU	53 99.2	47.2 117.6	↓ ↑	12
5	T4 lysozyme	Tm ΔHU	51.7 113	38.3 67	↓ ↓	13
6	T4 lysozyme	Tm ΔHU	65.3 130	63.5 130	↓ ↔	9
7	glucanases	Tm ΔHU	50 581	50 652	↔ ↑	8,14
8	CMTI-V	Tm ΔHU	70.9 267	71.7 242	↔ ↓	8,9
9	Ubq-UIM	Tm ΔHU	77±1 77±1	329±2 330±3	↔ ↔	17

Situation 1; (increased Tm and increased ΔHU), Ribonuclease T1 as an acidic protein with a pI 3.5 and a net charge of -6 at pH 7 is a first subject that is studied. The side chain of Asp49 is hyperexposed, without hydrogen bond. When Asp49His is formed in mutant type, stability increases so increment of Tm is about 44°C and □HU 43 kJ/mol (see situation1, table 1). Replacing negative charge Asp49 by positive charge of His lead to favorable ionic interactions so the more stable structure is formed and compactness is increased [7].

Situation 2; (increased Tm and decreased ΔHU), the three-dimensional structure of CryIIIA toxin from Bacillus Thuringiensis subspecies Tenebrionis has been determined by X-ray diffraction analysis. It has three domains that form wedge-shaped molecule. Tm and ΔHU for this protein in pH = 2.2 are 47°C and 840 kJ/mol respectively. The amount of ΔHU is decreased to 670kJ/mol while the Tm is increased to 70°C at pH 9.7-11.0 respectively (situation 2, Table 1). The unfolding of toxin molecule is a single transition at alkaline pH and double transitions are occurred at acidic pH. The reason for low value of ΔHU at alkaline pH (pH's 9.7-11.0) is due to loss of rigid tertiary structure of molecule by one of its domain during isolation, this domain leads to partial unfolding The melting of the native structure of CryIA(c) at pH 9.7-11.0 suggests that it consists of two domains. In CryIIIA from B. thuringiensis subspecies tenebrionis, the transition from the native to denatured state under alkaline conditions (pH 9.7-11.0) proceeds by the "two-state" principle; i.e., the protein melts as one

cooperative domain. The melting of the CryIIIA toxin at pH 2.2-3.5 is described by two transitions overlapping by temperatures, indicating the presence of two domains [10].

Situation 3; (increased Tm and constant ΔHU), the methionine residues in the calcium regulatory protein calmodulin (CaM) are structurally and functionally important. They are buried within the N- and C-domains of apo-CaM. Here Tm and ΔH parameters in apo-wt-CaM and apo-Nle-CaM in C-domain are compared. That amount of ΔH was almost similar while Tm is increased (see Situation 3, table 1). Here shown that The evidences show that methionine substitutions by norleucine do not impact the main chain structure of CaM, and has a very small effect on the global structures of apo-CaM because Nle side-chain structure is more similar to that of Me. These reasons consist to same enthalpy changes between two proteins. Met/Nle substitutions dramatically increased the Tm of the C-domain (+26C). Considering the two side chains, the non bulk side chain of Nle provides possibility [11].

Situation 4; (decreased Tm and increased ΔHU), There is a cavity in the hydrophobic core of Escherichia coli RNase HI, 1(a small globular protein) of more contact between the two domains C and N of protein so Tm value is increased. Two core residues, Ala52 and Val74, resided at both ends of this cavity. Since these cavities may be required to make the proteins highly functional, its cost leads to probably at reduced stability. Therefore, it seems likely that the tolerance of proteins to core mutations increases as the volume of the naturally existing cavity at the core increases. For analysis of the effect of the mutation at the cavity on the protein stability, Ala52 is replaced by the serine amino acid residue. This mutation increases the stability of protein by stabilization of cavity in spite of decrement of via change in protein frame lock. [12].

Situation 5; (decreased Tm and decreased ΔHU), In mutant form, Leu 84 that is located in Large cavity with channel to surface of T4 lysozyme is substituted with alanine. The Tm values for wild type protein and mutant protein are 51.7°C and 38.3°C and amounts of ΔHU for them are 113 kJ/mol and 67 kJ/mol respectively (Situation 5 , Table 1). Leu 84 is very close to the surface of the protein and, in addition, is contacted by Val 111 within helix F, which is one of the most mobile helices of the lysozyme structure. Thus, when Leu 84 is truncated to alanine, one of the major interactions stabilizing the position of helix F is removed, and the helix moves toward the position vacated by the leucine sidechain .The van der Waals interactions between the leucine sidechain and the atoms that surround it in the native structure. If the leucine is replaced by an alanine, a cavity is formed and the van der Waals interactions are lost[13].

Situation 6; (decreased Tm and constant ΔHU), Residues within the core of T4 lysozyme were replaced by methionine. Such variant are active and fold cooperatively with progressively reduced stability. The Tm values for wild type protein and mutation are 65.3°C and 63.5°C respectively and amounts of ΔHU for the both are 130 kJ/mol. (Situation 6 , Table 1). Therefore there is no suggestion that the substitution of methionine leads to disorder of the hydrophobic core, the loss in protein stability is understandable. For each methionine replacement there is a reduction in the solvent transfer free energy (about 0.6 kcal/mol for Leu to Met). Also the side chain of methionine has more degrees of freedom than do other hydrophobic core amino acids [9].

Situation 7; (constant Tm and increased ΔHU), enzyme (1,3-1,4)--glucanase from Bacillus Macerans (MAC) and the hybrid form of enzyme; H (A12-M) that containing 12 N-terminal amino acids drive from Bacillus Amyloliquefaciens (1,3-1,4)- glucanases show single two state transitions in DSC experiment. The Tm values for the both transitions are about 50°C and the amounts of HU for MAC and H (A12-M) are 581 kJ/mol and 652 kJ/mol respectively (Situation 7, Table 1). The crystal structure analysis has shown that the general fold state (topology) of H (A12-M) is similar to MAC (the same frame lock). The amount of half transition concentration of GdnHCl (C1/2) for two proteins is the same in the presence of Ca+2[14]. Therefore it can be concluded that the stability of the two proteins are the same but MAC having more flexibility related to H (A12-M) [14,8].

Situations 8; (constant Tm and decreased ΔHU), Cucurbita Maxima Tripsin Inhibitor–V (CMTI-V) is a small globular protein of 68 residues, including cys 3 – cys 48 link. CMTI-V has a large flexible and solvent – exposed loop whose amino acid residues are not involved in extensive interaction with others. Two hydrogen bonds, one between Thr43 and Arg52, and the other between Asp45 and Arg50- anchor the loop to the protein scaffold. The amounts of Tm and ΔHU for CMTI-V as well as mutant of proteins is given in (Situation8, Table 1). Deletion of hydrogen bond for Thr43Ala has no significant effect on stability of protein with having a little effect on Tm (the same frame lock) but observed increases the flexibility of the protein (because of decrease ΔHU), similar case is observed about Arg52Gln [8].

Situations 9; (constant Tm and constant ΔHU), The thermodynamic characterization of a Ubq-UIM fusion construct (Ubq-UIM), were monitored by DSC. Here we compared the melting profiles of four fusion proteins

contain Ubq-UIM1 and Ubq-UIM2 , Ubq-UIM3 and Ubq-UIM4 that amount of Tm for all was 771 and HU was 3292, 3303, respectively (see just two cases in table, Ubq-UIM1 and Ubq-UIM2)[17].

DISCUSSION AND CONCLUSION

When ΔHU,Tm or both parameters are increased for a certain protein in a new condition, it expresses that protein is stabilized[18]. In many cases it is reported that increment or decrement of ΔHU and Tm is not be consisted together. Sufficient evidences are presented and tabulated in table 1 for nine possible relationship between Tm and ΔHU . Therefore it can be concluded that ΔHU and Tm for a certain protein can be studied independent. In fact amount of ΔHU represent degree of overall intra molecular interactions so many interactions are accompanied with more enthalpy change [19]. and more compactness [20]. As it is shown in table 1 Tm parameter reflects local intramolecular interactions for molecule. If the part of protein that is involved in local intramolecular interaction be located in the critical region of molecule, Tm value will change significantly . Therefore Tm can be introduces as molecular frame lock. This lock may apply to compact or loss protein structure. Enhancement of Tm is accompanied with tightness of molecular frame lock which induces higher stability for protein. The increment of enthalpy of unfolding is consistent with compactness of protein structure. Based on this hypothesis new description of Tm and ΔHU can lead to better understanding of structural aspect of protein molecules.

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